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Timing dans le cortex moteur :
De l'anticipation d'un indice spatial à la préparation du mouvement

Timing in motor cortex: from cue anticipation to movement preparation

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Summary

Our actions are shaped by the spatial and temporal context in which they take place. With experience, we learn to extract the necessary spatial and temporal information, and use it to improve our sensorimotor behavior. During my thesis, I assessed the influence of the temporal context (i.e., the timing) on movement preparation, at the neuronal level. More specifically, I performed extracellular recordings in the motor cortex of two macaque monkeys while they were performing a delayed center-out reaching task. In our task, the cue carrying the spatial information about the movement and the cue requiring its execution were temporally distinct. Furthermore, their timing changed from trial to trial but was predictable, thus allowing the animals to anticipate their appearance. We recorded and analyzed about 1200 neurons and 650 local field potentials (LFPs). We found that prior to the onset of the cue, that is in absence of movement preparation, 40% of the motor cortical neurons showed an anticipatory activity, either ramping up or ramping down. The pattern of activity of these neurons (increasing or decreasing) was predictive of their activity during movement preparation, especially in the epoch following the spatial cue. The two categories of neurons were as much involved in motor preparation, as reflected in their degree of directional selectivity. In the period preceding the cue, neurons with the same pattern of anticipatory activity tended to co-vary positively from trial to trial, whereas neurons with opposite patterns were negatively correlated. We proposed the hypothesis that preceding the onset of an informative cue, the motor cortex enters into an optimal state, at the same time facilitating the processing of the cue and preventing the generation of an early unwanted movement. During movement preparation itself, between the spatial cue and the execution of the movement, the neuronal activity was deeply influenced by the delay duration. Specifically the LFP signals evoked by the spatial cue (Visual Evoked Potential, or VEP) and by the movement execution (Movement Related Potential, MRP) were inversely modulated by delay duration, the VEP being larger in short and the MRP larger in long delay trials. The firing rate modulation of neurons recorded during the same epoch followed the same pattern of delay selectivity, although not as strong as that of the LFP. The delay selectivity of both signals seemed rather independent of their selectivity to movement direction, but as important. However, the time course of the two selectivities was different, with the delay selectivity being more influent at the beginning of the delay, whereas the direction selectivity being more influent at the end of the delay. The delay selectivity may be the reflection of motor strategies or the influence of motivational factors. Lastly, we showed that in the context of a predictable GO signal, the ability to estimate the delay duration seems to be the best predictor for the variability of the animal's performance trial by trial.

Résumé

Toutes nos actions sont influencées par leur contexte spatial et temporel. Être capable d'extraire ces informations spatio-temporelles, puis les utiliser afin de prédire l'apparition d'événements probables nous permet d'avoir un comportement plus efficace. Pendant ma thèse, j'ai étudié l'influence du contexte temporal (ou “timing”) sur la préparation du mouvement, au niveau de l'activité neuronale. Plus précisément, j'ai enregistré l'activité extra-cellulaire des neurones du cortex moteur de deux macaques rhésus, pendant que ceux-ci réalisaient une tâche de pointage directionnel. Dans notre tâche, l'indice portant l'information concernant le mouvement et l'indice requérant l'exécution du mouvement étaient séparés dans le temps. De plus, leur timing changeait d'essai à essai mais était prédictible, permettant à l'animal d'anticiper leur apparition. Nous avons enregistré et analysé l'activité de plus de 1200 neurones et 650 potentiels de champs locaux (LFP). Nous avons tout d'abord trouvé que 40% des neurones des aires motrices modulent leur activité avant l'apparition de l'indice spatial, c'est à dire en absence de toute préparation motrice. Le profil de l'activité anticipatoire de ces neurones, croissant ou décroissant, était prédictif de leur activité pendant la préparation motrice (particulièrement dans la période suivant l'apparition de l'indice spatial). Les deux catégories de neurones (basées sur leur profil de décharge) semblaient par contre être autant impliquées l'une que l'autre dans la préparation du mouvement, basé sur leur degré de sélectivité pour la direction du mouvement. Dans la période précédant l'apparition de l'indice, l'activité des neurones montrant le même profil anticipatoire avaient tendance à être positivement co-modulée d'essai à essai, alors que l'activité des neurones montrant des profils opposés étaient négativement corrélée. Nous proposons que cette activité anticipatoire du cortex moteur reflète deux processus liés et complémentaires, renforçant la réponse à l'indice spatial tout en empêchant un mouvement involontaire d'être déclenché. Pendant la préparation du mouvement elle-même, c'est à dire entre l'apparition de l'indice spatial et l'exécution du mouvement, l'activité neuronale était profondément influencée par la durée du délai. Plus précisément, les potentiels du LFP évoqués) par l'indice spatial (VEP) et l'exécution du mouvement (MRP) étaient modulés inversement par la durée du délai. L'amplitude des VEP etait plus grande dans les essais courts, alors que celle des MRP était plus grande dans les essais longs. L'activité unitaire enregistrée pendant les même périodes de la tâche a été modulée de la même façon, mais à une intensité bien moindre. La sélectivité à la durée du délai de ces deux types de signaux semblait indépendante de leur sélectivité à la direction du mouvement, mais avait la même importance. Cependant, le décours temporel des deux sélectivités était différent, la durée du délai semblant plus influente au début du délai et la direction du mouvement à la fin. Cette sélectivité pour la durée du délai pourrait refléter une stratégie de préparation motrice, ou bien l'influence de facteurs motivationnels. Dernièrement, en étudiant la relation entre le taux de décharge et le temps de réaction de l'animal, nous avons montré que dans le contexte d'un signal GO fortement prédictible temporellement, la capacité à estimer la durée du délai est le meilleur prédicteur de la variabilité de la performance de l'animal.

