
What you see is what you get:
Brain mechanisms underlying
action-modulated perception

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ISBN: 978-90-8891-398-3
Printed by: Proefschriftmaken.nl
Published by: Uitgeverij BOXPress, Oisterwijk

The research in this thesis was made possible by an open competition grant from the Netherlands Organisation for Scientific Research (NWO 400-05-134).

Financial support by *SR Research Ltd.* for the publication of this thesis is gratefully acknowledged.

The research in this thesis was performed at the department of psychiatry, University Medical Center Utrecht, and at the division of experimental psychology, Utrecht University, The Netherlands.

What you see is what you get: Brain mechanisms underlying action-modulated perception

Je krijgt wat je pakt: hersenmechanismes achter actie-gemoduleerde perceptie

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 29 maart 2012 des middags te 12.45 uur

door

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geboren op 13 september 1981
te Vlissingen

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“If the doors of perception were cleansed, everything would appear as it is – infinite”

-William Blake

“Your focus determines your reality”

- Qui-Gon Jinn, Jedi Master

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Chapter 1

Introduction

One more cup of coffee for the road

Being a visuomotor scientist must be exhausting work, since they seem to be heavy coffee drinkers. All they seem to be talking about, is picking up cups of coffee from a table. Sadly, they never seem to be able to sit back and enjoy them. Mainly, because they seem to be fascinated by the processes that underlie the grasping of the cup and the subsequent motor behavior of lifting it off the table. Interestingly, herbal tea just doesn't seem to spark the same interest.

Being interested in visuomotor processes and a fan of freshly brewed coffee, I must succumb to the same behavior. So, imagine someone handing you a cup of coffee. The first thing you'll probably do, is look at it. The second thing you probably do is to grasp the cup. I spent the last few years trying to understand what happens when we perform those two little actions. Specifically, I have focused on what happens just before you do these things. Just before you look at the cup. Just before you grasp it.

Interesting things happen to your vision when you prepare to do these things. Just before you move your eyes toward the cup, your attention is already there, giving you a better image of the cup. Just before you actually grab the cup, your vision will focus on aspects of the cup that are important for grasping it, such as the angle the person is holding it and its size. This effect is what we like to call action-modulated perception. This thesis is all about that. But first, let me sit back and enjoy this cup of coffee.



1. Introduction

1.1 Action-modulated perception

‘Action-modulated perception’ refers to the process of perceptual enhancement of action-relevant features that occurs during the preparation of an action, before its actual execution. For instance, when preparing to grasp an object, certain features of the object that are relevant for the execution of the action (its orientation and size, but not its color) will be enhanced (the perception of these specific features is improved). Also, when preparing an eye movement, the intended target location will be enhanced (spatial location being the relevant ‘feature’). The behavioral advantages are easy to see, especially in the case of hand movements. If the visual system is made ‘ready’ to better process relevant parts of the incoming visual information, the execution of the intended action will improve.

This phenomenon is due to a close coupling of perception and action. Historically, many studies have reflected on the way action influences perception. Gibson [1] proposed the view that objects in the world are perceived by their ‘affordances’, that is, the actions that can be performed on the objects. Here, perception is directly coupled to possible actions, steering perception toward action relevance. The selection-for-action view [2] proposes that processing of visual information is targeted toward actions. That is, that whenever one looks at an object, it is processed with a focus on action-relevant features. This, in turn, can trigger effective execution of an action. The closest coupling between action and perception was formulated by the premotor theory of attention [3], which goes against the existence of a separate ‘attentional control’ mechanism. Instead, it states that the preparation of an action also entails its attentional preparation and even goes as far as saying that this is the same thing. This was initially formulated for eye movements, where covert shifts of attention are attributed to unexecuted eye movements.

This entails a strict coupling between spatial attention and eye movements. An important demonstration of this coupling was shown in a cued saccade paradigm by Deubel and Schneider [4]. Here, subjects were instructed to make an eye movement to a specific peripheral target. However, just before the actual execution of the eye movement, a discrimination target was briefly visible at a certain location. When the instructed eye movement was toward the location where the discrimination target would appear, performance was good. However, performance suffered dramatically when the instructed eye movement was toward a different location than the location where the discrimination target would appear. Crucially, the discrimination target occurred after a go-cue, but before any eye movement was executed. Thus, the preparation of the eye movement caused spatial attention to shift toward the eye movement target location, and that location only. This strong coupling has been shown repeatedly in existing literature [5–8]. This fits with the notion of action-modulated perception, where the preparation of an action (eye movement) modulates perception for the upcoming action.

Evidence for the existence of action preparation influences on perception has also been found for other actions, such as grasping and pointing actions. Initial evidence in a study by Craighero et al. [9] shows that grasping (onset) reaction times are reduced when a prepared grasp is congruent with a graspable visual stimulus that served as a go-cue. The authors attribute this to enhanced processing of action-relevant features of the go-cue. Another line of evidence comes from a study by Bekkering and Neggers [10] where eye movements scan paths were tracked while engaged in a feature conjunction search task (color, orientation). Here, less orientation errors were made (in the eye movement scan paths) when subjects were instructed to grasp a bar of a certain orientation and color than when instructed to point to the search target. This again was indicative of enhanced processing of orientation during grasping preparation. This effect has been replicated in similar designs [11–13]. Although encouraging, these paradigms did not provide direct evidence of a perceptual effect of action preparation, as the recorded performance measures were also motor acts. These results may have been caused or contaminated by interactions between motor systems, and not necessarily by interactions with perception.

It is likely that the action-modulated perception mechanism is mediated by cortical brain areas that are involved in motor planning and perception. Therefore, before proposing a neuronal model explaining action-modulated perception, I will briefly summarize the main perceptual and motor systems in the human brain.

1.2 Motor systems

The cortical motor network for both eye movements and hand movements consists of a fronto-parietal network ([14–17]). The most important areas involved in generating and executing eye movements are the *frontal eye fields* (FEF), (*lateral intraparietal sulcus* (LIP or the ‘parietal eye fields’, PEF, which is the part of the intraparietal sulcus that is engaged in eye movements) and the *superior colliculus* (SC) [15,18,19], see figure 1A. The FEF and IPS are heavily interconnected and are involved in preparing eye movements and they respond to behaviorally relevant visual stimuli. These areas project to the SC either directly or indirectly via the basal ganglia. In the SC, different inputs are integrated, and eye movements can be executed or suppressed through projections to the brainstem reticular formation [15].

A similar fronto-parietal network exists for reaching and grasping actions [16,17]. An obvious part of the network is the *primary motor cortex* (M1), which is the main motor output to the spinal cord. The frontal parts of the network are comprised of the *dorsal* and *ventral premotor areas* (PMd and PMv), which have differential connections to the parietal areas [20,21]. The parietal areas include the ‘*parietal reach region*’ (PRR), which is located at the medial bank of the IPS in the monkey and the *anterior intraparietal area* (AIP). As its name suggests, the PRR is primarily involved in reaching movements [22–24] and has strong connections with the dorsal premotor area (PMd). AIP, however, has strong connections with the ventral

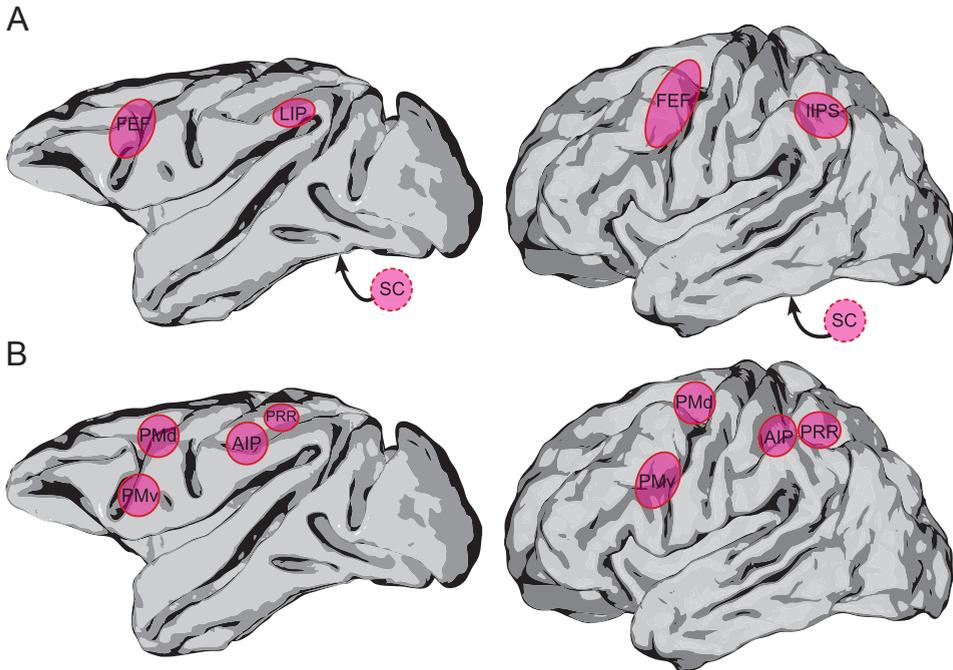


Figure 1. Schematic overview of the most important areas for eye movements (A) and grasping and reaching movements (B) for both the monkey (left) and human (right). abbreviations: FEF: frontal eye fields, LIP: lateral intraparietal area, SC: superior colliculus, IIPS: lateral intraparietal sulcus, PMd: dorsal premotor area, PMv: ventral premotor area, AIP: anterior intraparietal area, PRR: parietal reach region.

premotor area (PMv) [20] and is involved in the planning of grasping movements (in humans: [25–27], in monkeys: [28,29]). In this AIP-PMv grasping network, the PMv is thought to be mostly involved in selecting the grasp type and AIP in hand orientation [28,30,31]. Note that the AIP has primarily been investigated in monkeys, where the area has a clear definition. The human homologue of AIP has not clearly been established and is therefore sometimes referred to as hAIP (human AIP) or by its location aIPS (anterior intraparietal sulcus). For clarity, both AIP and its human homologue are referred to as ‘AIP’ for the remainder of this thesis.

1.3 Perception

Visual information that enters through our eyes is not processed by a single functional unit, but by plethora of specific visual areas. The first and foremost (or hindmost actually) is the primary visual area ‘V1’, which receives input from the retina through the lateral geniculate nucleus. V1 is the most basic processing area for visual information and is essential for conscious vision. It contains columns of orientation sensitive cells which are retinotopically organized to represent the entire visual field. From V1, visual information is projected to other visual areas which process this information in increasingly complex and specialized ways (aptly named V2, V3, and so on). Brain areas that are visually responsive extend well beyond

the occipital cortex, into parietal and temporal areas (for review, see [32]). While many visual areas have been identified, a clear functional specialization has yet to be discovered for a large number of these areas. It is clear though, that this intricate visual system has not evolved purely for the enjoyment of beautiful sunsets. An influential organization principle has been proposed that there are different ‘streams’ or pathways of processing in the visual cortex, namely the ‘dorsal’ and ‘ventral’ stream [33]. From V1, visual processing can either follow a ventral pathway, moving processing upstream toward the temporal cortex, or a dorsal pathway leading to the posterior parietal cortex. These streams are thought to reflect a division of labor within the perceptual system. Where the ventral stream is focused on perceptual processing toward the goal of object recognition and representation of the visual world, the dorsal stream is thought to process visual information for the sake of actions [33–35]. This dorsal stream processes incoming visual information in terms of action parameters and feeds it into ‘motor’ areas in the parietal areas, such as AIP and the lateral IPS (LIP). Thus serving mans most important function, motor output.

1.4 Top-down feedback

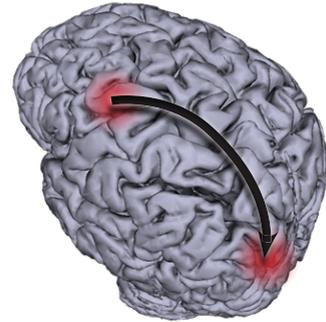
Specialized functional areas receive input from more basic processing units, but information also flows in the reverse direction. Projections from areas that are ‘higher’-order processing areas commonly project to ‘lower’ processing areas to influence processing at an earlier stage. These ‘top-down’ feedback projections enable dynamic processing of incoming information. This is an essential property as one needs to be able to dynamically react to incoming information. Factors such as expectations, current goals and context can be important in determining which parts of the incoming information are relevant (for review, see [36]). These may be implemented by simple shifts of spatial attention or complex modulations of specific features. A simple example is the enhanced processing of a stimulus at a cued location [37]. Here, spatial attention is deployed at a certain spatial location because the stimulus is expected there. These projections can have differential effects on processing, depending on the content of the projection. For instance, spatial attention modulates the amplitude of neuronal responses in a certain area in V1, but specific features can be enhanced by the sharpening of the tuning curves for that feature [38–41].

Perception is thus the result of a dynamic interaction between top-down (feedback) and bottom-up (input) processes. It has been suggested that this interaction is mediated by partially segregated networks. A fronto-parietal network, consisting of intraparietal and superior frontal areas, is thought to control goal-directed action and top-down control. The other network, consisting of temperoparietal and inferior frontal areas, is thought to be involved in the detection of behaviorally relevant bottom-up input and can act as a ‘circuit-breaker’ for the ‘top-down’ network [42]. This ‘top-down’ network shows great similarities with fronto-parietal networks engaged in motor planning and execution. Large overlaps

have been found between networks involved in attention and motor planning, especially for eye movements [14,43,44].

2. The neural mechanism underlying action-modulated perception

What neural mechanism might underlie action-modulated perception? Some evidence comes from the oculomotor system. Here, the FEF plays a critical role in generating voluntary eye movements. It is also strongly implicated in shifting spatial attention [5,45–48], which fits well with the idea of the premotor theory of attention. A strict interpretation of the premotor theory would entail that the attentional effects and the motor planning are implemented in the same region [14,43]. However, recent evidence suggests that the FEF exerts its attentional effects



through top-down feedback connections to the visual areas [49–52]. It may be that, during the preparation of an eye movement, the spatial attention shift, which is well established ([4–8]), is mediated by sending feedback signals to the early visual areas. During eye movement preparation, the FEF contains information that is relevant for perception, namely the spatial coordinates of the intended eye movement endpoint. This, combined with its connectivity with the visual areas, make it an ideal candidate for providing relevant feedback. Therefore, a likely implementation of action-modulated perception for eye movements would be through FEF-occipital feedback projections. Chapters 2 and 3 investigate whether this is indeed the case.

Where the FEF is a plausible cortical visuomotor area implementing action-modulated perception during the preparation of eye movements, which area might be responsible for this effect for actions such as grasping? This may be implemented in a similar manner, where a specialized (visuo-)motor area provides action-relevant feedback to the visual areas, depending on the motor parameters. A critical area for grasping actions is the anterior intraparietal area (AIP) [25–29]. In monkeys, this region has been shown to contain both motor and visually responsive neurons [29,53,54]. Therefore, being a cortical visuomotor area specific for grasping actions, AIP is a plausible mediator of action-modulated perception effects for grasping actions. To test this hypothesis, a behavioral paradigm was first established for evoking perceptual modulations during grasping preparation in chapter 4. Subsequently, the involvement of AIP in this paradigm was tested in chapter 5. Whether AIP exerts its effects through top-down feedback connections to the early visual areas was tested in chapter 6.

3. Methods

Following is a brief overview of the main methods employed in this thesis.

3.1 Electro-encephalography

Electro-encephalography (EEG) is the recording of volume conducted brain activity using scalp electrodes. In an experimental setup, a number of electrodes are placed on the scalp of a subject, usually by wearing a cap, along with some conductive gel to reduce impedance. The number of electrodes varies (e.g. 64 in our study, see chapter 2), but most current studies use 32, 64 or 128 scalp electrodes. A few electrodes are usually added to record eye movement activity and one or more reference sites. Since the electrical activity of single neurons is much too weak to pick up, each scalp electrode picks up underlying electrical activity from large bundles of synchronously active neurons. This electrical activity most likely stems from post synaptic potentials of cortical pyramidal neurons. EEG enables the recording of brain activity with high temporal (<ms) resolution, but low spatial resolution (>cm).

In this thesis (chapter 2), we use EEG for the recording of *event-related potentials* (ERPs). This type of analysis is focused on obtaining the brain activity pattern due to a certain event (e.g. a stimulus). Because the noise in the recording usually exceeds the signal of interest, multiple repetitions need to be averaged to obtain the ERP. While this ERP reflects brain activity with high temporal accuracy, the exact location of its source is difficult (if not impossible) to obtain. This is not just because of the low spatial resolution (due to electrode spacing and smearing of activity over the scalp), but because the obtained distribution of activity could have potentially been evoked by an infinite number of source configurations. Thus, EEG source localization can only obtain a 'best guess'. However, the millisecond precision recording of electrical brain activity makes it an invaluable research tool.

3.2 Transcranial Magnetic Stimulation

You could say that *transcranial magnetic stimulation* (TMS) is a sort of reverse EEG*, as you place a device on the scalp that can evoke electrical activity in underlying neuron bundles. A TMS pulse is generated by running a large current through a coil of wire. This produces a magnetic field which is orthogonal to the coil (usually about 1-3 Tesla). In turn, this generates an electric field in the underlying tissue through electromagnetic induction. This electric field stimulates a patch of neurons (by depolarization of their axons), given that the stimulation intensity is sufficient. In a standard setup, TMS can have effects up to 2-3 cm under the scalp, which limits stimulation to the cortical surface. Its focality lies in the vicinity of 1-2 cm² [55,56].

When applied to the primary motor area (for instance, the hand area), TMS has a directly visible effect in the form of muscle twitches. This is a common way to

* In fact, it is more like reverse MEG (*magneto-encephalography*)

determine the stimulation intensity needed to evoke a response in a certain subject (as this varies per person). When applied to the primary visual area, flashes of light, or phosphenes, are observed by the subject. Stimulation of other brain areas may not have such a directly observable effect. This stresses the need for accurate placement of the TMS coil (because there is no direct effect to test whether the target area is being stimulated). For the TMS studies in this thesis, stereotactical navigation was used that coregisters an individual MRI anatomical scan with the head of a subject to precisely locate the TMS target.

TMS has been described as a ‘virtual lesion’ technique, but whether its effect on brain function is disruptive or facilitating depends on a number of factors, such as stimulation intensity, (activity) state of the underlying area, stimulation protocol (single pulses or multiple pulses) and frequency, coil type and the task (if any) the subject is engaged in. And, of course, your personal definition of disruption or facilitation (after all, suppression of an interfering process may facilitate performance on a certain task). As a general principle, single pulses evoke activity in the underlying brain area, and thus have an excitatory effect. Repetitive stimulation seems to inhibit the activity in an area and the effect, depending on the duration and frequency, can last much longer (up to multiple hours [57]).

Regardless of the specific effect that TMS has on a brain area, it is a valuable tool for establishing causal involvement of brain areas in specific processes. By applying TMS to a brain area of interest or a control area during a certain task and compare task performance, one can draw solid conclusions on the involvement of this specific brain area, whether the effect be facilitating or disrupting. Additionally, by using single pulse TMS, one can not only ask whether an area is involved, but also when. Due to the short time frame of TMS pulses (<ms), time courses of events can be investigated with high spatial and temporal accuracy.

3.3 Psychophysics

Psychophysics investigates the relationship between a physical stimulus and its subjective effect. The most common psychophysical method of investigation, and the one employed in chapter 4, is based on signal detection theory. Signal detection theory provides a framework for studying decisions in uncertain or ambiguous situations [58]. As its name implies, this can be the detection of the presence of a certain weak signal (e.g. an auditory tone) in noise (e.g. auditory white noise). In a simple situation, an observer might listen to presentations of white noise, which could contain a target tone. The listener may correctly indicate that the tone is present in the presented sound (a ‘hit’), or fail to hear it (a ‘miss’). It could also occur that the listeners indicates the presence of the target tone, while it was not actually presented, this is a ‘false alarm’. Finally, the listener could correctly indicate that there is no tone, which is a ‘correct rejection’. Over trials, one can calculate the ‘*hit rate*’ (HR, the percentage of hits over all trials) and the ‘*false alarm rate*’ (FA). The miss rate and correct rejection rates are redundant, as they contain the same information as HR and FA (1-HR and 1-FA respectively). These measures (FA and HR) have a

special dependency, as the hit rate by itself can be unreliable if the false alarm rate is high. This is easy to see when imagining a situation where someone always indicates the presence of the target signal. The HR would be 100% (and a FA of 100%), but it would not be a true reflection of the detection performance of the observer. The tendency to say ‘yes’ (resulting in higher HR and FA) or ‘no’ (resulting in lower HR and FA) in detections is the response bias. A true measure of detection performance is free of response bias. This measure, d' , can be calculated by subtraction of the z-transformed HR and FA; $d' = Z(\text{HR}) - Z(\text{FA})$. This is under the assumption that the variance of the signal is equal to the variance of the noise. The parameter d' describes how well an observer can detect a signal from noise, where higher values indicate high separability from noise. In a perceptual detection experiment this can be taken as a clean measure of perceptual sensitivity.

3.4 Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging (fMRI) is an ‘attractive’ and relatively recent addition to the experimental arsenal, which enables insight in brain activity with high spatial accuracy. Basically, subjects lie in a MRI scanner which, for the most part, consists of a huge magnet. Because of the imposed magnetic field, which should be homogenous, a portion of the (hydrogen) protons will align with the magnetic field. By applying a radio frequency pulse of a certain resonance frequency, these protons will absorb this energy and lose alignment with the main magnetic field. In time, these protons will come back into alignment and form an ‘echo’. This echo is used to form an image of whatever was excited. A lot of information can be inferred from the echo, such as the different magnetic properties of the tissue that was excited. This is used for the ‘f’ part of fMRI, which is based on the different magnetic properties of oxygenated and de-oxygenated blood (specifically, hemoglobin). This is the *blood oxygenation level dependent* (BOLD) contrast and provides a measure of brain function through neurovascular coupling. As neurons use oxygen during activity, (oxygenated) blood flow will increase in this area, causing a difference in the oxygenated/deoxygenated blood levels and therewith a magnetic difference. By acquiring multiple scans with short intervals, the BOLD signal time course can be measured for each area, or voxel, in the brain. Importantly, the signal that is picked up with fMRI is not direct brain activity, but a reflection of this activity in blood oxygenation. The increase in oxygenated blood flow is a rather slow process (in the order of multiple seconds) and fMRI therefore has limited temporal resolution.

The strength of the magnet of the scanner is an important factor in fMRI. As the strength increases (common varieties are 1.5, 3 and 7 Tesla) the amount of aligned protons increases, enabling better signal to noise ratios. With higher field strength, one can either obtain the same signal in shorter time, or get the same signal at higher resolution. On the other side of the coin, the susceptibility for inhomogeneities in the magnetic field increases with field strength, making scans more prone to artifacts.

fMRI data can be analyzed in a variety of ways. The classic, *general linear model* (GLM), approach is to model the expected BOLD time course and fit it to the

data. Those voxels that shows a significant correspondence to the model are said to be ‘active’ during whatever condition the model was based on. By subtracting the active voxels over different conditions, brain activity corresponding to a certain experimental manipulation can be isolated.

A new analysis technique, which is used in chapter 6, is *multivariate* or *multivoxel pattern analysis* (MVPA). Here, the activity evoked by different conditions is not subtracted, but subjected to pattern classification. Here, a subset of voxels (a ‘pattern’) is extracted for each condition and used to train a classifier, such as a support vector machine. This classifier can learn to discern between activity patterns evoked by different conditions and generalize this to newly presented examples. The ‘prediction’ performance is taken as a measure of how well the different conditions can be separated. Accurate prediction scores indicate that there must be a consistent difference in activity between conditions for this voxel subset. The subsets can be different areas in the brain and reflect differential involvement of this area for the conditions. By analyzing the difference between voxel activity patterns, instead of amplitude differences between voxels, this technique is even able to pick up differences in activity that are below the spatial resolution for fMRI, such as activity differences in the orientation columns of V1 [59].

4. Outline of the thesis

The main aim of this thesis is to investigate the effects of action preparation on perception and the neural mechanisms that are involved in both the oculomotor (ch 2 & 3) and skeletomotor system (ch 4-6).

While the strong coupling between eye movement preparation and shifts of spatial attentional has been shown in a number of studies (e.g. [4–8]), it is unclear what the underlying neural mechanism is in humans. Therefore, **chapter two** investigates the areas involved in- and time course of- the spatial attention shifts that occur during eye movement preparation using EEG. **Chapter three** aims to establish the involvement of the FEF and IPS in this mechanism by applying TMS during eye movement preparation and assessing its effect on perception.

If action-modulated perception is a general phenomenon, it should also occur during the preparation of actions other than eye movements, such as grasping. Initial evidence that this may be so has been found [9–13], but only through indirect measures. Therefore, in **chapter four** a behavioral paradigm is established using a psychophysical measure of perception. This paradigm is used in **chapter five** in combination with TMS to investigate the involvement of AIP in action-modulated perception of grasping movements. Finally, **chapter six** investigates the effects of grasping preparation on the early visual areas using fMRI in combination with multivoxel pattern analysis techniques.

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Chapter 2

Lateralized frontal eye field activity precedes
occipital activity shortly before saccades:
evidence for cortico-cortical feedback as a
mechanism underlying covert attention shifts

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Journal of cognitive neuroscience (2010) 22(9), 1931-43

1. Abstract

When an eye movement is prepared, attention is shifted toward the saccade end-goal. This coupling of eye movements and spatial attention is thought to be mediated by cortical connections between the FEFs and the visual cortex. Here, we present evidence for the existence of these connections. A visual discrimination task was performed while recording the EEG. Discrimination performance was significantly improved when the discrimination target and the saccade target matched. EEG results show that frontal activity precedes occipital activity contralateral to saccade direction when the saccade is prepared but not yet executed; these effects were absent in fixation conditions. This is consistent with the idea that the FEF exerts a direct modulatory influence on the visual cortex and enhances perception at the saccade end-goal.

2. Introduction

When faced with an abundance of stimuli, the visual system must select certain stimuli over others for elaborated processing. This mechanism, selective attention, can be directed and biased in many ways, providing emphasis on certain perceptual components. Attention can be drawn toward an object or location by virtue of its characteristics and salience, which is referred to as stimulus-driven or bottom-up attention. When driven internally or top-down, attentional selection originates from an internal state. This type of attention is often driven by a specific goal or task, such as walking down the street or opening a door. It has been found that action and attention are closely coupled in the brain. In fact, selective visual attention in the absence of eye movements (covert attention) is often attributed to unexecuted eye-motor commands, a theory known as the premotor theory [1]. Numerous studies support this theory, or at least a large functional and anatomical overlap [2–7]; for a review, see [8]. This theory has been contested [9–12], in that attentional shifts do occur without eye movements and vice versa. Despite this, it is generally acknowledged that preparing an eye movement is a strong bias in the locus of visuospatial attention, albeit not the only one.

Deubel and Schneider (1996)[13] demonstrated a tight coupling between eye movements and spatial attention shifts. Performance on a discrimination task was enhanced only if the saccade end-goal and the target to be discriminated were at the same spatial location. Discrimination performance suffered severely when the saccade end-goal was different (although the target was spatially adjacent) from the discrimination target (DT). Importantly, no eye movements were yet made at the moment of DT presentation. This automatic coupling has also been shown in other studies [14–16]. Recent evidence suggests the existence of cortical (feedback) connections between motor and visual areas that could mediate attentional shifts. The FEFs are key cortical areas for saccade preparation and execution. Stimulation

of the primate FEF resulted in increased activity in the monkey visual area V4 when receptive fields matched [17] and increased discrimination performance [18–20]. Supèr and colleagues [20] found enhanced activity in the monkey primary visual cortex 100–200 ms before the initiation of a saccade when the recorded cells receptive field was in the saccade end-goal location.

In humans, evidence is emerging for these cortical connections. A study by Grosbras and Paus [4] showed increased visual detection performance when TMS on the FEF was applied 100–40 ms before target presentation. Lowered phosphene thresholds were found after TMS on the FEF in a study by Silvanto and colleagues [21]. A concurrent TMS–fMRI study by Ruff et al. (2006) [22] stimulated the right FEF while measuring BOLD response in posterior brain regions. It was found that FEF–TMS modulates activity in visual areas, including the primary visual cortex, which was confirmed by a recent EEG study Taylor and colleagues [23]. Although the studies by Ruff et al. and Moore and Armstrong (2003) found effects in the periphery, indicating that these effects subserved saccade planning (as saccades are made into the periphery), this retinotopy has not been consistently found [21,24].

Recently, in a study by Neggers et al. (2007)[14], a brief train of three TMS pulses was applied to the FEF just before subjects made a saccade to a DT (using the exact same paradigm as Deubel & Schneider, 1996 [13]). This diminished the known discrimination performance gains at future saccade targets (STs), although it did not disrupt saccade execution. This showed that by modulating activity in the FEF, discrimination performance gains (and therefore presumably attentional shifts) before saccades could be affected and that there is a tight coupling of saccade preparation and visual attention.

The present study elaborates on the results of the preceding TMS study. From that TMS–FEF study, it seems apparent that the FEF can induce visual processing changes in another region upstream in the visual processing pathways. Most likely this region entails the visual cortex, although that was not explicitly demonstrated (for other options, see Neggers et al., 2007[14]). Therefore, the same experimental design (the Deubel and Schneider task) was used while recording the EEG. This will give insight in the exact temporal order of activation for cortical regions contributing to attentional deployment during both directional cueing and saccade preparation. Specifically, after saccade-go but before the execution of the actual saccade, we expect to find activity that reflects the automatic coupling of eye movements and spatial attention. This would most likely involve the FEF, followed by activity in the visual cortex. The intraparietal sulcus (IPS) is also an important part of the attentional network [25–28]. However, on the basis of findings from a preceding TMS study from our group [29] and Ruff and colleagues (2008) [30], we expect no IPS involvement here because no influence of the IPS has been found in these studies.

In addition, EEG during the cue-go interval can reveal mechanisms involved in the control of attention as well. It has been shown that cueing the location of an upcoming DT elicits several distinct neural events, most notably the early directing

attention negativity (EDAN), the anterior direction attention negativity (ADAN), and the late directing attention positivity (LDAP) [25,31–34]. The EDAN is a lateralized occipital negativity that was originally thought to reflect attentional deployment; however, more recently it has been linked to the relevant part of the cue, that is, the part that signals the direction of attention [35]. The ADAN and the LDAP are thought to reflect (covert) attentional deployment and to originate from the FEF and the IPS, respectively [25]. In the current study, besides saccade-induced attentional shifts, we expect to find these established effects of attentional deployment during the cueing period in the FEF and IPS to assess the validity of the current paradigm.

3. Methods

Subjects were asked to make an eye movement toward a peripheral target while a DT appeared either at or near the ST. This experiment is a replication of Experiment 2 from Deubel and Schneider (1996) [13] with additional fixation conditions.

3.1 Participants

Thirteen right-handed subjects (8 women and 5 men) participated (including two of the authors). All participants had normal or corrected-to-normal vision. Subjects signed informed consent form prior to participation.

3.2 Apparatus

EEG was recorded using a 70-channel Biosemi ActiveTwo system, which has 64 active Ag–AgCl electrodes (10–10 system). Recordings were sampled at 2048 and 0.16–100 Hz band-pass filtered. Eye tracking was performed using the Eyelink II system (SR Research, Ottawa, Canada), positioned on the left eye, at a sampling rate of 500 Hz. The infrared camera was mounted on a rigid headrest and controlled by a dedicated PC. Subjects sat in a dimly lit room, secured in a chin- and headrest. Stimuli were presented on a 19-in. (40 × 30 cm) Iiyama monitor at 100 Hz and a resolution of 1024 × 768. The monitor was mounted 37 cm above a mirror at a 45° angle, which in turn was 35 cm away from the participant, yielding an effective viewing distance of 72 cm. Stimuli were generated and displayed using the Matlab psychophysics toolbox (version 7.0.4; [36,37]) on a Pentium PC. Both EEG and eye-tracker data were synchronized with stimulus presentation by TTL pulses from the stimulus presentation computer.

3.3 Behavioral paradigm and procedure

In this experiment, we used a modified version of Experiment 2 from Deubel and Schneider (1996)[13], which is a well-known demonstration of the coupling between saccade planning and discrimination performance. To be able to discern between saccade planning and other (task related) activity, two fixation conditions

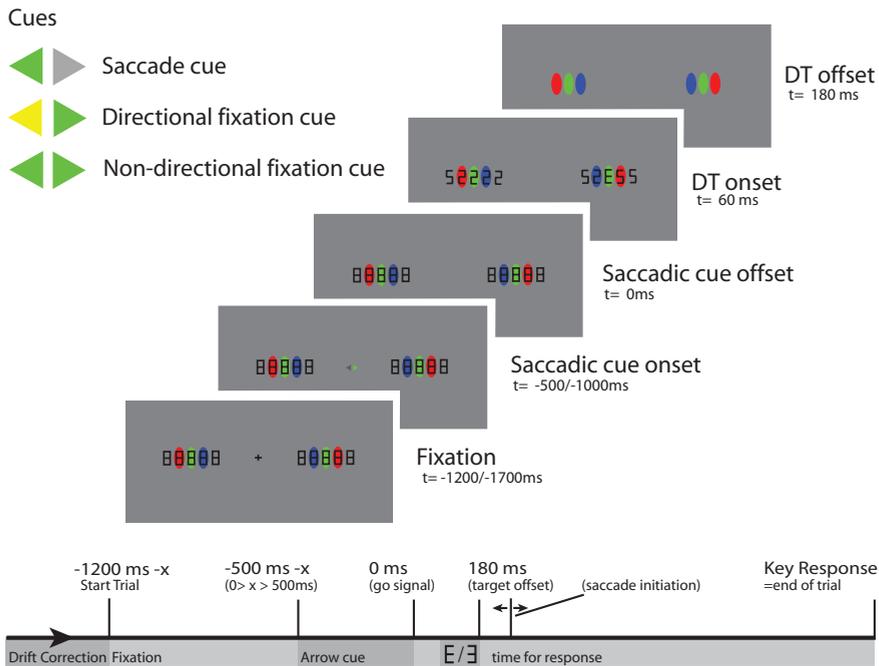


Figure 1. Schematic overview of the stimulus presentation. Different cues instructed the subject to either make an eye movement or covertly attend the cued direction. The sequence of events is shown from bottom left to top right. Each trial started with 6 masked colored ellipses. A cue indicated the type of trial and, in all cases except non-directional fixation, the direction of the upcoming discrimination target. Disappearance of the cue marked the go-signal in the saccade condition ($t=0$). However, before a saccade could be made, the targets unmasked, briefly showing distractors and the discrimination target (60ms), after which only the ellipses remained on-screen. Afterwards, subjects had ample time to respond. The bottom schematic depicts the time-course of events. The variability of the timing is represented by an 'x', which ranged between 0 and 500ms.

were randomly intermixed. Each trial started with a drift correction routine for the eye tracker. Trials did not start until the subjects gaze was centered and stable. At the start of every trial, a fixation cross ($0.52^\circ \times 0.52^\circ$) was presented for 700 ms with three colored ellipses on each side ($0.82^\circ \times 1.64^\circ$ per ellipse, spaced 5° from the fixation point and spaced 0.52° apart; see figure 1). These ellipses were blue (closest to fixation), green (center), and red (outer). Five premasks were overlaid (the center three coinciding with the ellipses) on each side in the shape of a digital 8. After fixation, the fixation cross was replaced by an informative arrow cue. The duration of this cue was randomly chosen between 500 and 1000 ms (step size = 1 ms) to avoid anticipation effects. There were three types of cues: saccade, directional fixation, and nondirectional fixation cues (see figure 1). Saccade cues were red, green, or blue and instructed the subject to saccade to the corresponding ellipse on the side the cue was pointing (the ST). Directional fixation cues were always yellow and indicated the side where a DT was going to be. Nondirectional fixation cues were also red, green, or blue but were double-sided arrows that did not indicate the direction of the upcoming DT. The color-coded cue made this condition comparable to the

stimuli in the saccade condition, apart from the fact that the cue did not indicate a direction. In both directional conditions (saccade and fixation), the colored arrow was accompanied by a gray arrow pointing in the opposite direction to control for visual stimulation differences between conditions. In saccade trials, the disappearance of the cue marked the saccade-go signal. Sixty milliseconds after cue disappearance, the premasks disappeared and the DT appeared (either a normal “E” or a mirrored version, “Э”) surrounded by randomly assigned distracters (digital 2s or 5s).

