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Time processing in visual cortices:

How the visual brain encodes and keeps track of time

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Tout commence par une interruption.

Paul Valéry

Abstract

Time is embedded in any sensory experience: the movements of a dance, the rhythm of a piece of music, the words of a speaker are all examples of temporally structured sensory events. In humans, if and how visual cortices perform temporal processing remains unclear. Here we show that both primary visual cortex (V1) and extrastriate area V5/MT are causally involved in encoding and keeping time in memory and that this involvement is independent from low-level visual processing. Most importantly we demonstrate that V1 and V5/MT are functionally linked and temporally synchronized during time encoding whereas they are functionally independent and operate serially (V1 followed by V5/MT) while maintaining temporal information in working memory. These data challenge the traditional view of V1 and V5/MT as visuo-spatial features detectors and highlight the functional contribution and the temporal dynamics of these brain regions in the processing of time in millisecond range.

The present project resulted in the paper entitled: '**How the visual brain encodes and keeps track of time**' by Paolo Salvioni, Lysiann Kalmbach, Micah Murray and Domenica Bueti that is now submitted for publication to the Journal of Neuroscience.

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I. Introduction

1. The sensory brain

Over the past decades, there has been a growing interest in understanding the different sensory modalities existing in the human brain. Indeed it is crucial for a human being to be able to cope with its environment. In order to properly interact with its surrounding environment, the human brain needs to acquire as much information as possible, and to do so it possesses different sensory modalities: somatosensory (touch, temperature, proprioception, and nociception), visual, auditory, vestibular and chemical (including taste and smell). All of these systems are functioning in a very similar way: a receptor converts the stimuli in a signal that is then transduced to the brain via different afferent pathways. In the brain the signal is then processed by specialized cortices that interpret the qualitative and quantitative properties of the stimulus (1). Time is a special feature of our sensory world, because unlike other features like color or pitch, it does not have a dedicated receptor system. Nevertheless temporal information is embedded in many aspects of our sensory experience; sensory events unfold in time and often acquire a particular meaning because of their specific temporal structure. The speed of a moving object, the words pronounced by a speaker and the tactile exploration of a texture, are all examples of temporally structured sensory inputs. Despite the ubiquitousness of the temporal dimension in our environment, our understanding of the functional and neural mechanisms underlying the temporal representation of sensory events remains controversial.

2. Time

Time is a central and ubiquitous player in the human environment. From controlling our movements to talking, playing sports, hearing music or dancing, a correct processing of time information is crucial. In that sense, humans possess a variety of systems to perceive time on different scales, ranging from the circadian rhythms to very precise millisecond timing (2–4).

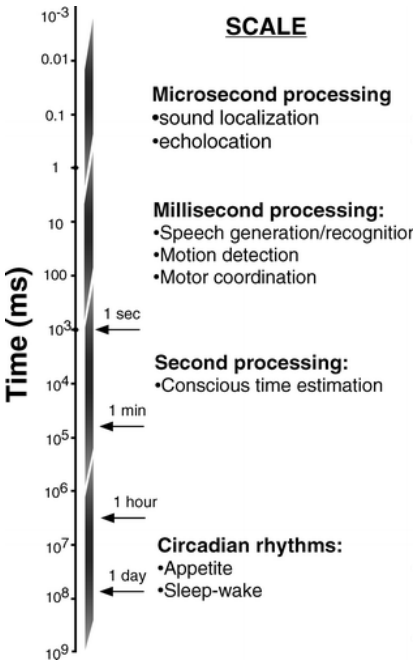

Fig. 1: Different timescales of temporal information and associated processes, adapted from (3)

Differently from circadian rhythms that are clearly under the control of the suprachiasmatic nucleus (SCN) in the hypothalamus, the neural basis of time in seconds/milliseconds range is still unclear. Two main models have been proposed to describe how the brain is able to represent temporal information: the pacemaker-accumulator model and a 'distributed' timing model. The idea that we time sensory signals via a single 'centralized' and 'amodal' clock dominated the field of temporal cognition over the last 30 years (5,6).In the central clock model time is represented in the integrated activity of a pacemaker and an oscillator. The pacemaker oscillates at a constant frequency and the beats are counted by the accumulator. The duration perceived is the result of the number of beats accumulated (2,4,7–9). As opposed to the internal clock model, there are the distributed' timing models. This is a broad class of models, which -although different regarding the mechanisms proposed for time processing- all together share the idea that we have multiple timing mechanisms 'distributed' across brain areas or circuits; and that the involvement of each single mechanism depends on the task, sensory modality and lengths of temporal intervals (2,4,10,11). Empirical findings up to now have failed to fully support these models. From the neurophysiological point of view, neuropsychological, electrophysiological, neuroimaging and magnetic stimulation studies have shown that many cortical (visual, auditory parietal, premotor and prefrontal cortices) and subcortical (basal ganglia and cerebellum) brain regions are involved in the processing of temporal information. However, the functional contribution of these different regions, as well as their interactions, is still subject of considerable debate (12–15)

3. The visual system

The primary visual cortex or striate cortex (V1), located around the calcarine sulcus in the occipital lobe, is the first cortical structure in the visual pathway. After leaving the rods and cones of the retina via the ganglion cells -whose axons form the optic nerve- visual information is first transmitted via the optic chiasm and the optic tract to the lateral geniculate nucleus (LGN). From there, the optic radiations, on both sides of the brain, carry visual information to V1 which is in turn connected to a vast network of secondary visual areas (V2, V3, V4 and V5/MT), collectively called extrastriate visual cortices.

