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# Timing of visual stimuli in V1 and V5/MT: fMRI and TMS

**Etudiant**

Michel Bornet

**Tuteur**

Prof. Micah Murray

Dpt de Radiologie et Dpt de Neurosciences Cliniques, CHUV

**Co-tutrice**

Dr Domenica Bueti

Dpt de Neurosciences Cliniques, CHUV

**Expert**

Dr David Benninger

Dpt de Neurosciences Cliniques, CHUV

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## ABSTRACT

Time perception is used in our day-to-day activities. While we understand quite well how our brain processes vision, touch or taste, brain mechanisms subserving time perception remain largely understudied.

In this study, we extended an experiment of previous master thesis run by Tatiana Kenel-Pierre. We focused on time perception in the range of milliseconds. Previous studies have demonstrated the involvement of visual areas V1 and V5/MT in the encoding of temporal information of visual stimuli. Based on these previous findings the aim of the present study was to understand if temporal information was encoded in V1 and extrastriate area V5/MT in different spatial frames i.e., head-centered versus eye-centered.

To this purpose we asked eleven healthy volunteers to perform a temporal discrimination task of visual stimuli. Stimuli were presented at 4 different spatial positions (i.e., different combinations of retinotopic and spatiotopic position). While participants were engaged in this task we interfered with the activity of the right dorsal V1 and the right V5/MT with transcranial magnetic stimulation (TMS). Our preliminary results showed that TMS over both V1 and V5/MT impaired temporal discrimination of visual stimuli presented at specific spatial coordinates. But whereas TMS over V1 impaired temporal discrimination of stimuli presented in the lower left quadrant, TMS over V5/MT affected temporal discrimination of stimuli presented at the top left quadrant.

Although it is always difficult to draw conclusions from preliminary results, we could tentatively say that our data seem to suggest that both V1 and V5/MT encode visual temporal information in specific spatial frames.

# INTRODUCTION

## 1.1 A point about time perception

### 1.1.1 Different models

Time perception is innate to human nature, and we are able to perceive the time in sensory events around us. While this perception of time remains fairly mysterious for science, a lot of studies already focused on how our brain processes time and how temporal data are involved in many cerebral capacities. Time is indeed involved in many cerebral activities, such as sound localisation that we use every day to drive a car or react to approaching hazards, perception of motion from static images passing before our eyes and coordination of muscles activation to perform physical activities. Even the functioning basis of the brain activity relies on temporal processing; the brain receives different signals from different modalities processed in distant neural areas, but these signals have to be correctly tagged to outside events and aligned in time to become useful as a whole.

In this study, we focused on time on a millisecond/second range. How our brain catches and processes time at that scale remains complex and controversial. Many laboratories around the world are working on this subject.

There are nowadays a few main theoretical models that hypothesize different timing mechanisms in a millisecond/second range.

**a. Internal clock model**

This model, developed on psychophysical and lesion studies, relies on the possible existence of one or multiple amodal neural clocks (i.e. which is engaged independently from the sensory modality, the task used or the length of the interval of a temporal task). It has been the dominating model for over 30 years. It suggests the existence of a dedicated timing mechanism that depends on one single specialized neural structure such as the cerebellum or the basal ganglia which is engaged in temporal processing of a wide range of timing behaviours and intervals (from hundreds of milliseconds to seconds).

Empirical support for this model has been given by recent fMRI studies (Lewis and Miall, 2003 (1)). These studies show the activation of a large neural network, including the supplementary motor area (SMA), the parietal and prefrontal cortices, the basal ganglia and the cerebellum across different tasks (motor, perceptual), durations (millisecond, second) and sensory modalities (visual and auditory).

**b. Ubiquitous timing model**

In this model (sometimes called "intrinsic") time encoding is considered inherent to multiple cortical circuits, meaning that every functional part of the brain could encode time on its own. This implies that perception of time duration during different tasks is an intrinsic property of cortical sensory-specific networks and excludes the existence of a neural circuit specialized in time perception. According to a 'state dependant network model', time encoding could be an emergent property of the pattern of activation of a population of neurons in different brain areas, and this pattern of activation is the result of time-dependant changes in synapses of the neural network involved in the task, like for example slow inhibitory post-synaptic potentials (IPSPs) or short-term synaptic plasticity (Buonomano and Maass, 2009 (2)). Empirical support for this idea comes from computational simulations and cell-culture recordings (Buonomano and Maass, 2009 (2) / Karmakar and Buonomano, 2007 (3))

**c. Partially shared timing model**

In this alternative model, temporal processing is supported neither by the intrinsic properties of neural networks nor by a single mechanism. Temporal processing is instead the result of interactions between multiple brain areas. Some regions (SMA and basal ganglia) have a dedicated role in timing, and others (like cortices and sensory areas) contribute to timing in a modality dependant way. Merchant et al., 2008 (4) studied timing with four different tasks requiring different sensorimotor processing and different modalities for the stimuli. Their results can be neither proof of the existence of a common timing mechanism nor interpreted as the result of multiple context-dependant timing mechanisms, suggesting that a distributed timing mechanism working together with core timing structures could be an explanation. In accordance with recent neuroimaging research by Buhushi and Meck, 2005 (5) and Coull et al., 2011 (6), these results suggest that time perception is probably the result of the interaction between core timing circuits within the cortico-thalamic basal ganglia (CTBG) and other brain regions providing additional information needed for time processing.