The DT always appeared at the green ellipse (which was known to the subjects). The DT and the distracters were shown for 120 ms, leaving an empty display with only the ellipses visible. As the average saccade latency is well above 250 ms, hardly any saccades were made before the DT disappeared (which disappeared 180 ms after saccade-go). After the saccade had been made, subjects had ample time to indicate whether they had seen an E or the mirrored version (a “Э”). Trials containing premature saccades (<200 ms) were removed from the data. In fixation trials, the same applied, but no saccade was made.

The experiment was divided in four blocks of 72 trials (lasting ~7–10 min) containing 24 (randomized) trials of each condition (saccade, directional fixation, and nondirectional fixation).

3.4 Analysis

Eye position data from the eye tracker were analyzed. Saccade onsets were detected using a velocity threshold of 30 deg/sec and an acceleration threshold of 8000 deg/sec². Rigorous analysis of the trials was essential to ensure that only correct saccades were allowed. Any trials with saccades before 200 ms after saccade-go (20 ms after DT disappearance) were discarded, as were trials in which saccades landed more than 50 pixels (2.07°) away from the ST. In fixation conditions, trials were rejected where the gaze position deviated more than 50 pixels from central fixation at any time during the entire trial.

EEG data were analyzed using EEGLAB (version 6.02b, [http://sccn.ucsd.edu/eeGLAB/\[38\]](http://sccn.ucsd.edu/eeGLAB/[38])), a Matlab toolbox. All signals were (off-line) average referenced. Both cue-onset- and offset (saccade-go)-locked analyses were made to assess both cue-related effects and saccade preparation activity. Cue-onset-locked epoch interval was -100 until 500 ms postcue, as this is the shortest interval of the variable cue-onset-offset duration. In the cue-offset/saccade-go-locked analyses, the epoch interval was -100 to 200 ms. For comparability, the same intervals were chosen for the fixation conditions.

Trials with premature saccades or small deviations from fixation were removed using data from the eye tracker. To ensure that no ocular artifacts (such as blinks) were left in the data built-in EEGLAB artifact rejection routines were used [38] as well as visual inspection of the data. Rigorous rejection ensured artifact and saccade-free epochs. This led to relatively low acceptance rates ($54 \pm 15.9\%$) in the cue-offset analysis. Acceptance rates did not differ significantly between conditions, ANOVA, $F(2, 24) = 1.535$ $p = .236$. Epoch acceptance rates for the cue-onset conditions were

higher ($81 \pm 8.8\%$) but differed between conditions, ANOVA, $F(2, 24) = 5.142$ $p = .014$. However, because the acceptance rates were high and the difference in acceptance rates is small (2–5%), it is unlikely that this will bias the data.

Separate analyses were done for cue-left and cue-right conditions. Data from the cue-left conditions were then mirrored with respect to the anterior–posterior midline and averaged with the cue-right conditions. This resulted in a scalp map, in which activity in the right hemisphere reflects activity ipsilateral relative to cue direction; the left hemisphere reflects activity contralateral to cue direction. For optimal comparability with the previous TMS study, time windows in the cue-offset condition were chosen on the basis of the TMS times of Neggers et al. (2007) [14], which had stimulation times at 60, 90, and 120ms after saccade-go. Average activity was extracted for time windows 60–90, 90–120, 120–150, and 150–180 ms for all cue-offset conditions at electrodes F7/8, FC3/4, P1/2, PO7/8, and O1/2.

To enable correlational analysis, average amplitudes were extracted for every subject over contralateral frontal electrodes FC3/4 for the early time windows (60–90 and 90–120 ms) and the last time window (150–180 ms) for occipital electrodes O1/2 from a saccade–directional fixation subtraction. As a control condition, the average peak of the N200 (O1/2 = 160–210 ms after cue onset) in the directional fixation condition was also extracted for all subjects.

Source localization was performed using the SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>) software package using an empirical Bayes approach. Here, electrode activity is projected onto a three-dimensional brain model, with a large number of fixed (location and orientation) dipoles, where the amplitude of the dipoles is the parameter to be optimized. The location and the orientation of the dipoles are fixed by assuming a standardized folded cortical surface, with dipoles orthogonal to the cortical sheet [39]. Saccade and directional fixation EEG data (after saccade-go) for each subject were converted from EEGLAB to the SPM data format. A contrast of interest was defined, which is the saccade–directional fixation subtraction. The data were coregistered to the Montreal Neurological Institute (MNI) space using default Biosemi electrode locations. Inverse reconstruction was performed using the classical linear approach [39] using multiple sparse priors with automatic relevance determination optimization [40] for the time window of interest (60–180 ms after saccade-go). This approach can test multiple restricted models and select the most relevant model using Bayesian model selection [41]. Therefore, a number of plausible models were defined, including bilateral and unilateral FEF, occipital area, and IPS (see figure 7). Coordinates were based on localizer findings of Neggers et al. (2007) [14] for the FEF and IPS and MNI electrode location of O1 and O2 for the occipital ROIs. The radius for these areas was a default of 32 mm.

4. Results

4.1 Discrimination performance

Averaged discrimination performance for all 13 participants is plotted in figure 2. A clear increase in performance can be observed when the ST coincides with the DT, ANOVA, $F(2, 22) = 15.11$, $p < .0001$; center/inner target, $t(12) = 3.62$, $p = .004$; center/outer target, $t(12) = 6.05$, $p < .0001$. No significant difference was found between both targets adjacent to the DT (paired samples t-test outer/inner target), $t(12) = 2.14$, $p = .056$. This is a replication of the results of Experiment 2 of Deubel and Schneider (1996)[13] and thus indicates that the subjects performed the task as instructed. Discrimination performance on the directionally cued fixation condition was (nearly) the same as the coinciding ST/DT saccade condition (paired samples t-test; $t = 1.98$, $p = .71$). Nondirectional cues impaired performance significantly compared with coinciding DT–ST trials in the saccade condition (paired samples t-test), $t(12) = 4.63$, $p = .001$, and trials in the directional fixation (paired samples t-test), $t(12) = 3.54$, $p = .004$.

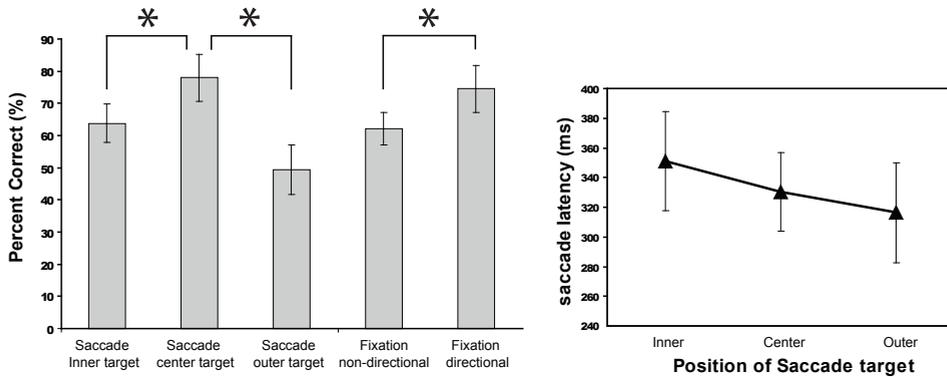


Figure 2. Behavioral performance. The left graph shows discrimination performance for all conditions. The left three bars display performance for the saccade conditions. As the discrimination target is always located at the center target, eye movements toward either inner or outer targets yielded lower performance. The two right bars display performance without eye movements, either with (right) or without (left) directional cueing. The right graph shows saccade latency for every saccade target. No significant differences were found between targets.

4.2 Saccade performance

Saccade latencies did not differ between the STs, $F(2, 22) = 1.289$, $p = .295$. On average, in $60.1 \pm 18.2\%$ of the trials, a saccade was made that was within the strict acceptance criteria (see methods). Subjects did not break fixation in $67.6 \pm 17.7\%$ of the fixation trials. The reason for this low performance is likely the intermixed saccade and fixation trials.

4.3 EEG—Cue-offset/Saccade-go-related effects

The cue-offset/saccade-go-locked analyses revealed saccade preparatory activity. Time windows (60–90, 90–120, 120–150, and 150–180 ms) were chosen to correspond to the preceding TMS article (Neggers et al., 2007). Because the only difference between the saccade and the directional fixation condition concerned the saccade preparation (with similar directional cues), the latter was used as a baseline condition. Grand averages from the directional fixation condition were thus subtracted from the saccade condition.

In turn, activity from the nondirectional fixation condition was subtracted from the directional fixation condition to remove irrelevant task-related activity, that is, correcting for all trial events except for the directionality of the cue possibly evoking covert spatial attention shifts. The latter contrast might therefore reveal lateralized EEG effects evoked by the directional cue itself.

Figure 3 shows saccade-go-locked ERPs for all selected electrode pairs. In the saccade condition (with directional fixation as baseline), a clear, lateralized, positive increase can be seen, contralateral to saccade direction at the frontal electrodes, peaking around 120–150 ms (see also figure 4). Time window analysis shows that this increase is significant at all time windows between 60 and 180 ms after cue offset for the electrode pair F7/8 (paired samples t-test), 60–90 ms, $t(12) = 2.16$, $p = .05$; 90–120 ms, $t(12) = 4.55$, $p < .001$; 120–150 ms, $t(12) = 4.63$, $p < .001$; and 150–180 ms, $t(12) = 4.30$, $p = .001$, and for the time window 90–120 ms for FC3/4 (paired samples t-test), 90–120 ms, $t(12) = 2.52$, $p = .03$. A later, negative occipital effect can be observed at electrode pair O1/2, reaching significance level at 120–150 ms after cue offset. Time window analysis shows that this effect is significant at time windows 120–150 and 150–180 ms (paired samples t-test), 120–150 ms, $t(12) = -2.47$, $p = .03$; and 150–180 ms, $t(12) = -3.01$, $p = .01$.

To check for possible baseline effects, the analysis was also performed using the nondirectional fixation condition as a baseline (see figure 3). Results were very similar for both baseline choices. Lateralized activity in frontocentral electrodes FC3/4 was slightly higher, resulting in more time windows reaching significance level (paired samples t-test), 60–90 ms, $t(12) = 4.06$, $p < .01$; 90–120 ms, $t(12) = 5.34$, $p < .001$; 120–150 ms, $t(12) = 3.63$, $p < .01$; and 150–180ms, $t(12) = 3.65$, $p < .01$. The occipital effect at time window 120–150 ms was not significant (paired samples t-test), 120–150 ms, $t(12) = -0.75$, $p = .47$, whereas it was significant when using the directional fixation condition as baseline, which indicates a possible baseline effect. This difference is reflected in the time window 120–150 ms in the directional–nondirection fixation subtraction (paired samples t-test), 120–150 ms, $t(12) = 3.16$, $p = .01$.

A lateralization difference was found for the occipital effect between saccade directions (see figure 5). When subjects were instructed to make an eye movement to a target on the right, a clear increase in negativity was observed in

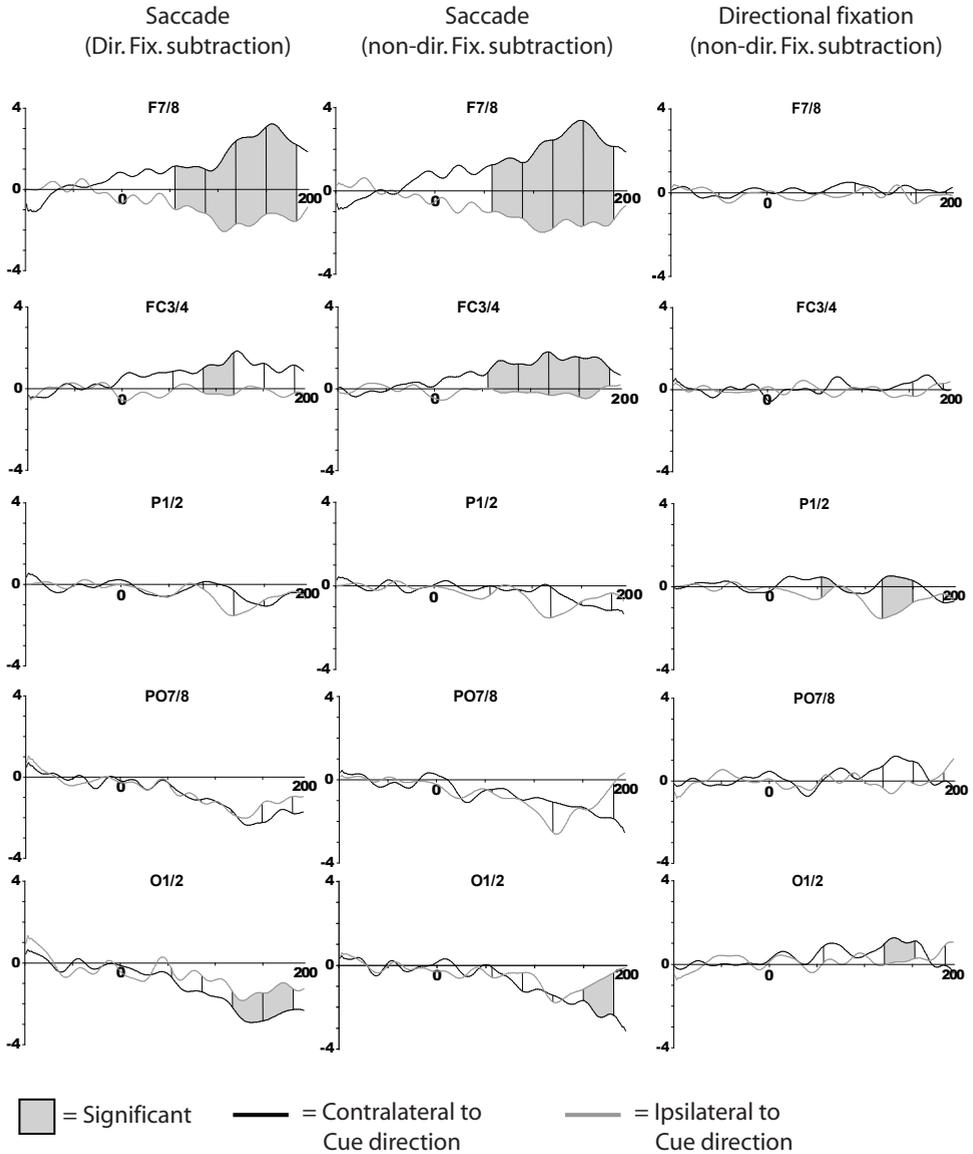


Figure 3. ERP plots after saccade-go (0 ms) for selected electrodes. Shown are plots for the saccade condition, with directional fixation subtracted (left); the saccade condition with nondirectional fixation subtracted (center) and the directional fixation condition with nondirectional fixation as baseline. Contralateral activity is shown in black, ipsilateral activity in gray. Significant differences between contra- and ipsilateral activity for time windows 60–90, 90–120, 120–150, and 150–180 ms are shown as gray-filled areas.



Figure 4. Double subtraction EEG topoplots for the saccade and directional fixation conditions for different time windows. To obtain the double subtraction plots, activity from the left and right hemispheres was subtracted for both conditions, and the resulting activity maps were subtracted again (saccade/cue-left minus saccade/cue-right). Activity in the left hemisphere reflects contralateral activity. Because of this subtraction, only lateralized activity is visible. Shown are plots for the saccade condition, with directional fixation subtracted (left); the saccade condition with nondirectional fixation subtracted (center) and the directional fixation condition with nondirectional fixation as baseline. Plots are averaged over the entire time window.

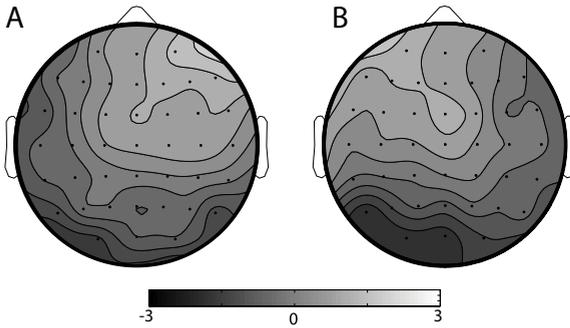


Figure 5. EEG topoplots for the saccade conditions (directional fixation subtraction), averaged over the entire 60- to 180-ms time windows. Shown are the plots for the saccade left (A) and saccade right (B) conditions. These plots were obtained without mirroring saccade directions. In the saccade left condition (A), activity can be seen in the right frontal and bilateral occipital areas; in the saccade right condition (B), activity can be seen in the left frontal and left occipital areas.

the contralateral (left) hemisphere. When instructed to make a saccade to the left, occipital negativity was observed in both hemispheres.

No significant effects were found at parietal electrodes P1/2 or parieto-occipital electrode PO7/8 in any time window.

4.4 EEG - Correlations

A significant negative correlation was found between the average amplitude of contralateral fronto-central electrode pair FC3/5 in early time window 60–90 ms and the late time window 150–180 ms of occipital electrode O1/2 (Pearson correlation FC3/5 contralateral O1/2 = -0.72, $p < .01$; see figure 6). No significant correlation was found between the second time window (90–120 ms) frontal time window

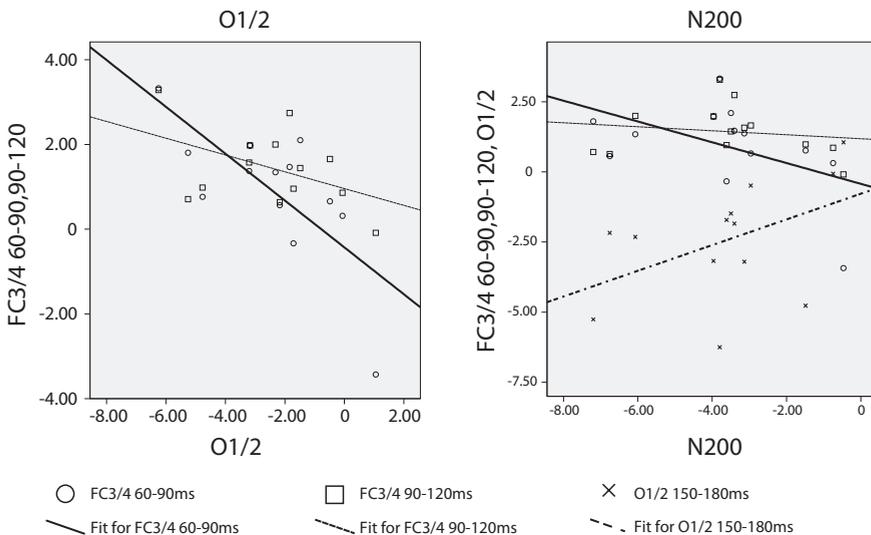


Figure 6. Scatterplots for the correlation analysis between electrodes O1/2 and FC3/4 for time windows 60–90 and 90–120 ms (shown left) and between the N200 peak and the O1/2 and FC3/4 (both time windows). A strong correlation can be seen between the early time window of electrode pair FC3/4 and O1/2. No significant correlations were found for any other combination of electrode/time window tested.

and the occipital electrode pair O1/2 (Pearson correlation FC3/5 contralateral O1/2 = -0.45 , $p = .12$). We recognize that the correlations could be confounded by differences in conductance per subject. A higher average amplitude due to, for instance, skull thickness could result in higher (or lower) frontal and occipital activity for a particular subject without a necessary neural link, which could artificially bias correlation coefficients. To control for this, the average N200 (160–210 ms) of electrodes O1/2 was extracted from the directional fixation condition during the cueing period as an individual measure of subject responsiveness. No significant correlation between occipital N200 and either occipital or frontal electrodes were found (Pearson correlation FC3/5 60–90 ms, N200 = -0.48 , $p = .10$; FC3/5 90–120 ms, N200 = -0.16 , $p = .60$; O1/2 150–180 ms, N200 = 0.46 $p = .12$). This suggests that the obtained correlations are not due to individual differences in conductance but due to actual neural activity.

4.5 EEG—Source localization

We performed source localization on the saccade–directional fixation subtraction to ensure that the observed activity originates from the proposed sources. Results can be found in table 1 and figure 7. Different models were tested, including an unconstrained model. The overall model evidence reflects the likelihood of this model, which incorporates the variance explained and model complexity (for details, see [42]). As expected, the unconstrained model can explain the most variance, as it has most degrees of freedom, which is also reflected in the model evidence. Despite this, we believe that this unconstrained model yields implausible results to maximize

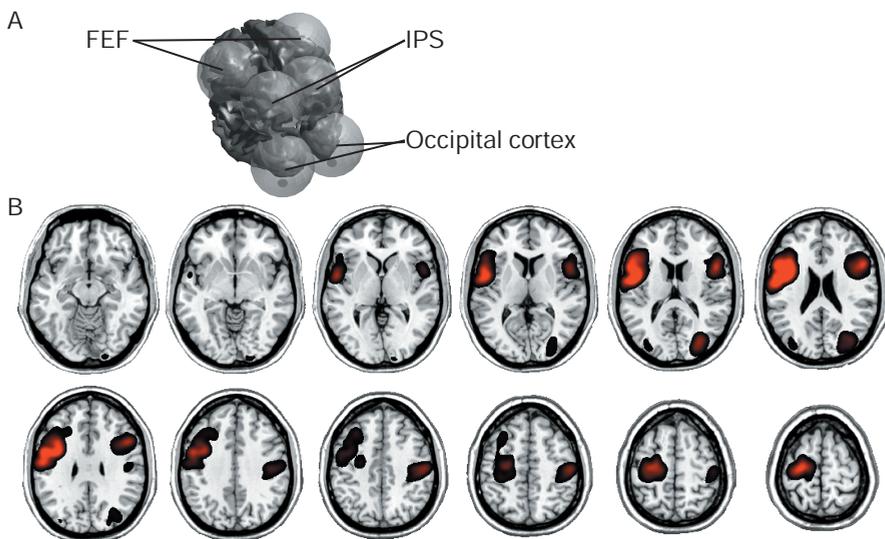


Figure 7. Localization of EEG activity during saccade preparation in the saccade condition (directional fixation subtraction). (A) Sources were restricted to spherical areas shown. Different combinations were used (see table 1). (B) Localization results obtained using the bilateral FEF–occipital model. Slices run from MNI $Z = -12$ (top left) up to $z = 72$ (bottom right).

Table 1. Localization results for different models tested

<i>Model</i>	<i>Explained variance (%)</i>	<i>Model evidence</i>
Unconstrained	95.97	187.4
FEF bilateral	93.58	152
IPS bilateral	79	89.8
FEF-occ bilateral	92.24	154.2
FEF-occ-IPS bilateral	91.33	145.8
FEF contralateral	89.66	141.6
FEF-occ contralateral	90.46	146.2

The unconstrained model does not restrict solutions to specified neural sources, only gray matter. The model evidence reflects the amount of explained variance against the model complexity. IPS = intraparietal sulcus; occ = occipital cortex.

the explained variance (for details, see the discussion section). The proposed models (including the FEF, the IPS, and the occipital sources) explain only a few percent less of the variance (95.97% unconstrained vs. 93.58% constrained) but are based on plausible sources. The model with the highest model evidence is the bilateral FEF–occipital model (154.2), followed by the bilateral FEF model (152.0). Adding the IPS to the model reduces model evidence (bilateral FEF–occipital–IPS = 145.8).

4.6 EEG—Cue offset in the fixation conditions

Time window analysis of the directional fixation condition revealed a significant, lateralized positivity at parietal electrode pair P1/2 (paired samples t-test) for time windows 60–90 ms, $t(12) = 2.34$ $p = .04$, and 120–150 ms, $t(12) = -2.54$, $p = .03$. Also, a lateralized occipital effect (O1/2) in the time window 120–150 ms was found (paired samples t-test), $t(12) = 2.97$, $p = .012$. However, this occipital effect is in the opposite direction compared with the occipital effect observed in the saccade condition: There is an increase in positivity contralateral to the cued direction (see figures 3 and 4). There were no lateralization differences between cue directions.

4.7 EEG—Cue-related effects

Cue-onset-locked ERP analysis was done to assess attentional effects during the cue–target interval [25,31–34], see figure 8). A lateralized increase in negativity was found for parieto-occipital electrode pair PO7/8 and occipital electrode pair O1/2 for the directional fixation condition, contralateral to cue direction, starting at ~200 ms. This was found to be significant in the time window 200–320 ms (postcue): PO7/8 paired samples t-test, $t(12) = -2.92$ $p = .01$; O1/2 paired samples t-test, $t(12) = -2.42$ $p = .03$. This is consistent with an EDAN, as found in previous studies. No significant effect was found at electrode pair P1/2 (paired samples t-test), $t(12) = -0.70$ $p = .50$. Similar activity was observed in the saccade condition for electrode pair O1/2 (paired samples t-test), $t(12) = -2.14$ $p = .05$. No significant differences were found in the nondirectional fixation condition.

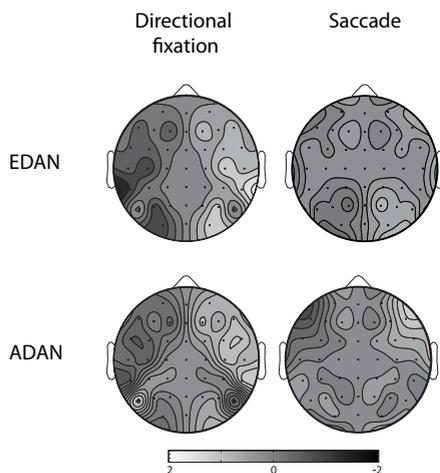


Figure 8. Double subtraction EEG topoplots for the directional fixation and saccade condition during the cueing period. To obtain the double subtraction plot, activity from the left and right hemispheres was subtracted for both conditions, and the resulting activity maps were subtracted again (cue left minus cue right). Activity in the left hemisphere reflects contralateral activity. Because of this subtraction, only lateralized activity is visible. The EDAN plots are averaged over a 200- to 320-ms time window; the ADAN plots are averaged over a 250- to 450-ms time window.

In a 250- to 450-ms time window, increased negativity was found at frontal electrode pair FC3/4 in the contralateral hemisphere (relative to cue/saccade direction) in the directional fixation condition (paired samples t-test), $t(12) = -2.56$ $p = .02$, and at electrode pair F7/8 for the saccade condition (paired samples t-test), $t(12) = -2.13$ $p = .05$. This is consistent with an anterior directing attention negativity (ADAN). No effects were found at other electrodes (P1/2;PO7/8;O1/2). No significant differences were found in the nondirectional fixation conditions.

No indications of an LDAP were observed for any time window, at any of the selected electrodes F7/8, FC3/4, P1/2, PO7/8, or O1/2. There was, however, a lateralized positivity at electrode pair P5/6 in the directional fixation condition, as can be seen in figure 8. However, this was just below significance at a 400- to 500-ms time window (paired samples t-test), $t(12) = 2.13$ $p = .055$. As this effect is only observed in this particular electrode, not typical for an LDAP, and does not seem to be very robust, it is uncertain whether this represents a true LDAP.

No differences in the lateralization were found in the ERP effects described previously between hemispheres for left- and rightward cues.

5. Discussion

Previous studies have established the coupling between eye movements and spatial attention [13,15,16] and the coupling of the FEF and the visual cortex [14,21–23]. This study provides support for the cortical FEF–VC feedback connection as a neuronal mechanism coupling saccade planning in the FEF to attentional processing in the occipital lobe, as has been hypothesized for human observers.

Namely, in the saccade condition, after cue offset, a transient lateralized frontal positivity was found, peaking around 120–150 ms after saccade-go signal. This was followed by an occipital negativity contralateral to the upcoming target. This

supports the view that saccade preparation, likely in the FEF, results in attentional shifts in the visual cortex [14] through FEF–VC connections [4,21–23].

Consistent with our expectations based on the preceding TMS study [14], significant frontal activity was observed contralateral to saccade direction during saccade preparation (after saccade-go). The frontal effect was slightly smaller when the directional fixation condition was used as a baseline. This may be due to the recruitment of the contralateral FEF (relative to cued direction) in the directional fixation condition, although this effect cannot be seen in the topography (see figure 3, right column).

It has been shown that TMS on these frontal areas, the FEF specifically, causes modulation in discrimination performance [4,14]. Lateralized frontal activity was found in the current study at the same time points as the TMS stimulation times of the Neggers et al. (2007) [14] study (see figures 3 and 4). It therefore seems plausible to associate this activity with the deployment of spatial attention shortly before saccade execution (note that during any of the analyzed time intervals, no saccades were made yet, as ensured by the rigorous analysis, excluding trials with premature saccades). This frontal effect does not occur in the fixation conditions after cue offset. This might be due to some hemispheric preparations already having taken place for the cued hemifield. This is reflected by the presence of the ADAN in the cueing period. It is interesting to note the difference in polarity between the ADAN in the cueing period and the frontal positivity after cue offset. Both may reflect preparatory processes in the FEF, but the ADAN might reflect inhibition of the saccade, whereas the frontal positivity might reflect saccade activation. This fits well with findings by Van der Lubbe et al. (2006) [25]. As the FEF are heavily implicated in attentional shifts [5,25,43], for a review see [44]), the combined TMS–EEG results suggest a strong (attention directing) influence of the contralateral FEFs on perceptual performance.

We observed a lateralized occipital negativity late in the saccade preparation period (120–180 ms after saccade-go). It is unlikely that this negativity is the result of target presentation. Activity from the directional fixation condition was used as a baseline, effectively subtracting any visual response to the presented stimuli. Also, target presentation was preceded by masking stimuli, resulting in low visual impact for the unmasking of the target stimulus (and simultaneous unmasking of distracters). Therefore, it is likely that this negativity is the result of an attentional shift, mediated by the FEF and not by the target presentation. A number of observations support this view: The observed negativity occurs approximately 60–120 ms after the onset of frontal positivity, which is in line with an estimated 100-ms conduction time of the FEF to the visual cortex (for details on this estimation, see methods section of Experiment 3 from Neggers et al., 2007 [14]).

Also, in the directional fixation condition, which is the same task in all respects except for the actual execution of the saccade, both the frontal positivity and the occipital negativity were absent after the go signal. This suggests that these processes are related and perhaps coupled. Furthermore, it can be noted that in the directional

fixation condition (nondirectional fixation subtraction), a significant effect is present in the time window 120–150 ms (see figure 3, right panel). Interestingly, this occipital effect is positive, whereas the occipital effect in the saccade condition is negative. This might be due to the different task instructions between these conditions. Whereas in the saccade condition an eye movement has to be executed after the go signal, any eye movements in the fixation conditions have to be suppressed (especially in the directional condition). This active suppression (as opposed to execution) may have differential effects on the occipital areas, perhaps also due to different modulations mediated by the FEF–VC connections.

Correlation analysis shows that the early frontal activity, contralateral to saccade direction, is significantly (negatively) correlated with the occipital activity seen late in the preparation period (150–180 ms after saccade-go). This means that more positive activity per subject is paired with more negative activity in occipital regions, as would be expected from FEF–occipital interactions. This, however, does not prove a causal relationship between frontal and occipital regions, as this cannot be inferred from the present data.

To justify our claims of source origins, a voxel-based source localization was performed on the saccade–directional fixation subtraction (see table 1 and figure 7). First, an unconstrained model was tested, which yielded the best results in terms of explained variance (and model evidence; see table 1). However, we believe that these sources are unlikely to be the true neural sources of our data. The source model places large sources in deep brain regions, for example, near the cerebellum, brainstem, and posterior OFC. These are not only unlikely to generate large cortical surface potentials but are also not likely to be involved in the current task. We therefore compared a number of constrained models on the basis of plausible neural sources in this task, including bi- and contralateral (to saccade direction) FEF, IPS, and occipital areas. The bilateral FEF–occipital model yielded the highest model evidence and explained only slightly less variance than the unconstrained model. This is a good indication that these areas are the actual sources of activity. It is interesting to note that the bilateral models outscore the contralateral models. Therefore, although the contralateral activity is stronger, there is some activity in the ipsilateral hemisphere. The addition of the IPS to the model results in lower model evidence, which confirms our previous results of (no) IPS involvement in this task [29] and the findings by [30]. This is again confirmed when models on the basis of bilateral FEF and IPS are compared (model evidence FEF = 152.0, IPS = 89.8).

There is a difference in the 120- to 150-ms time window in the saccade condition, depending on the baseline used. One might argue that the occipital effect in the saccade condition at 120–150 ms could reflect a baseline effect (see figure 3, bottom panel). For the final time window from 150 to 180 ms, however, this effect was not observed for the directional fixation condition (nondirectional fixation subtraction), and the saccade condition did show a significant difference for this time interval independent of baseline used. This means that the occipital effect at time window 150–180 ms is an effect of saccade preparation and is not a baseline effect.

Previous FEF-TMS studies [4,45] revealed a dominance of the right hemisphere in controlling visuospatial attention. Right FEF-TMS resulted in bilateral discrimination performance increases, where left FEF-TMS resulted only in unilateral (contralateral to cue direction) effects. Other more recent studies have found similar lateralization effects, such as Ruff et al. (2006)[22], who found bilateral effects in the visual cortex after right FEF stimulation using fMRI, and Silvanto et al. (2006) [21], who also found bilateral effects with right FEF-TMS and unilateral effects with left FEF-TMS. In the current study, a similar lateralization difference was found, where saccade preparation to the left invoked right frontal activity and a bilateral occipital effect (slightly lateralized to the right hemisphere). Preparation of saccades to the right invoked left frontal and left occipital activity. This striking similarity argues for an activation of the FEF-VC pathway in the current study.

Although in the current study we have emphasized the coupling between eye movements and attention, a number of studies report a dissociation [9,11,46–48]. For instance, Juan et al. (2004) [11] shows that the locus of attention can be dissociated from an evoked saccade in the FEF of the macaque monkey. This shows that covert attentional shifts are not strictly coupled to saccade preparation. This is likely the result of different neural populations in the FEFs that serve different functions over time. The role of the FEF in visual search, in the absence of eye movements, has been well established [24]. Although this is troublesome for the “hard” interpretation of the premotor theory of attention as posed by Corbetta et al. (1998) [5] and Rizzolatti et al. (1987) [1], this need not be a problem for the results obtained in the current study. The claim we make here is that just before the actual execution of the saccade, the attentional locus shifts toward the intended end point of the saccade and that this is mediated by the FEF. However, this does not imply that saccade preparation in the FEF is essential for attentional shifts; there could be other biasing sources, either within the FEF or elsewhere. It is very likely that the activity found in the current study in the frontal regions does not reflect activity from a homogenous neural population. Instead, it is likely that this is a cumulation of attention-related activity and saccade (motor) preparation. It is interesting to note that this is reflected in the correlation between frontal and occipital activity: Only the early frontal activity (60–90 ms after saccade-go) correlates significantly with the observed occipital effect (at 150–180 ms after saccade-go), not the later frontal time window (90–120 ms).

Cue-locked analyses revealed attentional preparation in the directional fixation and saccade conditions. In the directional fixation condition, activity patterns consistent with EDAN and ADAN (anterior attention direction negativity) were observed. The EDAN has been implicated as reflecting simple cue processing [49,50], and the ADAN indicates that covert spatial attention was allocated to the cued location. The presence of an ADAN supports attentional preparation in the frontal brain regions, likely originating from the FEF during the cueing period. No activity was found resembling an LDAP. This may be due to the short cueing interval used in the current study. These effects were absent in the nondirectional condition.

As expected, without directional cueing, no specific attentional preparation was made. Both EDAN and ADAN were found in the cueing period of the saccade condition. However, in the saccade condition, it was not as consistent as in the directional fixation condition. This may be due to a general reduction of attentional preparation, or it may be that subjects in some trials did not engage in attentional shifts until saccade execution. This does not rule out attentional shifts just before saccade execution, as can be seen in the poor behavioral results when ST and DT do not match.

As in a previous TMS study from our group using an identical procedure as in Neggers et al. (2007) [14,29], no evidence was found for the involvement of the IPS in attentional modulation during saccade preparation, as can also be clearly seen in figures 3 and 4.