List of common abbreviations

D1/D2	Delay 1 and 2 of our task (before and after SC)
DLPFC	Dorsolateral prefrontal cortex
EMG	Electromyogram
EEG	Electroencephalogram
EP	Evoked potential
FEF	Frontal eye field
GO	GO signal, requiring the execution of the movement
ICMS	Intracortical microstimulation
IT	Inferotemporal area
LFP	Local field potential
LIP	Lateral intraparietal cortex
M1	Primary motor cortex
MRP	Movement-related potential
MUA	Multi-unit activity
Mvt	Movement
PD	Preferred direction
PETH	Peri-event time histogram
PMd	Dorsal premotor cortex
PMv	Ventral premotor cortex
rms	Root mean square
RT	Reaction time
SC	Spatial cue
SEF	Supplementary eye field
SMA	Supplementary motor area
SUA	Single-unit activity
TC	Temporal cue
TMS	Transcranial magnetic stimulation
VEP	Visual evoked potential

1. General introduction

1.1. Scientific Context

1.1.1. Introduction

Interacting with our environment requires acquiring and processing information about the external world, interpreting it, and elaborating an appropriate behavioral response. In fact, cognition itself can be seen as a long sensorimotor loop, involving most parts of the brain. From the sensory to the motor cortex, complex interactions take place, progressively transforming information about the environment into motor actions, or inversely enhancing features of the environment to better match the behavioral requirements. Some experimental techniques enable the researcher to see how the whole brain works, either with great spatial but low temporal resolution like functional Magnetic Resonance Imaging (fMRI), or with opposite flaws and advantages, like recording the electroencephalogram (EEG). These techniques have the advantage of being non-invasive, permitting their use in human participants, and thus the study of complex cognitive functions. However, if one wants access to the fine-grained aspects of the neuronal signals, more local-scale and invasive methods are needed. Intracortical electrophysiology relies on microelectrodes sensing the activity of nearby neurons, and as such has a very limited (local) spatial scale. However, it allows us to directly record the neuronal activity of isolated neurons (spikes, or action potentials) but also of locally averaged activity (local field potentials, or LFPs).

When using such an invasive recording technique we have to deal with two major questions before starting our research. First, what is the animal model that best allows us to understand the specific aspect of cognition? Second, which cortical or subcortical area(s) is (are) likely to provide the best responses to our scientific question? Since the sensorimotor task that we used in the herein presented work was quite complex, the macaque monkey (*Macaca mulatta*, or rhesus monkey) was chosen as the best animal model. Furthermore, since we focus on motor behavior, we recorded the activity of the motor cortex, the area of the brain most directly connected to the spinal cord (Dum & Strick 2005).

In the next paragraphs, I will briefly describe the anatomy of the motor cortex in the macaque monkey and detail how this animal model fits to our study. Then I will present the two electrophysiological signals that will be analyzed in this manuscript: spiking activity and LFPs. Finally I will introduce the principles of the pre-cueing paradigm, which has been used since more than 30 years to understand how movements are prepared before being executed.

1.1.2. Anatomy of the motor cortex

In this paragraph, we will focus on the two cortical areas of our interest, the primary motor cortex (M1) and the dorsal premotor cortex (PMd). Most of the data were described in the two reviews by Dum & Strick (2005) and Geyer et al. (2000).

1.1.2.1. The primary motor cortex

The primary motor cortex (M1, or area F1, see Matelli et al. 1985, 1991) is located on the anterior bank of the central sulcus (in blue in Fig. 1.1), and corresponds to the cyto-architectonic area 4 (Brodmann 1909). M1 is defined by a thick layer 5 containing a large concentration of giant pyramidal neurons. Among these pyramidal neurons, the Betz cells project directly to the spinal cord, and are specific to the motor areas. However, they only constitute about 10% of the projections from M1 to the spinal cord (Lemon & Porter 1976). By stimulating electrically M1 intracortically, one can elicit muscle twitches with a current intensity of less than 20 µA (Asanuma & Rosén 1972). By comparison, when stimulating PMd, intensity has to be at least 30-40 µA to elicit a twitch and the probability to evoke it is overall lower (Weinrich & Wise 1982).