This shared model could also help explain time perception on the scale of seconds to minutes or more. The studies aforementioned (and most of the neurophysiological evidence for a modality-specific timing mechanism) concentrate on periods of hundreds of milliseconds. A more global model where temporal information computed by sensory cortices is sent to deeper specialized timing areas

that could integrate data to guide an action for example (Coull et al., 2011 (6)) could explain timing on larger scales than milliseconds. This possibility still needs to be elucidated and research focuses on whether these modality-dependent and modality-independent structures have dissociable or overlapping functions, and if their timing mechanism is the same or different. For example, Bologni et al. 2009 (7) and Kanai et al., 2011 (8) studied the auditory cortex and found that this structure is important for auditory temporal discrimination, but also for somatosensory and visual stimuli. They linked this discovery with the possible role of auditory-based mental representation for time estimation: timing information taken from visual stimuli could be converted into an auditory representation for temporal computation.

### 1.1.2 Neural correlates of time perception and involvement of visual areas

Since the end of the 19th century, many studies investigated parts of the brain that are engaged in millisecond/second time processing with different techniques: in humans with neuroimagery, transcranial magnetic stimulation, electro-encephalography or lesion studies and in animals with electrophysiology. All these studies (Koch et al., 2002 (9) / Jones et al., 2008 (10) / Ivry and Keele, 1989 (11)) have been looking for brain areas where time is processed. They found that many different brain regions are involved in temporal processing, like the cerebellum, the basal ganglia, the right prefrontal and parietal cortices, the SMA and the superior temporal gyrus.

However, some parts seem activated independently of the task used, the length of the temporal interval and the sensory modality of the stimuli (i.e., basal ganglia and cerebellum), and others are dependent of the sensory modality of the stimuli (i.e., visual and auditory cortices (Bueti et al., 2008 (12)).

This thesis will focus on time processing of visual stimuli in visual brain areas.

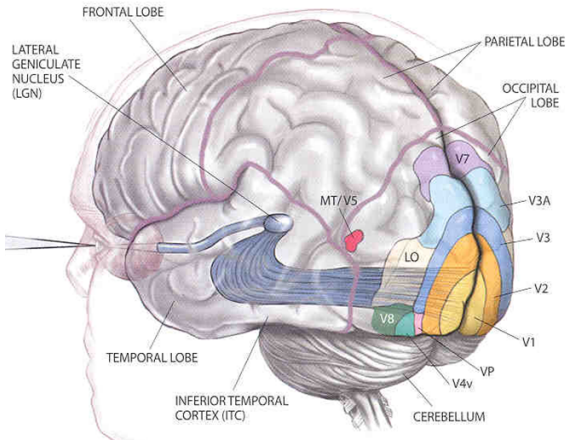


**Figure 1 : The visual system.** Retina senses the visual stimuli input and transforms visual data into electrical data. This electric impulsion is sent to visual primary cortex V1 via the optic nerve and the lateral geniculate nucleus of the thalamus. In V1, which corresponds to a retinotopic map of the contralateral hemifield, visual data is processed and sent to others secondary/tertiary visual areas like V5/MT (in the lateral occipital lobe, close to the temporal lobe).
From Terese Winslow, Harvard Medical School, *http://www.teresewinslow.com*

There is empirical evidence that suggests the involvement of visual regions in time processing. Electrophysiological studies on animals by Shuler and Bear 2006 (13) and Ghose and Maunsell 2002 (14) and neuroimaging and magnetic stimulation studies on humans by Bueti et al., 2010 (15) and 2012 (16) showed the engagement of visual cortices during the processing of the temporal dimension of visual stimuli. Primary visual cortex V1 and extrastriate visual areas like for example V5/MT seem to play a fundamental role in time processing of visual events.

Psychophysical observations (Morrone et al., 2005 (17) / Kanai et al., 2006 (18)) strongly implicate visual cortices in timing processes by demonstrating that perceived duration of a visual stimulus can be modified by visual motion or temporal frequency for example, which are modality-specific parts of the stimuli (i.e. are dependent of the modality used: motion on visual stimulus can only be linked to visual perception).

More recent studies using transcranial magnetic stimulation (TMS, see below for further information) showed that primary visual area V1 is necessary for time discrimination of visual stimuli (Kanai et al., 2011 (8)) and that V5/MT codes temporal data for moving stimuli as well as static events (Bosco et al., 2008 (19) / Bueti et al., 2008 (20)).

A paper by Salvioni, Murray, Kalmbach & Bueti, 2013 (21) showed that there is a causal relationship between the engagement of V1 and V5/MT during time processing at the encoding stage (i.e. when time is encoded from stimulus in neural

networks, in opposition to time maintenance in working memory). With five TMS experiments, they showed that V1 and V5/MT are involved in encoding and keeping time in memory independently from unspecific task requirements and low-level visual processing. More precisely, their study allowed determining the dynamics and functional association between V1 and V5/MT. These two regions were simultaneously engaged and functionally linked during time encoding, but V1 came into play before V5/MT during the memorisation of temporal information in working memory. They also hypothesized that this role at the encoding stage of temporal processing can be interpreted as a proof that time signals are generated locally in the neural activity of these areas.