The IPS has been implicated as an essential part of an attention controlling fronto-parietal network [25–28] and has been shown to have great functional overlap with the FEF [51–53]. Time window analysis of the electrodes located at or near the IPS (P1/P2) showed no significant, lateralized activity in any time window for the saccade condition. This was observed both during the cueing period and after saccade-go. Also, Bayesian model selection of source locations did not favor the IPS as a contributing source. However, a significant positive correlation was found between the early time window of the ipsilateral (to saccade direction) electrode pair P1/2 and ipsilateral occipital electrodes O1/2. As the effects are limited to the ipsilateral side and the activity is relatively small, we are unsure how to interpret these results. Also, significant lateralized activity was found after cue offset for the directional fixation condition. It may be that parietal areas are recruited for covert attentional shifts. These seem to be independent of frontal attention directing activity. In addition, frontal activity was followed by a lateralized occipital negativity, whereas parietal activity was followed by an occipital positivity. Thus, it seems that the effects are qualitatively different, and it is therefore likely that the IPS does not function as a relay station for frontal signal inducing attentional shifts. This is consistent with findings in a study by Ruff et al. (2008) [30] where TMS on the IPS evoked qualitatively different effect on the visual cortex than FEF-TMS in a preceding study [22].

In conclusion, the present study, combined with the previous TMS study by Neggers et al. (2007) [14], provides strong evidence for a crucial role of the FEF in spatial attention shifts before eye movements. Whether other areas such as the IPS and other frontal areas implicated in attentional shifts may be involved and what their contribution is in the current paradigm is still unclear. From the present results, it seems that they are not directly involved in the coupling of the locus of spatial attention to a saccade goal, as observed for the FEF.

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Chapter 3

fMRI-Guided TMS on cortical eye fields: the frontal but not intraparietal eye fields regulate the coupling between visuospatial attention and eye movements

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Journal of neurophysiology (2009) 102(6), 3469-80

1. Abstract

It is well known that parts of a visual scene are prioritized for visual processing, depending on the current situation. How the CNS moves this focus of attention across the visual image is largely unknown, although there is substantial evidence that preparation of an action is a key factor. Our results support the view that direct corticocortical feedback connections from frontal oculomotor areas to the visual cortex are responsible for the coupling between eye movements and shifts of visuospatial attention. Functional magnetic resonance imaging (fMRI)-guided transcranial magnetic stimulation (TMS) was applied to the frontal eye fields (FEFs) and intraparietal sulcus (IPS). A single pulse was delivered 60, 30, or 0 ms before a discrimination target was presented at, or next to, the target of a saccade in preparation. Results showed that the known enhancement of discrimination performance specific to locations to which eye movements are being prepared was enhanced by early TMS on the FEF contralateral to eye movement direction, whereas TMS on the IPS resulted in a general performance increase. The current findings indicate that the FEF affects selective visual processing within the visual cortex itself through direct feedback projections.

2. Introduction

By using selective visual processing, the human brain can filter relevant parts in a scene from a wealth of distracting information. Such selectivity is commonly referred to as visuospatial attention. Previous psychophysical studies demonstrated that visuospatial attention is tightly coupled to the preparation of saccadic eye movements; objects are perceived better when they are the target of a saccade in preparation [1–4]. Although it should be noted that saccade preparation and covert attentional shifts can be dissociated under the right circumstances [5,6], there is consensus that saccade preparation is an important regulatory factor in covert attentional shifts.

This coupling has been explained in broad terms by the premotor theory of attention [7], stating that shifting attention is related to preparing a saccade. Similar activity patterns during saccades and covert visual attention shifts in the ventral intraparietal sulcus (IPS) and the frontal eye fields (FEFs) support that notion [8–10]. The FEF and IPS are part of the oculomotor control pathway conveying signals from the visual cortex to the midbrain [11] and finally the brain stem [12].

More specifically, recent studies suggest that the FEF controls selective visual processing through reverse projections to the visual cortex. Moore and Armstrong (2003) [13] demonstrated that electrostimulation of monkey FEF sites elicited activity in V4 neurons with matching receptive fields. Supèr and colleagues (2004) [14] reported enhanced activation in monkey V2 neurons 100–200 ms before saccades into the receptive field. Interestingly, FEF electrical stimulation

enhances luminance discrimination performance [9,15], indicating that FEF projections indeed affect visual processing. Furthermore, Ekstrom and colleagues (2008)[16] demonstrated that electrical stimulation of monkey FEF modulated activity as measured by functional magnetic resonance imaging (fMRI) in early visual areas, but only when visual stimulation was present. These findings inspired several attempts to demonstrate the existence of similar networks in human subjects, mostly using noninvasive single-pulse transcranial magnetic stimulation (TMS) of the FEF and/or the IPS. TMS on the FEF or IPS during functional magnetic resonance imaging (fMRI) evokes occipital activation [17,18], albeit of a different nature for each stimulation site. Importantly, the latter studies also reported an increase in visual-discrimination performance along with the FEF-TMS-evoked occipital enhancements. TMS on the human FEF alters ipsilateral occipital electroencephalographic (EEG) signals [19] and enhances visual awareness [17,20,21]. Furthermore, TMS on the FEF lowers the threshold of a second TMS pulse on V4 needed for eliciting phosphenes, vivid TMS-evoked visual illusions [22]. It can be concluded that a single pulse of TMS on the FEF per behavioral trial can enhance visual processing, probably through connections to the visual cortex.

The reported reverse projections from the FEF back to the visual cortex can also explain the strong coupling between saccade programming and visual processing, as demonstrated by Deubel and Schneider (1996)[1], above and beyond the mere enhancement of visual cortical processing by signals from the FEF. That is, when saccade preparatory activation in the FEF, as reported not only for nonhuman [23] but also for human primates [10], is relayed to the visual cortex zones with a matching retinotopic topography, this could explain why perception is improved at locations of upcoming saccades. We could recently show using EEG and source localization that during the same paradigm as adopted by Deubel and Schneider (1996)[1], activation in the FEF contralateral to the saccade in preparation preceded similarly lateralized activation in the occipital lobe, well before saccade execution [24]. The temporal order of cortical activation in the latter study implicates a causal relationship between both areas during saccade preparation, which was further strengthened by a study adopting TMS on the FEF using the exact same behavioral paradigm [4]. The latter study administered a brief train of three TMS pulses onto the FEF just prior to presentation of a discrimination target (DT) at or next to the location to which a saccade was being prepared. TMS on the FEF contralateral to saccade direction disrupted the known coupling between saccade preparation and target discrimination. This was interpreted as TMS disturbing the modulating effect of signals from the FEF to the visual cortex. However, it could not be fully ruled out that the discrimination performance decreases after TMS on the FEF resulted from neural interference with visual processing in the FEF itself. That is, the effects of the TMS pulse train in the FEF might have outlasted the actual time of stimulation and would thus be able to disturb incoming visual information within the FEF. Furthermore, the pathway through which the FEF modulates the visual cortex is not known. The IPS might be included as an intermediate in the FEF projections

to the visual cortex, or the connections could be direct, bypassing the IPS. Ruff and colleagues (2008) [18] also demonstrated that the human IPS is connected to the visual cortex, even though TMS on the IPS yielded different effects compared with TMS onto the FEF.

The present study was performed to clarify the temporal characteristics of FEF involvement in visual attention shifts, as well as the possible pathways by which the FEF might exert its influence over the visual cortex. To establish the critical time window before saccade execution in which the human FEFs have an influence over visual processing, the left and right FEF and IPS were stimulated with a single TMS pulse at variable times during saccade preparation, using fMRI-guided stereotaxy. If fronto-occipital connections are indeed responsible for attentional modulations, TMS signals from the FEF and incoming visual signals from the retina should arrive in the visual cortex around the same time, to result in a maximal influence of TMS with visual processing. Therefore the optimal time from TMS stimulation to the moment of target presentation (which we defined as a moment halfway to DT presentation; see methods) should equal the electrical signal transmission from the FEF to the visual cortex (~100 ms; see following text) minus the transmission time from the retina to the visual cortex (~66 ms), yielding about 34 ms. The average visual latency for the primary visual cortex (V1) of macaque is about 66 ms [25]. This latency is compatible with the time range of the first visual response in human V1, as confirmed using concurrent EEG and fMRI [26]. We roughly estimated the FEF–visual cortex conduction time at about 100 ms based on the following two studies. First, monkey FEF presaccadic preparatory activation is reported to start about 50 to 60 ms after stimulus presentation in a study also using multiple-target configurations [27]. The discussed presaccadic activity in V1 is observed on average 156 ms after fixation point offset [14]. When indeed V1 presaccadic activity originates in the FEF, this would imply a conduction time of about 100 ms.

This estimate of time window relative to the appearance of a discriminated object in which the FEF is involved in attentional deployment is supported by a number of other studies. TMS of the FEF about 40 ms before presentation of a DT has been reported to be effective in changing perceptual performance [20] in humans. In Neggers et al. (2007) [4] a brief train of three consecutive pulses centered around 30 ms before DT presentation was also effective in disrupting the coupling between eye movements and visuospatial attention. Furthermore, the lateralized EEG potential preceding saccades as measured over the FEF in the study by Gutteling et al. (2010) [24], discussed earlier, was elevated around the same time shortly before and during DT presentation.

Therefore in the present study a single pulse is applied at either 60, 30, or 0 ms before the brief presentation of a DT (0 ms defined as halfway to the 120-ms duration of the DT). If we observe stronger effects for early rather than late TMS, the possibility of disturbed visual processing within the FEF itself as an explanation for the effects of TMS on the coupling of visual attention to eye movements can be ruled out with more confidence. If the IPS conveys the modulatory signals from

FEF to the visual cortex, TMS on the IPS should yield similar effects on visuospatial attention later during saccade preparation, compared with TMS on the FEF.

Finally, the study by Neggers et al. (2007) [4] offering a brief train of three TMS pulses to the FEF seems at odds compared with single-pulse FEF stimulation studies [17,20–22], who all report an enhancement of visual processing after stimulation of the FEF (albeit during visual fixation) instead of a disruption. At present, the precise effects of TMS on neuronal processing are not well understood and subject to considerable debate in the field, in that TMS can either facilitate or disrupt neuronal activation. This seems to be dependent on stimulation frequency [28] and the baseline level of excitability of the underlying cortex [29]. Low-frequency repetitive TMS (rTMS; between 1 and 5 Hz) has been shown to inhibit cortical tissue and decrease activation [28,30]. High-frequency rTMS, however, can facilitate neuronal processing in the stimulated tissue [31]. The short-lived effects of single-pulse stimulation (effect duration of ~10 ms according to [32]) can be either facilitating [33,34] or inhibiting (overview in [33,35]), depending on stimulation location and time of stimulation. For saccadic eye movements, either facilitating or inhibitory effects can be found when the FEF is stimulated at visual target onset or after target offset but before saccade execution, e.g., during saccade preparation [36]. Therefore in the present study we stimulated the FEF with a single pulse per trial rather than a brief train of pulses, as in Neggers et al. (2007)[4], otherwise using the same behavioral paradigm. When indeed a single pulse on the FEF can enhance visual processing (at saccade location) as reported by others, whereas a brief train disrupts it as in Neggers 2007, one might relate the present findings with other single-pulse TMS studies on the FEF with more confidence.

3. Methods

3.1 Participants

Ten right-handed participants (five male, five female) were selected for all experiments (three of the authors participated). All participants had normal or corrected-to-normal vision and were screened beforehand for metal implants and general MRI compliance (UMC Utrecht internal guidelines) and TMS compliance [37]. The TMS stimulation protocol remained within the internationally accepted safety limits [38]. All participants were mentally healthy and all provided signed informed consent. The procedures were approved by the Medical Ethical Committee of the University Medical Center Utrecht (protocol nr. 05-020).

Before participating in the TMS experiment, participants engaged in a practice session. They were required to complete the behavioral paradigm without TMS to check whether they were able to make voluntary saccades toward a cued target while performing the discrimination task.

3.2 Experiment 1: fMRI

The subjects first participated in a short fMRI experiment, in which they performed an eye-movement task alternated with rest blocks, to activate the cortical oculomotor regions including the IPS and FEF involved in voluntary saccades.

3.2.1 Apparatus

A 3T Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands) was used to acquire the functional and anatomical images. The scanner was equipped with an eight-channel independent receiver SENSE coil, allowing parallel imaging [39,40]. The stimuli were generated using the Presentation software (Neurobehavioral Systems) on a Plexiglas 1-m-wide screen. The screen was placed at about 2-m distance from the participants and viewed through a mirror mounted on the head coil.

3.2.2 MR data acquisition

In all, 830 functional T2*-weighted volumes were acquired using a PRESTO-SENSE acquisition scheme, effectively accelerating image acquisition by using sparse K-space sampling with SENSE acquisition. Further details are described in Neggers et al. (2008) [39].

The acquisition parameters for the functional images were repetition time (TR) = 21.75 ms; time to echo (TE) = 32.4 ms; field of view [FOV(ap, fh, rl)] = 224 × 256 × 128 mm; flip angle (FA) = 10°; matrix, 64 × 64; 40 slices; voxel size, 4 mm isotropic; 8-channel head coil; SENSE factor = 2 and 1.8 (in the left/right and anterior-posterior phase encoding directions, respectively). One volume was acquired in 609 ms.

After the functional sessions, an anatomical T1-weighted scan was acquired (TE/TR 4.6/9.87 ms; FA 8°; FOV 224 × 160 × 168 mm; matrix 256 × 256; slice thickness, 1 mm; slice gap, 0; voxel size, 0.875 × 0.875 × 1 mm). This scan was used for coregistration with the individual functional images.

3.2.3 Behavioral paradigm and procedure

The behavioral paradigm consisted of a central fixation cross and white circular dots appearing in the periphery to which a prosaccade (toward the dot) or an antisaccade (to the opposite direction) had to be made within 20 s. Peripheral targets were presented every 2 s during a saccade block. Such task blocks were alternated with mere fixation blocks of equal length. For details see Neggers et al. (2007) [4].

3.2.4 Data analysis

Details on the data analysis of the MR images are described in detail in experiment 2 in Neggers et al. (2007) [4] and briefly summarized in the following text.

3.2.5 *Spatial preprocessing*

The fMRI data were analyzed using SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm5/>). Functional scans were realigned to the first image to correct for head movement during the scans and registered to the individual T1-weighted anatomical image. The images were resliced at $4 \times 4 \times 4$ mm and smoothed with an 8-mm kernel full width half-maximum. No normalization was done since the activation maps were used only for individual neural navigation by the use of rigid-body stereotaxy on each subject's head in real space during TMS coil targeting [41]. Finally, the anatomical T1-weighted scans were also segmented, using a "unified segmentation" algorithm from SPM5 [42], for the purpose of reporting the TMS coordinates in a standardized stereotactic space. This algorithm estimates normalization parameters by matching the individual anatomy to gray and white matter Montreal Neurological Institute (MNI) templates using linear transformations and nonlinear warping. These parameters were used to transform the individual anatomical TMS coordinates to standard MNI space. This allowed comparison of the TMS locations over subjects (figure 2C) and with the literature.

3.2.6 *Statistical modeling*

To detect FEF and IPS activation during saccade blocks, a two-regressor general linear model (GLM) was fitted to the functional images per voxel. The first regressor was a boxcar regressor with a block length of 20 s and 20 s between blocks, convolved with the hemodynamic response function (two superimposed gamma functions) to predict blood oxygen level-dependent (BOLD) signal changes during saccade blocks. The second regressor was a constant modeling baseline. The regression coefficients for each voxel for the first regressor obtained by fitting the GLM to the data were statistically tested against zero using a one-sample t-test, with a $P < 0.05$ significance threshold (whole brain volume corrected for multiple comparisons according to random-fields theory [43]).

3.2.7 *Extraction of maximum activation coordinates*

The four native space locations of maximally activated (suprathreshold) voxels within the left and right FEF and IPS were used for TMS stimulation using fMRI-guided stereotaxy in experiment 2. When more than one focus of activation was observed in or near the FEF/IPS, the cluster with the strongest fMRI activation was selected. A cluster was considered to be part of the FEF/IPS when it was overlapping with the FEF/IPS probability map from the volume of interest BrainMap database [44].

3.3 **Experiment 2: TMS**

Our goal was to investigate the relative contributions of the frontal eye fields (FEFs) and the intraparietal sulcus (IPS) to modulation of target discrimination during the preparation of saccades, by stimulating these regions with TMS. Also, the critical time at which these regions have an effect on discrimination performance

was investigated. To investigate the link between preparation of saccades and visual attention shifts, a cue/postponed saccade task developed by Deubel and Schneider (1996)[1] was used.

3.3.1 *Apparatus*

The experiment took place in a darkened room. The participant's head was placed in a chin-rest with the forehead against a head-support 35 cm in front of a semisilvered mirror. The mirror was tilted 45° from horizontal with the near side being lowest and the computer monitor mounted 37 cm above the mirror. This resulted in a virtual image straight ahead of the participant at an effective distance of 72 cm. Presentation of stimuli was controlled using the Matlab Psychophysics Toolbox (version 7.0.4.; [45,46]) on a Pentium PC on a 19-in. color monitor (screen size, 40 × 30 cm; refresh rate, 100 Hz; resolution, 1,024 × 768).

Eye tracking was realized by recording the position of the right eye with the EyeLink II system (SR Research, Kanata, Ontario, Canada) at 500 Hz using infrared (IR) video oculography. The video camera was mounted on a rigid head support and controlled by a Matlab script using the EyeLink Toolbox from the Psychophysics Toolbox [47].

TMS pulses were administered by a Neopulse TMS device (Neotonus, Atlanta, GA) with an iron-core coil [48]. The pulses were triggered by the PC running the stimulation paradigm in Matlab through a TTL pulse over the parallel port. The placement of the TMS coil was stereotactically guided with help of the individual fMRI activation maps registered with individual MRI anatomical images. A frameless stereotactic technique developed in-house was used, based on calibration of a subject's craniotopic landmarks with the same landmarks in the individual MRI scan (for a detailed description see [41]). The activation t-maps obtained from the fMRI experiment (experiment 1) were superimposed on a three-dimensional (3D) rendering of the brain surface and skin for each individual in real time. The positions directly overlying the coordinates of maximum activation within FEF and IPS of both hemispheres as well as the vertex were pointed out with a 3D digitizer pen and marked on a tight-fitting cap placed on the participant's head. The accuracy of this procedure is around 3 mm [41], sufficient for TMS affecting about 1 to 2 cm of cortical tissue [49] fMRI-guided TMS was necessary because the interindividual locations of FEF and IPS are highly variable (see figure 2C).

3.3.2 *Behavioral paradigm and procedure*

Before starting the experiment, the TMS output intensity was determined for each participant. The motor threshold of each hemisphere was measured, which was determined as the minimal intensity of the TMS device at which the thumb showed visible twitches ≥ 5 out of 10 times after stimulating the cortical motor area for the thumb [50]. The latter procedure realizes individual TMS intensity adjustment correcting for differences in transfer of the magnetic field to the cortex and electric conductivity within. The TMS output intensity in the experiment was 120% of the

derived motor threshold (MT) (120% MT) for the corresponding hemisphere, whereas the vertex was stimulated at the same intensity as that of the hemisphere with the highest MT.

The TMS coil was stereotactically guided to one of the five stimulation areas for a particular session. The order in which areas of interest were stimulated was randomized over participants.

The experiment consisted of five stimulation sessions: left and right FEF, left and right IPS, and vertex. Each trial in a session consisted of a cue for the saccade, then presentation of the DT during which one TMS pulse was given. This was followed by a saccade and the subject's report of the nature of a DT. The TMS pulses were delivered 60, 30, or 0 ms before DT perception. Per session, 216 trials were used, resulting in 24 repetitions for each analyzed condition.

All stimuli were presented on a light gray background. Each trial was preceded by a drift correction, performed after 500 ms of stable fixation on a central black fixation cross ($0.52 \times 0.52^\circ$), which was repeated when not correctly executed. If the drift correction was successful, three colored ellipses of $0.82 \times 1.64^\circ$ appeared on each side of the fixation cross, 5° from the center and horizontally aligned with a spacing of 0.54° . The colors of the ellipses from the periphery toward the center were red, green, and blue. Five premasks were superimposed on the left and right ellipse arrays; the three middle ones overlapped the three ellipses. The premasks were black and resembled a digital "8" shape ($1.05 \times 0.52^\circ$) which are common on digital alarm clocks. Spacing between premasks was 1.09° . After a 700-ms delay the fixation cross was replaced by the saccadic cue (SC), a colored arrow in the shape of a triangle ($0.58 \times 0.52^\circ$). The arrow pointed in the direction in which the saccade should be made (left or right); the color referred to which ellipse the saccade should be made (red, green, or blue, isoluminant). This cue was presented with a random delay between 500 and 1,000 ms to avoid predictability of SC onset. The "Go signal" indicating the saccade could be made was marked by disappearance of the cue. At 60 ms after the Go signal, the premask in the green ellipse at the side of the cued saccade direction was replaced by a symbol resembling an "E" or a reversed version ("E") (randomly assigned), referred to as the DT. Simultaneously, the other premasks were replaced by randomly assigned distracters resembling a digital "2" or "5." The distracters and target were presented for 120 ms. See figure 1 A for an overview of the stimuli and figure 1B for the stimulation paradigm in time. The location of the DT was always the same (the green ellipse on the side to which the saccade should be made) and the participants were aware of this; 180 ms after the Go signal, the distracters and DT disappeared and only the colored ellipses remained visible.

The participant's cortex was stimulated with a single TMS pulse for each trial, at either 60, 30, or 0 ms before "DT presentation" (e.g., at 60, 90, or 120 ms after the Go signal). "Time of DT presentation" is defined here as half the time that DT was present on screen (e.g., 60 ms after DT onset). Since DT was presented from 60 to 180 ms after the Go signal, DT "presentation time" equals 120 ms after the Go signal. In contrast to other studies using visual targets (most notably [25]) the onset

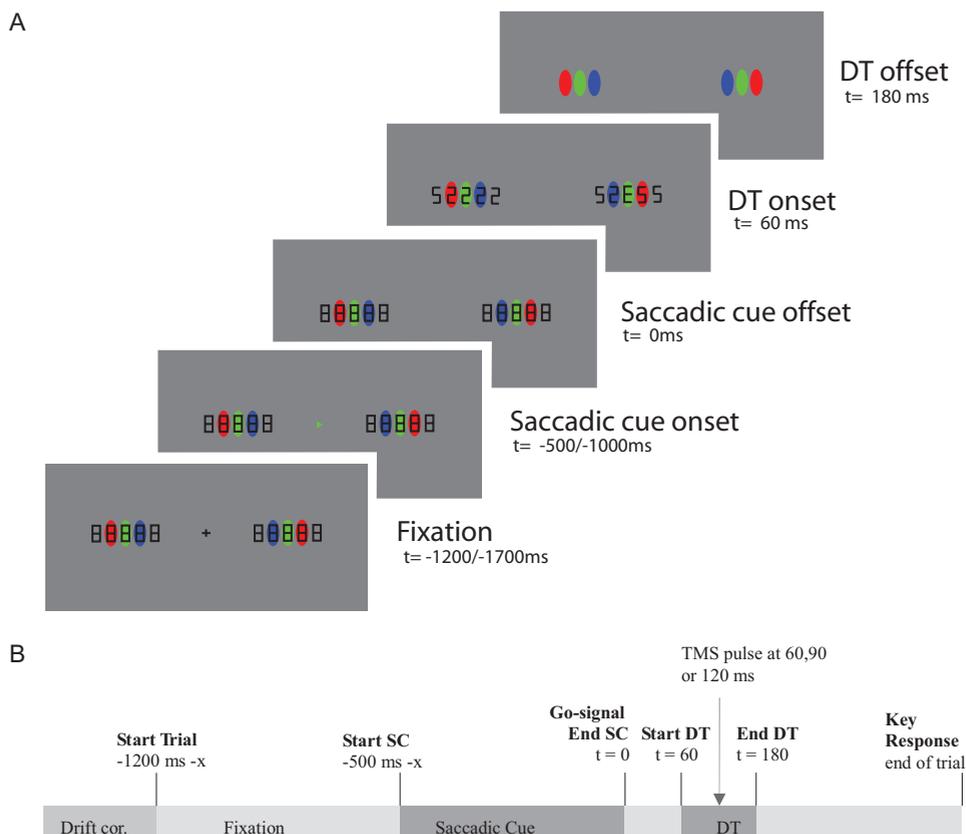


Figure 1. *A*: schematic overview of the different frames constituting the visual stimulation paradigm for the transcranial magnetic stimulation (TMS) experiment (experiment 2). After successful drift correction, 6 colored ellipses were shown containing 8-shaped masks. After 500 ms a central colored arrow appeared, indicating the target (saccade target [ST]) to which the saccade should be made. The arrow disappears after a randomized presentation interval of 500–1,000 ms (= Go signal). At 60 ms after the Go signal, the discrimination target (DT, consisting of an “E” or a “Э”) appeared at the left or right green ellipse location; the other masks were replaced by 2- or 5-shaped distracters. After disappearance of DT, the trial ended and the participant had to report the target identity by pressing a button without time pressure. *B*: timescale of the events during a trial in the TMS experiment (experiment 2). Drift Cor: drift correction, saccadic cue: colored arrow indicating ST (see A). DT, “E” or “Э”. The vertical arrow indicates the period at which the TMS pulse was delivered to the left or right frontal eye field (FEF), left or right intraparietal sulcus (IPS), or vertex, at 60, 30, or 0 ms before perceiving DT (determined as being halfway to DT presentation time). Times are expressed relative to the saccade Go signal (disappearance of the saccade cue).

of the display of the target and distracters as used in the present study did not result in a net luminance change. Thus the current design avoids any exogenous capture of attention by the target onset and tests presaccadic facilitation of visual discrimination prior to the onset of an endogenous saccade. The presently used display method most likely results in a different and less-vigorous onset of neuronal activity in visual cortex neurons, compared with sudden onsets with significant overall luminance changes

(see [25]). Therefore we assume that with respect to the net activation in the visual cortex elicited by the DT stimulus, the “presentation time” is best approximated by the moment halfway between the 60- to 180-ms presentation time of the target. The latter is important for our a priori choice of TMS–DT intervals (see introduction) based on neuronal transmission estimates.

Neggers and colleagues (2007)[4] deployed a train of three pulses at 60, 30, and 0 ms before DT presentation, to increase the likelihood of obtaining a TMS effect, which was estimated to be optimal around 34 ms before DT presentation (see introduction for the motivation). The present study aimed to determine which of those three pulses was responsible for the observed effects of performance disruption, thus deploying a single pulse on either of the three stimulation times (60, 30, or 0 ms before DT presentation). The stimulation times were pseudorandomized over the trials, resulting in an equal distribution of one third of the trials for each stimulation time.

The first task for the participants was to execute the saccade as cued. This meant they had to fixate their eyes in the middle of the screen and observe the arrow presented there until it disappeared—only then were the participants allowed to execute the saccade as prompted, in the cued direction and toward the cued colored ellipse. The second task for the participants was to report the identity of the DT (press the “f” key for an “E” or the “j” key for a “Э”). After reporting, the ellipses disappeared and the fixation cross reappeared, indicating the start of a new trial. The subjects were instructed to direct their saccades as correctly as possible to the instructed target, without attempting to look at any of the other objects.

The above-cited settings resulted in 36 possible conditions for a saccade trial per stimulation session of 216 trials: 2 cue sides (left or right), 3 locations (red, green, blue) \times 2 DT identities (“E” or “Э”), and 3 TMS times (60, 30, or 0 ms before DT presentation). The identity of the DT was not expected to yield a difference in performance and thus the corresponding data were pooled. Moreover, the present study aimed at comparing data from trials with contralateral versus trials with ipsilateral saccades, meaning that data from trials with leftward saccades during right-sided FEF stimulation could be pooled with data from rightward saccades during left-sided FEF stimulation for the contralateral condition. Pooling was performed vice versa for stimulation ipsilateral to saccade direction. Data from trials during vertex stimulation were neither contra- nor ipsilateral with respect to saccade direction and thus were also pooled. The resulting 9 conditions (3 locations \times 3 TMS times) were presented for each of the five sessions (contra- and ipsilateral FEF, contra- and ipsilateral IPS, and vertex stimulation). For 216 trials per session there were effectively 24 repetitions per condition.

3.3.3 Data analysis

To determine whether a proper saccade was made, the onset of a saccade was detected using a velocity threshold of 30°/s and an acceleration threshold of 8,000°/s². Premature saccades (arriving earlier than 20 ms after disappearance of the DT)

were excluded to prevent a foveal image of the target, since foveal vision of the DT would have influenced discrimination performance. Furthermore, saccades that landed $>1.2^\circ$ off target on either side, moved in the wrong direction, or with a reaction time >3 s were excluded. To ensure proper fixation, saccades starting $>1.2^\circ$ away from the fixation cross were also excluded.

For each participant the proportion of correct responses and the average saccade latency were calculated for the different experimental conditions. Saccade latency was defined as the time period between the Go signal (disappearance of the arrow cue) and saccade onset. The correct response ratios as well as the average individual saccade latencies were tested per stimulation sessions in repeated-measures ANOVAs for effects of saccade target position (TARGET), stimulation site (SITE: contra- or ipsilateral cortical stimulation with respect to saccade direction, or vertex), and the timing of TMS (TIME). Leftward and rightward saccades with the same target eccentricity (the same color ellipse as that of the cued target) were pooled because the previous study showed no significant difference between hemisphere stimulations [4]. The level of significance was set at $P < 0.05$.

4. Results

4.1 Experiment 1: fMRI

Ten participants took part in an fMRI experiment aimed at localizing the FEF and IPS for each subject individually. Here, participants made pro- and antisaccades in rapid succession in the scanner in a series of short blocks with fixation periods in between.

4.1.1 Saccade blocks versus fixation

Figure 2 A shows a 3D cortical rendering with superimposed suprathreshold FEF and IPS activity during the saccade blocks, compared with the fixation blocks, for a single representative participant, in native space. This is a screenshot from the stereotactic software as used during fMRI-guided stereotaxy [41]. Figure 2B depicts an axial slice through the FEF and IPS, with activation t-maps overlaid, for one participant.

Figure 2C depicts an average MNI normalized brain with the coordinates of maximum activation within the left and right FEFs and IPS, for all 10 participants. Although the maxima cluster around the zones is known to entail human FEF and IPS [51], there is considerable variability.

4.2 Experiment 2: TMS

In experiment 2, a TMS pulse was delivered over the individual left or right FEF, left or right IPS, and vertex, as localized using fMRI in experiment 1.

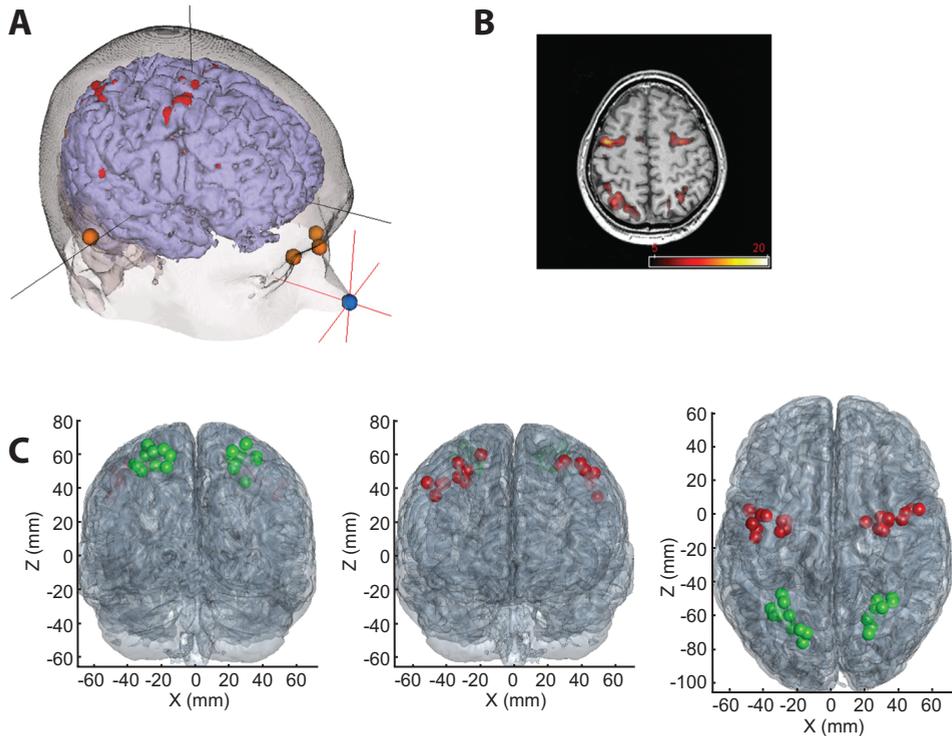


Figure 2. *A: 3-Dimensional (3D) cortical rendering with superimposed suprathreshold FEF and IPS activity during saccade blocks compared with fixation as determined by functional magnetic resonance imaging (fMRI) in experiment 1. This is a screenshot from the stereotactic software as used during fMRI-guided stereotaxy (Neggers et al. 2004). B: axial slice through the FEF and IPS for one individual participant, with participants' individual activation t -maps acquired in experiment 1 overlaid. C: average Montreal Neurological Institute (MNI) normalized brain with the coordinates of maximum fMRI activation from experiment 1 within the left and right FEF (red spheres) and left and right IPS (green spheres), from posterior, anterior, and superior views (from left to right). The depicted spheres are the coordinates of maximum activation for all 10 participants that were stimulated with TMS in experiment 2. FEF and IPS locations as indicated by these maxima vary considerably over subjects, even after normalization to MNI space (e.g., accounting for variability in gross cortical anatomy).*

4.2.1 Excluded trials

Trials were excluded from analyses when saccades were made too early (to prevent foveal vision of DT) or landed too far away from the intended saccade target (see methods for details). For one of the 10 participants <30% of trials remained for analysis only and thus this subject was excluded from analysis. For the remaining subjects, on average 51% of the trials entered analysis. The inclusion criteria for trials were strict because foveation of a DT due to saccades made too early would artificially improve discrimination performance. It is also important to ensure that indeed the instructed target was foveated to be able to validly attribute variation in target-discrimination performance to saccades to a certain target. Because our focus was on selective attention, it was of utmost importance that all saccades to

noninstructed targets were excluded. The number of trials excluded was independent of the area stimulated with TMS [ANOVA: $F(4,1) = 2.035$, $P = 0.296$]. For all the other experimental factors, only the factor `TARGET` resulted in a significant difference in the number of accepted trials [`TARGET`: $F(2,18) = 4.66$, $P < 0.05$]: slightly more trials were generally accepted for the central saccade target position (48 and 49% on average for inner or outer target positions, respectively, and 54% for the central target position).

4.2.2 *Discrimination performance compared for all TMS sites*

For the discrimination performance of all TMS sites and conditions, see Figs. 3 and 4 in the following text. First, we ran an omnibus ANOVA on all experimental factors to test whether effects of TMS on discrimination performance differed across the stimulated sites. The present experimental design did complicate a full-factorial ANOVA somewhat: the factor laterality (contra- or ipsilateral of TMS) cannot be included for vertex stimulation because the vertex is not hemisphere-bound, as opposed to the FEF and IPS. Therefore we ran an overall ANOVA with the factors `SITE` (IPS, FEF), `LATERALITY` (contra- and ipsilateral of stimulated hemisphere), `TARGET`, and `TIME`. This analysis ignores vertex stimulation data, but does allow a full-factorial ANOVA including laterality.

The quadratic contrast for the factor `TARGET` (modeling the reversed V-shape of the `TARGET` × performance graph) was significant [$F(1,8) = 30.73$, $P < 0.001$], indicating a clear coupling of saccade planning and spatial discrimination performance, consistent with previous findings [1,4]. This contrast is used as the main indication for the influence of saccade target location on discrimination performance in the following analyses. Here, the interaction `SITE` × `TARGET` × `LATERALITY` was significant [$F(2,16) = 3.892$; $P < 0.05$], implying that the specific and lateralized influence TMS has on the dependence of discrimination performance on saccade target is different for FEF compared with IPS stimulation. The latter justifies further testing of IPS and FEF stimulation data separately to determine what the specific effects of TMS are for combinations of experimental factors. In the subsequent-tests the effects are also tested against vertex stimulation.