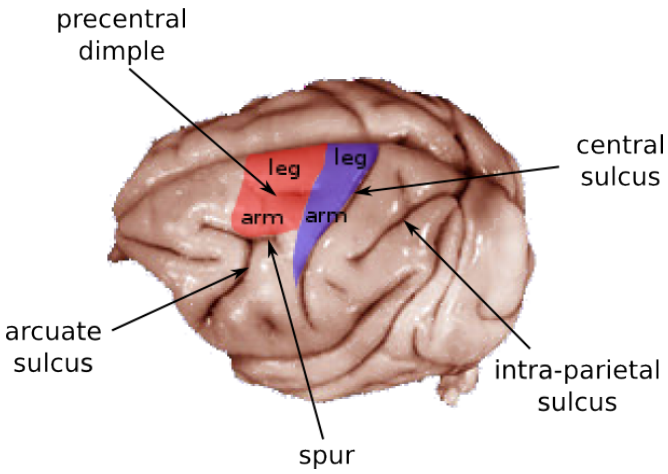


Figure 1.1: Brain of a macaque monkey and location of M1 and PMd. M1 is in blue, PMd in red. The broad subdivision of the somatotopy is indicated. Anterior part is on the left, posterior on the right (picture of the brain from the website of the Wisconsin-Madison Brain Collection).

With this method, a precise somatotopic organization of M1 has been discovered, corresponding to the main targets of these neurons in the spinal cord. Broadly, this map is organized along a centro-lateral gradient, with the leg located more centrally, then the arm and the face as one moves away from the midline (Penfield & Boldrey 1937). However, this somatotopy is not perfectly ordered. For example, areas projecting to the upper limb follow a "horseshoe" pattern, with the distal part in the middle (digits), the elbow on each side and the most proximal part (shoulders) surrounding them (Kwan et al. 1978; McGuiness et al. 1980). Furthermore, M1 spinal projections are highly divergent; there is no such thing as a "one neuron-one muscle pattern". Among the cortico-motoneurons (M1 neurons directly projecting to spinal motoneurons), 73% project to two or more

muscles (McKiernan et al. 1998), and 43% project to different spinal segments (Shinoda et al. 1979). However, projections to hand muscles tend to have a lesser degree of divergence (McKiernan et al. 1998), raising the idea that a finer control of the muscles requires a lesser branching of the cortical projections (see Lemon 2008). This idea is supported by the fact that the spatial pattern of M1 projections to the spinal cord changes tremendously across species (Lemon & Griffiths 2005), and correlates with the ability to move fingers independently. This point will be described in the next paragraph.

The projections to the spinal cord are not homogeneously distributed inside M1. The density of direct cortico-motoneuronal connections is much higher in the sulcus than on the bank of M1 (Rathelot & Strick 2009). This subdivision between sulcus and bank of M1 is accompanied by differences in neuronal responses during motor tasks. For example, most neurons whose firing rate is modulated by intrinsic motor properties (like load or arm posture) are located in the sulcus (see Kalaska 2009, for a review), and most neurons with responses to visual cues are located in the rostral part, on the bank (Weinrich et al. 1982, 1984; Crammond & Kalaska 1996, 2000; Johnson et al. 1999). Note that in this manuscript, the neuronal activity was recorded from the bank of M1, not from the sulcus. The firing properties of this region are intermediate between those of the caudal part of M1 and those of the caudal part of PMd (Crammond & Kalaska 1996, 2000).

M1 receives no direct input from the prefrontal cortex (Fig. 1.2), but has major reciprocal connections with the ventral and dorsal part of the premotor cortex (PMv and PMd), the caudal part of the supplementary motor area (SMA), and minor projections from the cingulate motor areas. Concerning the parietal lobe, M1 receives a major input from the somato-sensory cortex (area 1, 2, 3a), from the rostral part of the superior posterior parietal cortex (PE, or area 5), as well as from its component located in the intra-parietal sulcus (PEip). Additionally, M1 receives a major input from the substancia nigra, the pallidum and the cerebellum via the thalamus (reviewed in Dum & Strick 2005).

1.1.2.2. The dorsal premotor cortex:

The dorsal premotor cortex (in red in Fig.1.1, also named PMd or F2, see Matelli et al. 1985, 1991) is anatomically less well defined. It consists in the lateral part of the agranular cortex rostral to M1 (area 6), the medial part being SMA. More precisely, it extends from the midline to the spur of the arcuate sulcus, and stays caudally to the genu of the arcuate sulcus. Rostrally to F2 is F7 (also called "pre-PMd"), an area responsive to eye movement, without projections to the spinal cord (unlike F2). Here, what I will call PMd is thus F2, the caudal part of PMd in a broader sense (in red in Fig. 1.1). PMd is also organized somatotopically, but less clearly than M1, and lacks the representation of some parts of the body, like the face or digits. Each of these zones is bi-directionally connected with the corresponding areas inside M1, and projects directly to the spinal cord.

Indeed, contrarily to the popular beliefs, the premotor cortex has extensive direct connections to the spinal cord (He et al. 1993), especially to the cervical segments (control of the upper limb). Taken together, PMd, PMv and SMA projections to the spinal cord are as numerous as those from M1. However, unlike M1 cortico-motoneurons, their targets are restricted to the intermediate zone of the spinal cord (Dum & Strick 2002), where spinal interneurons are located. Thus, the influence of PMd on the spinal activity may be more modulatory than driving the activity of muscles.

PMd is highly connected to M1, SMA and the cingulate motor areas, but very weakly to PMv (dashed red arrow in Fig. 1.2). However, it also receives projections from the rostral part of SMA (preSMA) and from "prePMd" (or area F7, also called Supplementary Eye Field, or SEF), the area just rostral to PMd, which responds to eye movements. From the parietal cortex, it receives projections from the medial part of the intra-parietal sulcus (PEip and MIP) and from its superior posterior bank (PEc). Like M1 but in a lesser extent, PMd receives input from the substancia nigra, the pallidum and the cerebellum via the thalamus.