## GOALS OF THIS PROJECT

Until today, many studies focused on "where" time is encoded, leading us to find a lot of new structures involved in timing. In this thesis we are more on the "how" side of understanding timing: we work on structures known to be a part of the timing network and focus on understanding their role and the mechanisms they are involved in.

To explain the main goal of this project, we first have to differentiate retinotopy from spatiotopy. These are two different frames of reference our visual cortex uses to encode the spatial position of external objects. A retinotopic frame is eye-centered: visual objects are represented with respect to the position of their images on the viewer's retina. A spatiotopic map is head-centered: the spatial position of the objects is based on their relative position with respect to the position of the viewer's head (Melcher and Marrone, 2003 (22)).

Several studies (Binda et al., 2009 (23), Johnston et al., 2006 (24)) suggested the existence of spatially specific mechanisms in time encoding by showing that adaptation by fast-moving stimuli reduced the apparent duration of another stimulus presented in the same retinotopic or spatiotopic position. Lately, Burr et al., 2007 (25) showed that adaptation was more efficient if stimuli were corresponding on a spatiotopic map rather than a retinotopic one.

With this study, we went on with the experiment of another master thesis (Master Thesis in Biology, Faculty of Biology and Medicine, University of Lausanne) ran by Tatiana Kennel-Pierre. We focused on time perception of visual events in the range of milliseconds.

Based on the results of Salvioni et al., 2013, we tried to show if time encoding in visual areas V1 and V5/MT is embedded in retinotopically or spatiotopically organized neuronal networks.

We used paired-pulse TMS (based on the same methodology as Salvioni et al., 2013) applied over the right dorsal V1 and the right V5/MT during a temporal discrimination task to explore the spatial frame of reference of time encoding neurons of these two brain regions.

## METHODS AND MATERIALS

### 2.1 Subjects

The experiment was completed by 11 healthy volunteers (5 were tested during the thesis of Tatiana Kenel-Pierre and 6 during this thesis, 6 females, mean age: 24.9 / range: 21-32). All subjects had normal or corrected-to-normal vision and were given a written informed consent approved by the Ethics Committee of the Faculty of Biology and Medicine at the University of Lausanne.

### 2.2 TMS experiment

#### 2.2.1 Stimuli and procedure

Our experiment involved a temporal discrimination task of empty intervals. Subjects were seated in front of a computer screen (resolution 1024 x 768, 60 Hz refreshing rate) showing four sequential visual flashes as stimuli. The flashes were blue disks subtending 2° of visual angle at a 70 cm viewing distance. A temporal interval was defined as the empty interval between two successive flashes. Participants had to judge which one of two successive intervals was longer (the first or the second) and answer by pressing the one or two key on a keyboard. The stimuli were organised in trials. Within a trial the two temporal intervals were separated by a variable inter-stimulus interval ranging from 900 to 1200 ms. One of the temporal intervals was the "standard duration" and the other the "comparison duration". Standard duration T was always 200 ms and comparison duration was T + ΔT (ΔT was a variable value and was always positive). The order of standard and comparison interval was randomised.

An adaptive procedure (Bueti et al., 2012 (16)) changed the ΔT in order to obtain 79% of correct discrimination across all trials. If the answer was incorrect, the ΔT was increasing, and if it was correct 3 consecutive times, the ΔT decreased (ΔT changed in stages of 33.4ms for the three first modifications, and 16.7 for the others). A feedback (duration = 1 second) was given after each answer. The feedback was given through the fixation point (a black asterisk). The asterisk turned green in case of a correct answer, and red if the subject was mistaken. After the feedback there was an inter-trial interval of 1.8 to 2.5 seconds (chosen randomly). The adaptive procedure allowed us to obtain individual discrimination threshold that we expressed as a Weber fraction (i.e., ΔT/T). The discrimination threshold was the ΔT value leading to 79% of accurate response. We took the Weber Fraction as a measure of the participants' capacity in discriminating time.

Participants were asked to perform the task within peripheral vision. They fixated a black asterisk throughout the trial while the two intervals were flashed at one of 4 different spatial positions (see Fig.1): lower left quadrant (positions 1,2), lower right quadrant (position 3) and upper left quadrant (position 4). Each spatial position was tested in different blocks of trials. One block of consisted of 60 repetitions of the trial sequence described above.
In each of these spatial positions the distance between the flashes and the fixation point was kept constant and was 8° of visual angle. This means that in the 4 spatial positions the subjects performed the tasks with the gaze shifted according to the spatial position of the flashes.

We chose these four positions to have 4 combinations of retinotopic and spatiotopic positions (see Fig.2).

In particular our idea was to be able to disrupt:

a) With the TMS stimulation of the dorsal V1, temporal processing of stimuli in retinotopic position A. With respect to this goal, the retinotopic positions B and C represented two control conditions.

b) With TMS stimulation of the right V5/MT the temporal processing of stimuli presented in the spatiotopic position B. To this purpose the spatiotopic position C was a control condition.