4.2.3 *FEF stimulation: discrimination performance*

The percentage of correctly identified DTs was considered as a function of saccade target position, TMS location, and TMS timing in a separate ANOVA for FEF data only. TMS location (factor `SITE`) now could be FEF contra- or ipsilateral to saccade direction, or vertex. Figure 3A depicts the discrimination performance, averaged over nine subjects, for TMS on the FEF ipsi- or contralateral to saccade direction and for vertex stimulation.

4.2.4 *Influence of TMS site*

The effect of the target location of the saccade in preparation on discrimination of the letter probes was largest for TMS on the FEF contralateral to saccade

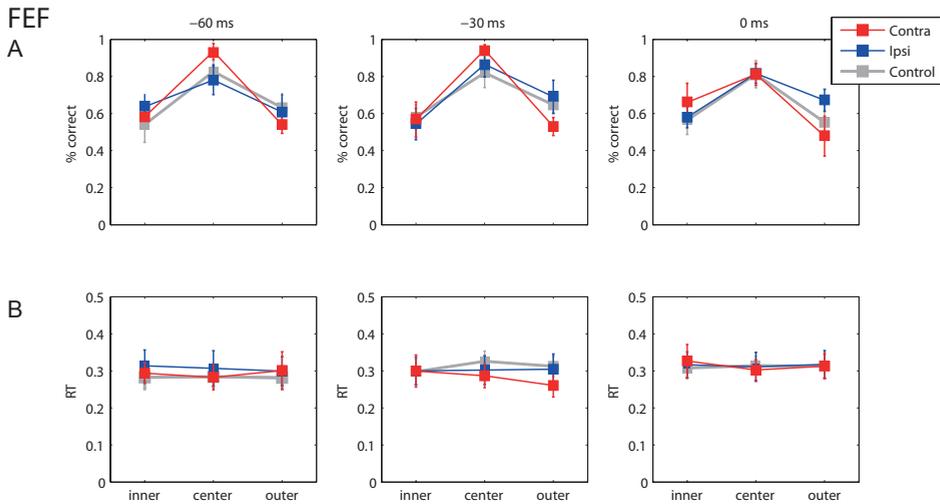


Figure 3. Performance of subjects after receiving TMS pulses on either contralateral FEF (red line), ipsilateral FEF (blue line), or on the vertex (gray line, control condition), for 9 subjects. Three separate graphs have been made for each stimulation time (from left to right: $t=60$, $t=30$, $t=0$; relative to presentation of DT). The 3 top graphs in A depict the discrimination performance, expressed in percentage correct DT identifications on the y-axis for the 3 different saccade locations on the x-axis (inner = blue ellipse; center = green ellipse; outer = red ellipse). The 3 bottom graphs in B depict the saccadic latencies expressed in reaction time in seconds for the same 3 saccade locations.

direction [see figure 3A; TARGET \times SITE: $F(1,8) = 5.8$; $P < 0.04$, quadratic contrast for TARGET].

Because the effect for the factor SITE was significant, separate ANOVAs were run for the comparison of contra- with ipsilateral TMS and for the comparison between contralateral and vertex stimulation. For the comparison contralateral and ipsilateral TMS, the quadratic interaction TARGET \times SITE was significant [$F(1,8) = 5.67$; $P < 0.05$]. For the comparison contralateral and vertex TMS, the quadratic interaction TARGET \times SITE was significant at the trend level [$F(1,8) = 4.095$; $P = 0.078$].

4.2.5 Influence of TMS time

As can be seen in figure 3A, the influence of TMS on the coupling between discrimination performance and saccade target seems to decrease with TMS time. This effect seems most profound for FEF stimulation contralateral compared with ipsilateral of saccade direction. These effects were investigated statistically for the comparison contralateral versus ipsilateral (to saccade direction) stimulation of the FEF and contralateral FEF versus vertex stimulation and separately for the comparison inner-central and outer-central saccade target locations.

The TMS-induced difference (contralateral vs. ipsilateral FEF) between the performance increase for coinciding ST and DT (DT is always located at the center ellipse), compared with the inner ST location, is strong for the first TMS stimulation

time, 60 ms before DT presentation, but decreases linearly with time until it is absent for 0 ms before DT presentation [$\text{TARGET} \times \text{SITE} \times \text{TIME}$: $F(1,8) = 7.08$; $P < 0.05$; linear contrast for the factor TIME ; the quadratic contrast for TIME was not significant: $F(1,8) = 0.004$]. For the comparison of discrimination performance for the outer versus central target this effect was neither linearly [$\text{TARGET} \times \text{SITE} \times \text{TIME}$: $F(1,8) = 0.31$; linear contrast for the factor TIME] nor nonlinearly dependent on time [$\text{TARGET} \times \text{SITE} \times \text{TIME}$: $F(1,8) = 0.38$; quadratic contrast].

The comparison of TMS-induced performance changes for contralateral FEF versus vertex stimulation yielded no significant influences for TMS timing, for either the comparison inner-central or outer-central discrimination performance increases.

4.2.6 FEF Stimulation: saccade latency

The latency of saccades was analyzed as a function of saccade target position, TMS location, and TMS timing. Figure 3B depicts the saccadic latencies, averaged over nine subjects, for TMS on the FEF ipsi- or contralateral to saccade direction.

Overall, the site of TMS (contra- or ipsilateral FEF stimulation, vertex) did not influence either the saccade latency [SITE : $F(2,18) = 0.90$] or the saccade target location [TARGET : $F(2,18) = 0.21$]. Saccade latency increased linearly with the time of TMS [linear contrast of TIME : $F(2,18) = 16.21$; $P < 0.001$; quadratic contrast n.s.: $F(2,18) = 0.88$]. Saccade latency changes over time depended, at the trend level, on the site of TMS [$\text{TIME} \times \text{SITE}$: $F(16,4) = 2.575$; $P = 0.056$], indicating that effects of TMS on saccade latency compared with control stimulation differ for each timing condition. Therefore three separate analyses were performed for each TMS timing level.

For TMS stimulation at -60 ms (i.e., 60 ms before DT presentation), no effects of site or saccade target on saccade latency were observed [SITE : $F(2,16) = 1.84$; $P = 0.19$; TARGET : $F(2,16) = 0.14$].

For TMS stimulation at -30 ms, mainly latencies for saccades contralateral to the stimulated FEF decreased compared with TMS on the vertex [SITE : $F(1,8) = 6.82$; $P < 0.05$]. The other combinations of sites (ipsilateral vs. vertex, ipsilateral vs. contralateral) did not yield significant effects.

For TMS stimulation at 0 ms, no effects of site or saccade target on saccade latency were observed [SITE : $F(2,16) = 0.39$; TARGET : $F(2,16) = 0.30$], nor did any of the interactions test as significant.

4.2.7 IPS stimulation: discrimination performance

Figure 4A depicts the discrimination performance, averaged over nine subjects, for TMS on the IPS ipsi- or contralateral to saccade direction and for TMS on the vertex (control site).

4.2.8 Influence of TMS site

The effects of TMS on the performance increase for coinciding ST and DT seem to be qualitatively different for IPS stimulation, as observed for FEF stimulation

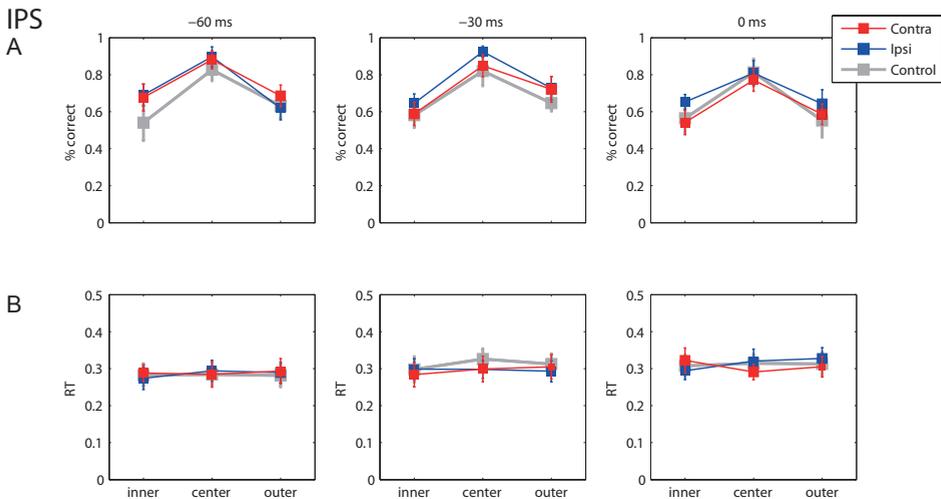


Figure 4. Performance of subjects after receiving TMS pulses on either contralateral IPS (red line), ipsilateral IPS (blue line), or on the vertex (gray line, control condition), for 9 subjects. Three separate graphs have been made for each stimulation time (from left to right: $t=60$, $t=30$, $t=0$; relative to presentation of DT). The 3 top graphs in A depict the discrimination performance, expressed as a percentage of correct DT identifications on the y-axis for the 3 different saccade locations on the x-axis (inner = blue ellipse; center = green ellipse; outer = red ellipse). The bottom graphs in B depict the saccadic latencies expressed in reaction time in seconds for the same 3 saccade locations.

(see figure 4A). This was confirmed by the overall full-factor ANOVA including both IPS and FEF stimulation data reported earlier. In general, the discrimination performance seems to increase for all three saccade target locations for IPS stimulation compared with vertex stimulation (“elevated V-shape”), whereas the advantage of coinciding ST and DT compared with other saccade target locations is comparable (“similar V-shape sharpness”). Note that for FEF stimulation, TMS induced a “sharpening” of the reverse V-shape and not a general “elevation.” Separate ANOVAs were conducted for the comparison of contra- versus ipsilateral TMS and between contralateral and vertex stimulation (the control condition).

Indeed, for the comparison ipsilateral versus vertex TMS, the site of TMS had a significant influence on discrimination performance [SITE: $F(1,8) = 7.24$; $P < 0.05$]; the quadratic interaction TARGET \times SITE was not significant [$F(1,8) = 0.18$]. No significant effects were found for the comparison contralateral versus ipsilateral TMS nor for contralateral versus vertex TMS.

4.2.9 Dependence on TMS time

In figure 4A, the reported influence of TMS on the general discrimination performance level (for ipsilateral IPS vs. vertex stimulation) seems to be strongest for TMS time at 30 ms before DT presentation. This is somewhat later compared

with TMS effects for FEF stimulation. However, none of the comparisons between stimulation sites and times that were tested for the FEF reached significance.

4.2.10 *IPS stimulation: Saccade latency*

The latency of saccades was analyzed as a function of saccade target position, TMS location, and TMS timing. Figure 4B depicts the saccadic latencies, averaged over nine subjects, for TMS on the IPS ipsi- or contralateral to saccade direction. The same combinations of factors as those for FEF stimulation were statistically assessed to detect an influence of TMS timing or site on saccade latency.

The only significant effect that was observed was that the time of TMS did influence saccade latency, reflecting a linear increase of saccade latency with TMS time [linear contrast of TIME: $F(2,18) = 77.24$; $P < 0.0001$] similar to that for FEF stimulation. This increase was not dependent on the site of TMS [TIME \times SITE: $F(16,4) = 1.09$; $P = 0.38$].

5. Discussion

The present study demonstrates that the FEF plays a key role in shifting focus of visual attention toward the target of a saccade, shortly before it is executed. The FEF and IPS were first individually localized with fMRI. It was observed that fMRI-guided TMS on the FEF contralateral to saccade direction 60 ms before the presentation of a DT can increase the discrimination performance of a target presented at the goal location of the upcoming saccade, thus strengthening the coupling between saccadic eye movement preparation and visuospatial attention. This effect was smaller at 30 ms and absent at 0 ms before DT presentation. Furthermore, saccade latency decreases were observed for TMS on the FEF 30 ms before DT presentation, again for saccades contralateral to the stimulated FEF. It is unlikely that the TMS-induced saccade latency decreases resulted in the enhanced discrimination performance for TMS on the contralateral FEF; the rigorous analysis ensured that trials were saccades arrived at the DT before DT offset were excluded from analyses and thus foveation of the DT in trials with shorter latencies can be ruled out. Furthermore, TMS on the FEF resulted in reduced latencies only 30 ms before DT presentation, whereas discrimination performance effects are maximal for TMS at 60 ms before DT presentation and decrease linearly with TMS timing.

The effects of IPS stimulation on discrimination performance differed substantially from effects of FEF stimulation. TMS on the IPS resulted in general ipsilateral increases in discrimination performance at all locations, irrespective of the target of the saccade, whereas TMS on the FEF led to enhanced performance only for targets at the upcoming saccade goal. Unlike for the FEF, effects of TMS on IPS were not dependent on TMS timing.

TMS stimulation on the FEF, IPS, and vertex induced an increase in saccade latency with an increasing Go signal TMS interval. This effect is therefore most

likely due to a general effect of TMS, for example, a warning effect increasing alertness induced by the audible or sensible nonneuronal effects of TMS.

5.1 Enhancement versus suppression of performance as a result of TMS

Interestingly, a preceding study [4], using a short train of three TMS pulses (at 110% MT) at 60, 30, and 0 ms before DT presentation in an otherwise identical stimulus paradigm, resulted in a decrease of discrimination performance gain for coinciding saccade and DTs, contralateral with respect to the stimulated FEF. The present study, however, applying only a single pulse at 120% MT at the contralateral FEF, reports an increase of discrimination performance. As stated in the introduction, effects of TMS on neuronal processing are reported to be highly variable. The inhibiting or enhancing effect of TMS is highly dependent on timing, location, duration, and—importantly—frequency or even an interaction of frequency and duration as shown by [52]. In a direct comparison study of single-pulse and repetitive stimulation, single-pulse TMS did not show any significant effect on motor-evoked potential size. This was in contrast to the observed decreased cortical excitability after 5 or 15 pulses [53] or as many as 1,600 pulses [54]. When regarding the three pulses applied in rapid succession in our preceding study [4] as a high-frequency–short-duration stimulation protocol, effects different from a single pulse of TMS as used in the present study could be expected [55]. Even though it is debatable whether a train of 3 pulses is comparable to longer high-frequency stimulation, it is clear from all preceding comparative studies that successive pulses can yield different or even opposite effects compared with single pulses. For single-pulse TMS the moment of stimulation in visual paradigms is of utmost importance. Whereas a single pulse during saccade execution can disrupt saccade performance, it has been repeatedly shown that single-pulse TMS applied before presenting a visual cue has facilitating effects, resulting in reduced reaction times in studies by Grosbras and Paus [20,56] and increased fMRI activity in the peripheral visual field in the occipital cortex [17]. It has been suggested that TMS actually adds noise to an intricate process of neuronal signaling reducing the processing efficacy, although that view has been challenged [57]. Only a few studies investigated interactions of a magnetic pulse with electrical neuronal signaling directly [33]. Much more research on physiological mechanisms underlying TMS effects is required to understand the effects of different TMS intensities and frequencies. Still, in the light of the studies discussed earlier, it can be argued that one realizes an enhancement of “normal” neuronal signaling when the current added by TMS somewhat resembles the natural neuronal process in the neuronal tissue under investigation. A disruption of function can be expected, however, when the TMS-induced current is remote from the operations the neuronal tissue under investigation normally performs. This might explain the reversal of the TMS effect in the present study compared with the preceding study [4]. That is, a brief but vigorous pulse of activity is normally observed in the primate FEF around saccadic eye movements [58], probably to some extent resembling the pattern a single TMS pulse evokes in the FEF (for evidence of

similarity between TMS and internally evoked BOLD responses in the motor cortex, see [59]). Therefore a single pulse could lead to facilitation of FEF functioning. A brief train of bursts, however, is not normally observed around a single saccade in the FEF, which therefore would suppress normal FEF functioning. Importantly, we do not imply that effects of a single TMS pulse resemble real neuronal saccade control processes in every way (otherwise TMS should be able to trigger overt saccades), but rather that the induced current resembles normal neural activation, albeit evoked in a larger, less-specified area.

5.2 Timing of the TMS effects: implications for pathways

It has been suggested that the influence of the FEF on early visual processing is realized by corticocortical connections between the FEF and early visual cortex [4,14,17,60]. That is, electrical stimulation of FEF neurons induces activation in V4 [13] for matching movement and receptive fields, V1 neurons show a clear modulation before a saccade is initiated [14] and TMS on the FEF during fMRI results in activation of V1 [17]. All the latter studies were performed during fixation. Recently, it was demonstrated that this feedback connection from the FEF to the visual cortex might subserve the well-known modulation of visual processing during saccade preparation, by disrupting this coupling with a short train of TMS to the FEF [4]. Importantly, TMS was administered before any information regarding the to-be discriminated target could have reached the FEF, indicating that the actual influence of the FEF over visual processing is exerted elsewhere, most likely the visual cortex. However, the direct neurophysiological effect of TMS might outlast the 30 ms we assumed as the time TMS affects neuronal processing [32]. In fact, longer-lasting neuronal TMS effects were recently reported [61,62]. This residual TMS signal could then disturb incoming information within the FEF, allowing the conclusion that attentional processing is implemented in the FEF itself rather than through recurrent connections to the visual cortex. The present results, however, contradict the latter: effects of TMS were strongest 60 ms before DT presentation and gradually disappeared for later time intervals. When neuronal effects induced by TMS stay present within the FEF for some time, the enhanced discrimination performance should have remained or increased for later TMS administration. Therefore the present study can conclude with more certainty that effects of FEF stimulation on the coupling of visuospatial attention to eye movement preparation are induced by the influence the FEF has over another region, most likely the visual cortex. Finally, we provided further support for this notion in a recent EEG study by Gutteling et al. (2010) [24], using the exact same behavioral paradigm as that in the present study. It was reported that presaccadic FEF activation lateralized to saccade direction preceded occipital activation lateralized in the same manner and peaked around the same time TMS on the FEF was most effective in the present study.

It should further be noted that FEF signals can reach the visual cortex not only directly [23], but also through known projections to the superior colliculus in the midbrain [63], from which these signals can be relayed to extrastriate areas.

5.3 Involvement of IPS

Previous reports argued that effects of FEF stimulation on the visual cortex are relayed through the IPS because effects were found only when a discrimination stimulus was presented and direct connections should have induced effects irrespective of visual stimulation [13,17]. In line with this, electrical stimulation of monkey FEF was found to modulate activity as measured by fMRI in early visual areas, but the effect was highly dependent on whether a visual stimulus was present [16]. However, in the present study, in contrast to TMS on the FEF, effects of IPS stimulation on visual discrimination performance were present for all conditions, irrespective of the target of the upcoming saccade. Furthermore, effects of IPS stimulation did not clearly depend on TMS timing. Finally, the only significant effect of IPS stimulation was observed for IPS stimulation ipsilateral to saccade direction, whereas contralateral effects were found for FEF stimulation. When the FEF indeed enforces a coupling of visuospatial attention to the saccade goal by means of projections to the visual cortex, TMS effects were expected mainly for saccades contralateral to the stimulated FEF. That is, the visual cortex codes contralateral retinotopic space, the FEF contralateral saccades [58], and the FEF projects to ipsilateral visual cortex [60]. As we observed clear differences in dependence of TMS effects on the local (e.g., the red, green, or blue) eye-movement target (clear dependence for FEF; no dependence for IPS) and in laterality of the effects one can argue that direct connections from the FEF to the visual cortex cause the reported influences, or some other pathway, but not a pathway including the IPS. Interestingly, in a recent study [18] concurrent fMRI/TMS revealed that FEF stimulation induced activation in the visual cortex, greatly depending on the coded visual eccentricity; TMS-evoked BOLD responses were large and positive for areas representing the visual periphery and negative for areas representing foveal vision. This supports the notion that the influence of the FEF over the visual cortex subserves saccade preparation: saccades are usually made into the periphery. IPS stimulation in the study by Ruff and colleagues (2008)[18], however, resulted in markedly different global effects—irrespective of the eccentricity coded in the specific zones of affected visual cortex. The latter is in agreement with the present report, observing a saccade-related enhancement of visual attention after FEF stimulation and a general improvement of visual performance for IPS stimulation. Finally, our recent EEG study by Gutteling et al. (2010) [24] mentioned earlier, using the exact same behavioral paradigm, did not detect any presaccadic IPS activation lateralized to saccade direction that was larger than a control (e.g., fixation) condition, as was found for the FEF and the occipital lobe.

5.4 Effects of TMS on saccade latencies

First, saccade latency increased slightly with later TMS times. Because this effect was the same for all TMS sites, including the vertex, it is most likely caused by a general, nonneuronal warning effect of the (audible and sensible) TMS pulse. Second,

saccade latencies were site-specifically but moderately shortened by TMS (only for FEF stimulation at $t = -30$ ms) in the present study, in contrast to the absence of any latency effect in Neggers et al. (2007)[4] using a similar paradigm with a brief train of TMS pulses at the same interval. The results, however, can be reconciled with other studies that did observe TMS effects on saccade latency (mainly increases) in specific and narrow time windows only. TMS on the FEF 60 ms before saccade initiation delays prosaccades [64]. Antisaccades were delayed when stimulating the FEF 100 ms after target presentation (~ 165 ms before saccade onset), but not at 80 or 120 ms [65], and prosaccades for TMS at that time interval were unaffected. In the present study TMS was delivered much earlier before saccade onset than 60 ms, known to delay prosaccades [64]. Possibly, the narrow time window in which TMS is effective in delaying saccades reflects buildup of a signal directly driving saccades rather than the earlier saccade preparation/anticipation-related signals known to exist in the FEF [66] that we might have tampered with, not necessarily leading to direct motor effects. This could explain the minimal effect on saccade latency in the present study, in the other direction than reported for TMS on the FEF later during the target-saccade interval [64].

5.5 Conclusions

The present study demonstrates that the FEFs are responsible for directing our visuospatial attention to the target of a saccade in preparation. TMS could enhance the coupling of spatial attention to the target of the saccade in preparation when administered to the FEF well before visual information regarding the discriminated probe could have reached the FEF—and this effect gradually disappeared for later stimulation times. This indicates that the FEF is probably the driving source of visuospatial attention shifts, although it cannot be the site at which visual information from the outer world is actually selectively filtered. Based on the present findings and other recent reports from single-cell neurophysiology, EEG, fMRI, and TMS mentioned earlier, we propose that the FEF directs selective visuospatial attention by influencing the visual cortex directly through recently discovered anatomical connections [13,14].

The effects of TMS on the IPS were a global improvement of visual performance, irrespective of the saccade target. Therefore the pathway from the FEF by which the visual cortex is influenced shortly before a saccade most likely does not involve the IPS.

5.6 Grants

This work was supported by a Netherlands Foundation for Scientific Research Open Competition Grant NWO 400-05-134.

5.7 Acknowledgements

We thank P. Lemmens for technical support and P. Bos for MR scanning.

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Chapter **4**

Grasping preparation enhances
orientation change detection

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PloS one (2011) 6(3), e17675

1. Abstract

Preparing a goal directed movement often requires detailed analysis of our environment. When picking up an object, its orientation, size and relative distance are relevant parameters when preparing a successful grasp. It would therefore be beneficial if the motor system is able to influence early perception such that information processing needs for action control are met at the earliest possible stage. However, only a few studies reported (indirect) evidence for action-induced visual perception improvements. We therefore aimed to provide direct evidence for a feature-specific perceptual modulation during the planning phase of a grasping action. Human subjects were instructed to either grasp or point to a bar while simultaneously performing an orientation discrimination task. The bar could slightly change its orientation during grasping preparation. By analyzing discrimination response probabilities, we found increased perceptual sensitivity to orientation changes when subjects were instructed to grasp the bar, rather than point to it. As a control experiment, the same experiment was repeated using bar luminance changes, a feature that is not relevant for either grasping or pointing. Here, no differences in visual sensitivity between grasping and pointing were found. The present results constitute first direct evidence for increased perceptual sensitivity to a visual feature that is relevant for a certain skeletomotor act during the movement preparation phase. We speculate that such action-induced perception improvements are controlled by neuronal feedback mechanisms from cortical motor planning areas to early visual cortex, similar to what was recently established for spatial perception improvements shortly before eye movements.

2. Introduction

In daily life, there is a constant interaction between what we do and what we see. Our actions are partly dictated by what we perceive, but the reverse also holds. When performing an action, our perception is focused toward those things in our visual experience that enable us to execute the action successfully. For instance, when picking up a book, its orientation, thickness and distance all determine grasping kinematics early during the movement. When the perceptual system would be 'primed' toward relevant features, such as the orientation or size of the book, the subsequent grasping action can be executed with increased accuracy and speed.

This effect of motor preparation on visual perception has been well studied for the oculomotor system. It is now well established that, shortly before the actual execution of an eye movement, spatial perception greatly improves at the eye movement target location [1–3]. Furthermore, recent evidence demonstrates that oculomotor areas in the (pre)motor cortex influence processing in the visual cortex during eye movement preparation [4–6]. This is a likely neuronal mechanism underlying the observed links between spatial attention and eye movements.

It would make sense that when preparing more complex actions with the skeletomotor system, such as grasping and manipulating objects, not only spatial perception but also the perception of object features relevant for the task at hand would be improved. However, where there is ample evidence for this phenomenon in the oculomotor system, few reports exist for the skeletomotor system. Among the scarce reports there is encouraging, but indirect, evidence from the analysis of eye movement scanpaths before grasping [7] and the influence of subconscious priming on grasping reaction times [8] that indeed object orientation is perceived in an enhanced manner during grasping preparation. Although these few findings support the influence of action preparation on perception in the skeletomotor system, the measures used are speeded motor responses or eye movement scanpaths that might reflect interactions within the motor control system itself. As such, it is difficult to tease apart the contributions of the motor acts on perception and vice versa.

We therefore aimed to provide a direct measure of visual performance during (skeletal) motor preparation. This was done by estimating the visual sensitivity (d') to slight orientation changes occurring during grasping and pointing preparation (just before the movement started). Visual discrimination performance was measured from non-speeded key presses well after the movement ended. Orientation was chosen as discrimination feature for its relevance for grasping acts, but not pointing acts. Should the preparation of a grasping act enhance the perception of relevant features, then sensitivity to orientation differences should be higher when preparing a grasping act, rather than a pointing act. As a control, luminance was chosen as a discrimination feature that is not relevant for either a grasping or pointing act. No differences in visual sensitivity were thus expected.

3. Methods

3.1 Ethics

This study was approved by the Medical ethical committee of the University Medical Center Utrecht. All subjects signed an informed consent form prior to participation.

3.2 Experiment I: Orientation

3.2.1 Participants

Sixteen subjects (11 women; mean age 25.9 SD4.4) with normal or corrected-to-normal vision participated in the first experiment. All were right handed, as checked by the Edinburgh handedness inventory (mean 85 SD22) [9].

3.2.2 Apparatus

Subjects sat in a dimly lit room in front of a Iiyama 17" (320×240 mm) monitor, with a resolution of 1024×768 pixels and a refresh rate of 100 Hz. They were

seated in a frame with head- and chinrest. Viewing distance was adjusted to enable comfortable pointing and grasping movements. Visual angle of the stimuli was kept constant by compensating the size of the stimuli relative to the viewing distance.

To ensure that grasping and pointing actions were executed correctly, motion tracking of the right hand (grasping/pointing hand) was performed using a 'driveBay' magnetic motion tracker (Ascension technology, Burlington, USA). Subjects wore a flexible, unrestrictive glove that was fitted with four motion sensors located at the tip of the thumb, tip of the index finger, back of the hand and at the wrist. Movement data were recorded from all sensors at 240 Hz.

3.2.3 Task

Subjects were instructed to perform an orientation discrimination task, see figure 1. Every trial started with a fixation spot (0.8° visual angle), after which a red rectangular bar appeared ($4^\circ \times 0.8^\circ$ visual angle) in either of four locations, equidistant (8.5°) to the fixation spot. This bar stayed on screen for 130 ms. After a brief disappearance (100 ms) the bar reappeared in the same location, either slightly rotated or having the same angle. The 100 ms blank interval was added to ensure that the discrimination was made based on the difference in orientation and not as a consequence of the sudden transition between orientations, which induced a motion-like rotation which was much easier to detect. Subjects indicated by pressing a key with their left (non-dominant) hand whether they observed a difference in orientation between the first and second presentation of the bar. The bar stayed on screen until a response was given. Fixation was required until the bar reappeared (see above), the fixation spot disappeared at that moment. Simultaneously, subjects were required to perform either a grasp or point action to the appearing bar, depending on the instruction at the start of the block. The go-cue for this action was the first appearance of the bar. Subjects were specifically instructed to initiate the action as soon as the first bar appeared. This realizes a situation where the to-be discriminated orientation change occurs during grasping preparation, as the orientation change occurs 230 ms after the grasping/pointing go-cue, which is well before the grasping/pointing movement onset (pointing/grasping movements have latencies of around 400 ms [10,11]). Grasping was performed by applying a precision grip in the length direction of the bar, i.e. to place index finger and thumb at the opposing short sides of the bar. The pointing action implied pointing to the center of the bar with the index finger.

The difference in orientation between the first and second bar could be either 'none', 'small' (2° rotation), 'medium' (4°) or 'large' (6°). The second bar was always oriented at either 45° or -45° (and hence the first bar at $\pm 39, 41, 43, 45, 47, 49$ or 51°). These differences occurred in both a clockwise and counter-clockwise direction. The second bar was always at the same orientation to avoid detection of the change after the movement preparation phase, as the second bar stayed on screen for the remainder of the trial. The magnitudes of change were small, as these were proven to evoke a stronger effect of action preparation in the pilot phase of the study.

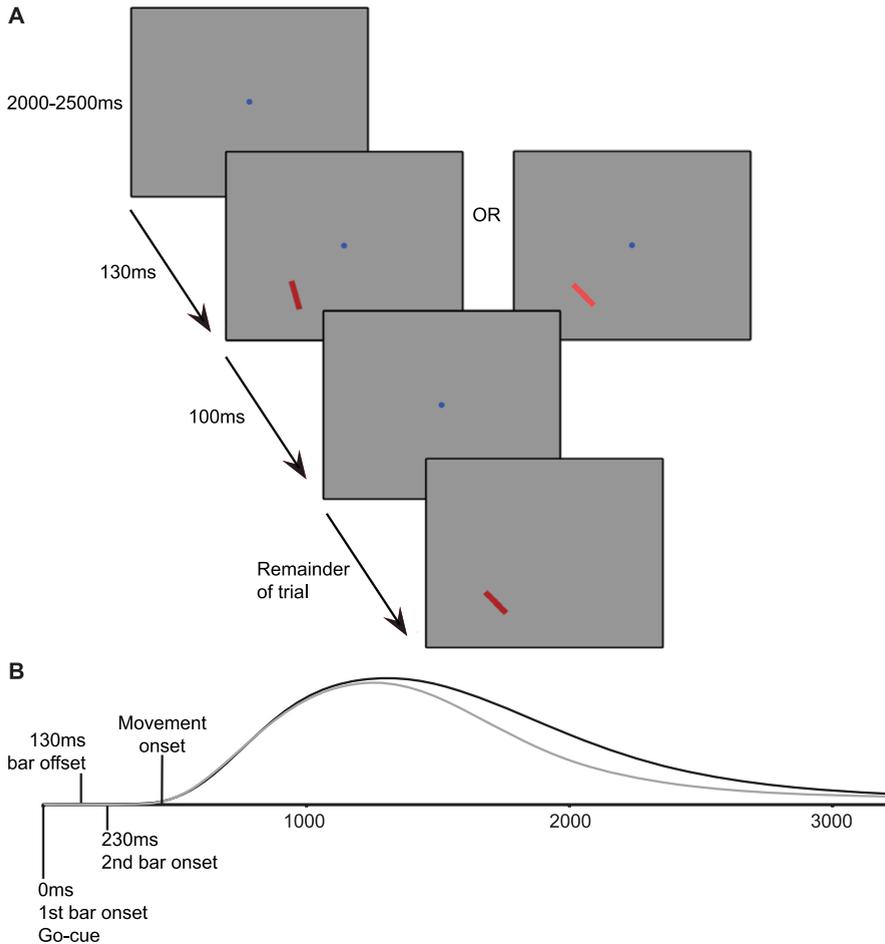


Figure 1. *Experimental paradigm. (A) Stimulus display used in experiment 1 (orientation) and 2 (luminance). A fixation spot was followed by the appearance of a bar that signaled the go-cue for the action to be executed (by instruction) and which could be either rotated slightly (left, experiment 1) or differ in luminance (right, experiment 2) from the subsequent second bar. A brief fixation period (100 ms) was present between the first and second bar presentation. Subjects responded by key-press after execution of the action. (B) Timeline representation of the paradigm. The top plot represents the grand mean average movement (distance to origin) for either grasping (black) or pointing (gray).*

Movement onset time (>0.15 m/s) was monitored to check whether no movement was made before the second bar appeared, to ensure that the discrimination was made in the action preparation phase. In case this was violated, the trial was discarded.

Subjects were trained to reach adequate detection performance levels and were grasping and reaching properly before starting the actual experiment. On average, subjects completed 2–3 training blocks before starting the actual experiment. After training, subjects performed 4 blocks, each consisting of 64 trials. Grasping and pointing blocks alternated and were counterbalanced across subjects.

Stimuli were presented using custom software ('Trackmagic', written in C++) that was able to interface with the movement tracker for synchronized data acquisition. Care was taken to ensure accurate timing of stimulus presentation by synchronizing to the screen refresh rate of the display monitor.

3.2.4 Analysis

All computational analyses were done using customized Matlab scripts (The Mathworks, Natick, USA). Sensitivity (d') for each 'magnitude of change' – 'action type' pair was estimated by subtracting z-transformed hit rates and block false alarm rate ($d' = Z(HR) - Z(FA)$, where HR = hit rate FA = false alarm rate and $Z()$ is the z-transformation). Sensitivity (d') represents how well one can detect a signal from noise, and thus is an estimation of the sensitivity to detect a certain stimulus, in this case an orientation change. This way, a measure of performance is obtained that is free of any response bias. For clarity, an indication of the response bias, in the form of $\log \beta$, was also calculated ($\log \beta = d'(\lambda - 1/2d')$, where d' is the sensitivity and λ the response criterion $-Z(FA)$) [12].

The d' values per condition were further analyzed in a repeated measures analysis of variance (ANOVA) with the factors ACTION (grasping/pointing) and CHANGE_MAGNITUDE (small/medium/large). The action counterbalancing order (grasping or pointing in the first block) was added as a covariate. A preceding pilot study showed that participants continued to show practice effects, even after training. These effects of training differed between grasping and pointing (see figure 2). We therefore treated the first 2 blocks as further training. The results section therefore describes the results of the remaining 2 blocks, as this is considered as representative data without practice effects.

As all actions were performed only with the right hand, but stimuli appeared in both left and right visual field, there might be an effect of hemifield. To this end, in a separate analysis, the data were divided by visual field in which the bar appeared and collapsed over the magnitude of orientation change (to retain sufficient trials to give a reliable sensitivity estimate).

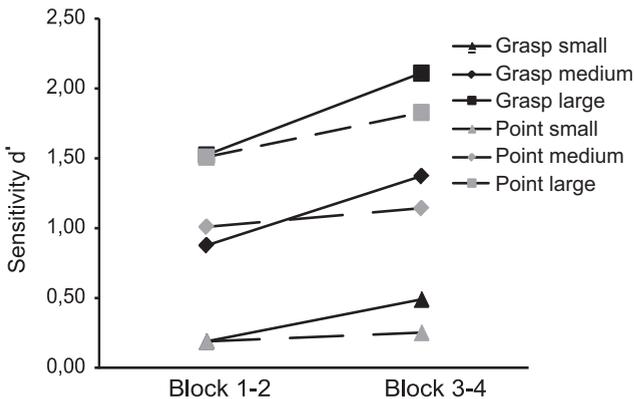


Figure 2. Effects of training. Separate analyses were performed on the first (block 1–2) and second half (block 3–4) of the first (orientation change) experiment. Differences in sensitivity due grasping or pointing preparation become apparent only in the second half of the orientation experiment (1).