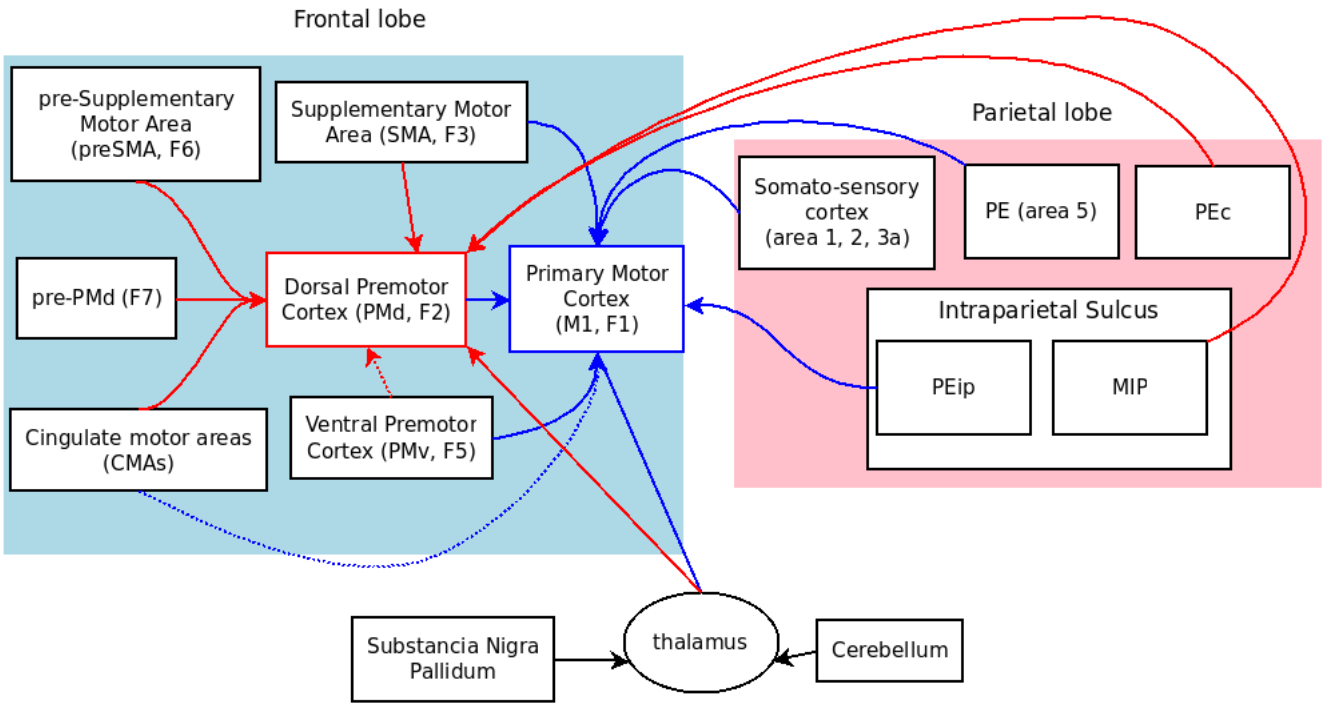


Figure 1.2: Connectivity of the motor cortex. Areas nomenclature from Matelli et al. (1985, 1991) is also indicated for frontal structures. All arrows are bidirectional. Dashed arrows are minor connections. Blue arrows are projections to M1, red arrows projections to PMd. The colored squares indicate the two major anatomical structures (frontal and parietal lobe).

1.1.3. The rhesus monkey as a model for the study of motor behaviors

The ultimate role of neuroscience is to understand human cognition. That is, an animal model allowing a more direct comparison with the human nervous system is of large interest. For example, studying reaching movements in the cat may provide many insightful data about the nervous system. However, the corticospinal organization of this mammal differs strongly from that of primates and *a fortiori* humans that the results cannot be transferred across species. Lemon & Griffiths (2005) conducted an extensive review of the differences in the corticospinal organization of different species. They show that the spinal targets of the cortico-motoneuronal projections vary tremendously between species (see Fig. 1.3). For species like the opossum or the cat, most projections end in the dorsal or intermediate zone of the spinal cord (where are located somatosensory input and spinal interneurons projecting to several motoneurons, see Maier et al. 2002; Takei & Seki 2010). By contrast, in humans, chimps and to a lesser extent in macaques, M1 also projects directly to the ventral horn (where motoneurons are found) (Kuypers 1981). This proportion of direct projections to the motoneurons of the ventral horn correlates well with the index of dexterity of each species; the denser the direct projections to spinal motoneurons, the better the animal is at independently moving its digits. That is not to say that these direct cortico-motoneuronal projections are restricted to the control of digits, many of them project to more or less distal or proximal muscles of the upper limb (Buys et al. 1986 in macaques; Nielsen & Petersen 1995 in humans). From this point of view, humans are closer to macaques than to any other animal model usually used in research (rats, mice, cats).