Position 1: retinotopic position A – spatiotopic position A
Position 2: retinotopic position A – spatiotopic position B
Position 3: retinotopic position B – spatiotopic position B
Position 4: retinotopic position C – spatiotopic position C

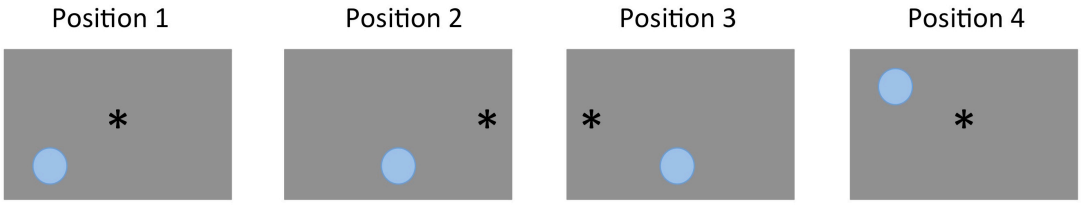


**Figure 2: Stimuli presentation.** Stimuli were presented in four different spatial positions. The asterisk represents the centre of fixation also used for feedback.
adapted from Kenel-Pierre T., 2014

For each stimulation site (V1, V5/MT and vertex), we ran 4 blocks of TMS, each one corresponding to a different combination of retinotopic/spatiotopic positions (sequence was randomized) for a total of 12 blocks.

In addition to these 12 blocks, we began with 4 blocks without TMS to allow the subjects to get used to the task and give a stable performance and to help us obtain individual thresholds.

### 2.2.2 Transcranial magnetic stimulation

First described by Arsène D'Arsonval in 1896, transcranial magnetic stimulation (TMS) is used in clinical neurosciences since the 1980s. The main fields where TMS is used are research in the field of neuroscience, where its effects can lead to a better understanding of how some brain areas function, and in the treatment of several neurological or psychiatric pathologies such as fibromyalgia, deep depression or schizophrenia.

Nowadays, TMS is widely used in cognitive neurosciences to understand the function of brain regions. TMS pulses are delivered on a particular brain area while volunteers perform a given task that is supposed to involve the target area. By interfering with the normal neural processing of the target area, TMS can show if that specific brain region is important for a given cognitive function or when (at which processing stage) this region is involved.

A stimulator induces a strong and quickly changing magnetic field in a coil placed near the subject's head. This field will induce, according to Lenz-Faraday's law, a current in the cells located nearby the coil, causing changes in their polarization, thereby interfering with the normal activity of the target brain area. Because the strength of the magnetic field decreases when distance from the coil increases, parameters that affect TMS effects are for example thickness of the scalp or deepness of the stimulation point.

TMS works by inducing magnetic pulses that causes these changes in the polarization of the membranes of neurons. Pulses can be unique, paired or repetitive: while single pulse (spTMS) and paired-pulse (ppTMS) are used for their high temporal and spatial resolution (i.e., physiological effects are limited in space and time), repetitive stimulation (rTMS) where a few pulses are delivered on a constant fast rhythm is preferred when longer-lasting behavioural effects are needed and temporal resolution is not crucial.

Because TMS is interfering with normal brain's activity, safety is important. Rossi et al., 2009 (26) described the most frequent safety issues. As TMS induces erratic neural activation, possibility of inducing seizures is a major concern when using the technique, and all patients that have a family history of epilepsy or are epileptic should avoid TMS testing.

In our experiment, we used TMS as a way to interfere with the normal function of V1 and V5/MT by increasing neural noise (Walsh & Pascual-Leone, 2003). This noise corresponds to the random activation of both inhibitory and excitatory neurons located at the stimulation point: TMS created a kind of transient and reversible "virtual lesion". During the performance of our task, this "virtual lesion" has a disruptive effect on V1 and V5/MT because the random activity interferes with the normal neural activity. Therefore we expected to find worse discrimination thresholds after TMS of V1 and V5/MT only at certain spatial position.

### 2.2.3 TMS methods

For the TMS experiment, we used a Magstim Rapid$^2$ Stimulator coupled with two different coils. The first one was a 70 mm figure-of-eight shaped coil used at 50% of maximal stimulation output. The second one was a 40 mm figure-of-eight shaped branding iron coil used at 63% of maximal stimulation output.

We used ppTMS for its stronger effect on the target area compared to spTMS (i.e. paired pulses effects are added up), without affecting spatial or temporal resolution (defined by the temporal distance between the two pulses, Silvanto et al., 2005 (27)). TMS pulses were delivered 120-155 milliseconds after the onset of the first flashing blue disk i.e. at the onset of the first temporal interval. This stimulation timing was proved to interfere with the time encoding phase (Salvioni et al., 2013 (21)). The inter-pulse interval used was 35 ms (this was a hardware limitation).

We chose as target areas the right dorsal V1 and the right V5/MT. We also used the stimulation of the vertex as a control site. We used it to obtain temporal discrimination thresholds in the exact TMS context as the ones obtained during the V1 and V5/MT blocks. To determine the exact vertex stimulation site, we took the point situated at half the inion-to-nasion distance and half the ear-to-ear distance.