Additional parameters were extracted from the acquired movement data, including movement onset and duration, grasp angle and aperture. Movement onset threshold was set at 0.15 m/s. Trials with a movement onset before second bar appearance or two standard deviations beyond the subject mean movement onset were excluded from behavioral analysis, as it is unlikely that the discriminations in this case were made during action preparation.

The grasping angle was defined as the angle between the line defined by the DriveBay probes attached to the index finger and thumb, and an imaginary line along the vertical edge of the screen. This angle was extracted for every sample during every trial and sorted by target angle. For statistical testing purposes, angle timeseries were normalized to movement duration and divided in 25 equal time windows. A bonferroni adjusted significance threshold of $p < 0.002$ was used.

All statistical analyses on the aforementioned parameters were performed using SPSS (15.0, SPSS inc., Chicago).

3.3 Experiment II: luminance

Experiment 2 was identical to experiment 1, except as described below.

3.3.1 Participants

Sixteen subjects (13 women; mean age 25.6 SD3.5) participated in the second experiment, 12 of which had participated in experiment 1. All were right handed, as checked by the Edinburgh handedness inventory (mean 86 SD22) [9]. Subjects signed an informed consent prior to participation.

3.3.2 Task

The task was identical to experiment I, except the discrimination feature was luminance instead of orientation. Again, four levels of luminance changes were used: 'none', 'small' ($\pm 2.7\%$), 'medium' ($\pm 4.7\%$) and 'large' ($\pm 6.6\%$). These levels were chosen to match the difficulty of the orientation discrimination task, based on hit rates during grasping and pointing trials in the pilot phase of the experiment. Luminance levels of the first bar were either incremented or decremented, where the second bar always had the same luminance level.

4. Results

We found that sensitivity to orientation changes increased when grasping, rather than pointing, see figure 3 and table 1. An analysis of (co)variance (ANCOVA) was conducted with factors ACTION (grasping/pointing), CHANGE_MAGNITUDE (small/medium/large change) and covariate 'order' (grasping or pointing first). This yielded a significant main effect of ACTION ($F(1,14) = 6.56$, $p = 0.023$, partial $\eta^2 = 0.32$), indicating that the visual sensitivity significantly differed, depending on the

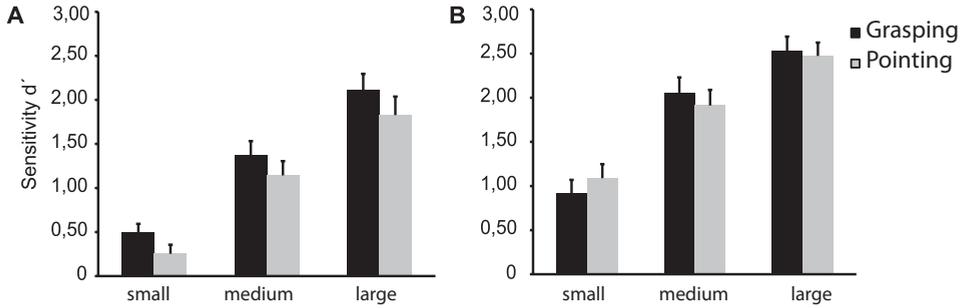


Figure 3. Main findings. (A) In the orientation change discrimination experiment (1), performance is increased when a grasping action is prepared. This effect occurs for all magnitudes of change tested. (B) No such consistent change in performance was found when luminance was used instead of orientation as a feature to-be discriminated.

instruction to grasp or point. The mean overall sensitivity for grasping (1.32 SD 0.60; Hit rate 59.9% SD 16.1; false alarm rate 16.4% SD 14.4, bias $\log \beta$ 0.08 SD 0.53) was higher than the sensitivity for pointing (1.07 SD 0.63; Hit rate 57.9% SD 20.1; false alarm rate 22.6% SD 15.4, bias $\log \beta$ 0.15 SD 0.62).

Also, a significant main effect of CHANGE_MAGNITUDE ($F(2,28) = 24.82$, $p < 0.001$, partial $\eta^2 = 0.79$) was found, showing that subjects sensitivity depended on the magnitude of the orientation change, as expected. No interactions between factors reached significance levels (ACTION \times CHANGE_MAGNITUDE; $F(2,28) = 1.10$, $p = 0.51$, partial $\eta^2 = 0.046$).

To test for possible effects of visual field in which the bar appeared, a separate ANCOVA with factors HEMIFIELD (left/right), ACTION (grasp/point) and covariate 'order' was performed. This yielded a main effect of ACTION ($F(1,14) = 5.73$, $p =$

Table 1. Behavioral performance for all conditions of experiment 1 (orientation).

Action/Change	Hits (%)	FA rate	d'	$\log \beta$
Grasping		16.4		0.08
Small	32.1		0.49	(0.15)
Medium	62.9		1.37	(0.31)
Large	84.8		2.11	(20.22)
Pointing		22.6		0.15
Small	31		0.25	(0.3)
Medium	60.3		1.14	(0.18)
Large	82.5		1.83	(20.04)

'Hits': Percentage of correct detections. 'FA rate': Percentage of false alarms (indications of change when no change was present). d' : Measure of perceptual sensitivity. $\log \beta$: Measure of response bias toward either a change or no-change response.

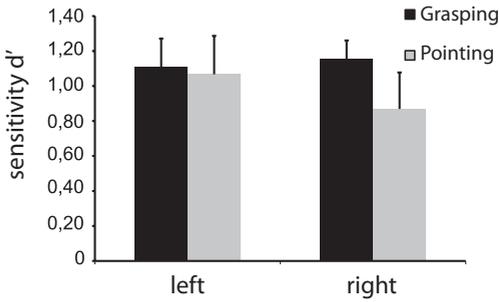


Figure 4. Visual hemifield differences in grasping and pointing performance. Differences in sensitivity between grasping and pointing are prominent when the stimulus is shown in the right visual field, but not when the stimulus appears in the left visual field.

0.031, partial $\eta^2 = 0.29$) and a significant ACTION \times HEMIFIELD interaction ($F(1,14) = 5.10$, $p = 0.040$, partial $\eta^2 = 0.27$), see figure 4. This indicated that the effect depends on the visual field where the discrimination is made and the action performed. The sensitivity values in the left hemisphere show very little difference between actions (grasping: 1.11, pointing: 1.07), whereas the sensitivity values in the right hemisphere do (grasping: 1.15, pointing: 0.87). The increase in performance due to grasping preparation thus only seems to occur for stimuli in the right visual field, or the ipsilateral hemifield with respect to the performing hand.

Data rejection due to premature or late movement onset (beyond two standard deviations of the subject mean) was 3.0% (SD 1.5) on average. Rejection rates did not differ between actions (paired samples t-test, $t(15) = 0.11$, $p = 0.91$). Mean button press response time in the grasping condition was 1127 ms (SD 597) and 1096 ms (SD 568) in the pointing condition.

As a control, the same experiment was repeated using luminance as the discrimination feature instead of orientation, see figure 3 and table 2. The same ANCOVA was performed as in the orientation experiment: ACTION (grasping/

Table 2. Behavioral performance for all conditions of experiment 2 (luminance).

Action/Change	Hits (%)	FA rate	d'	$\log \beta$
Grasping		7.3		0.46
Small	33.0		0.91	(0.72)
Medium	73.7		2.05	(0.60)
Large	86.5		2.52	(0.05)
Pointing		8.2		0.53
Small	38.1		1.09	(0.73)
Medium	70.1		1.91	(0.59)
Large	87.3		2.47	(0.27)

'Hits': Percentage of correct detections. 'FA rate': Percentage of false alarms (indications of change when no change was present). d' : Measure of perceptual sensitivity. $\log \beta$: Measure of response bias toward either a change or no-change response.

pointing), CHANGE_MAGNITUDE (small/medium/large) and covariate ‘order’ (grasping or pointing first). This yielded only a significant main effect of CHANGE_MAGNITUDE ($F(2,28) = 37.75, p < 0.001, \text{partial } \eta^2 = 0.85$). No significant effect was found for ACTION ($F(1,14) = 0.40, p = 0.54, \text{partial } \eta^2 = 0.027$), or any interaction between factors (ACTION \times CHANGE_MAGNITUDE; $F(2,28) = 0.44, p = 0.65, \text{partial } \eta^2 = 0.035$). Thus, no difference in visual sensitivity was found between grasping (mean $d' = 1.83$ SD 0.67; hit rate 64.4% SD20.5; false alarm rate 7.3% SD8.3, bias $\log \beta$ 0.46 SD 0.62) and pointing (mean $d' = 1.82$ SD 0.65; hit rate 65.2% SD19.7; false alarm rate 8.2% SD8.0, bias $\log \beta$ 0.53 SD 0.46) when using a feature that is not relevant for the action in preparation.

Again, an ANCOVA with factors HEMIFIELD, ACTION and covariate ‘order’ was performed. No significant main effects or interactions were found.

Data rejection due to premature or late movement onset (beyond two standard deviations of the subject mean) was 3.3% (SD 1.6) on average. Rejection rates did not differ between actions (paired samples t -test, $t(15) = 0.0, p = 1.0$). Mean button press response time in the grasping condition was 1100 ms (SD 692) and 968 ms (SD 569) in the pointing condition.

4.1 Movement parameters

See table 3 for an overview of the extracted movement parameters and figure 5 for example kinematic data. No significant difference in movement onset was found between grasping and pointing (paired samples t -test, $t = -1.16, p = 0.27$) for the orientation experiment or for the luminance experiment (paired samples t -test, $t = -0.55, p = 0.59$). However, movement duration was significantly shorter for pointing (565 ms) than for grasping (610 ms) (paired samples t -test, $t = 2.27, p = 0.039$). This was also the case in the luminance experiment (paired samples t -test, $t = 4.19, p = 0.001$) for pointing (525 ms) and grasping (566 ms) durations.

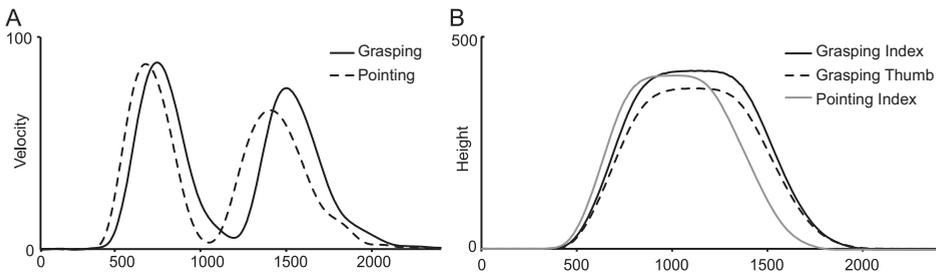


Figure 5. Kinematic data example. Exemplar data from grasping and pointing from a single subject, for a single bar position. (A) Velocity profile is taken from the wrist position. The first peak in velocity reflects the initial transport to the screen, whereas the second peak is caused by the retraction from the screen after the grasping/pointing action to the rest position. (B) Height profile is extracted from the thumb and index positions. Here, maximum height is reached when the subjects points to/grasps the bar on screen. Differences in thumb-index height in the grasping condition reflect the grasping aperture.

Table 3. Mean movement parameters extracted from the movement tracker.

Action/Parameter (ms)	Movement onset	Movement duration	Angle preshaping
Orientation			
Grasping	550	610	.176
Pointing	576	565	NA
Luminance			
Grasping	517	566	.113
Pointing	513	525	NA

Movement onset times (ms) is the time between the go-cue (onset of the first bar) and the actual initiation of movement. Duration of movement (ms) is defined as the time between movement onset and movement offset (when the object on screen is grasped or pointed at). Angle preshaping time (ms) is the time point where a significant difference is observed in thumb-index angle between 45 and 245 degree target bars.

To test for proper angle pre-shaping of the hand during grasping (that is, the alignment of the orientation of the hand with respect to the target in-flight), angle timeseries were separated for target angles of 45 and -45 degrees. These timeseries were divided in 25 time windows (where time windows 1 is movement onset and time window 25 is movement offset) and tested for significant deviation. This yields the time point in which the angle of the target bar influences the grasping action. The preshaping timecourse was averaged over all bar positions. For the orientation experiment, the difference between target angles reached significance from time window 7 (of 25) onwards ($t = 4.97$, $p < 0.001$). This means pre-shaping of the hand was differentiated to target orientation from 28–32% of the grasping movement duration and onwards, which corresponds to 171–196 ms after movement onset, as the mean movement duration is 610 ms. In the luminance experiment it was slightly

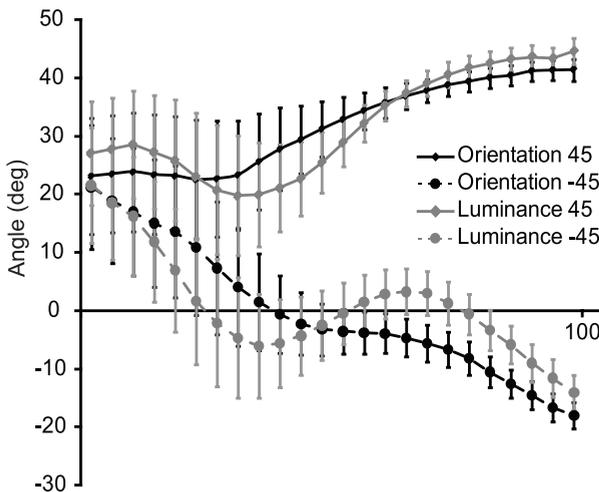


Figure 6. Grasping angle preshaping. Mean orientation of the thumb-index vector, as a function of target bar orientation (45 or 245 deg) and experiment (orientation/luminance) in the grasping condition. The horizontal axis represents the percent movement completed (0–100%), where 0% is movement onset and 100% is the point where the bar on screen is grasped. Error bars represent the standard error (SE).

earlier, in time window 5 ($t(15) = 3.92, p = 0.001$; 20–24% of the grasping duration, 566 ms, or 113–136 ms after movement onset). See also figure 6 for the time course of grasping preshaping to both bar orientations.

As the change in orientation may have influenced grasping angle preshaping, a similar analysis was performed on the angle timelines (divided over 25 time bins). Here, angle time courses were separated by orientation change condition (small, medium, large or none) and target angle (45 or -45 degrees) and tested for significant deviation as a function of magnitude of orientation change. In a $\text{CHANGE_MAGNITUDE}(4) \times \text{TIME_BIN}(25)$ ANOVA, no significant effect of CHANGE_MAGNITUDE was found for either the 45deg target orientation ($F(3,45) = 0.88, p = 0.46$, partial $\eta^2 = 0.055$) or the -45deg target orientation ($F(3,45) = 2.15, p = 0.11$, partial $\eta^2 = 0.13$), indicating that we found no influence of orientation change on grasping preshaping angle. Similarly, angle preshaping time courses were analyzed as a function of the given response (change/no change) instead of change magnitude. Again, no effect of the given response (45deg: $F(1,15) = 1.31, p = 0.27$, partial $\eta^2 = 0.08$; -45deg: $F(1,15) = 0.11, p = 0.74$, partial $\eta^2 = 0.008$) was found in the data.

5. Discussion

In the current study, we found direct evidence for a perceptual enhancement of a specific, relevant feature when preparing a motor act. Visual sensitivity to object orientation change was increased when subjects prepared a grasping action (for which orientation is a relevant parameter) relative to preparing a pointing action (for which orientation is irrelevant). However, no differences in sensitivity were found between grasping and pointing preparation when a luminance change of the target object had to be discriminated, a feature that is irrelevant for both actions. Luminance is not an object feature that must be incorporated in a grasping action, unlike orientation. It is, however, an object feature that is similar to orientation for all other aspects of the task, thus controlling for non-specific effects. The critical difference between orientation and luminance is its relevance for the upcoming action. Take together with the existing literature, this strongly supports a specific action-relevant modulation of perception during action preparation (for encouraging results on grasping preparation and ‘size’ as the relevant feature, see [13,14]). A direct measure of visual sensitivity was obtained by using non-speeded key-press responses occurring well after the grasping or pointing action, where the key-press reactions indicated a discrimination of visual changes that happened during action preparation. This way, we ensured there was no interference between two different active motor systems (key-presses and grasping/pointing) causing the observed influence of action on perceptual discrimination. This is supported by the finding that there was no influence of the magnitude of object orientation change on the preshaping angle of the hand during grasping and also, no difference in the grasping angle preshaping time course between ‘change’ and no-change’ responses.

Analysis of the grasping and pointing movements revealed no differences in movement onset. This implies that the time course of the planning phase was similar, and that the discrimination was made during the same phase of action planning. An influence of target bar orientation (+ or - 45 degrees) on the grasping preshaping angle was found within the first third of the grasping movement, implying that the orientation of the object was an important factor in preparing the grasp.

Interestingly, the effect of action preparation on perception differed between visual hemifields. The effect of enhanced grasping performance was only present when the discrimination target was presented in the right visual field. This may be linked to the hand that was used to perform the action, which, in the current paradigm, was the right hand in combination with central fixation. Neuronal processes in motor related brain areas may only induce changes in perceptual areas within the same cerebral hemisphere, which might explain this effect (see the discussion of feedback based neuronal mechanisms below). One must note though, that there seems to be a non-specific overall increase in visual performance for both grasping and pointing movements in the left visual field. The improvement in discrimination performance for grasping movements we report here is always expressed relative to discrimination performance for pointing movements. This hemifield difference may be attributed to a general difference in performance between visual fields. It has been shown that different parameters of a visual stimulus (i.e. stimulus eccentricity, spatial frequency, perceptual demand) have differential effects on processing efficiency of the left and right hemisphere (for review, see [15]). In the current paradigm, the right hemisphere in right handed individuals (processing information from the left visual field) may be better suited to make the type of spatial discriminations required in the current task.

As mentioned in the method section and in figure 2, training effects were still present after initial training and they seemed to differentiate between actions. To accommodate this, the first two blocks of the experiment were discarded and a 'starting-action' covariate (whether the subject started with either a grasping or a pointing block) was added to the analysis. It is interesting to note that the effects of action specific perceptual enhancement improved with training. It is likely that, especially for grasping, the artificiality of the current setup may have counteracted or occluded any performance gain at first. With practice, the actions became more automatic, as they are in daily life.

In general, discrimination performance during the luminance change experiment was slightly higher than during the orientation change experiment, despite efforts to match the difficulty. This may have impaired comparability between experiments. However, the orientation change experiment shows the effect of action preparation for all change magnitudes and thus seems independent of difficulty. Furthermore, the effect of action preparation does not show for the smaller change magnitudes in the luminance experiment, and therefore it is unlikely that the slight mismatch between orientation and luminance change discriminability explains why the

effects of action-modulated perception were only found in the orientation change experiment.

Our current results agree well with existing literature. It fits with the idea that selection of visual processing (selective visual attention) is based on the intended action to be executed, that is, as a selection-for-action mechanism (e.g. [16]). This selection of action relevant information and the planning of this action may be implemented in a common mechanism (e.g. [17,18]). This idea is closely related to the influential pre-motor theory of attention [19], stating that the preparation of a motor act is essentially identical to the attentional preparation that facilitates the action. Originally this was formulated for the oculomotor system, explaining covert shifts of attention as unexecuted eye movements. Later the theory was expanded to incorporate skeletomotor acts as well (e.g. [8]). In the latter study by Craighero et al., subjects had to execute a grasping action to a (real) bar object, triggered by a go-cue (a bar of matching or non-matching orientation) on a computer screen. When the bar on screen (go-cue) and the bar to-be-grasped had a matching orientation, movement onset times were reduced compared to incongruent orientations. The authors attributed this effect to enhanced visual processing of the go-cue due to the preparation of the grasping action. Although the latter explanation is likely, the results by Craighero et al. entail an enhanced orientation perception only for matching orientations. That is, the preparation of a grasping action facilitates the visual processing of objects with an orientation matching the prepared grasping action. This might be due to the specificity of the prepared grasping action, which was known before the appearance of the go-cue on screen in the study by Craighero et al. Therefore, this effect could also be related to a feed-forward process that perceives cues congruent to the instructed action in an enhanced way, rather than movement preparation effects that improve orientation perception.

In a similar fashion, Symes and colleagues [14] showed reduced reaction times in a change blindness paradigm if the changing object was congruent, rather than incongruent with a planned grasp (precision/power grasp and small/large objects).

The effect of action-modulated perception has also been shown to facilitate visual search for grasping-relevant features such as bar orientation. In a study by Bekkering and Neggers ([7], see also [20]), subjects had to grasp or point to an object of a certain orientation and color among other objects. The saccadic eye movements that naturally precede the grasping or pointing action were analyzed. Fewer eye movements were made to wrong orientations when subject had to grasp the object rather than point to it. Increased peripheral sensitivity to orientation, as was found in our study, can account for the performance improvement observed by Bekkering and Neggers. It is interesting to note that the visual search enhancement seemed to disappear with smaller set sizes. In the current study, three levels of difficulty were used, covering a wide range of performance. Here the effect did not disappear with decreasing difficulty, but remained consistent across difficulty levels. It may be that the current paradigm is more sensitive to performance differences, even when the task is relatively easy. Furthermore, the effect by Bekkering and Neggers can also

be explained by interaction between two motor processes, grasping preparation and saccade scanpaths (patterns we are often not aware of). The present influence of grasping preparation on visual discrimination judgments, which we are fully aware of, cannot be explained this way.

One can speculate on the neuronal mechanism underlying action-modulated perception. First, the current observed effect may be very similar to action-induced perceptual enhancements in the oculomotor system, where strong links have been found between spatial attention and eye movement preparations. Namely, Deubel and Schneider [1] showed that spatial attention is greatly increased at the target position of the upcoming eye movement. This has been interpreted as support for the influential pre-motor theory of attention [19]. Recently, the neural mechanisms underlying this effect have been studied in more detail. It is becoming clear that the effect is mediated by cortical feedback connections from the oculomotor areas (specifically the frontal eye fields) to occipital areas shortly before an eye movement [4–6,21]. Such connections allow preparatory activation in motor control areas to modulate early visual processing in the occipital lobe. It may very well be so that this current form of ‘action-modulated perception’ is mediated by similar mechanisms in the skeletomotor domain. Cortical (pre)motor areas, specific for the action to be performed, might modulate visual processing through feedback connections to occipital areas. For instance, the anterior intraparietal area (AIP) would be an ideal candidate to fulfil such a function, as it is heavily implicated in the planning and execution of grasping actions [22,23]. A recent EEG study [24] shows some initial evidence for such a mechanism. Here, grasping preparation elicited an enhanced occipital selection negativity that was absent in pointing preparation. This is indicative of an early modulation of visual processing specific to the preparation of a grasping action. Interestingly, when such feedback from AIP to the occipital lobe would occur within each cerebral hemisphere only, this would explain why we mainly find effects of grasping on perception in the right visual field. When assuming that left AIP is activated for grasping with the right hand, this induces changes in the left occipital lobe which in turn leads to action-modulated perception in the right (contralateral) visual field. Further studies are needed to unveil the exact neuronal mechanism driving the enhancement of action relevant features during action preparation.

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Chapter 5

TMS of the anterior intraparietal area
selectively modulates orientation change
detection during action preparation

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revision submitted

1. Abstract

Perception of relevant visual object features can be modulated by the preparation of an action toward it, such as the orientation of a book when grasping it. However, the underlying neuronal mechanisms are poorly understood. We argue that brain areas controlling arm movements are involved in establishing this effect through top-down feedback to early visual areas, similar to the neuronal mechanisms linking visual attention and eye movements. To investigate this involvement we applied transcranial magnetic stimulation (TMS) to a grasping motor area, the anterior intraparietal area (AIP), during grasping preparation. Concurrently, an orientation change detection task was performed. This was contrasted with the preparation of a pointing action. As a control area the vertex was stimulated.

With TMS applied to the vertex, the expected effect of enhanced orientation detection performance while grasping was observed. However, when TMS was applied to the AIP, this effect was disrupted.

We argue that AIP is a critical part of the mechanism underlying perceptual modulations during grasping preparation. The present results and recent literature suggests that this action-modulated perception is implemented through a cortical feedback connection between AIP and early visual areas.

2. Introduction

In order to successfully execute our actions in daily life, it is beneficial to focus on specific features of the incoming visual information. Picking up a pen from the table might be more successful when one is focused on its orientation, rather than its color. Obviously, it is advantageous to induce such selective perception automatically, before the actual execution of the action. Evidence toward such an action-modulated perception has accumulated over recent years [1–5].

Specifically, in a recent experiment [6], we could demonstrate such an effect using a direct measure of visual performance. Subjects performed an orientation change detection task, while preparing either a grasping- or a pointing action toward a bar object. Orientation change perception improved significantly when preparing a grasping action, rather than a pointing action. As orientation is a relevant feature for grasping actions, but not for pointing actions, we attribute this visual performance gain to perceptual enhancement of action relevant features during motor preparation.

However, much is unclear about the neural implementation of such a mechanism. Recent studies have shown that the frontal eye fields are responsible for shifts of spatial attention known to precede eye movements through connections with early occipital visual areas [7–11]. Here the modulation of perception is driven by motor preparation in an area that is specialized for a certain action: eye movements. For other actions, such as grasping, this could function similarly: action preparation processes in a specialized cortical motor area might drive perceptual changes in the

visual cortex through feedback connections. A recent EEG study [12] found initial evidence for modulation of occipital activity shortly before grasping, confirming this notion.

One cortical motor area, the anterior part of the intraparietal sulcus (AIP), is clearly involved in controlling grasping movements, both in monkeys (e.g. [13]) and humans [14,15]. As this area is connected to visual areas [16–18], including ventral stream areas [19], it is a likely mediator of enhanced orientation sensitivity due to grasping preparation.

To test the involvement of AIP in the modulation of orientation perception during action preparation, we aimed to test whether we can disturb the grasping induced improvement of orientation perception with TMS on AIP. Therefore, AIP was stimulated during action preparation while subjects performed an orientation change detection task. If AIP is involved in the modulation of perception during the action preparation phase, stimulation during this phase should interfere with the enhancement of relevant features induced by grasping preparation.

3. Materials and methods

3.1 Participants

16 healthy subjects (8 women; mean age 25.6 SD3.4) with normal or corrected-to-normal vision participated in the experiment. All were right handed, as checked by the Edinburgh handedness inventory (mean 81.6 SD24.1) [20]. Subjects signed an informed consent and went through a TMS safety screening prior to participation. All procedures were approved by the medical ethical committee of the UMC Utrecht. TMS (resting) motor thresholds were determined per subject by applying TMS to primary motor cortex. Stimulation intensity was decreased whenever a reliable motor response was observed (visually) in any of the fingers for at least 5 out of 10 stimulations (or increased when no such response was found). This was repeated until the motor threshold was reached [21]. Mean motor threshold was 56.6% (SD8.4) of the maximum machine output.

3.2 Apparatus

Subjects sat in a dimly lit room in front of an Iiyama 17" (320x240mm) monitor, with a resolution of 1024x768 pixels and a refresh rate of 100Hz. They were seated in a frame with head- and chinrest. To minimize head motion during the experiment, a flexible strap was applied around the back of the head, assisting the participant to keep their head in the chin- and headrest.

The distance of the frame to the pointing/grasping targets was adjusted per subject to enable comfortable pointing and grasping movements. Visual angles of the stimuli were kept constant by compensating the size of the stimuli relative to the viewing distance.

To ensure that grasping and pointing actions were executed correctly, motion tracking of the right hand (grasping/pointing hand) was performed using the ‘driveBay’ magnetic motion tracker. Subjects wore a flexible, unrestrictive glove that was fitted with four motion sensors located at the tip of the thumb, tip of the index finger, back of the hand and at the wrist. Movement data were recorded from all sensors at 240Hz.

TMS was applied using a Magstim Rapid² (The Magstim company, ltd. Wales, UK) with a 70mm figure-of-eight coil. During the experiment, subjects received single pulse (biphasic) stimulation at 110% of their individual motor threshold.

3.3 TMS site localization

MRI guided navigation was used to place the TMS coil on the appropriate scalp location, such that the TMS pulse was directly targeting the individual AIP area. To this end, T1-weighted anatomical MRI scans were obtained from a Philips 3T Achieva scanner (Philips medical systems, Best) for every participant (scan parameters: TE/TR 4.6/9.87 ms; FA 8°; FOV 224 x 160 x 168 mm; matrix 256 x 256; slice thickness, 1 mm; no slice gap; voxel size 0.875 x 0.875 x 1 mm). As AIP target coordinates, we used Talairach group activation maximum coordinates (MNI: x: -37, y:-45, z: 37) reported in an fMRI study by [14] investigating human AIP. In this study, grasping and reaching actions toward three dimensional objects were contrasted, which resulted in activity in the anterior intraparietal area. These coordinates, after conversion to MNI space, were transformed to native space (i.e. not normalized) of individual subjects’ brains by inverse normalization, based on the ‘unified segmentation’ algorithm [22]. The latter procedure matches standardized MNI space brain atlas coordinates to individual MRI brain coordinates as accurately as possible, yielding approximate AIP coordinates that can be used for individual stereotactical navigation of AIP. After conversion of the standardized MNI space coordinates to personal (native) space, the personalized AIP target coordinates were entered into our stereotactic neuronavigation software (NeNa 2.0, Brain Science Tools, Utrecht). These were then manually adjusted (individually) by aid of cortical landmarks also described by Cavina-Pratesi and colleagues. That is, the initial approximate AIP coordinate was lifted along the banks of the anterior parietal sulcus toward the surface of the brain for each participant, as that is the part of the cortex one can affect with TMS. AIP was found to be reliably located at the junction of the anterior end of the intraparietal sulcus and the postcentral sulcus [14]. See figure 1 for an impression of the original and adjusted individual AIP coordinates. The vertex coordinates were determined purely based on anatomical landmarks and was defined as the top-most part of the head, between hemispheres. This usually overlays the leg-motor areas, although these areas are not stimulated effectively.

Having determined the target coordinates, the anatomical scan was registered with the subject’s actual head, using the stereotactic neural navigation system in combination with the ‘driveBay’ magnetic tracker (Ascension technology, Burlington, USA), for the measurement of facial landmarks. Coil placement markings were

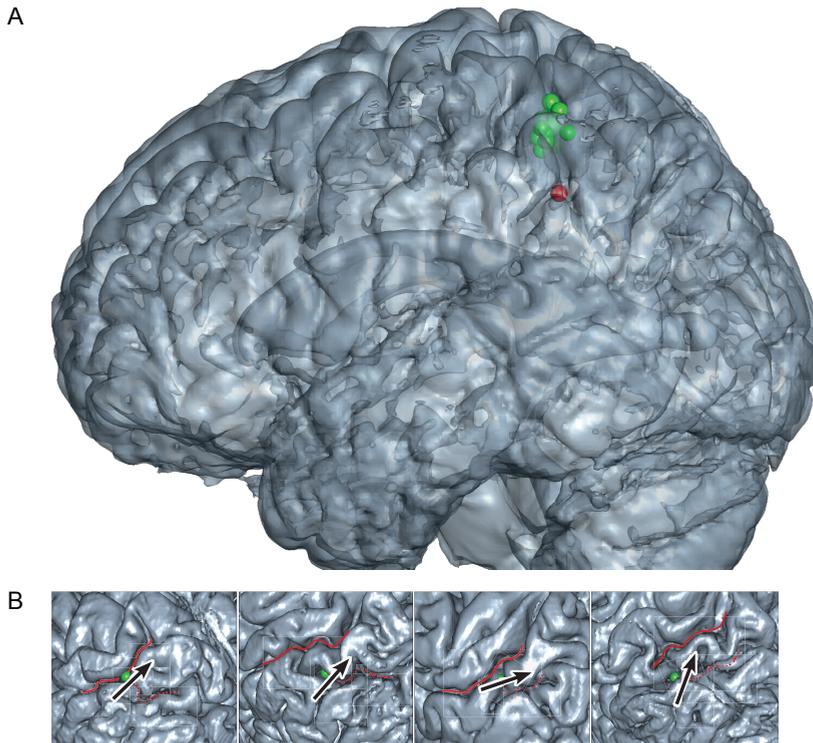


Figure 1 A) Rendering of the re-normalized TMS targets for all participants on a translucent normalized cortical surface (see methods), showing individual variability of the TMS targets. The original AIP coordinate from the literature is shown in red.

B) Renderings of individual TMS target locations, used to guide the TMS coil, and cortical surface for four representative subjects. The TMS target was placed at the junction of the anterior intraparietal sulcus and the postcentral sulcus. The cortical renderings were directly created from the gray matter segmentation derived from the individual subjects' MRI scan. TMS target locations are shown as green spheres. Dashed lines represent the intraparietal sulcus (IPS). Solid lines represent the postcentral sulcus (PCS). Arrows indicate the estimated optimal TMS current direction to which the TMS coil was aligned during placement.

drawn on a tight fitting swimming cap covering the subject's head. In addition, the orientation of the anterior part of the intraparietal sulcus was marked on the swimming cap. In the AIP stimulation condition, the coil was placed such that it was tangential to the scalp and the current induced in the brain is perpendicular to the sulcal direction to optimize stimulation efficiency. It is known that the direction of induced current and the general direction of the underlying pyramidal neuron tracts are roughly the same in this situation, yielding optimal TMS effects [23,24]. In the vertex (control) condition, the coil was placed on the top of the head, with the focal point of stimulation in between hemispheres and the induced current directed along the hemispheric division.

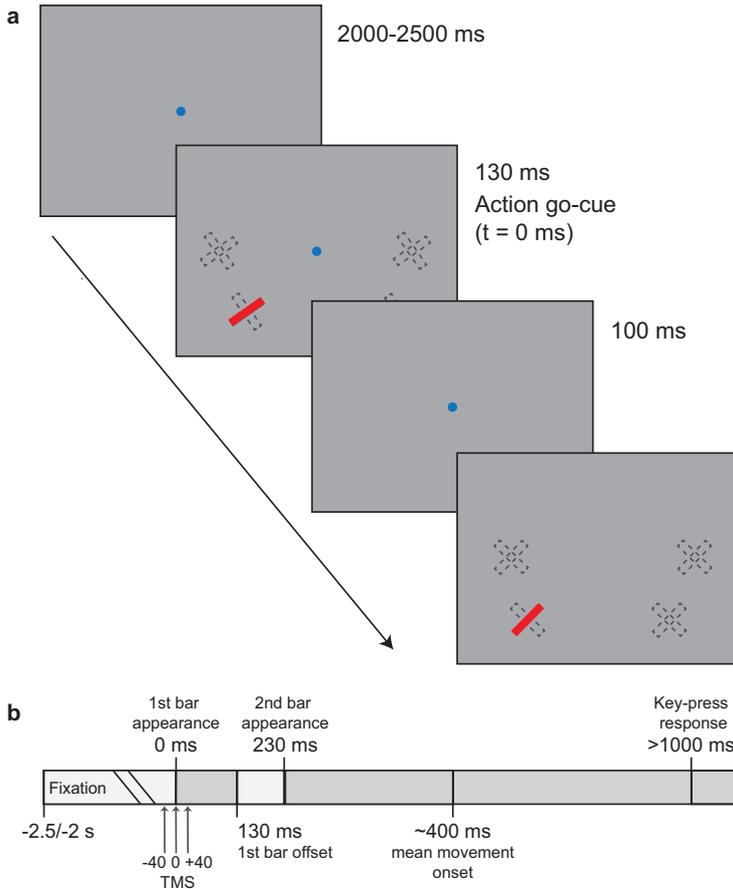


Figure 2 A) Schematic representation of the orientation change detection task. Subjects were presented with a blank screen for 2000–2500 ms (random length) after which the first bar appeared at either of four locations, equidistant to the fixation spot (indicated by the dashed outlines), which served as the grasping or pointing go-cue. This bar could have an orientation differing from ± 45 deg and disappeared after 130ms. After a blank screen for 100ms, another bar appeared and stayed on screen which was always ± 45 deg. Due to grasping/pointing latencies, the instructed action was not executed until after the appearance of the second bar. After execution of the action, subjects could respond by key press whether they had perceived a change, without time pressure.

B) Timeline of events. $t=0$ represents the action go-cue. Single pulse TMS was delivered at either $t=-40$, 0 or $+40$, relative to the go-cue (1st bar appearance).