However, the corticospinal projections of macaque monkeys and humans are far from being identical. One of their major differences is the influence of the rubrospinal and propriospinal pathways in the control of the upper limb. Indeed, in all species, direct projections to the spinal motoneurons sprout from the red nucleus, located in the midbrain, next to the substancia nigra. However, this pathway seems to lose its importance as the direct cortico-motoneuronal pathway develops. Whereas it is almost vestigial in human beings, it seems to be more important in macaque monkeys. Similarly, an alternative route connecting the cortex to the motoneurons exists, called the propriospinal pathway. It involves neurons from the motor cortex, projecting onto propriospinal interneurons in the C3-C4 vertebrae, which in turn project to the motoneurons innervating the muscles in the C5-Th1 vertebrae. This system has been shown to be present and active in lower mammals and some primate species like the squirrel monkey, but seems to lose its importance as the direct cortico-motoneuronal system becomes more prominent. Whether it is actively used or not in macaques and human beings is still the matter of a heated debate (Lemon & Griffiths 2005; Lemon 2008; Alstermark & Isa 2012). However, Kinoshita et al. (2012) recently showed that specifically inactivating this subpopulation of spinal

interneurons strongly impaired the grasping ability of macaque monkeys. In summary, these two systems, the rubro- and propriospinal pathways seem to have different importance in humans and macaque monkeys. However, their function is mainly the control of digits in grasping behaviors (Kinoshita et al. 2012 for the propriospinal; van Kan & McCurdy 2001 for the red nucleus). Since our task does not concern grasping, but only 2-dimensional reaching movements, we do not think that this difference in corticospinal architecture is a great obstacle to the interpretation of the data.

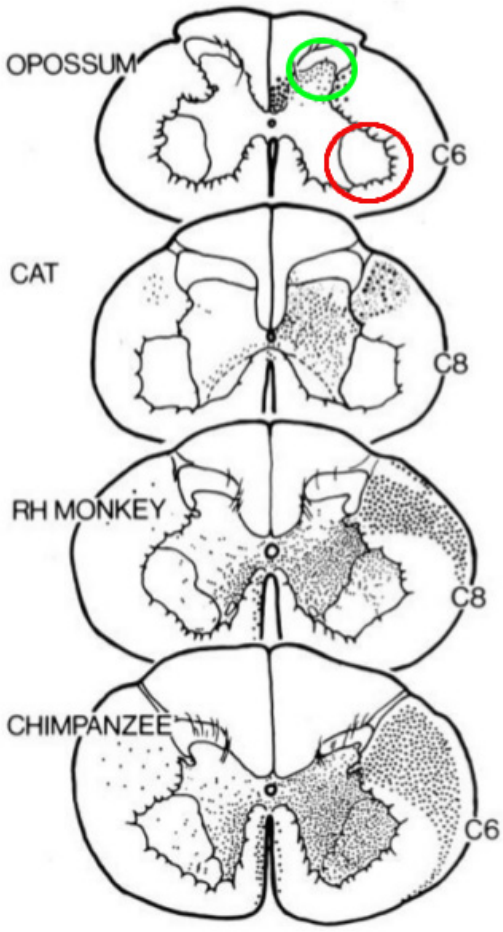


Figure 1.3: cortico-spinal projections in four species. Results were obtained by staining degenerated fibers after lesions of the contralateral motor cortex. RH: rhesus. The dorsal horn is indicated by a green circle (location of most somatosensory afferents), the ventral horn by a red circle (where the spinal motoneurons are located). The zone in between is called "intermediate". Note that from top to bottom, motor cortex projects less and less to the dorsal horn, and more and more to the ventral horn. This may indicate that throughout evolution, motor control shifted from a modulation of the sensory input and spinal reflexes (dorsal horn) to a more direct cortical control of the motoneurons (ventral horn). Note that even though the rhesus monkey does not have as many projections to the ventral horn than does the chimpanzee, it is still much more than for the other mammals. Modified from Kuypers (1981).

Another aspect of our task is the timing. Zarco et al. (2009) directly compared the ability of macaques and humans subjects to estimate delay durations of 450ms to 1 second. They found noticeable differences across species. Monkeys tend to underestimate durations more than humans, show less inter-modality differences in synchronization to visual and auditory stimuli than humans, and have overall a lower temporal precision and higher variability than humans. However, their performances were in general modulated by the same task parameters, they both displayed scalar property of timing (the variance of the estimated intervals is proportional to their mean, Gibbon 1977), and most importantly, their performances were similar for the

production of intervals. Since it is this aspect of timing that our task exploited, we consider that macaque monkeys are well suited to the study of timing during motor behaviors.

1.1.4. Physiological bases of spikes and LFPs

Since the end of the XVIIIth century and the work of Luigi Galvani, we know that electric stimulation can induce muscular activity. Much later, in 1928, Edgar Adrian reported the first recordings from a nervous cell in the "The basis of sensation". More than ten years later, the first recordings of pyramidal neurons in the cat hippocampus (Renshaw et al. 1940), then recordings from the cortical surface via platinum wires (Woldring & Dirken 1950) showed that it was possible to obtain the activity from single neurons using micro-electrodes, as well as "slow oscillations". Soon afterward, Hodgkin & Huxley (1952) published a work uncovering the physiological bases of the action potential via intracellular recordings in the squid giant axons, which earned them the Nobel Prize in medicine. Since then, the technology of extracellular recordings kept evolving, starting from the Lippman electrometer of Edgar Adrian to the Utah array or the Michigan probe used nowadays (Jones et al. 1992; Hoogerwerf & Wise 1994; Nordhausen et al. 1996; Maynard et al. 1997; Vetter et al. 2004), allowing the simultaneous recording of more than 100 neurons.