The spatial resolution of the 40 mm coil is much better due to its smaller size and its light coating which allows the coil to be closer to the scalp and therefore to the target area. Because of these properties, we preferred this coil on V1 and V5/MT and used the 70 mm one on the vertex, allowing us to switch between the two coils. This approach allowed us to have some breaks during the testing phase to allow the 40 mm coil to cool down without increasing the overall duration of the experiment.

The coil was held on the scalp with a multiple degrees of freedom mechanical arm (Magic Arm, Manfrotto).

Because TMS pulses produce a sound and are only delivered during the first empty interval, we recorded a TMS sound and played it during the presentation of the second interval after the same 120-155ms delay. (Bueti et al., 2008 (20), Salvioni et al., 2013 (21)) Moreover to further reduce the acoustic noise of TMS, participants wore earplugs and noise protection headphones to minimize acoustic impact of TMS and recorded sounds. This technique was also used by Salvioni et al., 2013, based on psychophysical observation (Treisman et al., 1990) that showed that trains of acoustic stimuli played regularly before the presentation of a stimulus can bias the duration perception of that stimulus.

## 2.2 Functional MRI acquisition

In this part of the study, we acquired functional and structural MRI data that helped us to precisely locate and the target sites right dorsal V1 and right V5/MT for each subject.

Blood oxygen level-dependent (BOLD) fMRI and structural MRI data were acquired with a 32-channel head coil on a Siemens Trio 3T MRI scanner. Functional images were acquired using an echo-planar imaging (EPI) sequence (TR: 3.03s, echo spacing: 560 x $10^{-3}$ ms, matrix size: 96x96, ascending slice acquisition) of 2 x 2 x 2 mm resolution (0.3 mm inter-slice gap). In addition to functional images, a high-resolution T1-weighted anatomical volume was acquired for each participant (MPRAGE, 160 slices, 1 x 1 x 1 mm voxel size).

### 2.2.1 Retinotopic mapping

Sereno et al, 1995, described phase-encoded retinotopic mapping of visual areas in the human brain in a classic study used today by many teams around the world.

With the fMRI, we acquired data allowing us to draw a retinotopic map of each subject's occipital cortex.

The stimuli used were high-contrast checkerboard patterns on a grey background (contrast polarity was reversing at a frequency of 4 Hz), projected on a screen (resolution 1280 x 800) placed outside the scanner at a distance of 1 meter from subjects eyes (dimension of image: 13/11° of visual angle at 1m). Subjects could see the screen via mirrors.

We used two kinds of stimuli: a polar and an eccentricity one. The polar stimulus was a 40° sector rotating in a clockwise or anticlockwise direction, covering 8° of visual angle at a 1 meter viewing distance. The eccentricity stimulus consisted of a ring of 2° of visual angle in width expanding or contracting from the centre of fixation to a maximum of 11° of visual angle.

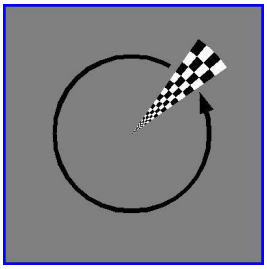

**Figure 3: Retinotopic mapping stimuli.** Rotating clockwise and anticlockwise wedge.
From http://sampendu.wordpress.com/retinotopy-tutorial/

During all acquisition duration, subjects were asked to fixate a cross at the centre of the screen without moving their eyes. To help them concentrate, subjects had to press a key on a keypad whenever they saw a grey circle (0.5° diameter) appearing randomly for 200ms within the stimuli.

Acquisition was made in 5 blocks put in a random order. One block consists in 10 consecutive repetitions of a stimulus (e.g. 10 clockwise revolutions of the rotating wedge), meaning that we had four blocks of stimuli: two polar ones (clockwise and anticlockwise rotating wedge) and two for eccentricity (expanding and contracting wedge). The fifth block was the acquisition of the structural high-definition MPRAGE image.

fMRI data acquired with this procedure were analysed on a single subject level. The first step of pre-processing was slice time corrections and realignment in SPM8 (www.fil.ion.ucl.ac.uk/spm/software/spm8). The four realigned files obtained (polar clockwise/anticlockwise and eccentricity expanding/contracting) were passed through a fast Fourier transformation in MATLAB (MathWorks) to extract phase and power at the stimulation frequency (here 10 cycles per block) for each block. By dividing the power found at the fundamental frequency of the stimulus by the average power

across all frequencies, a variable was calculated to indicate the significance of the visual response for each voxel.

We had then to eliminate the lag due to the fMRI technique itself, that is to say the lag in the BOLD response. To do so we averaged the phase maps obtained with clockwise/expanding with respectively anti-clockwise/contracting.

The next step consisted in creating a surface to display these averaged maps. In order to do this we used a standard pipeline of the Freesurfer 11 software (surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferWiki). Freesurfer enabled us to construct an inflated surface of the grey-white matter boundaries and to flatten it.

We so obtained two retinotopic maps: one for the polar stimuli and one for the eccentricity ones.