3.4 Task

Subjects were instructed to perform an orientation change detection task (see figure 2) while performing grasping or pointing actions, similar to the task described in the previous chapter [6]. Every trial started with a blue fixation spot (2000–2500ms, 0.7° diameter of visual angle), after which a red rectangular bar appeared ($0.8^\circ \times 4^\circ$ visual angle) for 130ms in either of four locations, equidistant (9° from fixation to bar center) to the fixation spot. After a brief disappearance (100ms) the

bar reappeared in the same location, either slightly rotated, or having the same angle. At this point the fixation spot disappeared and fixation was no longer required. Without time pressure, subjects indicated by pressing one of two keys with their left hand whether they observed a difference in orientation between the first and second presentation of the bar or not. The bar stayed on screen until a response was given. Simultaneously, subjects were required to perform either a grasp or point action to the appearing bar, depending on the instruction at the start of the block. The go-cue for this action was the first appearance of the bar. Subjects were specifically instructed to initiate the action as soon as the first bar appeared. This realizes a situation where the to-be discriminated orientation change occurs during the grasping preparation phase, as the orientation change occurs well before the grasping/pointing movement onset (pointing/grasping movements have latencies of around 400 ms [25,26]). Grasping was performed by applying a 'precision grip' in the length direction of the bar, i.e. to place index finger and thumb at the opposing short sides of the bar. As subjects are not grasping a real-life object (it is merely drawn on the screen), the precision grip cannot be fully applied, but we verified previously [6] that the kinematics in this situation are comparable to a real precision grip. The pointing action implied pointing to the center of the bar with the index finger. When a successful grasping or pointing action was performed, the bar turned green and a sound was played. This was done to motivate participants to make correct grasping or pointing movements. Online analysis of the grasping or pointing movement data (acquired by the motion tracker) was performed to determine whether the action was executed correctly.

The difference in orientation between the first and second bar could be either 'none', 'small' (3° rotation) or 'large' (5°). The second bar was always oriented at either 45° or -45° (and hence the first bar at +/- 40, 50, 42, 48 or 45°). In half of the trials, an orientation difference occurred, either small or large (also distributed equally). These differences occurred in both a clockwise and counter-clockwise direction. It is important to note that the second bar appearance was essential to be able to make the orientation difference detection (although the second bar was always oriented at 45 or -45 degrees). Movement onset time (>0.15m/s) was monitored to check whether no movement was made before the second bar appeared, to ensure that the discrimination was made in the action preparation phase. In case this was violated, the trial was discarded during the analysis.

In every trial, a single pulse of TMS was applied at either one of three different time points around the onset of the first bar: Either 40ms before (-40), during (0ms) or 40 ms after (+40) the first bar appearance. The first bar, rather than the second bar, was chosen because the first bar contains the information necessary to make the orientation change detection, i.e. only the first bar could have an orientation deviating from +/- 45 degrees. The second bar is always oriented at +/- 45 degrees, to ensure that the grasping itself was always to a bar oriented at either -45 or 45 degrees.

The stimulation times were chosen based on estimated cortical conduction times for visual input (the time between presentation of the first bar stimulus and its arrival in the visual cortex) and between AIP and the visual areas. We estimated these times to be roughly equal (see [27] for a similar reasoning), i.e. assuming feedback connections between AIP and the visual cortex, a TMS pulse and a visual stimulus at time 0 should arrive simultaneously in the visual cortex. Given the hypothesis that a feedback signal arises from AIP during early action preparation that subsequently modulates perception in the visual cortex before or during visual input, we centered the timing of the TMS pulses on 0ms (onset of the first bar stimulus).

Before starting the actual experiment, subjects were trained to reach adequate orientation change detection performance levels. On average, subjects completed 4-5 training blocks (32 trials each) before starting the actual experiment. Detection performance was checked after each training block. Criteria for 'adequate performance' were a hit rate in the small change condition of 25% or better; better performance on the 'large change' condition (large change > small change) and a false alarm rate of less than 40%. After training, subjects completed 8 blocks of 64 trials each. Site of stimulation (AIP, vertex) was changed after 4 blocks and was counterbalanced across subjects. Grasping and pointing blocks alternated and the order was also counterbalanced across subjects.

Stimuli were presented using custom software ('Trackmagic', written in C++) that was also able to interface both with the movement tracker for synchronized data acquisition and the TMS device for triggering the TMS pulse. Care was taken to ensure accurate timing of stimulus presentation and pulse triggering by synchronizing to the screen refresh rate of the display monitor.

3.5 Behavioral analysis

All analyses were done using custom Matlab scripts (The Mathworks, Natick, USA). Statistical analyses were performed using SPSS (15.0, SPSS inc., Chicago).

Sensitivity (d') for each TMS site/TMS timing/action combination was estimated by subtracting z -transformed hit- and false alarm rates ($d' = Z(\text{HR}) - Z(\text{FA})$, where HR=hit rate FA=false alarm rate and $Z()$ is the z -transformation, which is the inverse cumulative normal distribution under the equal variance assumption). Sensitivity values were calculated individually per subject and separately for every block. Values for the same conditions were averaged over blocks afterwards. This was done to prevent any difference in response bias between blocks from contaminating the performance measure.

These d' values were used analyzed in a repeated measures ANOVA with the factors TMS SITE (AIP, vertex), TMS TIMING (-40, 0, +40) and ACTION (grasping/pointing).

3.6 Kinematics

To test whether stimulation of AIP had direct effects on the grasping and pointing movements, motion tracking of the grasping/pointing hand was performed

during the performance of the task. Kinematic data were obtained using a magnetic motion tracker (DriveBay, Ascension technology, Burlington, USA).

Parameters were extracted from the acquired movement data, including movement onset, duration, (time to) peak velocity, grasp angle and aperture. Grasp angle was calculated as a two dimensional projected angle between the index finger-thumb pair and an imaginary vertical line on the monitor. Grasping aperture was the distance between these fingers. The grasping angle time course (from movement onset to offset) was divided in 24 time bins and tested for significant differences between AIP and vertex stimulation conditions per timing and target orientation.

Remaining parameters were extracted from the wrist probe. Movement onset threshold was set at 0.15m/s.

All parameters were entered in an ANOVA with factors TMS SITE, TIMING and ACTION.

3.7 Visual hemifield

In the preceding study [6] we found an effect of visual hemifield, in that the enhancement in orientation detection due to grasping preparation was only found for the right visual field. To test whether this effect is present in the current data and whether it is affected by TMS, the current data were also divided by hemifield in which the stimulus appeared. As fixation was required until the second bar appeared, stimuli presented on the left side of the screen appeared in the subjects left visual field (and vice versa). The data were divided by visual hemifield (left/right), site of stimulation (AIP/vertex) and action (grasping/pointing), but collapsed over TMS timing (-40/0/+40) to retain sufficient trials to estimate a reliable sensitivity measure. These d' values were used in a repeated measures ANOVA with the factors HEMIFIELD, TMS SITE and ACTION.

4. Results

4.1 Subjects

Initial analysis of individual discrimination performance revealed that four of the sixteen participants showed the opposite effect of action preparation on discrimination performance in the control condition, i.e. their discrimination performance was enhanced during pointing preparation, rather than grasping preparation, see figure 3. The data for these four subjects are directly opposite to what was found in an earlier study using the same paradigm [6]. We expected to replicate these results in the control stimulation condition, as vertex stimulation should not specifically affect discrimination performance. The current study aims to use TMS to modulate the specific effect of grasping-induced orientation discrimination changes and thus these four subjects, not showing the effect at baseline, were eliminated from further analyses. This did not alter the direction of the effects found in the initial analyses, but did increase specificity of the effects.

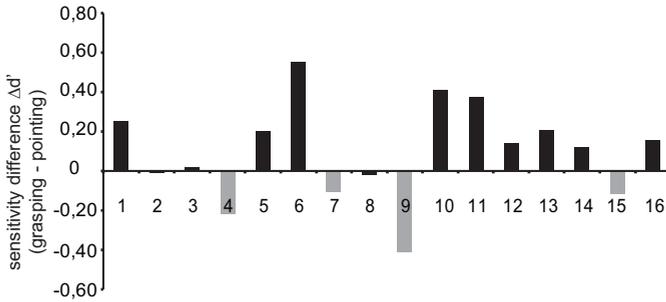


Figure 3. Individual subject performance in the vertex (control) stimulation condition. Values on the ordinate represent difference in orientation change sensitivity ($\Delta d'$) between grasping and pointing ($d'_{\text{grasping}} - d'_{\text{pointing}}$). Rejected subjects are marked in gray. The abscissa represents subject number.

Analysis of data rejection, based on movement onset times, yielded no significant differences in trial rejection rates, between sites (AIP, vertex) or actions (grasping, pointing). The mean trial rejection rate in the AIP stimulation condition was 2.93% (SD 1.41) for grasping and 2.24% (SD 1.27) for pointing. In the vertex condition the mean rejection rate was 2.44% (SD 0.94) for grasping and 2.39% (SD 1.58) for pointing.

4.2 TMS site localization

TMS target coordinates (MNI: x: -37, y:-45, z: 37) were obtained from MNI atlas space group activation coordinates reported in an fMRI study [14]. After transformation to native space, the individual AIP coordinates were manually adjusted to better align with superficial anatomical landmarks visible in individual 3D brain renderings (see methods and figure 1). This adjusted location was entered in the neuronavigation software and used to guide the focus of the TMS coil to AIP. Only to accurately report individually adjusted AIP coordinates in figure 1, the native space target coordinates were (re-)normalized to MNI space. All TMS targets fitted within a sphere with radius 6.3mm (maximum distance to mean coordinates), see figure 1 for the distribution of individual AIP coordinates in MNI space. The mean stimulation coordinates (MNI: X: -42.5 Y: -42.8 Z: 51.9) were located 16.1mm (Euclidean distance) from the original coordinate (MNI: x: -37, y:-45, z: 37) from the literature, mainly due to the elevation toward the surface of the brain.

4.3 Main Analysis

A significant SITE \times ACTION interaction was found ($F(2,22)=6.97$, $p=0.023$, partial $\eta^2=0.39$), see figure 4. This indicates that, as hypothesized, perceptual sensitivity significantly differs between actions, depending on the site of stimulation. Mean sensitivity values, pooled over timing, show that in the control condition (vertex), discrimination performance is higher when preparing a grasping action than a pointing action (grasping $d'=0.49$, pointing $d'=0.34$). However, with AIP

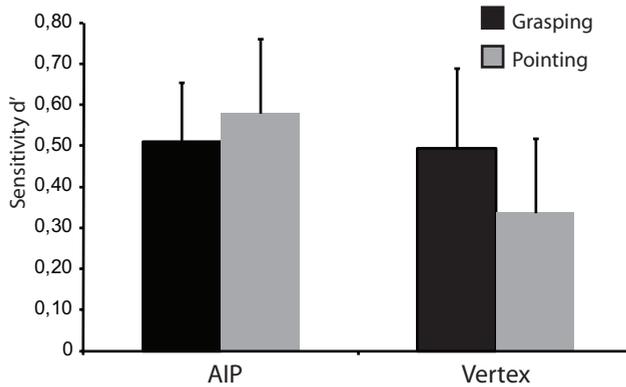


Figure 4. Mean sensitivity values (d') for the AIP and Vertex stimulation conditions, during grasping and pointing preparation. Error bars represent the standard error.

stimulation, performance in the grasping condition is not higher than in the pointing condition (grasping $d' = 0.51$; pointing $d' = 0.58$), effectively negating the grasping induced perceptual improvements observed in our previous study and in the (vertex) control condition.

Although mean sensitivity in the AIP stimulation condition seemed higher than in the vertex condition (AIP: 0.54 SD 0.19, Vertex: 0.41 SD 0.22), this effect was not significant as no significant main effect of SITE was found ($F(1,11) = 1.56$, $p = 0.24$, partial $\eta^2 = 0.12$).

In addition, a significant main effect of TIMING was found ($F(2,22) = 5.00$, $p = 0.019$, partial $\eta^2 = 0.31$), indicating that there was a significant difference in visual perception performance depending on the time of stimulation. Post-hoc pairwise comparisons showed that performance in the 'stimulus onset' (0ms, $d' = 0.66$ SD 0.65) stimulation condition was significantly increased, relative to early (-40, $d' = 0.36$ SD 0.55) stimulation ($p = 0.021$) and late (+40, $d' = 0.42$ SD 0.61) stimulation ($p = 0.039$). However, there was no significant interaction with stimulation site and it is therefore unlikely to be a neural effect of interest.

4.4 Effects of visual hemifield

In a separate analysis, the data were pooled over timings and separated by hemifield where the stimulus appeared, to investigate the effect of visual field on discrimination performance. This ANOVA yielded a significant SITE x ACTION

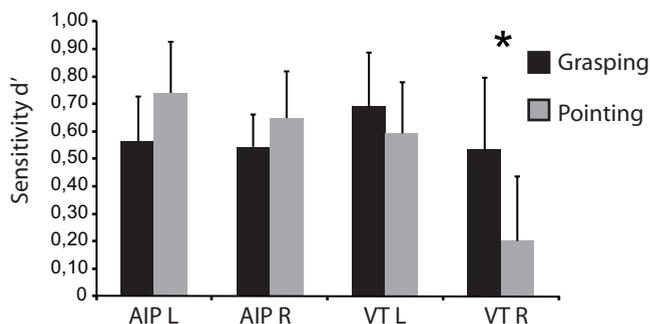


Figure 5. Mean sensitivity values (d' , ordinate) separated by site of stimulation and visual field that the stimulus was presented in. Error bars represent the standard error.

interaction ($F(1,11)=5.83$, $p=0.034$, partial $\eta^2=0.35$), as was found in the main analysis (see above). In addition, a main effect of hemifield was found at trend level ($F(1,11)=3.82$, $p=0.076$, partial $\eta^2=0.26$), where sensitivity was generally higher for stimuli in the right visual field (LEFT: 0.65, RIGHT: 0.48). To further test the effect of action preparation per hemifield and stimulation site, grasping and pointing sensitivity values were compared in one-tailed paired sample tests per hemifield, see figure 5. A significant difference ($t=2.02$, $p=0.034$) between grasping and pointing performance was only found in the vertex-right condition (in favor of grasping), indicating that the effect of action preparation may only occur in the right hemifield.

4.5 Hand movement kinematics

Analysis of the extracted movement parameters, using an ANOVA with the same factors as the main analysis above, yielded a significant main effect of TIMING for ‘movement onset’ ($F(2,,10)=4.80$, $p=0.019$, partial $\eta=0.30$). Post hoc pairwise comparisons showed that stimulation in the +40 condition resulted in a delayed movement initiation (610ms for +40, compared to 587ms for -40 and 585ms for 0ms). As there is no effect of stimulation site, this likely reflects a secondary non-neuronal TMS effect such as a general increase in movement initiation time simply due to the warning signal provided by the late TMS pulse relative to the go-cue. Movement onset times did not differ significantly between grasping and pointing actions, suggesting a similar motor preparation time course.

Peak velocity values significantly differed between grasping and pointing actions (main effect of ACTION: $F(1,11)=17.34$, $p=0.002$, partial $\eta^2=0.61$), where grasping (71.2 cm/s SD16.9) was faster than pointing (63.6 cm/s SD17.7).

A significant TIMING x SITE interaction was found for the ‘time to peak velocity’ parameter ($F(2,30)=5.53$, $p=0.016$, partial $\eta^2=0.27$). This however, did not differ between actions and is therefore not a factor in grasping/pointing performance differences.

No significant factors were found in the ‘maximum grasping aperture’ or ‘movement duration’ ANOVAs. Analysis of grasping angle time courses did not reveal any significant differences between AIP and control stimulation for any timing or target angle, i.e. no effect of TMS was found on the angle preshaping during the grasping action.

In summary, for the timings tested, no significant effects of AIP stimulation were observed on grasping or pointing kinematics specifically.

5. Discussion

In the current study we aimed to investigate the neural mechanism underlying action-modulated perception phenomena that have been reported for arm and hand movements in several studies [1,5,6,28]. We hypothesized that the anterior intraparietal area (AIP) is not only involved in the preparation and control of

grasping actions, but also in the modulation of perception of visual object features that are relevant for the upcoming grasp. We found that stimulation of AIP, but not a control area (vertex), during action preparation disrupts the selective enhancement of orientation sensitivity normally observed shortly before grasping. This argues for the involvement of AIP as a critical area in the mechanism underlying the effect of action-modulated perception for grasping movements and that AIP might influence perception through cortical feedback connections to the visual areas.

Stimulation of AIP affected object perception, but not the execution of grasping or pointing itself. Effects of AIP TMS on grasping kinematics have been shown previously, both during the movement [15,29,30] and at the grasping go-cue [31]. However, no effects on kinematics have been reported using unilateral stimulation at the go-cue, as is the case in the current study. It is likely that we stimulated AIP too early in the grasping preparation period to significantly affect the kinematics.

As in the previous study [6], an effect of visual hemifield was found in the vertex stimulation condition, where the selective enhancement due to grasping preparation was only present when stimuli were presented in the right visual field, possibly because subjects grasped with their right hand. In the AIP stimulation condition, no significant effect of motor preparation on detection performance was found for either left or right visual field. Obviously, as no effect of motor preparation was found in the left visual field, there was no disruption. The disruptive effect of TMS on AIP reported in the current study is predominantly based on effects in the right visual field. This might not be surprising, as the left AIP was stimulated and all movements were made with the right hand. We assume that neuronal signals from AIP, contralateral to the moving hand, are relayed to the visual cortex in the same hemisphere to manipulate visual processing during action preparation. Influences of TMS on action-modulated perception can then be expected in the hemifield ipsilateral to the moving hand and contralateral to the stimulated hemisphere.

It is unlikely that the differences in detection performance found in the current study are due to differences in the difficulty of grasping or pointing actions. No differences in sensitivity between grasping and pointing conditions occur when an action-irrelevant feature (luminance) is used as the to-be discriminated feature [6].

The observed disruption of grasping-induced orientation discrimination improvements when stimulating AIP is compatible with the idea of neuronal feedback connections from AIP to the visual cortex modulating perception, as put forward in the introduction. In this respect it is interesting to note that, although not reaching significance level, mean overall discrimination sensitivity, irrespective of the action being prepared, was also found to be higher when stimulating AIP as compared to vertex stimulation as can be seen in figure 4. These observed effects can be explained in two ways.

First, AIP stimulation may cause a general increase in performance (i.e. elevated baseline performance for both grasping and pointing) by connections from the intraparietal sulcus (IPS) to the visual cortex, combined with a disruption of the signal that causes grasping-induced perceptual enhancement through another

feedback channel. In our own lab we have found general improvements of discrimination performance due to single pulse stimulation of the IPS before [9], which is consistent with existing literature [17,18]. Similarly, perceptual discrimination improvements have been found for TMS of the frontal eye fields as well [8,9,11,32].

Second, performance may be enhanced only in the pointing condition. TMS on area AIP may add a feedback signal during pointing preparation to the visual cortex, similar to the AIP signal naturally occurring during grasping preparation. Thus, the stimulation of AIP during pointing preparation may mimic the feedback signals normally sent to the visual cortex during grasping preparation. This can result in similar discrimination performance increases during pointing preparation as is usually observed during grasping preparation. Consequently, this diminishes the performance differences between pointing and grasping in the AIP stimulation condition.

Both these possible explanations fit with the idea of cortical feedback connections between motor area AIP and visual areas that are activated when preparing an action. Recently, an EEG study [12] demonstrated enhanced activation in the visual cortex for grasping as compared to pointing, which can be regarded as first evidence for influences of motor areas controlling grasping on processing in the visual cortex. This mechanism may be similar to the cortical feedback connections between the frontal eye fields and occipital areas, enabling an enhancement of spatial perception during the preparation of an eye movement [7,9,33]. Both types of action preparation (for eye movements and grasping movements) may induce some sort of ‘action-modulated perception’, where the preparation of a certain action also entails the perceptual enhancement of features that are relevant for the upcoming action, increasing its chance of success.

The current study shows that the anterior intraparietal area is involved in the perceptual modulation preceding a grasping action, just as the frontal eye fields are involved in the modulation of perception preceding eye movements. Further studies are necessary, not only to show the exact mechanism that drives the perceptual changes due to grasping preparation, but also to identify other motor areas involved in the general mechanism of action-modulated perception.

5.1 Acknowledgements

This work was supported by a Netherlands Foundation for Scientific Research (NWO) Open Competition Grant NWO 400-05-134.

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Chapter 6

Grasping the intention and getting the point: Decoding the influence of action preparation on perception

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In preparation

1. Abstract

The preparation of an action has been shown to enhance action-relevant visual features, before the execution of the action. This may be implemented by cortical feedback connections between motor areas and early visual areas. In the current study we investigate the effects of action preparation on perception. A grasping action has different visual requirements than a pointing action, such as the orientation of the object to-be grasped. Therefore, the preparation of a grasping action should have different effects on visual processing than pointing. To investigate this, subjects ($n=6$) performed a grasping and pointing task to real bar objects in a 7T MRI scanner. Multivoxel pattern analysis (MVPA) techniques were used to analyze visual activity during action preparation, as these are sensitive to subtle modulations of activity. The analysis was restricted to voxels that showed significant classification scores for the bar orientations used in the grasping/pointing experiment. Results show high classification scores for grasping versus pointing in early visual areas V1-3 and the anterior intraparietal area when preparing an action, in both go and no-go conditions. This suggests that the preparation of an action, even without execution, modulates activity in the early visual areas, which may be due to changes in orientation tuning.

2. Introduction

When picking up an object, our vision guides the action. It tells us where it is located, its size and orientation. It is essential that these properties are accurately assessed before starting the action. It would therefore be very beneficial to focus on such relevant features of the object while planning the action. So far, several studies have shown (indirect) evidence for an ‘action-modulated perception’ mechanism that automatically enhances relevant features during action preparation [1–4] using motor output as a performance measure. A recent study [5] showed more directly that orientation perception is enhanced during the preparation period of a grasping action, compared to a pointing action (for which the orientation of the bar is irrelevant for the execution of the action). No such enhancement was found when an action-irrelevant discrimination feature was used (luminance). This supports the idea that perceptual features that are relevant for the upcoming action (e.g. orientation and size of an object for a grasping action) are enhanced when preparing this action.

Currently, we believe that such effects are mediated by feedback connections between cortical motor areas and early visual areas. Some initial evidence for this idea was found in an EEG study by van Elk and colleagues [6], where preparatory activity was found in the visual areas during grasping preparation (see also [7,8], where activity was found in the visual areas for grasping>reaching). Additionally, disruption of action-modulated perception was found in a recent transcranial magnetic stimulation (TMS) study [9] when applied to the anterior intraparietal area (AIP) during grasping preparation. AIP is an area involved in the planning and

updating of grasping actions [7,10–13] and thus a likely source of top-down feedback to the visual areas that underlies action-modulated perception. The enhancement of orientation perception that normally occurs when preparing a grasping movement [5] was absent when AIP was stimulated during the action preparation period [9]. This provides strong support that AIP is involved, not only in the preparation of grasping movements, but also the perceptual preparation that precedes the grasping movement, enhancing action relevant visual features.

While AIP is a likely source of the top-down feedback during grasping preparation, its target of modulation is less established. As the action-modulated perception effect is a swift and automatic process occurring before action execution, modulation of early (lower order), rather than higher order visual areas is a plausible target for effective behavioral benefit [6].

When a spatial location is attended, a general amplitude increases of neuronal responses within the corresponding receptive fields in the visual cortex is observed [14–16]. This way, spatial attention is applied and processing of visual input from this location is enhanced. Specific features, such as orientation, can also be enhanced. In the early visual cortex, specifically V1, neurons are orientation tuned, i.e. they respond most vigorously to a certain orientation. Attention to orientation is thought to alter (e.g. sharpen) this orientation tuning [17–20]. These orientation selective neurons are organized in columns, which contain all orientations for a certain (small) spatial location. The human orientation columns are a very fine grained, sub-millimeter structure. Thus, these shifts in tuning are difficult to pick up using conventional human imaging techniques, such as fMRI. Many columns with different orientation tunings fall within a voxel of typical size. Modulations of specific features is thus difficult (but not impossible, see [21]) to pick up when looking at the amplitude response of single voxels, as in a standard general linear model approach (GLM). However, recent studies have shown that these modulations can be picked up using multi-voxel pattern analysis (MVPA) techniques [22–24]. These are sensitive to subtle shifts in underlying neuronal responses, even below the spatial sampling frequency, as they combine changes in groups of neighboring voxels in order to detect differences between brain states in a multivariate framework. This should especially work well at higher MR field strengths where one can acquire signal changes at or below 1mm^3 . It has even been demonstrated that the orientation columns can be mapped in humans at 7T [25].

In the current study we aim to uncover influences of action preparation in the early visual areas using MVPA techniques on 7T fMRI data. Given that orientation perception is enhanced during grasping preparation, but not pointing preparation [5], we hypothesize that the preparation of a grasping action enhances orientation sensitivity in the early visual areas, whereas a pointing action would not. If so, it should be possible to pick up these modulations using pattern analysis techniques to obtain above chance classification performance for action (grasping/pointing) preparation in the early visual areas. We will use standard retinotopic mapping techniques to extract regions of interest (ROIs) of the early visual areas

V1-3. Furthermore, in this ROI analysis we will restrict the input to voxels that show selectivity for the orientations used in the grasping/pointing task in order to associate action classification in the visual areas to orientation perception. To this end, we combine the grasping/pointing experiment in the scanner with an orientation perception experiment.

3. Methods

3.1 Subjects

Six healthy subjects (2 male, mean age 25.8 SD 2.5) with normal or corrected-to-normal vision participated in this study. Five out of the six subjects were right handed. This study was approved by the Medical ethical committee of the University Medical Center Utrecht. All subjects signed an informed consent prior to participation.

3.2 Tasks

Subjects performed three tasks in two separate sessions while lying in the 7T scanner. In one session, subjects performed a grasping and pointing task. In the other session, subjects engaged in a passive viewing orientation task and a standard retinotopic mapping task.

3.3 Grasping/pointing task

Subjects lay in the MRI scanner with a custom made MR compatible 'grasping/pointing device' that enabled subjects to perform grasping or pointing actions to black bar objects mounted on a white background. Two protruding bars were placed on either side of a fixation cross (6cm from fixation cross to bar center, bar width 1.5cm, length 6cm), within easy reaching distance for the participant. The bars were oriented either at -45° (left) and $+45^\circ$ (right), or vice versa. This was counterbalanced across participants. See figure 1 for a graphical depiction of the setup. The display was visible through prism glasses, worn by the subject while lying supine. Due to the use of prism glasses, no visual transformations were necessary as the obtained view is without mirror image flips. It is thus as if the subjects were looking down at their hands in a normal situation. Visual color cues were given by a back projection system, illuminating the white bar display. Auditory cues were given by the stimulus presentation software (Presentation v14.9, Neurobehavioral systems, Albany, USA) and presented through soft tubes inserted near the ear canal and closed off by moldable ear plugs. The upper part of the right arm was restrained by a soft elastic band wrapped around the upper torso. This restricted movement in the upper part of the arm and thus the head, minimizing MR artifacts induced by a moving arm and head in the scanner. Subjects were required to perform the grasping and pointing

actions with the lower part of the arm, which was possible due to the relatively small movement amplitudes.

Subjects performed 4 grasping and 4 pointing blocks, with a 20 second pause between blocks and a longer break after 4 blocks. Total duration was approximately 35 minutes. Every block started with an instruction of the action to-be performed in the upcoming block (grasping or pointing) by a color cue (red or green). Actions alternated for every block, resulting in 4 pointing and 4 grasping blocks. Within each 4-minute block, the subject performed 20 trials (a trial contains 1 grasp or pointing movement) of 12 seconds each. Each trial started with an instructional cue, indicating whether subjects should perform their action toward the left or right bar object. After 3.5-4.5 seconds (random interval), an auditory cue instructed to perform the action (single beep, go cue). When a second beep sounded shortly afterwards, the movement that was prepared, had to be withheld (double beep, no-go cue). The no-go condition was added to be able to measure effects of movement preparation without execution and exclude any sources of noise due to movement in

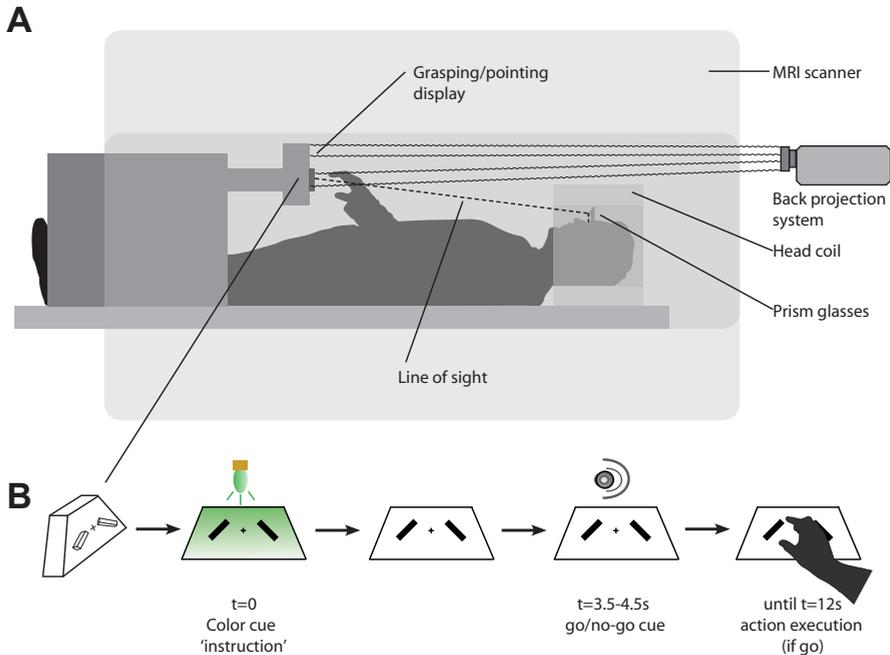


Figure 1. Schematic depiction of the experimental setup and procedure. *A)* Graphic depiction of the grasping/pointing setup in the MRI scanner. Subjects lay supine in the MRI bore. Subjects were able to see the grasping/pointing display (depicted in B) through prism glasses. Color cues were given through a back projection system, illuminating the grasping/pointing display. When not giving cues, the back projection illuminated the grasping/pointing display (white). *B)* Time course of the grasping/pointing experiment. Every trial started with an 'instruction' color cue (red/green, 1 second duration) indicating whether the action should be performed on the left or right bar. After a 2.5-3.5s interval, an auditory cue instructed the subject to either perform the action (single beep) or withhold it (double beep). In case of a go cue, the subject performed the action that was instructed at the start of the block of trials.

our findings. Although considerable care was taken that movements were minimal (small amplitude, restraining upper arm band), it cannot be fully ruled out that even small movements induce confounding artifacts in brain activation. This confound cannot be present in the no-go condition, which is therefore an important additional condition.

This resulted in six possible events per trial: grasping instruction, grasping go, grasping no-go, pointing instruction, pointing go and pointing no-go. Subjects were asked to keep fixating throughout the experiment. When pointing, subjects were asked to point to the center point of the bar object. When instructed to grasp, subjects grasped the bar object in the longitudinal direction (thumb and index fingers were to be placed at the short sides of the bar). Hand movements were monitored with a MR compatible dataglove (Fifth Dimension technologies, 5DT Inc, Irvine, USA) that tracked the bend fraction of the individual fingers through fiber optic tubes in the dataglove.

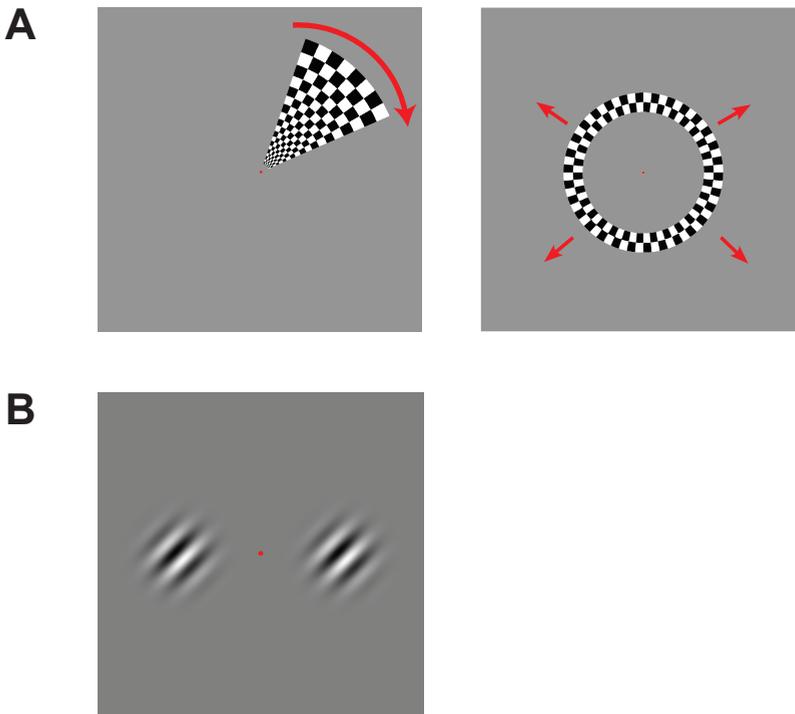


Figure 2. Stimuli used in the retinotopic mapping procedure (A) and orientation paradigm (B).
A) Retinotopic mapping consisted of a rotating checkerboard wedge (left) and an expanding checkered ring stimulus (right). The red arrows did not appear in the actual stimulus presentation.
B) Orientation grating stimuli. Two gratings of equal orientation appeared on either side of a fixation point for two seconds, followed by eight seconds of rest.

3.4 Orientation task

In a separate session, subjects performed a passive viewing orientation ‘task’. Stimuli were presented using a back projection system, visible through a mirror system and prism glasses. Subjects viewed a fixation spot, flanked by two oriented gratings, either 45° or -45° , see figure 2 (B). The spatial frequency of the grating patterns was aimed to be roughly equal to the spatial frequency of the oriented bars in the grasping/pointing task. The task duration was 8 minutes and contained 48 repetitions (10 seconds each), equally divided between 45° and -45° gratings. At the start of each trial, the gratings were presented for 2 seconds, followed by 8 seconds of rest.

3.5 Retinotopy

In the same setup, subjects engaged in a standard retinotopic mapping protocol [26,27], see figure 2 (A). Subjects viewed a rotating checkerboard wedge to obtain a polar angle map. The protocol started with 30 seconds of rest, followed by 7 rotations of the wedge (thickness 48°), each one minute long, and ended with another 30 seconds of rest. The eccentricity mapping was identical, with the exception that the main stimulus did not rotate, but expanded from the center outwards once per cycle (7).

3.6 Acquisition

A high resolution 3D EPI sequence (voxel size: 1.25mm isotropic; acquisition time: 2.88s; 35 slices,) was used for all experiments. A posterior field of view (FOV) was scanned, positioned such that the primary visual areas up to the anterior parietal regions were included, see figure 3. We took care that besides the entire visual cortex, AIP was included for all subjects. The volume was locally shimmed per

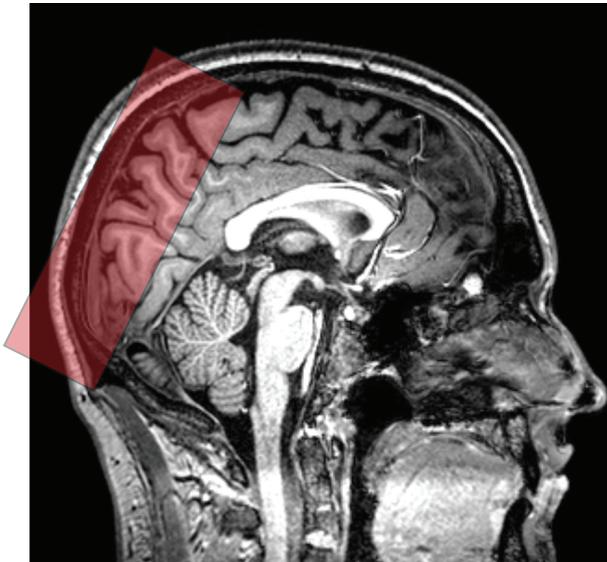


Figure 3. Scanned functional volume used for all tasks, depicted on a single subject anatomy. The partial volume was chosen to encompass both the early visual areas and the anterior parietal areas.

subject to minimize geometric distortions in the functional volumes. All functional scans were acquired using a Philips 7T scanner (Philips Medical Systems, Best, The Netherlands), with a 32-channel head coil, located at the University medical center Utrecht. For the grasping/pointing task, 720 volumes were acquired and for the orientation task and each retinotopy task, 165 volumes. Whole brain T1-weighted anatomy scans were acquired on a Philips 3T Achieva scanner (Philips medical systems, Best, Netherlands; voxel size 0.875 x 0.875 x 1 mm). For registration purposes, a partial T1 was acquired for each subject at the 7T scanner (voxel size: 0.97x0.97mm; slice thickness 1 mm, 44 slices).