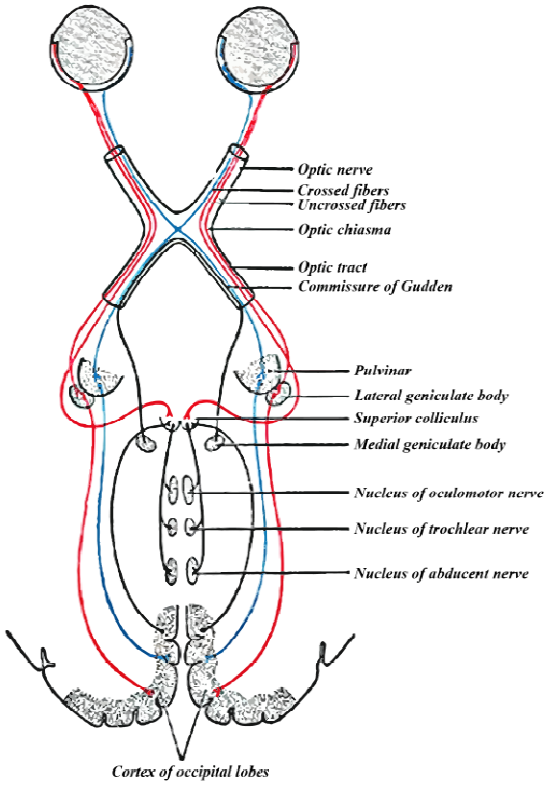

Fig. 2: The early visual system, adapted from (16)

From that point, the complexity of neural representations increases and neurons not only respond to simple stimulus features like orientation or directional motion but also to more complex signals like human faces. To explain the levels of specialization of the associative visual cortices, the Two Streams hypothesis was developed by Ungerleider and Mishkin in 1982. This hypothesis divides visual information arising from V1 into two distinct pathways: a dorsal “where” pathway involved in spatial information processing (that includes the V5/MT region and communicates with motor regions) and a ventral “what” pathway specialized in identifying and characterizing visual stimuli (1,17).

4. Visual timing

According to ‘distributed’ timing models, sensory-specific cortices should play a role in temporal computations since temporal mechanisms are hypothesized to be ‘modality specific’ (10,18). This idea is supported by many studies showing, for example, that the ability to discriminate a sensory event can be distorted by modality-specific characteristics of the stimuli such as temporal and spatial frequency (19) and that transcranial magnetic stimulation (TMS) over auditory areas impairs time estimation without affecting pitch perception (20). In a similar study, disruption of the auditory cortex was shown to deteriorate auditory and visual time perception whereas TMS on the primary visual cortex affected only visual temporal judgments (21). In addition the role of primary visual cortex and extrastriate visual regions in temporal computations has been shown by neurophysiological (22–24) and neuroimaging studies (21,25).

Taken together, these findings support the existence of modality-specific temporal mechanisms. However, the functional organization of these mechanisms in sensory specific areas in general and in visual cortices (striate and extrastriate) in particular, remains unclear. Using TMS in healthy volunteers, we explored the differential contributions of V1 and V5/MT to the encoding and the working memory of visual durations.

5. Clinical relevance

Living in the XXIst century makes it easy to understand how important and precious time is for our everyday life. In order to cope with the numerous inputs we receive, it is crucial to estimate time correctly, not only to process incoming sensory information but also to control our motor behavior. A large number of studies have suggested strong links between brain lesions and impaired time perception (13). A classical example is patients with cerebellar lesions that are characterized by the impossibility to synchronize muscular activity. These patients, for example, have problems in throwing a ball because of the increased variability in the timing of the opening of the hand with the

arm rotation (26,27). These patients were also shown to have an increased variability in rhythmic tapping and in the production of isolated movements of a given duration (28). Lesion studies also highlighted the role of basal ganglia in temporal processing: Parkinson patients were shown to have time perception deficits (29,30), although some controversies persist (31). The role of the basal ganglia in temporal computation has also been suggested by dopaminergic manipulations in healthy individuals. Haloperidol, a dopaminergic receptor antagonist, was shown to alter brief temporal perception while other neurotransmitter modulators like scopolamine and midazolam didn't (32).

As discussed above, most of the lesion studies focused mainly on the cerebellum and basal ganglia. However, there are a few studies showing time perception deficits also in patients with right hemispheric lesions (33,34). These patients have problems not only in estimating time but also in processing information over time (35) and in judging the temporal order of events (36). They often have lesions centered on the parietal cortex and suffer from spatial attentional deficits. However, none of these works has clearly shown that an ischemic lesion in the extrastriate cortices affects time perception or has determined precisely how spatial and temporal deficits interact in these patients.