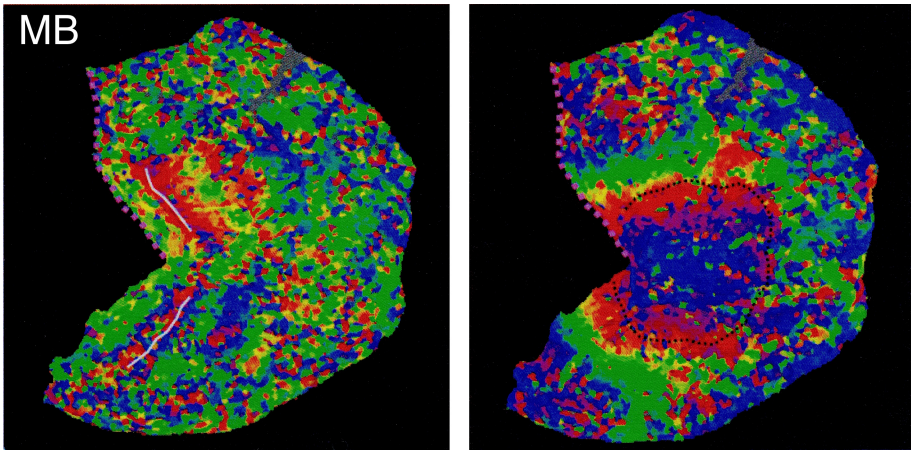


**Figure 2 : Retinotopic map.** Polar (left) and eccentricity (right) retinotopic maps. V1 borders are represented with the white lines on the polar map and the dashed black line represents the iso-eccentric line at 8° of visual angle from the fovea. Pink dots represent the calcarine sulcus.
From Kenel-Pierre T, Time encoding in the visual cortex, School of Biology, University of Lausanne

We determined stereotaxic coordinates of V1 for each subject by overlapping the two maps: we were looking for the point that corresponded to the lower left visual quadrant (using the polar map) at an eccentricity of 8° of visual angle from the fovea (using eccentricity map). This point was chosen according to the stimuli we used during the temporal discrimination task.

Stereotaxic Talairach coordinates of right V5/MT were taken from Dumoulin et al., 2000 (28): x=44, y=67, z=0.

Codes used during this procedure (stimuli, fast Fourier transform, phase average) and tutorials for retinotopic mapping are free and public on Sam Schwarzkopf's website (www.sampendu.wordpress.com)

## 2.3 Co-registration

In this part of the study, we used a neuronavigation software (BrainVoyager, Brain Innovation, Maastricht, www.brainvoyager.com) coupled with a stereotaxic co-registration system (using an ultrasound camera and ultrasound transmitters on subjects heads) to get a correspondence between the coordinates obtained using fMRI data and real world coordinates. This was a necessary step to find the exact TMS stimulation points on volunteers' scalp.

The first step was to represent our target areas in a computed representation of the real world: the goal was to find as precisely as possible where TMS stimulation had to be delivered on the scalp to target V1 and V5/MT.

We determined for each subject a Volume of Interest (VOI) that represented a sphere of 5 mm of diameter around the stereotaxic coordinates of V1 and V5/MT found in part I. Then we created two meshes: one surface mesh that represents the scalp of the subject, and one brain mesh that corresponds to the white/grey matter boundary of the right hemisphere. We converted the VOIs into patches of interest (POIs) that represented the patches of surface mesh that were within a 2 mm marge from the VOI.

We now had one brain mesh that showed V1 and V5/MT and one surface mesh that gave us visible landmarks of the subjects' heads. Using a co-registration toolbox of BrainVoyager and an ultrasound camera (Zebris CMS20S-TMS, Zebris Medical GmBH), we could match POIs to reality. To do so, we first set head mesh fiducials, which are points marked on the head mesh that corresponds to easy-to-find anatomical landmarks on the subjects heads. We co-registered the head-mesh to reality by placing three ultrasound emitters on subjects' heads and pointing with a stereotaxic ultrasound pen the head fiducials. BrainVoyager now correlates position of the three emitters to the position of the head fiducials, and shows the pen at its exact position over the head mesh.

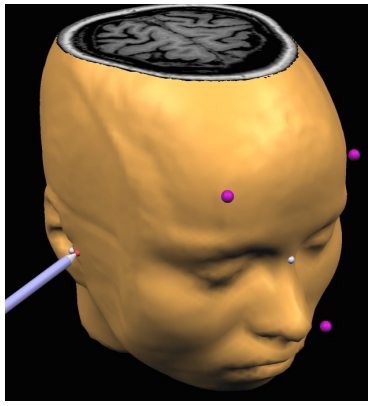

**Figure 5 : Coregistration in BrainVoyager.** In white are the head fiducials, in pink the three ultrasound emitters. Once these coordinates are co-registered, pen can point any place on the head mesh, making a correspondence between mesh and reality

From http://support.brainvoyager.com/tms/76-co-register-head/208-users-guide-head-fiducial-digitization.html, 12.01.2015

We displayed the head mesh as a grid so we could see the brain mesh below it, and by pointing POIs with the pen we were able to precisely mark on subjects' heads the correct position of right dorsal V1 (we chose the portion of V1 that codes visual information coming from the lower left quadrant according to the stimuli used during the experiment) and right V5/MT.

## 2.4 Expected results

If V1 encodes time in a retinotopic reference frame we expect to find worse temporal discrimination performance after TMS on V1 only for position 1 and 2 because retinotopic representation of the stimuli is represented in the lower left quadrant for these two positions.