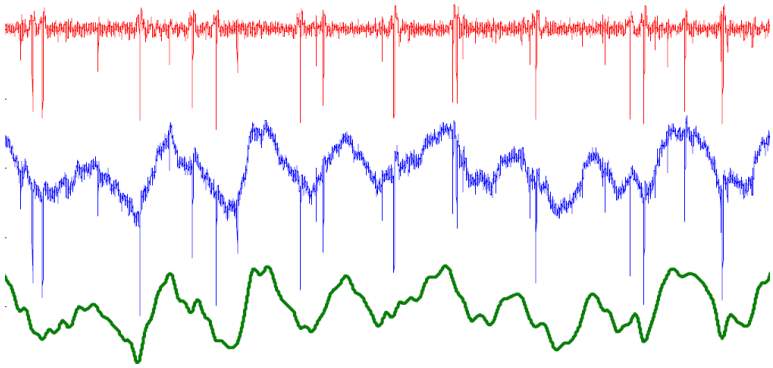


Figure 1.4: Electrophysiological signals recorded on one electrode. In blue is the raw signal (from 1Hz to 10kHz), in red the high frequency signal (0.3-10kHz), from which the individual spike shapes are detected (see Materials and Methods), and in green the low-frequency signal (1-250Hz), called the LFP.

These extracellular electrodes allow the recording of action potentials (spikes) of single neurons, as well as the low-frequency activity of local populations of neurons (LFPs). The extracellular spikes that we record in motor cortex are short-lasting (1-3ms), and are caused by fast inward currents of sodium ions. Spikes are emitted when the summed post-synaptic activity (excitatory- and inhibitory post-synaptic potentials, EPSPs and IPSPs) reaches a given threshold. In essence they are only informative about the output of the neuron, and not about its subthreshold activity. The tip of an electrode located near (~50 µm) the soma of a neuron enables to accurately record the shape and latency of an action potential (Harris et al. 2000). However, the probability to be recorded is not equivalent for all neurons. Stone (1973) showed that there is a strong bias toward large

neurons (hence, pyramidal neurons). Indeed, neurons with a large cell body (>20-30µm in diameter) emit spikes leading to an extracellular potential of >100µV in an electrode whose tip is located at >50µm from the soma. The size of this potential decreases quickly with distance (Abeles 1982 shows that the recorded potential becomes <100µV after 30µm away from the cell body). At 140µm from the soma, the spikes are no more distinguishable from the background noise (Rall et al. 1962). Thus, spikes emitted by large neurons will be detected from a farther distance than those emitted by small neurons, strongly biasing the odds in their favor. This is especially true in the behaving animal, in which small movements will slightly move the electrode (Fried et al. 1997). The spikes from small neurons will easily be "lost" (i.e., small movements will move the electrode away from the neuron, and the spikes it emits won't be large "heard" by the electrode anymore, as they will blend in the background noise), whereas spikes from large neurons will not. The consequence of these biases is that single-neuron recordings are mainly obtained from a small subtype of neurons (in motor cortex, the pyramidal neurons of layer 5). By contrast, other neuronal subtypes are usually overlooked, like inhibitory interneurons (see Logothetis et al. 2003). In summary, single-neuron recordings are mainly informative about the suprathreshold activity of pyramidal neurons. Practically, the spiking activity of a single neuron is extracted from the high-frequency component of the raw electrophysiological signal (in red in Fig 1.4). The shape of the spikes of a given neuron is usually quite easy to differentiate from the ones emitted by nearby neurons or from the background noise. One uses an algorithm to select one of the spike shapes and track its frequency and timing of appearance (see Materials and Methods chapter for a description of the spike sorting procedure). Like that, the modulations of firing rate of a single neuron can be computed and related to the events of the task. As the recorded spikes are supposed to be emitted by a single neuron, one also calls this signal Single-Unit Activity (or SUA). By opposition, certain studies also use the Multi-Unit Activitity, or MUA, which is the unsorted spiking activity of a local neuronal population. The classical way of computing the MUA is to set a threshold on the high-frequency activity (in red in Fig. 1.4), and to gather all the spikes crossing the threshold as if they came from a single source. Another way, introduced by Stark & Abeles (2007), consists of computing the envelope of the high frequency component of the raw neuronal signal. This procedure allows obtaining a continuous signal of the spiking activity of many neurons instead of a discrete point process of a single neuron. It is noteworthy that one can decode different amounts of information from the SUA and the MUA regarding the on-line control of hand trajectories (Stark & Abeles 2007).

Another electrophysiological signal is the LFP, which is supposed to reflect the averaged activity of slow neuronal currents of local populations of neurons (in green in Fig. 1.4). The most ubiquitous of these currents are the post-synaptic potentials, supposed to form the main part of the LFP signal (Buzsáki et al. 2012). However, other slow currents may also influence it. As reviewed by Logothetis et al. (2003) and Buzsáki et al.