II. Materials and Methods

Many different methods can be used in cognitive neurosciences, ranging from fMRI (functional magnetic resonance imagery) to simple psychophysical tests. In this research we decided to use TMS (transcranial magnetic stimulation) because of its high spatio-temporal resolution and its ability to create virtual lesions (37). We took advantage of these features to clarify the contributions of V1 and V5/MT in visual time discrimination.

During TMS stimulation, a coil is held over a subject's head and produces an electric current in the subject's brain through electro-magnetic induction. This current in turn depolarizes neurons and generates various physiological and behavioral effects depending on the target area and on the TMS parameters (i.e. intensity and frequency of stimulation (37,38)).

The spatial resolution of a TMS stimulus, depending whether it is a rTMS (repetitive-pulse TMS) or a single-pulse TMS, has been shown to be between 5 to 22 mm, depending on the measurement method used (PET, fMRI or EMG) (37). It is therefore very focal. In this respect it is worth emphasizing here that the intensity of the induced electric field has an exponential decrease from coil to the periphery. Furthermore the timing is also very precise, a TMS pulse has a rise time of approximately 200µs and a duration of 1ms (37).

In clinics, TMS has been used to explore the excitability of the corticospinal tract and more precisely for diagnostic purposes. Used in combination with electromyography (EMG), low intensity TMS stimulation of the motor cortex evokes a controlateral motor evoked potential (MEP), thought to originate from transsynaptic excitation of corticospinal tract neurons. The measure of MEP is therefore very useful for the differential diagnosis and grading of some diseases like multiple sclerosis, compressive myelopathies, atrophic lateral sclerosis (ALS), Parkinson disease and many others (39,40). As a therapeutic tool, TMS has been used first in depression and more specifically in drug resistant major depression (41,42). It has also been tried as therapy for post-traumatic stress disorder, auditory hallucinations, chronic pain, aphasia and many other neuropsychiatric disorders. The efficacy of TMS as therapeutic tool remains controversial (40).

On the other hand, TMS has also been widely used in research. The first use of TMS has been in the motor system where applying the coil on the primary motor cortex increased massively reaction time after an auditory “go” signal (43). This study opened up a very broad literature of motor studies involving TMS. Another classical stimulation site is the visual cortex, where TMS stimulation produces phosphenes (i.e. brief flashes of light). According to the area stimulated the phosphenes

can be perceived as moving, if the site of stimulation is V5, or static if V1 is stimulated (37). Speech production has also been disrupted with repetitive TMS (rTMS) applied over the frontal lobe (37).

Further elements regarding the Methodology (subjects, stimuli, procedures and data analysis) can be found in the article presented.

III. Results

This chapter is developed in the following article:

How the visual brain encodes and keeps track of time

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Abstract

Time is embedded in any sensory experience: the movements of a dance, the rhythm of a piece of music, the words of a speaker are all examples of temporally structured sensory events. In humans, if and how visual cortices perform temporal processing remains unclear. Here we show that both primary visual cortex (V1) and extrastriate area V5/MT are causally involved in encoding and keeping time in memory and that this involvement is independent from low-level visual processing. Most importantly we demonstrate that V1 and V5/MT are functionally linked and temporally synchronized during time encoding whereas they are functionally independent and operate serially (V1 followed by V5/MT) while maintaining temporal information in working memory. These data challenge the traditional view of V1 and V5/MT as visuo-spatial features detectors and highlight the functional contribution and the temporal dynamics of these brain regions in the processing of time in millisecond range.

Introduction

Time in millisecond range is a key feature of any sensory experience. Sensory events unfold in time and the way we perceive this temporal unfolding is often crucial for our understanding of these events. For example, the movement of a hand approaching a face can be seen as a slap or a caress depending on the fast or slow rate, i.e. the tempo, of the movement. Despite the importance of the temporal dimension for our perceptions, if and how, time in millisecond range is processed by our sensory systems is controversial. The controversy relates to both the actual engagement of sensory-specific cortical regions in the processing of temporal information and the underlying neurophysiological mechanisms. The contribution of visual cortices to temporal computations has been recently suggested by psychophysical observations (Morrone et al., 2005; Kanai et al., 2006; Heron et al., 2012) showing for example that the perceived duration of a visual stimulus can be distorted by modality-specific features of the stimulus like visual motion and/or temporal frequency, and that some of these temporal biases occur for specific retinotopic and spatiotopic position (Burr et al., 2007; Ayhan et al., 2009). Empirical support to the role played by visual cortices in temporal computations comes from both electrophysiological data in animals and neuroimaging findings in humans. Electrophysiological studies have found that the firing rate of V1 neurons in rats and V4 neurons in monkeys is modulated by the time of the expected reward (Ghose and Maunsell, 2002; Shuler and Bear, 2006). Whereas neuroimaging works have shown activations in both V1 and extrastriate visual regions correlating with the temporal expectation of a given visual event (i.e. a stimulus changing in colour) and with the representation of a learned temporal interval (Bueti et al., 2010; Bueti et al., 2012). The correlational nature of both electrophysiological and neuroimaging measurements does not allow though to infer any causal contribution of these visual regions to temporal computations. The very few existing magnetic stimulation studies on the subject highlight the importance of area V5/MT in temporal judgments of visual moving and static stimuli, but leave open the issues of the causal involvement of primary visual cortex in temporal computations and that of the independence of this contribution from low level visual processing (Bosco et al., 2008; Bueti et al., 2008; Kanai et al., 2011). But most importantly, to date, none of the lines of evidence described above has determined precisely to which temporal computations V1 and V5/MT are contributing (i.e. duration encoding versus duration short-memory), the temporal dynamics of these computations within each region and the extent of functional interplay between them.