Oppositely, if there is a spatiotopic frame of reference of time encoding in V5/MT, we expect to find worse scores on thresholds on positions 2 and 3 because stimuli have the same spatiotopic position, which is head-centred.

We also have different control mechanisms. First the vertex position allows us to have a baseline threshold, which corresponds to a subject's temporal discrimination performance. Second (and more important), is the position 4 stimulus. Using a totally different spatiotopic and retinotopic position that did not correspond to the portion of V1 stimulated by TMS gave us a second solid control.

## RESULTS

### 3.1 Statistical analysis

To check for differences in discrimination thresholds obtained across the four different spatial conditions, we entered individual discrimination threshold (Weber fractions) values into a site (V1, V5/MT, Vertex) by position (1-4) ANOVA. We explored effects of position, site and the interaction between both factors. In order to further investigate the differences between each stimulation site and each position, paired as well as one sample t-tests were carried out as post-hoc tests, the alpha level was set to 0.05.

### 3.2 Results

The ANOVA site x positions revealed only a marginally significant effect of stimulation site ($F_{(2,20)}$=2.08 p=0.15). We found that independently of spatial positions, discrimination thresholds were higher (i.e., worse performance) after TMS of V5/MT compared to the Vertex stimulation (p=0.058). Concerning the effects of spatial positions (site x positions interaction, $F_{(6,60)}$=1.15 p=0.35), we found that compared to vertex stimulation, discrimination thresholds were worse:

1) after V1 TMS in position 2 : retinotopic position A / spatiotopic position B, vertex position 2 *vs..* V1 position 2 (p=0.07) (i.e., for visual stimuli displayed in the lower left quadrant)

2) after V5/MT TMS in position 4 : retinotopic position C / spatiotopic position C, vertex position 4 *vs.* V5/MT position 4, (p=0.11) (i.e., for visual stimuli presented in the upper left quadrant)

The V1 and V5/MT effects for, respectively, position 2 and 4 were also confirmed by one-sample t-tests performed on normalized data (V1 position 2 $t_{(10)}$=2.11 p=0.06, V5/MT position 4 $t_{(10)}$=2.30 p=0.04).

Consistent with these results we found a significant difference between V1 and V5/MT stimulation for discrimination thresholds obtained at position 4 (paired t-test t10= p=0.05). No difference was observed between the two areas for all the other spatial positions. We did not observe any difference between discrimination thresholds obtained at the 4 different spatial positions independently of space (main effect of position: $F_{(3,30)}$=1.22 p=0.32).