3.7 Analysis

3.7.1 Grasping/pointing task

All acquired functional volumes were realigned using rigid body transformations. The partial T1, acquired at 7T and encompassing the same volume as the functional images, was coregistered with the functional scans using normalized mutual information. The local shimming procedure of the functional images during acquisition eliminated nearly all spatial distortions, which can be severe at high field strength and small voxel sizes. Therefore, coregistration of anatomical and functional images was near perfect. Subsequently, the whole brain T1-weighted anatomy obtained at the 3T scanner was coregistered to the 7T partial T1. The whole brain 3T scan was essential for the surface extraction and flat mapping procedures described later.

The multivoxel pattern analysis is based on classification. Classifiers need multiple instances of each ‘class’ (in this case grasping and pointing) to train a model. To this end, one can either take the activity of a single (grasping or pointing) trial, or take the average of a number of repetitions. The former has the advantage of a large training set (as many as there are trials), but suffers from noise. Therefore, in this study, the latter option was taken and four repetitions (e.g. grasping) were averaged to reduce noise, while still retaining a large enough training set. To obtain the pattern data set, a separate GLM analysis was run for all conditions of interest, i.e. the instruction, go and no-go condition. Each of these GLM analyses contained a single regressor for each of the conditions of non-interest and multiple regressors for the condition of interest (e.g. no-go grasping and pointing). For instance, the GLM analysis for the no-go condition contained one grasping instruction regressor, one pointing instruction, one grasping go, one pointing go, ten grasping no-go and ten pointing no-go regressors. So, instead of modeling all repetitions in one regressor, one regressor was put into the GLM for every four repetitions of one condition. These repetitions per regressor were spread out over the different blocks, to avoid bias. This resulted in ten beta images per action (grasping/pointing) for every condition, reflecting percent global signal change for these conditions. These twenty beta images were used in the MVPA analysis in order to build a model by estimating a hyperplane (see below). Movement parameters and two white matter

regressors (one for each hemisphere) were also added as nuisance regressors to the GLM in order to eliminate possible movement noise.

MVPA distinguishes between two conditions by analyzing groups of voxel values ('patterns'). The selection of these groups can be done in various ways. A group of voxels taken from an area that is involved in one of the two conditions (e.g. grasping and AIP) will show higher classification scores than a group of voxels taken from a task-irrelevant area. To get an estimate of the spatial distribution of areas with high or low classification performance, a 'searchlight' method can be used. Here, a group of voxels is chosen around a center voxel. Classification is performed on this group and the classification accuracy is attributed to the center voxel. This procedure is then repeated for each voxel in the volume. Traditional searchlight methods use a spherical volume around the center voxel [28], including every voxel that is within a certain radius (Euclidean distance) of the center voxel. Recently, surface based searchlights were introduced [29], which use circular patches of cortical surface, see figure 4. The latter was used in the current study.

For this, surface reconstruction was needed. Therefore, two segmentations of the whole brain anatomical scan were made. First, the anatomical T1 weighted scan obtained in the 3T scanner was segmented using CARET software [30]. This created a gray matter mask. Second, the same T1 scan was also segmented using unified segmentation [31] in SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>) to obtain a probabilistic gray matter map for each hemisphere. The CARET based segmentation was used as a mask for the probabilistic gray matter map, to restrict surfaces to a single hemisphere, remove the cerebellum and remove any overlap between gyri. This combined probabilistic gray matter map was subsequently used to create a surface using SPM8 routines, expressed as a triangulation of the gray matter surface consisting of nodes (3D coordinates) and edges (connections, forming triangles).

For every node in the gray matter surface, a circular surface patch (6mm radius) around it was calculated. Surface patches avoid the problems posed by spherical volumetric searchlights, which selects voxels that are close in Euclidean distance, but may be part of a different gyrus (and thus very likely functionally distinct) or even white matter and CSF, thus obscuring effects of interest. Surface patches only select voxels that are close when the surface is unfolded, and thus only selects connected voxels which form a more coherent and functionally homogenous unit, consisting entirely of gray matter. See figure 4 for an example illustration.

The patches, calculated as a set of connected nodes, were interpolated to a set of voxels using a nearest neighbor algorithm. The surface based searchlight was iteratively moved over the entire gray matter surface coinciding with the scanned volume of the fMRI sequence. The MVPA analysis was performed separately for every condition (instruction/go/no-go). For each condition, beta values for all repetitions were extracted for grasping and pointing for every surface patch of voxels. This resulted in 10 voxel patterns ('data instances') for grasping and 10 for pointing per condition. The data instances were divided in a 'training set' (18

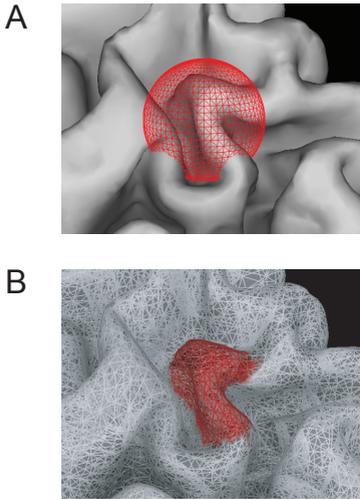


Figure 4. Illustration of the searchlight types. *A) The spherical searchlight places a sphere with a certain radius around the center voxel. All voxels that are contained within this sphere are selected as input for the classifier. Notice that this can span more than one gyrus and is not restricted to gray matter. B) Surface based searchlight. This searchlight selects only voxels that are connected to the center voxel with a distance less than a certain radius. On a flattened surface, the patch of selected voxels is circular. This way, a more homogenous selection of voxels is obtained which contains only gray matter.*

patterns) and a ‘test set’ (2 patterns). A linear support vector machine (LIBSVM implementation, <http://www.csie.ntu.edu.tw/~cjlin/libsvm/>), with a constant penalty parameter $C=1$ was trained on the 18 training patterns (9 grasping, 9 pointing) and tested using the test set. It is essential to test classification performance on data instances that were not used for the estimation of the classifier model. This was repeated 20 times with different (unique) train/test set divisions to remove possible biases from test and train set selection and obtain a reliable measure of classification performance. This cross-validation (e.g. [32]) is a standard procedure to obtain a measure of the generalization performance, instead of the separability of the specific data instances. The resulting accuracy was attributed to the center voxel for each patch. This procedure was repeated for every voxel in the scanned volume to obtain a classification accuracy map for all conditions (instruction/go/no-go) and all subjects.

In summary, this procedure yields the strength at which our MVPA analysis can distinguish between grasping and pointing trials based on all the percent signal change values of the voxels in the surrounding circular patch of cortical surface. This implies the information content in this patch is able to distinguish between both conditions, rather than single voxel activation magnitudes alone, as in classical GLM analysis.

3.7.2 Orientation task

The surface based searchlight procedure was also applied to the functional volumes obtained from the orientation task. Here, beta images were also estimated based on four repetitions, but as there were 48 repetitions in total, this resulted in a total of 12 beta images (6 for -45° , 6 for 45°). Surface based searchlight classification was performed on the categories $-45^\circ/45^\circ$.

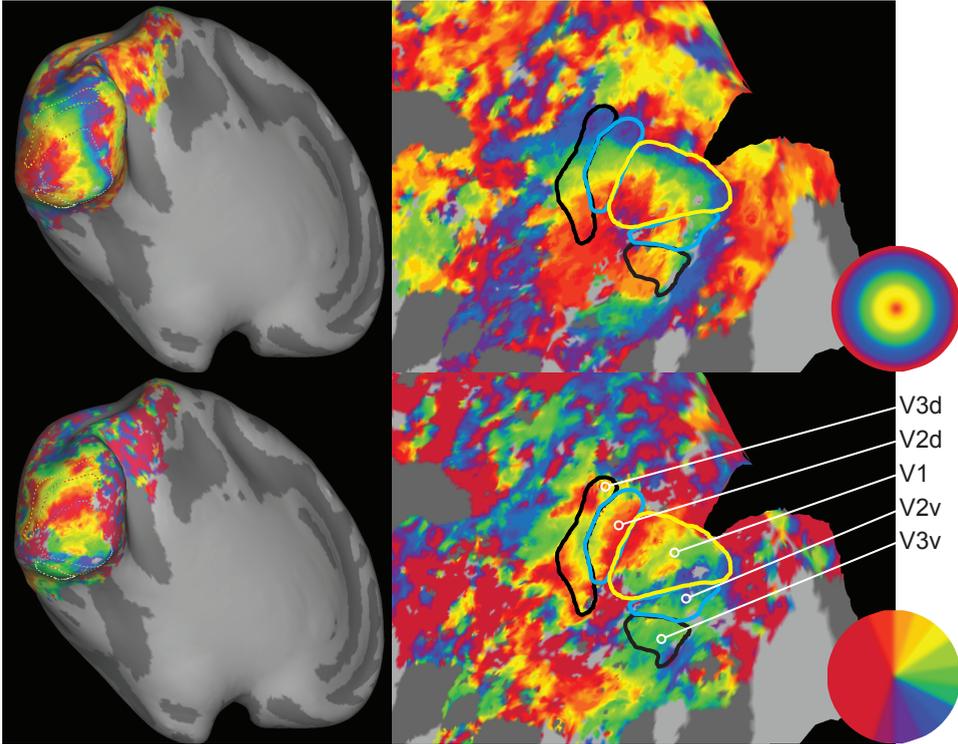


Figure 5. Retinotopic mapping results for a single subject. TOP: eccentricity map projected on an inflated brain (left) and flat map (right). BOTTOM: results from the polar angle mapping procedure on an inflated brain (left) and flat map (right).

3.7.3 Retinotopy

Processing of both eccentricity and polar angle functional data was performed in the same way. The volumes were realigned and coregistered with the full T1 anatomical scans (acquired at 3T) using the partial T1 from the 7T as an intermediate. The data were smoothed with a 4mm FWHM Gaussian kernel. In SPM, the data were fitted to a sine and cosine regressor. For every pair of beta voxel values (corresponding to the sine and cosine regressor), the arctangent was calculated to obtain an eccentricity/polar angle phase map [26,27].

These polar angle and eccentricity maps were projected to the CARET generated surface representation and warped to a flat map. Using the polar angle phase reversals and direction of eccentricity, regions of interest (ROI) were manually created for early visual areas V1-V3 (see figure 5). Furthermore, a ROI of the anterior intraparietal area (AIP) was created based on cortical landmarks (the confluence of the anterior end of the intraparietal sulcus and the postcentral gyrus [7,8]).

3.7.4 ROI analysis

The extracted gray matter ROIs for V1-V3 and AIP were used in a ROI analysis of the grasping/pointing task. For every ROI, only those voxels were selected that

exhibited significant above chance classification performance in the orientation task (and can hence discriminate between $\pm 45^\circ$). This was done to restrict the analysis to orientation selective voxels, which are thought to be modulated by grasping preparation. This allows one to not only draw conclusions on what areas are modulated for grasping versus pointing, but also on which type of information this is based (orientation processing).

To this end, first the classification accuracy that is significantly above chance was established (see below). Second, a mask was created for each ROI that only contained those voxels that were significantly above chance for the orientation data. Third, these masks were used to extract the surface based searchlight accuracy values from the grasping pointing task. These grasping versus pointing discrimination values were averaged, to obtain the mean accuracy for each ROI, condition and subject separately. See figure 6 for an overview of the ROI analysis procedure.

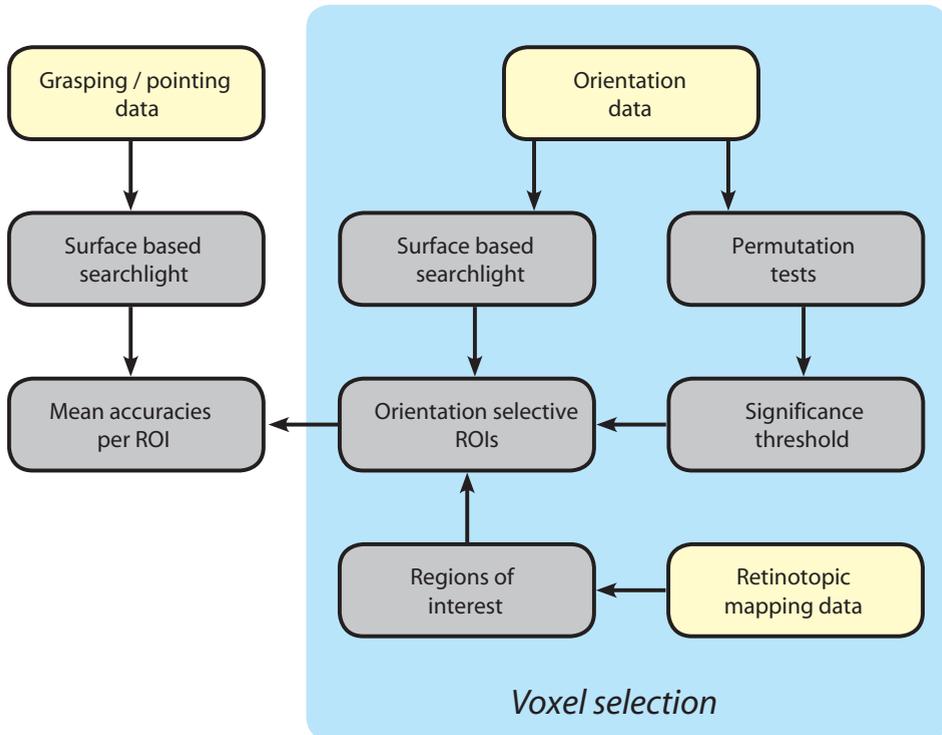


Figure 6. Schematic overview of the ROI analysis procedure. Voxel selection was based on the orientation task data, where the significant voxels of the (orientation) surface based searchlight analysis were selected. Significance threshold was calculated using permutation tests on the orientation data. ROIs were created from the retinotopic mapping data, and applied to the (thresholded) surface based searchlight analysis of the orientation data. This resulted in ROI masks of the early visual areas and AIP, restricted to orientation selective voxels. These masks were applied to the results of the searchlight analysis of the grasping/pointing data and averaged across subjects to obtain the final results of the ROI analysis.

The theoretical chance level of a classifier with two classes is 50%. However, due to variance in the data and the amount of independent classifications performed (for every voxel), some classification values above 50% may be due to chance (i.e. false positives). To establish the classification accuracy that is significantly above chance performance at the single subject level, permutation tests were performed on the orientation data [33] for every subject. To this end, 10,000 voxels and their corresponding surface patches were randomly selected for permutation tests. Class labels (i.e. grasping or pointing) were randomly permuted 250 times for each patch. After each permutation, classification was performed using the random labels, with 20-fold cross validation. With random labels, any obtained classification score is determined by chance.

This enables the estimation of the probability density function when no effect should be present, which should thus constitute the null-distribution [33]. The significance threshold was set at the Bonferroni corrected alpha threshold of 0.05 (that is, divided by the total number of voxels in the ROIs, which was $\sim 20,000$ per subject on average). This way, a significance threshold could be estimated in terms of classification accuracy. This procedure implies that we accept a probability of type II errors (an effect arising by chance) of 5% for the entire volume of voxels tested, and is similar to a 'whole brain correction' for classical GLM analysis, except that we had to estimate the null-distribution for our MVPA analysis numerically.

At the group level, individual classification accuracies were tested against theoretical chance level (50%) in one sample t-tests. Due to the small group size, both corrected (Bonferroni) and uncorrected (<0.05) results are included.

4. Results

4.1 Searchlight decoding

Surface based searchlight decoding (6mm) was performed for all conditions of the grasping/pointing task for all subjects. As the scanned volume only encompassed the posterior part of the brain only parietal and occipital areas were analyzed. See figure 7 for an overview of the results for grasping and pointing (go/no-go). Normalized accuracy maps were smoothed and averaged for display purposes only. In general, high grasping versus pointing decoding performance was found throughout the parietal and occipital areas for both go and no-go conditions. High classification scores are found mostly in the superior parietal areas, extending into the intraparietal sulcus. In the occipital lobe, high classification accuracies were found along the calcarine sulcus, lateral occipital areas and the parieto-occipital junction.

For the orientation task, classification performance was generally lower. Additionally, there is considerable variation in classification maps between subjects, yielding little overlap in the mean surface map. Therefore, results from a single subject are shown in figure 8. Higher orientation classification scores were found

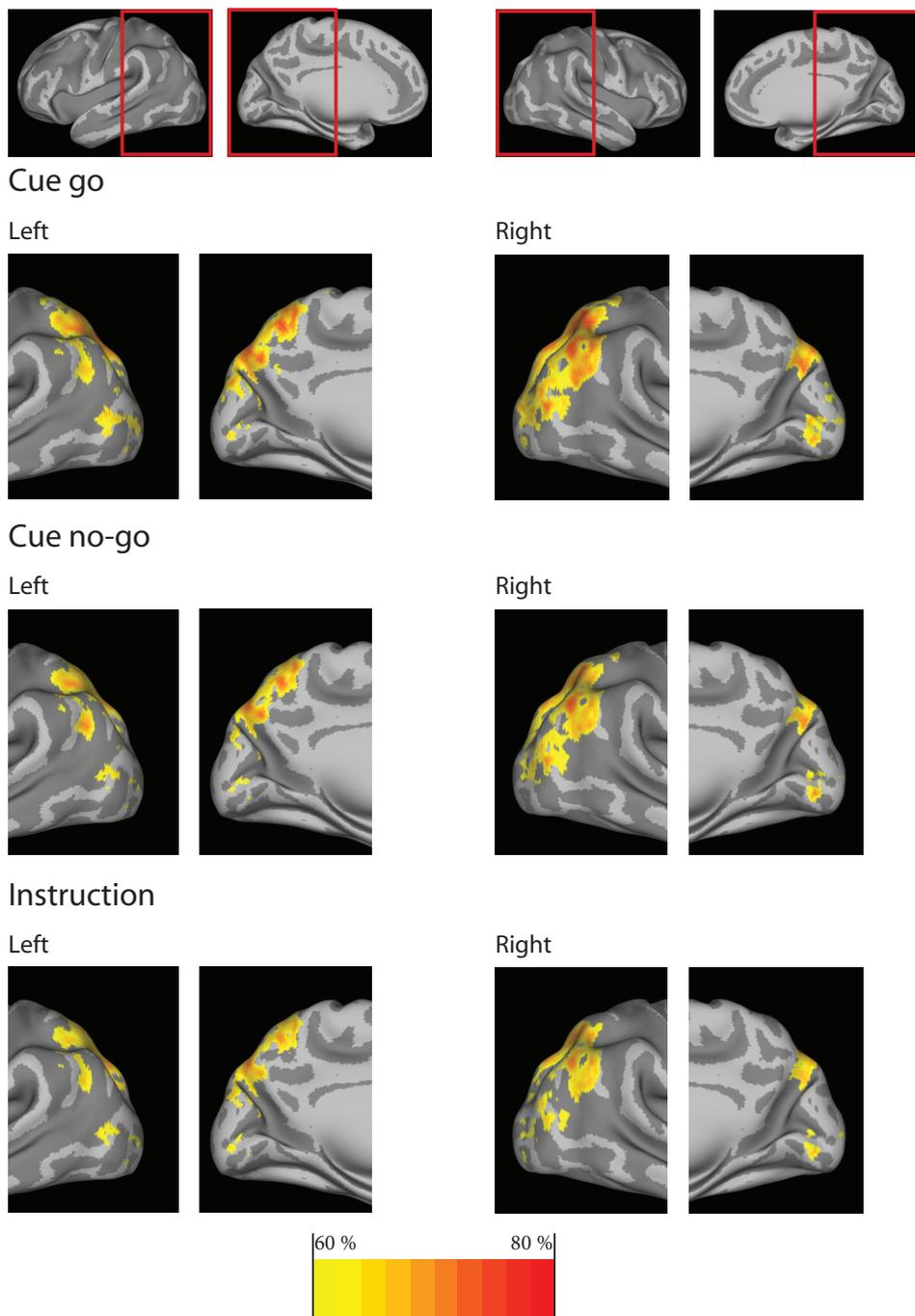


Figure 7. Group results for the surface based searchlight grasping/pointing analysis on a normalized brain. The individual searchlight results were normalized, averaged across participants and smoothed for display purposes. The left hemisphere is depicted on the left (lateral and medial view) and the right hemisphere on the right.

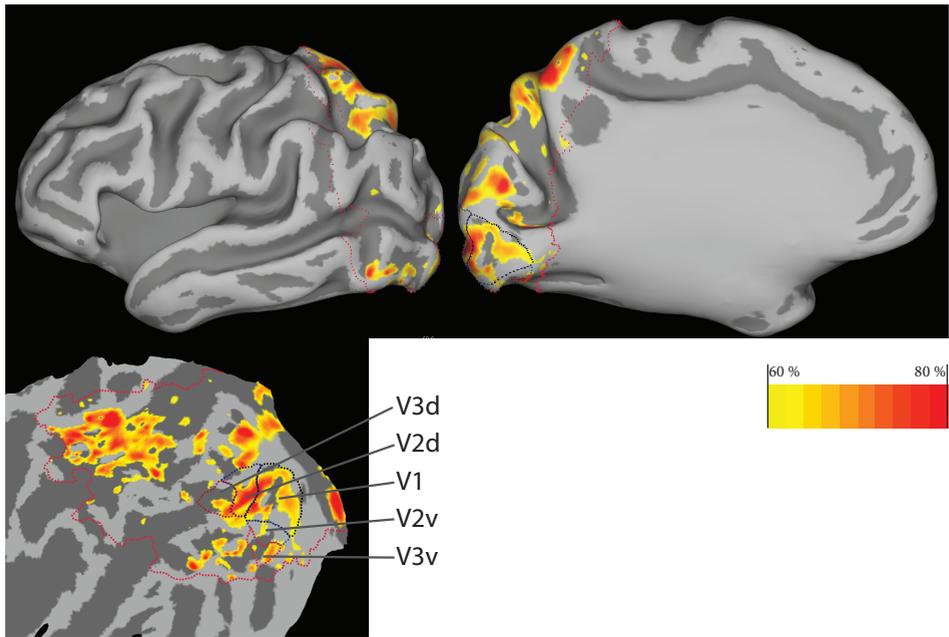


Figure 8. Single subject results of the surface based searchlight analysis of the orientation task in a lateral view, medial view and flat map. The dotted red line indicates the edge of the scanned volume. Individual regions of interest of early visual areas V1-3 are marked.

mostly in the superior parietal and intraparietal areas and the posterior occipital area.

4.2 ROI analysis

The ROI analysis was performed to examine the effects of action preparation on the early visual areas and AIP that are associated with the specific bar orientations used in this study. Therefore, Single subject regions of interest were created based on the retinotopy (see figure 5). Within these ROIs, classification accuracy values of grasping or pointing were extracted for voxels that showed significant above chance orientation classification performance at single subject level (See figure 6). Results are shown in figure 9. The significance levels for the orientation classification varied only marginally across subjects (mean: 72.2%, SD: 0.4). Results show that classification performance for grasping and pointing is above theoretical chance (50%) for all regions of interest.

In the cue go condition (where an actual movement was executed), the left anterior intraparietal area shows higher classification accuracy than the right AIP. All movements were executed with the right hand, so the left AIP represents the hemisphere contralateral to movement execution. The primary visual area V1 shows high classification accuracy bilaterally.

In the no-go condition, where the action was suppressed, classification scores are generally lower. However, grasping versus pointing classification scores in V1,

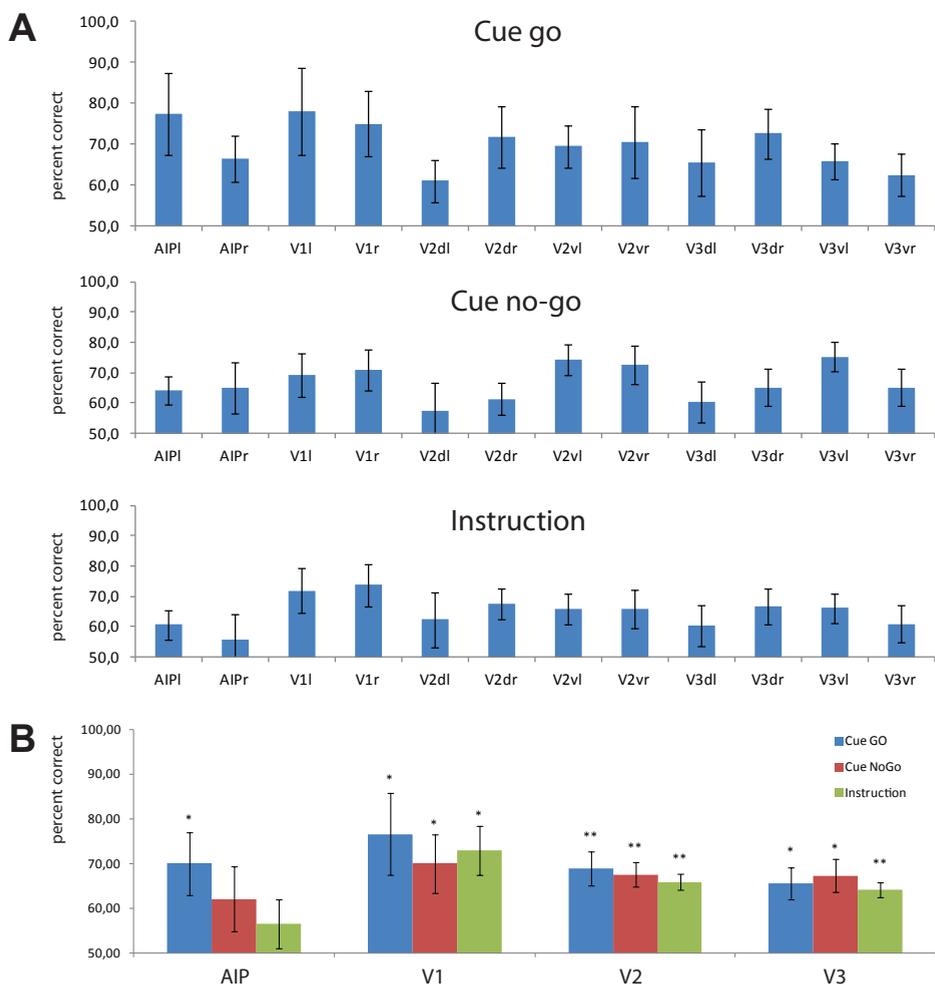


Figure 9. Results from the region of interest analysis for all conditions.

A) Classification scores of the grasping/pointing task for every ROI in the analysis. Suffix 'l' or 'r' indicates the hemisphere of the ROI, 'v' or 'd' indicates either the 'dorsal' or 'ventral' portion of the ROI for V2 and V3.

B) Mean results per area, collapsed over hemisphere and 'dorsal/ventral' portion (if applicable). Single asterisk denotes one sample *t*-test significance (against the theoretical chance level of 50%) at uncorrected level ($p < 0.05$) and double asterisk at corrected level ($p < 0.0042$, Bonferroni).

ventral V2 (both hemispheres) and ventral V3 (left, contralateral) are consistently high across subjects.

Interestingly, when looking at the classification performance for the instruction condition (instructing the grasping/pointing target, approximately 3 seconds before the go or no-go cue) high classification scores are also observed for these areas (bilateral V1, ventral V2 and left ventral V3).

When looking at the group results, collapsed over hemisphere and dorsal/ventral distinction, it is interesting to note that AIP is only significantly involved in the cue go condition. Classification scores are higher in V1, but more consistent across subjects in V2 and V3.

5. Discussion

In the current study, we were able to predict whether a subject was going to grasp or point to a bar object from activity in the early visual areas using multivoxel pattern analysis (MVPA). We hypothesized that if action preparation, in particular grasping preparation, modulates activity in the early visual areas, a classifier should be able to pick up these modulations and be able to discern between grasping and pointing preparation. As we found above chance classification scores in all early visual areas for grasping and pointing, the results obtained in this study support the idea that action preparation modulates early visual processing in the occipital lobe.

As an additional constraint, only voxels that are able to classify the different orientations used in this study (45 and -45 degrees) with significant above chance accuracy, were included in the ROI analysis. Although classification scores did not increase due to this (compared to the searchlight analysis), the specificity did. Orientation sensitivity increases when preparing a grasping action [5] and thus we expect that those voxels that are highly sensitive to the orientations used in this study, would also show the greatest modulation due to action preparation. As results show, all regions included in the ROI analysis still yielded above chance classification scores. Particularly the primary visual area V1 shows high scores across conditions. This supports the idea that particularly the early visual areas are the target of these modulations, which enhance further processing at later stages of visual processing.

Classification scores are especially high in the cue go condition. That is, at the go cue for the actual execution of the action. This is perhaps not remarkable, as this is the most engaging condition. Here, both V1 and the intraparietal area (AIP) showed the highest classification scores in the contralateral hemisphere (relative to the hand used to perform the action). This is interesting, given that we believe these feedback connections to be within the hemisphere (remain ipsilateral and thus not cross over). This is congruent with findings in our previous TMS study, where stimulation of the left AIP disturbed action-modulated perception in the right visual field shortly before executing the action [9].

In the no-go condition the action was prepared but not executed. This enables insight into preparation processes only, which is of interest in the current study. Here, classification scores were also clearly above chance level, although less pronounced than in the go condition. Although the classification scores are generally lower, the differences between ROIs are very similar to the go condition. However, the classification score in the contralateral AIP was notably lower when no action was actually executed. Perhaps this is due to the lack of actual execution. This does not necessarily mean that AIP is not involved here, as it may be that it is still involved in generating a feedback signal to the visual areas (as classification scores in both AIP and the visual areas are still well above chance). The reduction may be due to the absence of the actual motor process, while the feedback mechanisms are still effective. Seeing that the grasping and pointing can still be robustly classified in the absence of the actual action, the preparation itself seems sufficient to enhance visual processing. The occipital effects during action preparation reported here could very well be the neurophysiological basis of the action-modulated perception phenomena that were reported in recent years [1,4,5,9,34]. This supports the notion that it is the preparation of an action that enhances perception for the upcoming action.

Even in the instruction condition, the intended action can be decoded with above chance accuracy. That is, during the instruction phase (grasp/point left or right), which occurs several seconds before the go or no-go cue, the action to-be performed can be classified. During this instruction phase, high classification scores are most pronounced in V1. This is interesting, as the classification scores in AIP are notably lower than in the other (cue) conditions. While in this case there may not be a direct driving input, modulating the activity in V1, there may be a baseline shift. As grasping and pointing was performed in separate blocks, instead of an intermixed design, subjects may have been in ‘grasping-mode’. That is, a steady-state of perceptual sensitivity for grasping relevant features, allowing for classification of action intention, several seconds before the go (or no-go) cue.

The early visual areas were not the only parts of the scanned volume that showed high classification scores. The surface based searchlight analysis revealed that large parts of the superior parietal and intraparietal areas yielded equal or higher classification scores than the visual areas. This may also not be surprising, as these areas are involved in the dorsal stream of the the fronto-parietal reaching and grasping network [7,35–37]. As the anterior end of the intraparietal sulcus (AIP) is our main parietal area of interest (because of its implication in grasping preparation [7,8]), we included it in the ROI analysis. It may be however, considering the current results, that other parietal areas are involved in the feedback mechanism underlying action-modulated perception. Any area that has a different activity pattern for grasping actions than for pointing actions will show above chance classification. Thus, areas specifically involved in pointing movements will also show high classification accuracy. MVPA techniques are more sensitive to differences between conditions (grasping and pointing in this case) and may thus implicate areas that were previously not found in a direct contrast using a conventional GLM approach.

In fact, pattern analysis techniques are very sensitive to any kind of consistent difference between conditions. A pattern classifier is therefore also sensitive to noise patterns that consistently differ between conditions. In the current study, movement of an arm in the MR scanner may be a possible source of such noise, and could explain some of our results if this noise significantly differs between grasping and pointing. First, there is the direct effect of movement in the magnetic field of the scanner, causing inhomogeneities of the B₀-field. This, however, is an instantaneous effect, which can affect the volume acquisition only during this movement. In the go condition, the actual movement was unlikely to co-occur with the BOLD response of interest that is delayed for several seconds [38]. Furthermore, in the no-go condition, the movement was suppressed. In some cases, this resulted in very slight movements of the hands at the no-go cue, but this was short lived and equal between grasping and pointing. Second, the head movement as a result of arm movement may also be a source of noise. Care was taken during the acquisition and analysis to eliminate this as a possible confound. During acquisition, the upper arm was strapped to the torso, to avoid movement being carried over to the head. In the analysis, both realignment parameters and two white matter regressors (one for each hemisphere) were added as nuisance regressors. While this noise source may have affected classification scores in the go condition, the no-go trials contained little to no movement. The results from the no-go condition show similar results as the go condition. Importantly, classification scores were high even in the instruction condition, which occurred several seconds before the go or no-go cue. Here, it is very unlikely that confounds related to movement induced signal distortions played any role (let alone consistently differ between grasping and pointing). We thus believe that movement played only a marginal role, if any, in obtaining the high classification scores found in the current study.

There may be other sources of information that enable classification of grasping and pointing conditions. For classification in the visual areas, the question arises whether differences in visual input may have played a role in the high classification accuracy of grasping and pointing. In the current setup, this is very unlikely. The color cue that was used in the instruction phase was the same for grasping and pointing. Also, left and right cues were averaged together to obtain the beta estimates for the instruction condition. The (no-)go cue was chosen to be an auditory signal for this reason, i.e. there is no visual stimulation. However, the hand was in the field of view during the performance of the action. This may have caused some slight differences in visual input after the go-cue, but only in go trials. As stated before, the similarity between the go and no-go conditions render it unlikely that the classification in the go condition was based on noise sources such as this. The same reasoning holds for eye movements, which could have been made during action execution. Subjects were required to keep fixating at all times, but the execution of the action could have occluded the fixation spot. This, however, did not occur during no-go and instruction conditions.

Given the above reasoning, it is plausible to assume that the high classification scores in V1 and the early visual areas V2 and V3 are due to modulatory influences of action preparation. Given that the analysis was restricted to voxels that are sensitive to the orientations used in the current study, this modulation may well be a shift in orientation tuning. The preceding literature implicates AIP in action-modulated perception when grasping [9], and the current results show above chance classification scores for this area. Therefore, the anterior intraparietal area is a plausible source of the feedback signals. However, given the large spread of parietal areas that show high classification scores (and are thus differentially active for grasping and pointing) other sources of feedback cannot be excluded. Taken together, the current results show strong evidence that grasping preparation modulates orientation selectivity in the early visual areas, especially V1, enabling action-modulated perception.

6. References

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Chapter 7

Summary & general discussion

The general aim of this thesis was to study the effects of action preparation on perception, which we refer to as ‘action-modulated perception’, and its implementation in the brain. This was studied in both the oculomotor and skeletomotor system. Perceptual action preparation effects were found for both these motor systems, as well as evidence for its implementation in a top-down feedback model.

1. Summary of findings

The first part of the thesis involved studies in the oculomotor system. In **chapter two**, the time course of events preceding eye movements was studied using electroencephalography (EEG). In a cued saccade task [1], frontal activity, contralateral to the eye movement direction was found, shortly after the go-cue. This was followed by occipital activity, also contralateral to eye movement direction. Importantly, the amplitude of the early frontal activity, in the 60-90ms (after go-cue) time window, was significantly correlated with the amplitude of the late (150-180ms) occipital activity. This indicates that it may be the early activity that reflects the perceptual preparation. Source localization implicates the frontal eye fields (FEF) and early occipital areas as areas of signal origin. This implicates a role for the FEF in driving spatial attention shifts by means of top-down feedback projections to the early visual areas during eye movement preparation.