Across four paired-pulse transcranial magnetic stimulation (TMS) experiments we sought to address these unresolved issues. Specifically we asked: first, whether and *when* V1 and V5/MT play a *causal* role in discriminating temporal intervals independent of the processing of low-level visual features of the interval. Second, the extent to which V1 and V5/MT contribute to the encoding of temporal intervals and/or their maintenance in working memory, and third, the functional interplay vs. independence of V1 and V5/MT in these temporal computations.

Materials and Methods

Subjects

Participants in Experiments 1-4 respectively included 14,14,10 and 14 healthy right-handed adults (9, 10, 9 and 8 females; mean age was 25.1, 26.2, 26.5, and 26.1 yrs, range was 28-22, 38-22, 38-21, 38-22 yrs for Experiment 1, 2, 3 and 4, respectively) with normal or corrected-to-normal vision. All gave written informed consent to participate in this study, which was approved by the Ethics Committee of the Faculty of Biology and Medicine at the University Hospital Center and University of Lausanne. Among the tested participants, 5 participated in Experiments 1 and 3 and 6 participated in Experiments 2 and 4.

Stimuli and Procedure

Experiments 1-2 involved a temporal discrimination task of empty intervals, each marked by two brief (16.7 ms each) light blue disks (0.78 deg of diameter) presented at the center of the screen (resolution was 1024 x 768 pixels and refresh rate was 60 Hz). A black asterisk (0.39 deg of visual angle) - presented 0.78 deg above the center of the screen - served as the fixation point and was continuously displayed for the entire duration of the trial. Each trial consisted of the sequential presentation of the two temporal intervals separated by a brief gap (i.e. a random value taken from a uniform distribution ranging from 900 ms to 1200 ms); one of the two intervals was the “standard duration” and the other the “comparison duration”. The duration of the standard interval (T) was fixed (200 ms). The duration of the comparison interval was the standard plus a variable, always positive, ΔT value (i.e. comparison duration=T+ΔT). The presentation order of the standard and the comparison intervals was randomized and counterbalanced across trials. In half of the trials the standard was presented first, in the other half it was presented second. The volunteers performed an interval-discrimination task that consisted in judging which one of the two intervals had lasted longer (first or second). Subjects responded by pressing one of two keys on the keyboard (see Figure1A for a schematic representation of a trial sequence). Visual feedback was provided at the end of each trial: the fixation asterisk turned green or red signaling whether the response was correct or incorrect. The duration of the feedback was 1 sec, whereas the duration of the inter-trial interval was a random value taken from a uniform distribution ranging from 1.8 to 2.5 seconds. The duration of the comparison interval (T+ΔT) was adjusted adaptively across trials, in order to obtain the ΔT threshold leading to 79% correct discrimination. For this, the duration of the comparison interval was adjusted by decreasing the ΔT after every three consecutive correct responses and increasing the ΔT after each incorrect response. The ΔT was changed is steps of 32 ms until the third reversal and 16 ms thereafter. The ΔT values at which the direction of the change was reversed (decreasing to increasing or vice-versa) were noted. The first three reversals of each block of trials were discarded, and the 79% correct point on the psychometric function was estimated by taking the average value of the remaining reversals (Levitt, 1971; Bueti et al., 2012). To ensure reliability, no estimate was retained if there were fewer than four reversals. The final threshold was

expressed as a Weber fraction, i.e. the ΔT needed to achieve 79% correct discrimination divided by T. In Experiments 3 and 4 we used the same task structure as Experiments 1 and 2; the only differences are that we kept the interval length constant (i.e. 200 ms), we changed the brightness of one of the four disks, and asked participants to decide which pair of disks was on average brighter. The disk that changed in brightness could be in either the first or the second pair of flashes and be either the first or the second flash within each pair. The position of the changed disk was randomized and counterbalanced across trials. In order to obtain individual discrimination thresholds leading to 79% correct discrimination, we used the same adaptive procedure (i.e. rule 'three up one down') used in Experiments 1-2. The brightness was changed decreasing the original luminance value (358 cd/m^2) by 5% until the third reversal and 1% thereafter. The luminance of the monitor background was (182 cd/m^2).

In each experimental session of all the four experiments, participants performed a minimum of 12 blocks (60 trials each) of the visual task. Of the 12 blocks, eight were with TMS (i.e. 2 sites x 3 delays plus 2 blocks of vertex stimulation), the remaining were without TMS. The no TMS blocks were used to obtain stable and reliable individual discrimination thresholds before applying TMS. Each participant performed at least 4 blocks (range 4-8) of the task without TMS plus 20 initial practice trials to familiarize with the procedure. The task was performed in an acoustically isolated and dark room, sitting 45 cm from the computer's monitor.