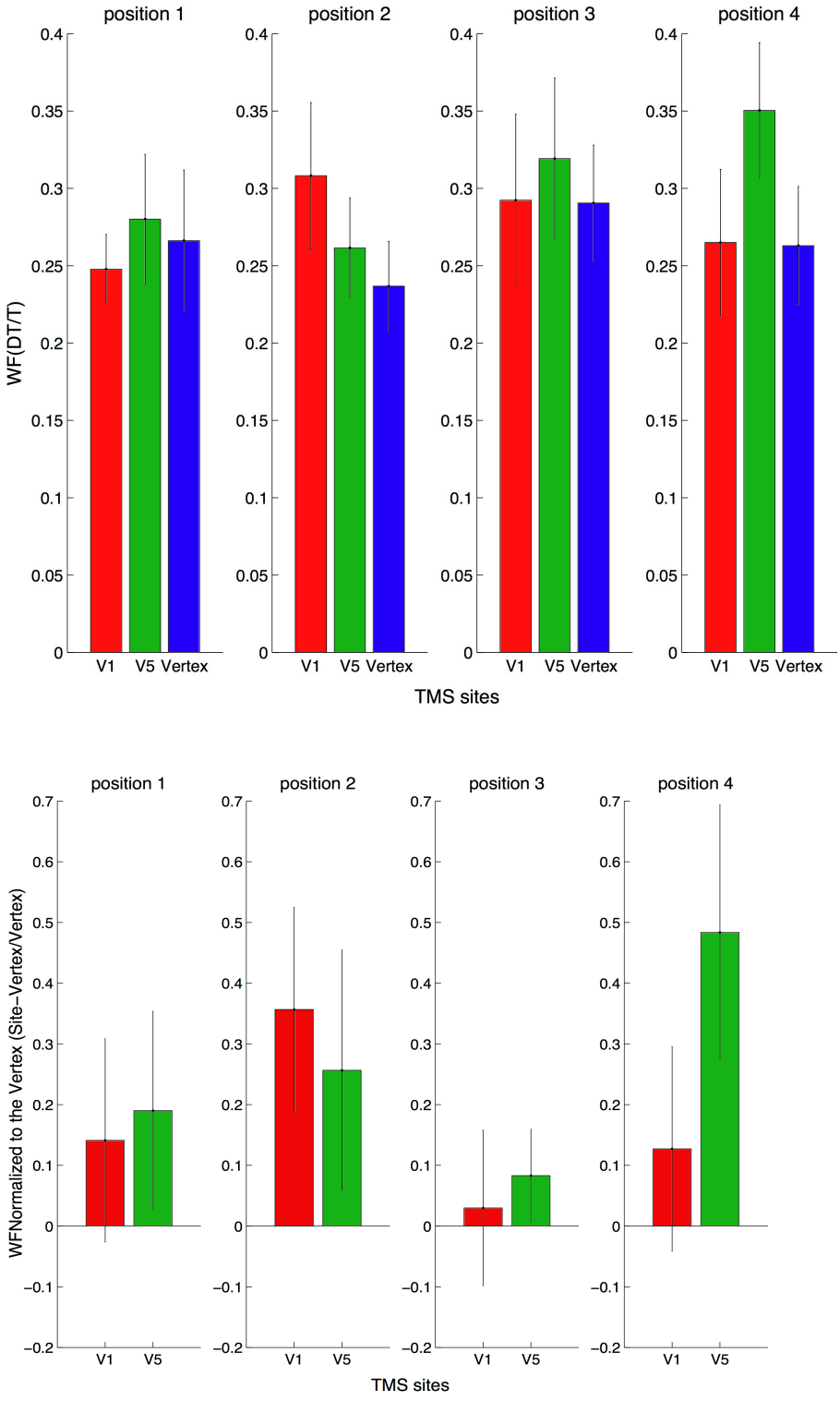


**Figure 3 : Temporal discrimination normalized results.**

1) Individual discrimination thresholds (Weber fractions) after paired-pulse TMS over V1, V5 and the vertex

2) Individual discrimination thresholds (Weber fractions) after paired-pulse TMS over V1 and V5. For each position, results were normalized to non-TMS condition (i.e. vertex, averaged across positions) as fallows: site-vertex/vertex.

## DISCUSSION

With this experiment we aimed to investigate the spatial organization of neural networks implicated in the encoding of temporal intervals in V1 and V5/MT. In positions 1 and 2, the visual stimuli shared the same retinotopic coordinates whereas in positions 2 and 3 they were displayed at the same spatiotopic coordinates. Positions 3 and 4 served as controls for V1 TMS stimulations whereas positions 1 and 4 served as controls for V5/MT.

Although the data presented are still preliminary, we found that the spatial positions of the visual stimuli affected the capacity of discriminating their temporal properties. In particular we found that both V1 and V5/MT were sensitive to the visual quadrant in which the stimuli were displayed. The right dorsal V1 seems to be involved in the temporal discrimination of stimuli presented in the lower left visual quadrant (retinotopic position A, position 2), whereas the right V5/MT seems to be engaged in the temporal discrimination of stimuli presented in the upper left visual quadrant. These effects seem to be quadrant specific because in case of V1 were present only for the lower left visual quadrant (position 2) and absent for the upper left quadrant (position 4) and in case of right V5/MT were present for the upper left (position 4) but not for the lower left (positions 1 and 2) visual quadrant. Importantly both the right dorsal V1 and the right V5/MT were not affected by TMS stimulation when the stimuli were in the right visual hemifield (position 3, ipsilateral to the side of the stimulated visual field).

Concerning the V1 effect we were puzzled by the absence of effect for the position 1 which was retinotopically identical to position 2 (i.e., retinotopic position A). The only difference between position 1 and 2 was the gaze direction of the participants. In position 1 volunteer stared at a fixation asterisk that was aligned with the body mid-line whereas the stimulus was presented 8° leftwards. In position two the gaze was shifted to the right compared to the volunteers midline and the stimulus was presented centrally. It seems then that, at least from these preliminary results, temporal discrimination in V1 engages neural populations encoding specific portions of the space (a visual quadrant) and that are sensitive to the position of the eye in orbit. Contrary to our prediction and to previous literature, time encoding in V1 seems to be not strictly retinotopic. However our experimental design allowed us to rule out the possibility that in V1 time is encoded in spatiotopic coordinates. V1 effect was indeed observed only for position 2 (retinotopic position A/spatiotopic position B) and not for

position 3 (retinotopic position B/spatiotopic position B) which shared the same spatiotopic coordinates of position 2.

Concerning V5/MT effect we were not able to find a modulation of temporal discrimination thresholds linked to the spatiotopic position B. Similarly to the V1 results, the effect observed after V5/MT TMS was quadrant specific. However differently from V1, here we could not determine whether this effect was retinotopic or spatiotopic, because for position 4 (i.e., upper left quadrant) we did not have a control condition with identical spatiotopic coordinates but different retinotopic coordinates. Moreover we should point out here that with respect to V5/MT our methodology has a strong limitation; the V5/MT target in our group of subjects was chosen based on averaged stereotaxic coordinates. This did not allowed us to know which portion i.e., retinotopic or spatiotopic of V5/MT we were targeting. For this reason it was impossible to predict which portion of the visual space we could potentially target with TMS.

There is another consideration to make on our present results. Data so far have been very noisy i.e., highly variable. This high variability was probably due to the very challenging method of dorsal V1 localization and stimulation. It is worth noting here that we were trying to stimulate a small portion of V1, an area not easily reachable with TMS because it lies on the medial surface of primary visual cortex. Moreover, differently from previous studies (Salvioni et al. 2013 (21), Bueti et al. 2008 (20)), the temporal stimuli here were not presented within the fovea but 8° far from the fixation. These differences in stimulus presentation and TMS stimulation might be the cause of this high variability and these, so far, unclear results.

For all these reasons we think that it is difficult to draw strong conclusions from these preliminary results. Additional data collection is therefore warranted.

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