(2012), long-lasting calcium spikes (10-100ms), spike afterhyperpolarisation, resonance properties of the neuronal membrane, etc, could also enter into the LFP signal. For example, part of the delta oscillations observed during the slow-wave sleep can be explained by the synchronized spike afterhyperpolarisations of pyramidal cells (Buzsáki et al. 1988). The LFP recorded on one electrode was proposed to reflect the averaged post-synaptic activity of the cortex within 0.5-3mm around the electrode tip (Mitzdorf 1987). This estimate is supported by Kreiman et al. (2006), who found that the selectivity of the LFP signal was best correlated with that of the local MUA if the latter was averaged over 3mm around the site of the LFPs recording. However, this view was recently challenged by Katzner et al. (2009) in a task specifically designed to study the spatial extent of the LFP signal. In the primary visual cortex of anesthetized cats, they showed that the correlation between the directional selectivity of the LFP response evoked by a visual stimulus was best predicted by a weighted average of the membrane potentials recorded 250µm around the electrode. This study would thus support a rather reduced spread of the LFP signal. A modeling study by Lindén et al. (2011) could reconcile these contradictory findings. The authors showed that the size of the cortical surface generating a LFP depends on the degree of correlation of the activity of the local neuronal population. For an uncorrelated activity, the cortical surface generating the LFP will not extend farther than a few hundreds of micrometers. However, correlated activity will influence the LFP at a larger distance. The correlation of the local neuronal activity could depend on the microstructure of the recorded areas: the columnar organization of V1 could thus explain the results of Katzner et al. (2009). More generally, the microstructure of the motor cortex (agranular cortex, and lack of a clear columnar organization) differs radically from that of the areas in which Kreiman et al. (2006) and Katzner et al. (2009) recorded. One can thus ask to what extent these results can be transferred to motor cortex.

Unlike spikes, the LFP signal does not seem to be influenced by the size of cell bodies, but rather by the dendritic architecture. If the local population of neurons follow the same vertical disposition, with the cell body on one side and the dendritic tree on the other (like pyramidal neurons), synchronous somatic input will create a strong dendrite-to-soma current. This disposition is called "open-field", and participates greatly to the LFP signal (Logothetis 2003). If, by contrast, the dendritic tree is arranged horizontally or evenly spread around the soma, the dipoles will cancel each other out. Such "closed-loop" architecture will hardly be visible in the LFP signal. Consequently, in primates, the concave part of a gyrus will create a higher current density compared to its convex part, because the folding of the tissue brings the dendrites closer to each other (Nunez & Srinivasan, 2006). The other main factor determining the amplitude of LFPs is the synchronization of the spiking activity (Denker et al. 2011). As explained above, a synchronized input over a neuronal population will create a larger dipole than a scattered one, giving rise to a larger potential (Lindén et al. 2011; Buzsáki et al. 2012). In

summary, the main parameters which will influence the amplitude and frequency of the LFP signal are the size and architecture of the neuronal population, and its degree of synchrony.

The LFP oscillates at different frequencies, depending on the behavioral state of the subject (for example, asleep vs. awake), or the site of recording. The frequency bands of these oscillations have been given names, from the EEG litterature: delta for 1-4Hz, theta for 4-8Hz, alpha for 8-12Hz, beta for 12-24Hz, gamma for 24-40/80Hz (Logothetis 2003), or even "high gamma" (up to 100-300Hz), which can be highly correlated with MUA activity (Kreiman et al. 2006; Mollazadeh et al. 2011). These frequency bands are rather arbitrary conventions, and what is called gamma in one study can be labeled beta in another (e.g., Kilavik et al. 2012a). In addition to oscillations, the LFP signal also contains "evoked potentials" (EPs, or ERPs for "event-related potential"), complex modulations of several successive components, evoked by behavioral events, such as a visual signal (visual evoked potential, or VEP) or the initiation of a movement (movement-related potential, or MRP, sometimes called in the literature MEP, movement-evoked potential, Donchin et al. 2001). The different components of the EPs can be modulated by different parameters of the task. For example, the first positive component of the MRP was shown to be specifically modulated by the expectancy of a GO signal (Roux et al. 2006). Chapter 3 of this report will focus on the modulation of these two types of EPs during a sensorimotor task.

1.1.5. Pre-cueing and the paradigm of movement preparation

The study of movement preparation used to belong to the field of cognitive psychology, with the study of reaction time (RT) as a main tool (e.g. Niemi & Näätänen 1981). The rationale behind it goes back to Sternberg (1969) and his "additive factor method", and is based the seminal works of Donders (1868). It postulates that RT is determined by a set of discrete and serial processes, where each of them can be manipulated independently from each other, modulating RT. More simply put, and regardless of the validity of Sternberg's theories, the less the subject knows in advance about the action to be performed (its duration, extent, timing, etc), the longer the RT will be; RT is thus tightly linked to the concept of uncertainty. In this theoretical framework, Rosenbaum (1983) developed the pre-cueing paradigm, very well described by Jean Requin (Requin et al. 1991): "*The rationale of studies devoted to preparatory processes is to manipulate the subject's expectancy about the stimulus which is to occur and thus the response to be performed, by providing advance information about these events. This is accomplished by introducing a preparatory signal (S1) which provides prior information about either the timing and/or the features of the expected imperative signal (S2) and associated response (R)*" (p. 363-364). This first signal will decrease the "event uncertainty" and/or the "temporal uncertainty" of S2, thus shortening RT. Note, that in our manuscript we will call S1 the "cue" and S2

the "GO signal". Prior information mainly impacts the preparation of a movement and not its execution since, unlike the RT, the movement time stays more or less constant whatever the amount of prior information (Riehle 2005).