Transcranial Magnetic Stimulation

In all experiments TMS was delivered by a Magstim Rapid^2 Stimulator and by a 70-mm figure-of-eight coil. We used paired-pulse TMS to take advantage of the summation properties of TMS pulses — double-pulse TMS gives larger effects than single-pulse, but still provides a reasonable temporal resolution defined by the temporal distance between the two pulses (Walsh and Pascual Leone, 2003; Silvanto et al., 2005). In different blocks of trials, paired-pulse TMS was applied over V1 and right V5/MT at three different delays (50-85 ms, 85-120 ms, 120-155 ms) from the offset of the first flash (i.e. beginning of the first interval) in Experiments 1 and 3 and from the offset of the second flash (i.e. end of the first interval and beginning of the retention period) in Experiments 2 and 4. Each experimental session had a total of 6 TMS blocks i.e. 2 sites by 3 different delays plus two additional blocks of vertex stimulation used as control site for nonspecific effects of TMS such as acoustic and somatosensory artifacts. The vertex blocks were always the first and the last TMS block, whereas the order of V1 and V5/MT blocks were randomized across subjects. During vertex stimulation the TMS pulses were delivered randomly at the three different delays from either interval onset (Experiments 1 and 3) and from interval offset (Experiments 2 and 4). To prevent any temporal bias that might be induced by the acoustic artifacts of TMS (Treisman et al., 1990), we recorded the sound of a TMS pulse and played it twice via loudspeakers at either the onset (Experiments 1 and 3) or the offset (Experiments 2 and 4) of the second interval. The timings at which this 'fake' paired-pulse TMS was played was always congruent with the timing of

the real pulses (i.e. 50-85 ms, 85-120 ms, 120-155 ms). Finally, in order to minimize the acoustic impact of both real and 'fake' TMS, participants wore both earplugs and headphones.

The TMS stimulus intensity was arbitrarily set to 55% of the maximum stimulator output; this intensity when applied to V1 and V5/MT did not produce phosphenes that obscured the stimuli. The coil handle was oriented upward for V1 stimulation and leftwards for V5/MT stimulation. Both V1 and V5/MT were localized using a functional method that consisted in eliciting phosphenes from the site (for a discussion of this method, see Walsh and Pascual-Leone, 2003). For V1, the starting point of stimulation was 2 cm dorsal from the inion and for V5/MT it was 2 cm dorsal and 4 cm lateral from the inion. The coil was then moved slightly to find a region from which the clearest static (V1) or moving (V5/MT) phosphenes could be obtained. This location was an average 2.0 cm dorsal and 0.5 cm lateral from the inion for V1 and 3 cm dorsal and 5 cm lateral to the inion for V5/MT. Initially, the intensity of stimulation was 65% of the stimulator output, and it was increased if participants failed to perceive any phosphene.

Data Analysis

For each experiment the individual discrimination thresholds (i.e. Weber fractions: $\Delta T/T$) obtained in each V1 and V5/MT TMS block were used to calculate an index of change with respect to the vertex stimulation (i.e. the average of the two vertex blocks) as follows: site-vertex/vertex. The resulting values were then entered into a site (V1, V5/MT) by delay (50-85 ms, 85-120 ms, 120-155 ms) repeated measures ANOVA. As post-hoc tests we used one-sample t-test (two-tailed) to control for differences against the vertex stimulation and paired t-test (two-tailed) to control for possible differences between the three delays. The alpha value was set to 0.05 and the Bonferroni correction for multiple comparisons was applied (3 comparisons leads to a p-corrected <0.016).

Results

In Experiments 1 and 2 healthy participants performed a temporal discrimination task. Experiment 1 (N=14) investigated the functional role and the temporal interplay between V1 and V5/MT during the encoding of temporal intervals by applying paired-pulse TMS (pulses separated by 35 ms) on different blocks of trials over V1 and V5/MT at three different delays (50, 85 and 120 ms) from the *onset* of the first interval (i.e. offset of the first flash). Compared to vertex stimulation and to the earliest stimulation delay (50 ms), discrimination thresholds were significantly higher following TMS of both V1 and V5/MT at delays of 85 and 120 ms (main effect of timing: $F_{2,18}=18.39$ $p<0.001$; Figure 1B-C). These changes in discrimination threshold following stimulation of V1 and V5/MT were positively correlated across subjects (see Figure 1D leftmost panel; $r=0.36$, $p=0.10$ and $r=0.68$, $p=0.004$ for the 85 and 120 ms delays, respectively) indicating a possible functional coupling between these two areas.