The role of the FEF and intraparietal sulcus (IPS) in mediating spatial attention shifts shortly before eye movements was investigated in **chapter three**. Using the same task as in chapter two [1], single pulse transcranial magnetic stimulation (TMS) was applied either 60, 90 or 120ms after an eye movement go-signal (but before the actual execution). Different effects were found on perception, depending on the timing and site of stimulation. Stimulation of the FEF enhanced discrimination performance specifically at the eye movement end point, when applied shortly after the go-signal and contralateral to the direction of the eye movement. This effect on perception was most pronounced for the early pulse timing (60ms). Congruent with the findings in the previous chapter, this indicates that it is the early FEF activity that may drive top-down feedback to the visual areas. A general perceptual enhancement was found with IPS stimulation, but without a significant dependence on timing or laterality. This implies that the (contralateral) FEF is involved in the modulation of perception at the location to which an eye movement is being prepared. Due to the qualitative differences between FEF and IPS stimulation, it is likely that the FEF mediated this effect through direct projections to the visual areas, instead of through the IPS.

The second part of the thesis studied the effects of action preparation on perception in the skeletomotor system, specifically grasping actions. To this end, a behavioral paradigm was developed in **chapter four** that established the effects of grasping preparation on visual perception using psychophysics. Subjects performed

an orientation change discrimination task during the preparation of an action. This task was designed to be analogous to the task used for eye movements and suitable as a TMS paradigm (see chapter five). When preparing an action, subjects were better able to detect changes in the orientation of a bar that was to be grasped, than when preparing to point to this bar. This is consistent with the idea of action-modulated perception, as orientation is a relevant feature for grasping actions, but not pointing actions. When using luminance as the feature to-be discriminated, no perceptual enhancement was found for grasping or pointing, showing that only action-relevant features are enhanced. This study confirms, using a measure of visual sensitivity, that effects of action preparation on perception also occur in the skeletomotor system.

This experimental paradigm was also used in **chapter five**, which investigates the role of the anterior intraparietal area (AIP) in mediating action-modulated perception. AIP is, similar to the FEF, a visuomotor area involved in planning and executing a specific action, in this case grasping [2–4]. During the orientation change detection task, area AIP was stimulated after the action go-cue, but before the orientation change and actual execution of the action. This disrupted the increase in orientation sensitivity with grasping preparation over pointing preparation. When stimulating a control site, the normal enhancement due to grasping preparation was found, as in chapter four. This shows that AIP is an essential component in establishing enhanced perception for grasping actions. This also implicates AIP as a source of top-down feedback to the early visual areas.

Finally, in **chapter six**, the effects of action preparation on the early visual areas were investigated using functional magnetic resonance imaging (fMRI) and multivoxel pattern analysis techniques (MVPA). This was done by having subjects prepare and perform grasping and pointing actions to actual bar objects in the 7T MRI scanner (both with and without actual execution of the action, i.e. 'go/no-go conditions'). Orientation selective voxels (determined using a separate orientation task) in the early visual areas (determined by retinotopic mapping procedures) and AIP were entered in the MVPA. Results show that the action in preparation (without actual execution of the action) can be reliably predicted from the activity in these visual areas (and AIP), indicating that action preparation has a profound effect on early visual processing. This effect was strongest in V1 and less, but still reliable, in V2 and V3. As MPVA picks up on sub-resolution signals, the effects in the visual cortex could be due to modulation of the orientation columns. Taken together, this suggests that the preparation of an action modulates action relevant features in the early visual areas through top-down feedback, enabling action-modulated perception.

2. The emerging theory of action-modulated perception

Based on the results obtained in this thesis, one can speculate on the generality of action-modulated perception effects. Effects of action preparation on perception

have been found in both the oculomotor and skeletomotor system. For both eye movements and grasping actions, a visuomotor area, specifically involved in preparing this action, was found to also be involved in the preparation of perception for this action.

2.1 The general principle

This general principle may hold for other actions than eye movements or grasping actions as well. Numerous specific effects of action on perceptual judgments have been found in daily life (see [5] for review). The different perceptual needs for different actions may be implemented in the brain regions specialized in generating the action parameters for this action. Motor acts are not mediated by a single brain area, but by a network of cooperating areas. The division of labor for action preparation in different motor areas may also hold for specifying the perceptual needs. For instance, different perceptual effects have been found for the preparation of different grasp types (precision grip / power grip) [6]. One could hypothesize that this effect is mediated by the ventral premotor area, as the functioning of this area has been associated with grip type more than AIP [7]. The role of a certain motor area might depend on the level of abstraction of motor commands. An effector independent, high level motor planning area might relay a different aspect of the perceptual needs than a low-level motor area that implements a highly specific part of a motor act. However, it may be the intermediate visuomotor areas, such as AIP and the FEF, that are most essential in relaying the proper perceptual needs.

2.2 Implementation

While the premotor theory [8] states that the motor preparation and attentional preparation are implemented in the same area (see also [9]), the results in this thesis point toward a top-down feedback mechanism. Thus, while the specification of action-relevant visual needs may be performed in the specific motor areas, its effect is mediated by connections to the early visual areas. Modulatory feedback connections are abundant in the brain, and seem to be an organizational principle, rather than an occasional mechanism [10]. This is a very efficient mechanism that facilitates rapid and accurate action execution. Instead of a hierarchical visual processing system, the incoming information is processed with a focus on the needs of current goals by combining top-down and bottom-up input in the early visual areas. This, in turn, enhances feedforward visual input to the motor areas. As the modulation seems to occur at a very early stage, this may affect processing in both the dorsal and ventral stream. This eliminates the need for a top-level ‘supervising’ attentional system (at least for simple motor acts), as the perceptual needs are specified by the same areas that mediate the motor goals. This way, allocation of perceptual resources is computed in a distributed network that already contains the relevant action plans.

3. Limitations and future directions

The above framework is speculative, and much research is still needed to both fully explore the extent of action-modulated perception and its implementation. At a behavioral level, it is unclear what the specificity is of the modulations that occur with action preparation. Specifically, the range of features that can be enhanced this way and which actions are associated with these features.

An interesting finding that needs further exploration is the hemifield effect that was observed for grasping actions. Enhancements of orientation sensitivity were only found in the right visual field, when performing the action with the (dominant) right hand. This effect was found in chapter four, and replicated in chapter five. It is unclear whether this reflects an effect of hand dominance, hand of execution, or another factor. It would be interesting to investigate whether this effect holds for left handed subjects, and/or left handed grasping. Similarly, it would be interesting to test the roles of the left and right AIP in this hemifield effect. In chapter five, only the left AIP was stimulated (along with right handed action execution), and therefore this question cannot be answered with the available data.

On the level of implementation this thesis focuses on the FEF for eye movements and AIP for grasping movements. It is unlikely that these are the only motor areas involved in action-modulated perception. For eye movements, both the FEF and IPS were investigated using TMS. This resulted in qualitatively different perceptual effects. For grasping actions, only AIP was stimulated. It may be that stimulation of another grasping motor area, such as the ventral premotor area, also results in qualitatively different perceptual effects. Likely, it is not a single area, but an entire network of brain regions that provide feedback to visual areas as a function of motor preparation. These areas, and their connectivity, need to be uncovered to produce a complete view of the interactions between motor planning and perception. This may be done using emerging network analyses techniques, such as dynamic causal modeling or graph theory approaches in conjunction with visual and motor tasks.

The high field imaging at 7T used in chapter 6 has its benefits, but also imposes some limitations. The higher field strength allows for the acquisition of small voxels sizes (1.25mm isotropic in our study) while still retaining sufficient signal. This gives very detailed functional maps, but poses problems for group analysis. The high spatial resolution makes it possible to look at detailed activity patterns within a certain area (e.g. V1). However, the overlap of these maps between subjects, even with accurate normalization procedures, is likely to be very small. While this can be amended with smoothing, this negates the high-resolution advantage. We therefore chose to perform single subject analyses (taking advantage of the high spatial resolution) and extract hyperparameters for group analysis. Future techniques may enable the construction of high resolution functional maps at group level, to obtain reliable and generalizable organization principles.

A promising emerging imaging analysis technique is multivoxel pattern analysis (MVPA), which was used in the last chapter to study the effects of action

preparation on the early visual areas. By analyzing patterns of activity, instead of amplitude changes of single voxels, this technique is much more sensitive to subtle (sub-resolution) differences in underlying activity between conditions. This provides a powerful way of identifying the information content of a certain area. While this may not be advantageous for all paradigms, it provides a distinct advantage when studying areas comprised of differentially responsive neuronal sub populations, such as visuomotor integration areas. This way, the specific contributions of certain areas to action preparation can be disentangled (e.g. [11]). In light of the current topic, this is highly relevant, as brain areas at the convergence of perception and action are prime candidates for mediating action-relevant feedback to benefit perception.

In this thesis, two important areas have been identified that contribute to the enhancement of action-relevant perceptual features during action preparation, through top-down feedback connections to the early visual areas. Further research is needed to elucidate the network underlying action-modulated perception.

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Chapter 8

Nederlandse samenvatting

1. Inleiding

Stel je voor dat iemand je een kopje koffie aanreikt. De gemiddelde mens denkt dan: “Ha, lekker, koffie!”. De gemiddelde ‘perceptie en actie’ wetenschapper denkt echter aan alle processen die ten grondslag liggen aan het aannemen van dit kopje koffie. Zelf denk ik: “Hè nee, niet weer dat voorbeeld met dat kopje koffie”.

Als je immers dat kopje aangereikt krijgt, zal je waarschijnlijk eerst naar dit kopje kijken, om het vervolgens aan te pakken. Dit proefschrift gaat over de hersenprocessen die zich afspelen vlak voor het kijken naar het kopje en vlak voor het pakken van het kopje. Het blijkt namelijk zo te zijn dat vlak voor het kijken, je aandacht verschuift naar het kopje om je er een beter beeld van te geven. Vlak voor het grijpen van het kopje zal je automatisch beter de oriëntatie en de grootte van het kopje verwerken. Dit komt omdat de voorbereiding van een actie (iets grijpen, of ergens naar kijken) je perceptie aanpast om die actie vervolgens beter te kunnen uitvoeren. Dit gebeurt door aspecten van de binnenkomende visuele informatie te benadrukken die relevant zijn voor de actie die je aan het voorbereiden bent. Dit effect noemen we actie-gemoduleerde perceptie.

2. Samenvatting

Het doel van dit proefschrift is het onderzoeken van de invloed van actie voorbereiding op perceptie en het onderliggende mechanisme bij zowel oogbewegingen (hoofdstukken 2 en 3) als grijpbewegingen (hoofdstukken 4 tot 6).

In **hoofdstuk twee** is het tijdsverloop van neurale processen, voorafgaand aan de uitvoering van een oogbeweging, onderzocht met behulp van elektro-encefalografie (EEG). Dit werd gemeten tijdens de uitvoering van een oogbewegingstaak. Deze taak laat verbeterde waarneming op de plek van de geplande oogbeweging zien. Vlak na het startsignaal voor het uitvoeren van een oogbeweging (maar voor de daadwerkelijke uitvoering) was er frontale hersenactiviteit te zien, contralateraal aan de richting van de oogbeweging. Dit werd later gevolgd door occipitale hersenactiviteit, tevens contralateraal aan de richting van de oogbeweging. De amplitude van de vroege frontale activiteit (60-90ms na het startsignaal) was significant gecorreleerd met de amplitude van de latere occipitale negativiteit (150-180ms na het startsignaal). Hieruit kan worden opgemaakt dat de frontale activiteit betrokken is bij de perceptuele voorbereiding tijdens de oogbewegingspreparatie. Bronlokalisatie van de EEG signalen duidt de frontale oogvelden (‘frontal eye fields’, FEF) en de vroege visuele gebieden aan als bronnen van de gemeten signalen. Dit impliceert een rol voor de FEF in het bewerkstelligen van spatiële aandachtsverschuivingen door middel van feedback projecties naar de vroege visuele gebieden tijdens het voorbereiden van oogbewegingen.

De rol van de FEF en de intrapariëtale sulcus (IPS) bij aandachtsverschuivingen tijdens de voorbereiding van oogbewegingen werd verder onderzocht in **hoofdstuk**

drie. Tijdens het uitvoeren van dezelfde taak als in hoofdstuk twee werd er transcraniële magnetische stimulatie (TMS) toegepast na het startsignaal, maar voor de daadwerkelijke uitvoering van een oogbeweging. Afzonderlijke stimulatie pulsen werden 60, 90 of 120ms na het startsignaal voor de oogbeweging gegeven. Verschillende effecten van deze stimulatie werden gevonden, afhankelijk van zowel het tijdstip en de plek (FEF of IPS) van de stimulatie. Stimulatie van de FEF verbeterde het discriminatievermogen op de plek waar de oogbeweging naar toe gemaakt moest worden, wanneer de puls vroeg (vooral 60ms) na het startsignaal en contralateraal aan de oogbewegingsrichting werd toegepast. Dit is consistent met de bevindingen in hoofdstuk twee, en duidt aan dat vroege FEF activiteit de feedback naar de visuele gebieden bewerkstelligt, die de waarneming verbeterd op de plek waar de oogbeweging naar toe gepland is. Met stimulatie van de IPS werd een algemene verbetering gevonden in het discriminatievermogen, die niet afhankelijk was van het tijdstip of hersenhelft van stimulatie. Vanwege deze kwalitatieve verschillen tussen FEF en IPS stimulatie, is het waarschijnlijk dat de FEF direct naar de vroege visuele gebieden projecteert, in plaats van via de IPS.

In het tweede deel van dit proefschrift werd het effect bestudeerd van actie voorbereiding op perceptie in het skeletomotor systeem en grijpbewegingen in het bijzonder. Om dit te kunnen bestuderen werd er in **hoofdstuk vier** een gedragsmaat ontwikkeld die deze effecten van actie voorbereiding op perceptie lieten zien, met behulp van psychofysica. Proefpersonen moesten in deze taak verschillen in oriëntatie detecteren tijdens het voorbereiden van een actie. Deze taak was ontworpen om zoveel mogelijk te lijken op de oogbewegingstaak die gebruikt werd in het eerste deel van het proefschrift en ook geschikt te zijn in combinatie met TMS (zie hoofdstuk vijf). Tijdens de voorbereiding van een grijpactie waren proefpersonen beter in het detecteren van oriëntatieveranderingen in een balkje dan wanneer er naar gewezen moest worden. Dit is consistent met de effecten van actie-gemoduleerde perceptie, aangezien de oriëntatie van een object relevant is voor grijpacties, maar niet voor wijsacties. Wanneer er veranderingen in luminantie van het balkje gedetecteerd moesten worden, waren er geen verschillen tussen grijp- of wijs acties. Dit geeft aan dat het verbeteringen alleen optreden voor visuele aspecten die relevant zijn voor de actie die voorbereid wordt. Deze studie bevestigt, gebruikmakend van een psychofysische maat van visuele gevoeligheid, dat effecten van actie voorbereiding op perceptie ook voorkomen in het skeletomotor systeem.

In **hoofdstuk vijf** werd dit experimentele paradigma gebruikt om de rol van de anterieure intrapariëtale sulcus (AIP) te bestuderen in actie-gemoduleerde perceptie. AIP is, net als de FEF, een visueel-motorisch hersengebied, betrokken bij het voorbereiden en uitvoeren van een specifieke actie, in dit geval grijpen. AIP werd gestimuleerd met TMS tijdens het uitvoeren van de oriëntatie detectie taak (zoals beschreven in hoofdstuk vier), net na het startsignaal voor de actie uitvoering (maar voor de daadwerkelijke beweging). Dit verstoort de verbeterde oriëntatie detectie tijdens grijpvorbereiding (relatief aan wijsvorbereiding). Wanneer er, in plaats van AIP, een controle gebied werd gestimuleerd, werd de normale verbetering

door grijpvoorbereiding op perceptie gevonden, als in hoofdstuk vier. Dit toont aan dat AIP een essentieel onderdeel is voor het verbeteren van perceptie bij grijpacties. Tevens impliceert dit dat AIP een bron is van feedback naar de vroege visuele gebieden.

In **hoofdstuk zes** werden de effecten van actie voorbereiding op de visuele gebieden onderzocht met functionele kernspintomografie ('functional magnetic resonance imaging', fMRI) en multivoxel patroon analyse technieken ('multivoxel pattern analysis', MVPA). Proefpersonen moesten hierbij grijp- en wijsbewegingen voorbereiden en uitvoeren naar balkjes in de 7 Tesla MRI scanner. Soms moest de uitvoering onderdrukt worden en werd de actie alleen maar voorbereid. Patroon analyse werd uitgevoerd op activiteitspatronen van de vroege visuele gebieden (V1-3, bepaald met behulp van retinotopie taken) en AIP. Slechts die delen (voxels) van deze gebieden werden gebruikt die gevoelig waren voor de oriëntaties die gebruikt werden in deze studie (aan de hand van een aparte oriëntatie taak). Uit de resultaten blijkt dat aan de hand van de activiteit in de vroege visuele gebieden kan worden voorspeld welke actie er in voorbereiding was (ook wanneer er geen uitvoering van de actie plaatsvond). Dit betekent dat de voorbereiding van een actie een substantieel effect heeft op de activiteit in de vroege visuele gebieden. Dit effect was het sterkst in V1 en minder sterk (hoewel duidelijk aanwezig) in V2 en V3. Aangezien MVPA technieken gevoelig zijn voor signalen die onder de meetresolutie liggen, zou het zo kunnen zijn dat de effecten in de visuele cortex een modulatie van de oriëntatiekolommen weerspiegelen.

De voorgaande resultaten ondersteunen het idee dat de voorbereiding van een actie relevante aspecten van perceptie verbeterd door feedback te sturen naar de vroege visuele gebieden, hetgeen resulteert in actie-gemoduleerde perceptie.

3. Discussie

De resultaten in dit proefschrift maken het aannemelijk dat actie-gemoduleerde perceptie een algemeen fenomeen is, dat niet beperkt is tot oogbewegingen en grijpacties. De uitvoering van een actie is het gevolg van een samenwerking van verschillende hersengebieden, met elk een eigen specialisatie. Het is goed mogelijk dat deze specialisatie niet alleen geldt voor de voorbereiding van de motorische aspecten, maar ook voor de visuele aspecten. Het lijkt logisch dat het hersengebied dat specifieke informatie bevat over een motorisch aspect, ook de visuele gebieden informeert over de visuele behoeftes die hieraan verbonden zijn. Uit dit proefschrift blijkt dat het perceptuele aspect van actie voorbereiding niet geïmplementeerd is in het motor gebied zelf, maar door middel van feedback verbindingen naar de visuele gebieden wordt bewerkstelligd. Hierdoor wordt de binnenkomende visuele informatie verwerkt, gericht op de behoeftes van de huidige (actie) doelstellingen. Op deze manier kunnen acties efficiënter worden uitgevoerd,

omdat de visuele informatie die bij de motorgebieden binnenkomt geoptimaliseerd is.

In dit proefschrift zijn twee belangrijke hersengebieden geïdentificeerd die tijdens de voorbereidingsfase voor een actie bijdragen aan de verbetering van actierelevante aspecten van perceptie, door middel van feedback koppelingen met de visuele gebieden. Verder onderzoek is nodig om het netwerk in kaart te brengen dat aan de basis ligt van actie-gemoduleerde perceptie.

Chapter 9

Dankwoord

Time is an illusion. Lunchtime doubly so.
- Douglas Adams

Waar is de tijd gebleven? Werktijd, lunchtijd, koffietijd, congrestitijd, funcotijd, scantijd, borreltijd.. Dik vier jaar voorbij. Vier jaar aio-schap. Vier jaar onderzoek doen aan het UMCU. Het leek een eeuwigheid in november 2007. Nu lijkt het in een vloek en een zucht voorbij gegaan te zijn. Hoe komt dat? *Time flies when you're having fun*. Onderstaande mensen hebben er waarschijnlijk veel mee te maken.

Ten eerste natuurlijk Bas. Co-promotor en mastermind achter de projecten in dit proefschrift. Het is eigenlijk niet te doen om in een paar zinnnetjes over te brengen hoe fijn het was om jou als begeleider te hebben. Altijd belangeloos bereid om te helpen, altijd tijd om het een en ander te bespreken. Heel veel dank voor alle vrijheid die ik heb mogen genieten de afgelopen jaren; ik heb altijd het gevoel gehad samen te werken, in plaats van voor je te werken. Ik heb bijzonder veel geleerd van je inzichten, kennis van zaken, optimisme en schijnbaar onuitputtende stroom Duitse wetenswaardigheden. Maar het was niet alleen prettig op wetenschappelijk vlak, ook persoonlijk was het een bijzonder prettige tijd. Ontelbare lunch gesprekken, borrels (met luxe bittergarnituur), lange autoritten over de Canadese snelweg, verregende Franse steden, tuffende Westfalia campers, fietstochten over de Golden Gate en ritjes in volgepakte Londense metros. Het was gezellig.

Leon Kenemans, promotor, bedankt voor het heldere, inzichtvolle commentaar op de manuscripten en experimenten in wording. Het was fijn om binding te hebben met de UU, waar ik ook tijdens mijn master-stage een erg leuke tijd had.

René Kahn, tevens promotor. Bedankt dat ik mijn project kon uitvoeren bij psychiatrie op het UMCU, en alle vrijheid die ik daarbij gekregen heb.

Mijn dank gaat ook uit naar de leden van mijn leescommissie: De professoren Sarah Durston, Pieter Medendorp, Frans Verstraten en Chantal Kemner. And many thanks to Dr. Jody Culham, coming all the way from Canada; it's an honor having you here. Thanks also for the warm welcome Bas and I received last year, when we visited!

Mijn paranimfen. Mariët: wat begon met lichte wederzijdse irritatie over de temperatuur van de kantoortuin, is omgeslagen tot bijzonder fijne vriendschap. Wat was het gezellig in Washington, San Francisco en vooral Quebec. Bedankt voor alle gesprekken, van onzinnige observaties tot diepzinnige gedachten. Bedankt voor je steun tijdens de wat mindere dagen (soms te merken aan de muziekkeuze) en je enthousiasme tijdens de betere (ook te merken aan de muziekkeuze). De afgelopen maanden voelde op het werk verschijnen meer als thuiskomen in ons gezellige

kantoortje, doordrenkt met de geur van Bengal Spice (“*die penetrante pepernotengeur*”). Ik zal het missen. Wat fijn dat we straks weer collega’s zijn in Nijmegen!

Alyanne, na een korte afwezigheid weer terug in de wetenschap, een aanwinst! Bedankt voor alle gezelligheid, ook tijdens de master. Je hebt me door een moeilijke periode gesleept, misschien zonder dat je het wist. Bedankt voor je fijne kijk op de dingen van het leven. En altijd fantastisch om een mede-Monkey Island nerd te hebben ;). Wat ontzettend leuk dat je mijn paranimf wil zijn.

Met plezier naar aan het werk gaan staat of valt met het hebben van leuke collega’s. In dit geval stond het. Allemaal heel erg bedankt daarvoor. Mocht ik hieronder iemand vergeten: je bent niet vergeten, alleen per ongeluk niet in mijn dankwoord beland.

Antoin, dude. Zoals een wijs man ooit zei (die met die Duitse verhalen): “Waar Antoin is, is de gezelligheid”. Hoe waar. Het was echt heel erg gezellig de afgelopen jaren, van carnaval vrijdagmiddalborrels tot BBQ met epische steaks in San Diego. Jij begrijpt tenminste dat je geen (werk) inspanning kan leveren zonder de nodige ontspanning. Dat onderzoek doen leuk is, maar ergens moet je een grens trekken. Daarbij moet ik ook zeggen dat die instelling blijkbaar loont, want je bent ook een bijzonder goede onderzoeker (hé, wie staat er nou in de quest!?). Dat we elkaar nog vaak tegenkomen op congressen. Veel succes in Oxford!

De mensen van kantoortuin 1 (helaas meer kantoor dan tuin) *past en present*. Soms was het stil, soms levendig, soms koud, soms warm, soms leeg, soms vol, maar de mensen die er zitten en zaten, waren altijd erg fijn om mee op een kamer te zitten! Cédric, uit een tijd waar nog een fatsoenlijke temperatuur heerste in de kantoortuin. Het was gezellig in de kantoortuin én op het balkon in San Francisco (oa). Bram, altijd goed voor zowel een wetenschappelijke als PC vs MAC discussie, ik kom nog eens een ritje maken in je pickup; Daan, Thomas G, Maartje A, Geartsje. Nieuwere kantoortuin gangers, Janna Marie, Esther (succes als kantoortuin oudste!), Florian, Thalia, Sven, Marc (dank voor de hulp bij het behalen van de dagelijkse portie woorden) en Sanne: Jullie brachten een stuk meer levendigheid naar de kantoortuin, wat misschien niet zo goed was voor de productiviteit, maar des te meer voor de gezelligheid.

Dan nog de rest van de collega’s van het UMC, al dan niet geaffilieerd met een kantoortuin. Bedankt voor alle gezelligheid en discussies bij de random encounters in de wandelgangen, koffieautomaten of onder het genot van wat zachtkrokante bitterballen van de inmiddels ter ziele gegane vrijdagmiddagborrel: Mirjam (Bloemeling..ik lig nog steeds in een scheur als ik terugdenk aan de keer dat we vast zaten in de NeNa pointer, we worden binnenkort weer collega’s!), Remko (nog ééntje dan?), Dora (met dank voor de Nieuw-Zeeland connectie!), Mireille (mede-classificator), Kelly (je mag m’n promotie vergeten), Anouk (3T is voor watjes),

Sanne vR (ik zal bij deze de gehaktballen niet noemen), Mitzy (wees vooral heel erg jezelf), Matthijs V, Mathijs R, Gayane, Richard, Heleen F(tack!), Fiona (weet waar je aan begint met je master, voor je het weet zit je ook zo'n boekje te schrijven), Maartje S (wordt vast een smakelijke promotie!), Sanne K (Sanne één), Kirstin, Sanne V, Yumas (bedankt dat ik als eerste op de nieuwe Hulk mocht spelen), Myrte, Frank, Esther vdZ (we'll always have the Integratron), Jeroen S, Max (monsieur de Lyon), Petar, Lisette, Anca, Erika, Matthijs B, Martijn M, René M, Marinka, Lucija, Guusje, Willemijn, Dienneke, Anna, Janna.. *et al.*

Ook moet ik natuurlijk nog de mensen op de universiteit, afdeling psychonomie (ehm psychologische functieleer) bedanken. Was altijd weer leuk wanneer ik even langs 'moest' komen voor het een of ander. Het voelde toch een beetje als terugkomen 'op het oude nest'. Dus bedankt Stijn, Floris, Alyanne (nog een keer), Branka (ex-colleague, current colleague and future colleague!), Joke, Jelmer, Marijn, Ben (viva Las Vegas!), Serge en Stefan (bedankt dat we af en toe wat speelgoed van je mochten lenen). Nisan, dank je voor het me bijbrengen van de eerste fundamenten van het onderzoek in de praktijk, en het doorsturen van het vacature mailtje van Bas. Je ziet wat een mailtje teweeg kan brengen.

En een speciaal bedankje aan Erno, die het met mij en Bas in een oud Duits busje zonder airco door de californische woestijn vol hield met ZZ Top door de speakers. Klasse. Je weet me straks te vinden in Nijmegen, als je nog eens wat echte muziek wil horen.

Natuurlijk bedankt aan alle (co-)auteurs op mijn papers (voor zover nog niet genoemd) in dit proefschrift voor alle fijne samenwerking: Helene, bedankt voor het mooie derde hoofdstuk en het de hulp tijdens de inwerkfase! Willem, dank je voor het leggen van de basis van dit proefschrift (leuk je weer eens tegen te komen in Quebec!). Natalia, thanks a lot for all the support on the 7T project, we would've been hopelessly lost without your guidance. Serge & Ben, bedankt voor alle hulp bij het opstarten van het 7T project en de introductie in de wereld van het retinotopische mappen. Erik, bedankt dat we met stijl konden grijpen in de scanner.

Rosanne, stagiaire extraordinaire, ex-collega, mede-aio. Het was erg fijn om samen met je aan een project te werken, al moesten we (vooral jij eigenlijk) dan af en toe in wat stinksokken knijpen. Daarnaast was het ook gewoon buitengewoon gezellig, toch? (zeg maar ja). Super dat je een mooie aio plek hebt, we komen elkaar nog wel tegen!

Soon, my other intern. Thanks for all the hours in the lab, and bringing one of the most culturally diverse subject populations. It made the results that much more generalizable!

Muriel and Sharika: It was great having a small international TMS team for a little while! Thanks for the nice times we had in and out of the lab. Sharika, thanks for expanding my music knowledge.. and let's get that paper to a nice journal soon! Muriel, thanks for expanding my cooking knowledge! I'm sure I'll bump into you at a conference soon (or in -rainy?- Lyon ;).

Roeland en Erik, bedankt voor de filmweekendjes, ze waren altijd gezellig. Fijn om eraan herinnerd te worden dat er meer is dan wetenschap. Laten we altijd zorgen dat het IBI niet te lang wordt!

Aan mijn familie: Het was altijd erg fijn om weer even thuis te zijn. Om weer even echt bij te praten over van alles en nog wat. De Wondergemmetjes: bedankt voor jullie aanhoudende interesse voor mijn bezigheden in Utrecht en buitenlandse reisjes! Lieve Oma: Afgelopen tijd was weer een bijzonder moeilijke periode voor je, maar je blijft stug vol houden, zonder op te geven. Ik bewonder je! Bouwe, broer, en wat een bijzonder fijne broer om te hebben. We zien elkaar veel te weinig, maar als we elkaar zien, is het alsof we niet weg geweest zijn. Jij en Carolien geven me de hoop dat het allemaal nog wel goed komt met mij ;). Tenslotte nog mijn lieve ouders: ik zeg het nooit, maar wat hou ik van jullie! Jullie hebben me onvoorwaardelijk gesteund in alles wat ik deed, zelfs als dat betekende dat ik een tijdje aan de andere kant van de wereld zat (om naar balkjes te kijken). Zonder jullie had ik nooit zover gekomen, aan het eind van dit 'opstel'. Ik kan me geen betere ouders wensen, jullie zijn fantastisch! Bedankt voor alles.

Chapter 10

List of publications

1. Journal Articles

Gutteling TP., Petridou N., Harvey BM., Aarnoutse EJ., Kenemans JL. & Neggers SFW. Grasping the intention and getting the point: Decoding the influence of action preparation on perception. (in preparation)

Sharika KM., Neggers SFW., **Gutteling TP.**, Van der Stigchel S., Dijkerman HC. & Murthy A. Role of supplementary eye fields in the proactive control of sequential saccades: a single pulse TMS study in humans. (in preparation)

Gutteling TP., Park SY., Kenemans JL. & Neggers SFW. TMS of the anterior intraparietal area selectively modulates orientation change detection during action preparation. (under review)

Neggers SFW., van Diepen RM., Zandbelt BB., Vink M., Mandl RCW. & **Gutteling TP.** (2012). A functional and structural investigation of the human fronto-basal volitional saccade network. *PLoS ONE* 7(1): e29517.

Panouillères M., Neggers, SFW., **Gutteling, TP.**, Salemme R., van der Stigchel S., van der Geest, JN., Frens, MA. & Pélisson D. (2011). Transcranial magnetic stimulation and motor plasticity in human lateral cerebellum: Dual effect on saccadic adaptation. Human brain mapping.

Gutteling TP., Kenemans JL. & Neggers SFW. (2011). Grasping preparation enhances orientation change detection. *PloS ONE*, 6(3), e17675.

Gutteling TP., van Ettinger-Veenstra HM., Kenemans JL. & Neggers SFW. (2010). Lateralized frontal eye field activity precedes occipital activity shortly before saccades: evidence for cortico-cortical feedback as a mechanism underlying covert attention shifts. *Journal of cognitive neuroscience*, 22(9), 1931-43.

Van Ettinger-Veenstra HM., Huijbers W., **Gutteling TP.**, Vink M., Kenemans JL. & Neggers, SFW. (2009). fMRI-guided TMS on cortical eye fields: the frontal but not intraparietal eye fields regulate the coupling between visuospatial attention and eye movements. *Journal of neurophysiology*, 102(6), 3469-80.

2. Conference abstracts

Gutteling TP., Kenemans JL., Park SY. & Neggers SFW. TMS on AIP disrupts perceptual enhancement of action relevant features. Poster presentation at the Annual international Human Brain Mapping conference 2011, Quebec city, QC, Canada

Gutteling TP., Park SY., Kahn RS., Kenemans JL. & Neggers SFW. Action preparation increases sensitivity to relevant features. Oral presentation at the international Society for Neuroscience annual meeting 2010, San Diego, CA.

Gutteling TP., Van Ettinger-Veenstra HM., Kenemans JL. & Neggers SFW. The frontal eye fields drive shifts of visuospatial attention before eye movements by connections to the visual cortex: evidence from EEG, TMS and fMRI. Poster presentation at the Annual international Human Brain Mapping conference 2009, San Francisco, CA.

Gutteling TP., Van Ettinger-Veenstra HM, Kenemans JL. & Neggers SFW. The frontal eye fields drive shifts of visuospatial attention before eye movements by connections to the visual cortex: evidence from EEG, TMS and fMRI. Poster presentation at the Annual international Human Brain Mapping conference 2008, Washington, DC.

Chapter **11**

Curriculum Vitae

Tjerk Peter Gutteling was born on Sunday, September 13th 1981 in Vlissingen. After some essential growing-up, he attended Scheldemonde college, where he obtained his VWO diploma in 2000. Unable to decide whether he was more interested in computer science or neuroscience, he combined interests and studied cognitive artificial intelligence at Utrecht University. After obtaining his master's degree in 2005 he was accepted into the Neuroscience and Cognition master program in Utrecht. His first internship was performed at the (then) psychonomics department under the guidance of Nisan Mol, dr. Joke Baas and Prof. Leon Kenemans studying the effects of distractor anticipation with fMRI. This was also the historical point of first contact with dr. Bas Nèggers (in the role of supporting supervisor). The second internship was performed at the University of Auckland in New Zealand, under the supervision of dr. Jeff Hamm, studying the neural correlates of illusory motion perception with EEG. Upon returning he was offered a PhD position by dr. Bas Nèggers, now at the UMCU, which culminated in the current thesis. Starting March 2012, he will start at the Donders Institute in Nijmegen as a post-doctoral researcher at the sensorimotor lab, led by Prof. Pieter Medendorp.

Tjerk Peter Gutteling werd geboren op zondag 13 september, 1981 in Vlissingen. Na wat opgroeien heeft hij zijn VWO diploma gehaald aan het Scheldemonde college in 2000. Omdat hij geen keuze kon maken tussen informatica en neurowetenschappen, besloot hij cognitieve kunstmatige intelligentie te gaan studeren aan de Universiteit Utrecht. Na het behalen van zijn master diploma in 2005 werd hij toegelaten tot de Neuroscience & cognition master in Utrecht. Tijdens zijn eerste stage, bij de afdeling psychonomie onder begeleiding van Nisan Mol, dr. Joke Baas en Prof. Leon Kenemans onderzocht hij het effect van distractor anticipatie met fMRI. Dit was ook het historische moment waarop hij dr. Bas Nèggers voor het eerste ontmoette (in de rol van ondersteunend begeleider). De tweede stage deed hij aan de Universiteit van Auckland in Nieuw-Zeeland, onder begeleiding van dr. Jeff Hamm, waar hij neurale correlaten bestudeerde van bewegingsillussies met EEG. Bij terugkomst in Nederland kreeg hij een aio-positie aangeboden door dr. Bas Nèggers, inmiddels op het UMCU, hetgeen resulteerde in het huidige proefschrift. Vanaf maart 2012 zal hij als post-doctoraal onderzoeker starten aan het Donders instituut in Nijmegen in het sensorimotor lab van Prof. Pieter Medendorp.

GUYBRUSH:

- *“How can you see without eyeballs?”*

MURRAY THE DEMONIC SKULL:

- *“How can you walk around without a brain? Some things no one can answer.”*

from ‘The Curse of Monkey Island’