While Experiment 1 showed that V1 and V5/MT are both causally involved in the accurate encoding of temporal intervals in a functionally-coupled and temporally-synchronized manner, they do not clarify whether these areas also play a role in the retention period, when the first interval has just been presented and needs to be stored in memory before the presentation of the second interval. To address this issue we asked a different group of healthy participants (N=14) to perform the same discrimination task, with the sole difference being that paired-pulse TMS was applied at three different delays from the *offset* of the first interval (i.e. offset of the second flash). Compared to vertex stimulation and to different stimulation delays we observed higher discrimination thresholds following TMS over V1 at the 50 ms stimulation delay and following TMS over V5/MT at the 85 ms stimulation delay (site × timing interaction: $F_{2,26}$=6.75, p=0.004). In contrast to the results during the encoding phase, there was no evidence of correlated effects across subjects (r=0.18 p=0.27, Figure1D), suggesting functional independence of these two areas during working memory maintenance of temporal information.

To ensure that the observed effects during time encoding and working memory maintenance were not caused by interference with either low-level visual processing or with unspecific task requirements, we conducted two additional experiments. Using the same task structure as in Experiments 1 and 2 but this time changing adaptively the brightness of one of the four visual markers, we asked participants to decide which pair of disks was on average brighter. Paired-pulse TMS was applied at either the encoding (Experiment 3; N=10) or the working memory (Experiment 4; N=14) stage of the task. In both experiments we found that TMS was ineffective in disrupting brightness discrimination threshold at any site or stimulation delay (see Figure 1B-C rightmost panels, Experiment 3: main effect of site $F_{1,9}$=0.57, p=0.47; main effect of delay $F_{2,18}$=0.40, p=0.68; interaction site by delay $F_{2,18}$=0.26, p=0.77; Experiment 4: main effect of site $F_{1,13}$=2.05, p=0.17; main effect of delay $F_{2,26}$=1.06, p=0.36; interaction site by delay $F_{2,26}$=0.59, p=0.56). These results indicate that the effects observed in Experiments 1 and 2 were not simply due to an interference with low-level visual processing. Instead and in general, the role played by both V1 and V5/MT during encoding and working memory appears genuinely linked to temporal processing and not to aspects of the task common to any discrimination processes.

Discussion

The results of the four experiments show that primary visual cortex and extrastriate area V5/MT are causally involved in encoding and keeping time in memory and that this involvement is independent from low-level visual processing. Moreover we found that V1 and V5/MT are functionally linked and temporally synchronized during time encoding whereas they are functionally independent and operate serially (V1 followed by V5/MT) while maintaining temporal information in working memory.

Compared to previous neuroimaging and electrophysiological works (Ghose and Maunsell, 2002; Shuler and Bear, 2006; Bueti et al., 2010), here we were able to show the *causal* contribution of both V1 and V5/MT to different aspects of temporal processing i.e. temporal encoding and working memory maintenance. The contribution of distinct brain regions to these two different functional stages of temporal computation has not been studied before. The engagement of V1 and V5/MT in duration *encoding* has been suggested by previous electrophysiological and neuroimaging data (Ghose and Maunsell, 2002; Shuler and Bear, 2006; Bueti et al., 2010). However none of these previous works has clarified that V1 and V5/MT play a causal role in temporal processing, that this role is at the encoding stage and it is independent of low level visual processing. Only two previous TMS studies have shown a causal relationship between V5/MT activity and temporal discrimination judgments (Bosco et al., 2008; Bueti et al., 2008). However, the first of these studies fails to clarify the computational stage at which V5/MT is engaged during duration discrimination (Bueti et al., 2008); whereas the second does not specify whether the role played by V5/MT during stimulus encoding is time specific and it is independent from the presence of visual motion in the stimuli (Bosco et al., 2008). Our results here go beyond these previous observations demonstrating that the role of V5/MT during stimulus encoding takes place at a specific time (i.e. around 85-155 ms) after interval onset and that this contribution is independent of stimulus motion. Whereas the causal engagement of V5/MT during interval encoding was anticipated by previous TMS works, the causal involvement of V1 is a new finding; this result represents indeed the first demonstration that primary visual cortex causally contributes within a certain temporal window (from 85 to 155 ms after interval onset) to temporal encoding, and that this contribution is time specific.

During interval encoding V1 and V5/MT exhibited functionally-coupled and temporally-synchronized effects at delays of 85 and 120 ms after interval onset. This early (from 85 ms up to 155 ms from interval onset) and synchronous engagement seems to suggest that time signals are generated locally in these areas. Moreover the timing and the progressive increase of these effects with longer delays that approach the end of the to-be-encoded interval (i.e. either 200 or 200 + ΔT) is consistent with the hypothesis that activity in these regions increases monotonically throughout the interval until its end and with the notion that time is encoded in the firing rate of neurons via 'accumulation' mechanisms (Ghose and Maunsell, 2002; Durstewitz, 2003; Bueti et al., 2010). Electrophysiological data in monkeys and neuroimaging findings in humans have shown indeed that activity in visual cortices (i.e. the firing rate of V4 neurons and the BOLD response in V1 and V2/V3) increases monotonically while temporal information is tracked and pecks at the expected time of a behavioral relevant event (i.e. a stimulus changing in orientation or color; Ghose and Maunsell, 2002; Bueti et al., 2010). The redundancy of this temporal encoding is also congruent with the same neuroimaging data showing that V1 but also extrastriate areas V2/V3 respond to temporal expectations with analogous activation profiles (Bueti et al., 2010). The reason for this redundancy is unclear; a tentative but yet highly speculative hypothesis is that time is encoded in different spatial frames in

these two visual regions (i.e. retinotopic versus spatiotopic). The functional interplay between V1 and V5/MT during interval encoding seems also to speak in favor of the idea that visual areas encode time as part of circuit where the different nodes process different aspects of the temporal information.

The involvement of V1 and V5/MT during the *short-term memory* stage of the task is also a new finding. Previous electrophysiological works have mainly focused on the encoding stage of temporal computation (i.e. the time after stimulus onset, Ghose and Maunsell, 2002; Leon and Shadlen, 2003; Shuler and Bear, 2006) whereas neuroimaging studies, due to the poor temporal resolution of the technique, have failed to draw a clear distinction between time encoding and short-term memory (Rao et al., 2001; Coull et al., 2008; Bueti et al., 2012). Although it has never been shown before, the role of sensory specific cortices in temporal short-term memory has been suggested by many psychophysical observations showing that the capacity to keep time in memory depends on the sensory modality of the temporal signals (Penney et al., 2000; Gamache and Grondin, 2010a; Ogden et al., 2010; Rattat and Picard, 2012; Takahashi and Watanabe, 2012). For example, it has been shown that the working memory span is shorter for visual compared to auditory time signals (Gamache and Grondin, 2010a, b; Takahashi and Watanabe, 2012), and that capacity to keep in memory multiple intervals is better for multi-modal (audio-visual) rather than unimodal (visual-visual, audio-audio) signals. This latter result indicating the existence of distinct memory resources for different sensory modalities (Gamache and Grondin, 2010b). Our data, in line with these behavioral findings, represent the first demonstration that sensory specific visual regions V1 and V5/MT are engaged in the short-tem memory of visual temporal intervals.

While V1 and V5/MT are functionally-coupled during interval encoding, they seem to work independently and with different temporal dynamics (i.e. first V1 and subsequently V5/MT) during the working memory stage of the task. These differences might be linked to the different strategies used to keep time in memory, which may in turn reflect the existence of separate and independent routes for temporal memory (Takahashi and Watanabe, 2012). Further experiments are necessary to better specify the nature of these different memory pathways and to clarify the potential differences between V1 and V5/MT at the encoding stage.

Overall the present findings challenge the traditional view of V1 and V5/MT as visuo-spatial features detectors and highlight the temporal dynamics and the functional contribution of V1 and V5/MT to both encoding and working memory of temporal information in millisecond range.

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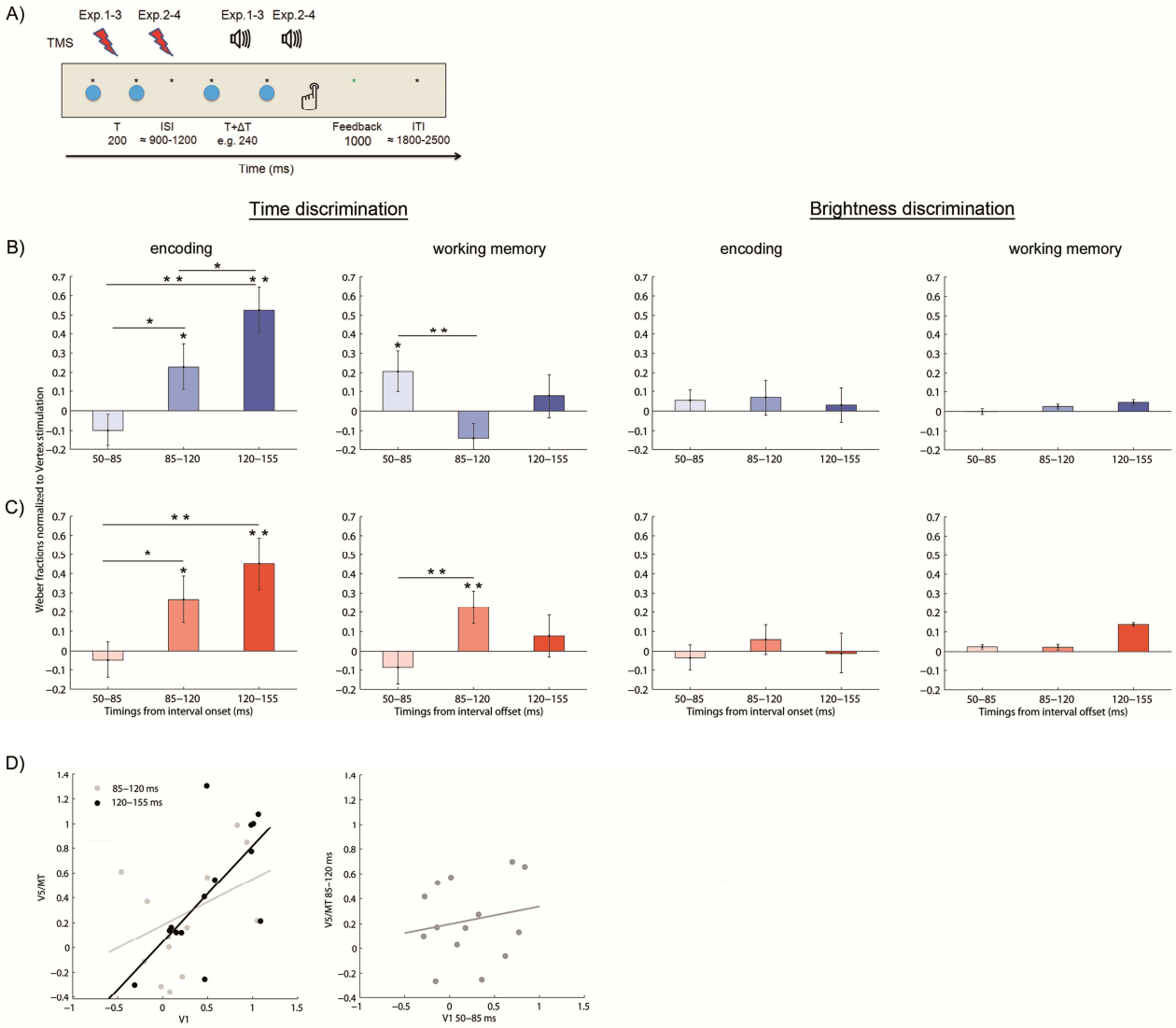

Figure 1:A Schematic representation of the experimental paradigm (detailed in Materials and Methods). B-C. Average (s.e.m. indicated) of individual discrimination thresholds (i.e. Weber fractions: ΔT/T) after paired-pulse TMS of V1 (B) and V5/MT (C) at three different delays (different shades of blue and red for V1 and V5/MT, respectively) after either interval onset (encoding) or offset (working memory). Thresholds are indexed as the change with respect to the vertex stimulation (i.e. average of the two vertex blocks) as follows: site-vertex/vertex. D leftmost panel: correlations across subjects between discrimination thresholds obtained after TMS of V1 and V5/MT at 85 ms (light grey) and 120 ms (black) stimulation delays after interval onset. D middle panel: correlations across subjects between discrimination thresholds obtained after TMS of V1 at the 50 ms delay and V5/MT at the 85 ms stimulation delay after interval offset. (*) significant at $p<0.05$, (**) significant at $p<0.01$.

IV. Conclusion

As already discussed in the article, there seems to be a functional link between V1 and V5/MT during the encoding process, while during interval working memory these areas seem to work independently and with different temporal dynamics, suggesting the existence of distinct routes for temporal memory.

For the encoding process, it is surprising that two distinct areas, one primary and one associative area, react with the same timing to the same stimulus and the question of redundancy certainly arises. Moreover, as suggested in recent data, the apparent duration of a visual stimulus can be distorted in spatially restricted regions of the visual field (19,44). In this respect, we could also ask ourselves whether there is a retinotopic or spatiotopic preference for one or the other route/area. In fact, we used an empty time interval marked by two light blue disks and even though spatial coordinates of these disks were conserved among participants, they might have generated redundant routes. The arising question would be to repeat the task but with different spatial coordinates and see if one or the other route takes over.

Regarding the working memory process, the reason of having 2 functionally independent and serially working areas remains unclear. As already suggested in some psychophysical studies (45–47), sensory-specific cortices can play a role in short-term memory. Here we were able to show that visual timing information can be stored in short-tem memory in two distinct visual areas. It would certainly be of great interest to further analyze these possible different routes and whether they're part of a single or of distinct pathways/ strategies.

Furthermore, it would be interesting to address the role/implication of magnocellular and parvocellular pathways in temporal memory. Are there any differences or specificities? Knowing that the magnocellular system responds transiently to the presentation of visual stimuli and that damage to the magnocellular layers dramatically reduces the ability to perceive rapidly changing stimuli, the contribution of the magnocellular system in visual timing should be considered. In order to test this hypothesis, one could use isoluminant chromatic stimuli to reduce the magnocellular system's implication and achromatic low contrast stimuli to minimize parvocellular's contribution.

As a more general conclusion, this work supports the theoretical model suggesting the existence of modality-specific distributed temporal mechanisms and challenges the traditional view of V1 and V5/MT as simple visuo-spatial features detectors.

Yet, we conducted here a functional experiment, where we used double-pulse TMS stimulation to impair a task performed by a specific area. This allowed us to highlight what could be a potential mechanism of visual time processing that includes V1 and V5/MT. It would be therefore interesting

to continue analyzing time processing with a functional perspective (performing functional experiments rather than neuroimaging) and try to see if a functional link exists between V5/MT and other associative areas as the auditory cortex, known to play a role in visual timing (20).

Another interesting perspective would be to address whether anatomical substrates can be found/verified in patients with deficits in temporal cognition. Parietal lesions and spatial attention deficits would certainly be relevant, also in order to see if an association exists between spatial and temporal deficits. Knowing the role of auditory cortex in visual timing it would also be of great interest to study deaf patients and more precisely see if differences in visual time processing arise between congenital deaf patients and acquired hearing impaired patients.

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