This paradigm was adapted to monkey experiments (e.g., Evarts & Tanji 1974,1976; reviewed in Evarts et al. 1984), and has been used extensively since then. For example, Riehle and collaborators (1989, 1993, 1994, 1995, reviewed in Riehle 2005) showed that providing prior information about the movement direction reduces RT, and induces a directionally selective preparatory activity in a large proportion of motor cortical neurons. By contrast, providing prior information about force or extent of the movement only weakly reduces RT, and only few neurons show a preparatory activity selective related to those parameters (Riehle & Requin 1989; Kurata 1993). However, many neurons were selective for force or extent during movement execution itself in their tasks, as direction was also known at this point (review: Riehle 2005). Another example is described by Weinrich & Wise (1982), who temporally dissociated the cue indicating movement direction from the one indicating its execution. They demonstrated that neurons from PMd discharge in response to the visual cue, during the delay, or during movement execution. These two examples illustrate the rationale of many cognitive neuroscience studies (including the present report). By dissociating the cue providing prior information about the forthcoming movement from its execution, one expects to temporally separate modulations of activity that were usually confounded, and to observe their potential selectivity for a particular movement parameter. Even though this approach has been fruitful by allowing gathering a wealth of data about the neuronal activity during cognitive tasks, one has to be careful in their interpretation. Since the data are usually interpreted *a posteriori*, their relation to the task parameters is correlational and not causal. Furthermore, relating the modulation of neuronal activity to the task parameters in an inductive way is never straightforward, as one never knows the hidden variables being manipulated by changing the task parameters (this will be illustrated in the discussion of chapter 3). The interpretation of the data will there be biased by our *a priori* ideas about the task, which may depend, for example, on the area of recording (Riehle 2005). Keeping these limitations in mind, in the next section I propose to quickly review what this paradigm had taught us about the neuronal activity of the motor cortical areas during movement preparation.

1.2. Movement preparation in motor cortex

1.2.1. Activity of the motor cortex during movement execution

The neuronal activity of the motor cortex during movement execution has been studied since decades (reviewed in Hepp-Reymond 1988). It has been shown to covary with numerous motor variables like the force output (Evarts 1963, 1964), but also to more complex kinematic variables involving several joints, like the

direction of a reaching movement (Georgopoulos 1994). This property of motor cortex will be briefly described below.

1.2.1.1. Directional selectivity of the firing rate

During the execution of a reaching movement, many motor cortical neurons change their firing rate in relation to the direction of the movement. Using a center-out pointing task in eight possible directions in the horizontal plane, Georgopoulos et al. (1982) showed for the first time that more than half of the motor cortical neurons discharged most in relation to their preferred direction (PD), and much less during the others. Specifically, the mean firing rate computed during each movement direction followed a cosine curve (Fig. 1.5 A-B). This property was called "directional tuning". Using a reaching task in 20 different directions, the fine properties of the tuning curve were better assessed (Amirikian & Georgopoulos 2000). It was shown that the width of the tuning was quite variable from one neuron to the other, and that the median width was about 60°, which is much narrower than previously thought (90°). Furthermore, the authors showed that the vast majority of the tuning curves were symmetrical. By computing the vector sum of the preferred directions of all neurons, each of them weighted by their firing rate in the current movement direction, one can obtain the "population vector" (Georgopoulos et al. 1983), which predicts the direction of this movement (Fig. 1.5 C). This method was then adapted to compute the time-resolved population vector (Schwartz et al. 1988), and thus provide an insight in the modulation of the population vector in real time. This property was used to decode movement trajectories in real time (reviewed in Schwartz 2007), but also to assess the covert processes of movement preparation (e.g. Shen & Alexander 1997a,b).

However, measuring the tuning curves of motor cortical neurons in two dimensions is artificial, given that most movements are performed in 3D. Accordingly, it was shown that the tuning curve of M1 neurons also contains a vertical dimension (Georgopoulos et al. 1986; Caminiti et al. 1990), and that the distribution of all neurons' PDs cover the entire 360° in rotation and 180° in elevation. The distribution of PDs (in 3D) is nevertheless not completely homogeneous. Even though the deviation from homogeneity is very weak, more neurons "point" in direction of reaching away from the body (and in a lesser extent, toward the body) than would be expected by chance (Naselaris et al. 2006a). The authors hypothesized that this feature was caused by the naturally occurring higher frequency of reaching movements in these directions compared to others. At a large scale, the PDs are evenly distributed over the surface of M1. It was estimated that the entire angular (spherical) range could be covered by the neurons recorded in 3mm², and each of these sub-populations showed a slight bias in direction away from the body (Naselaris et al. 2006b).

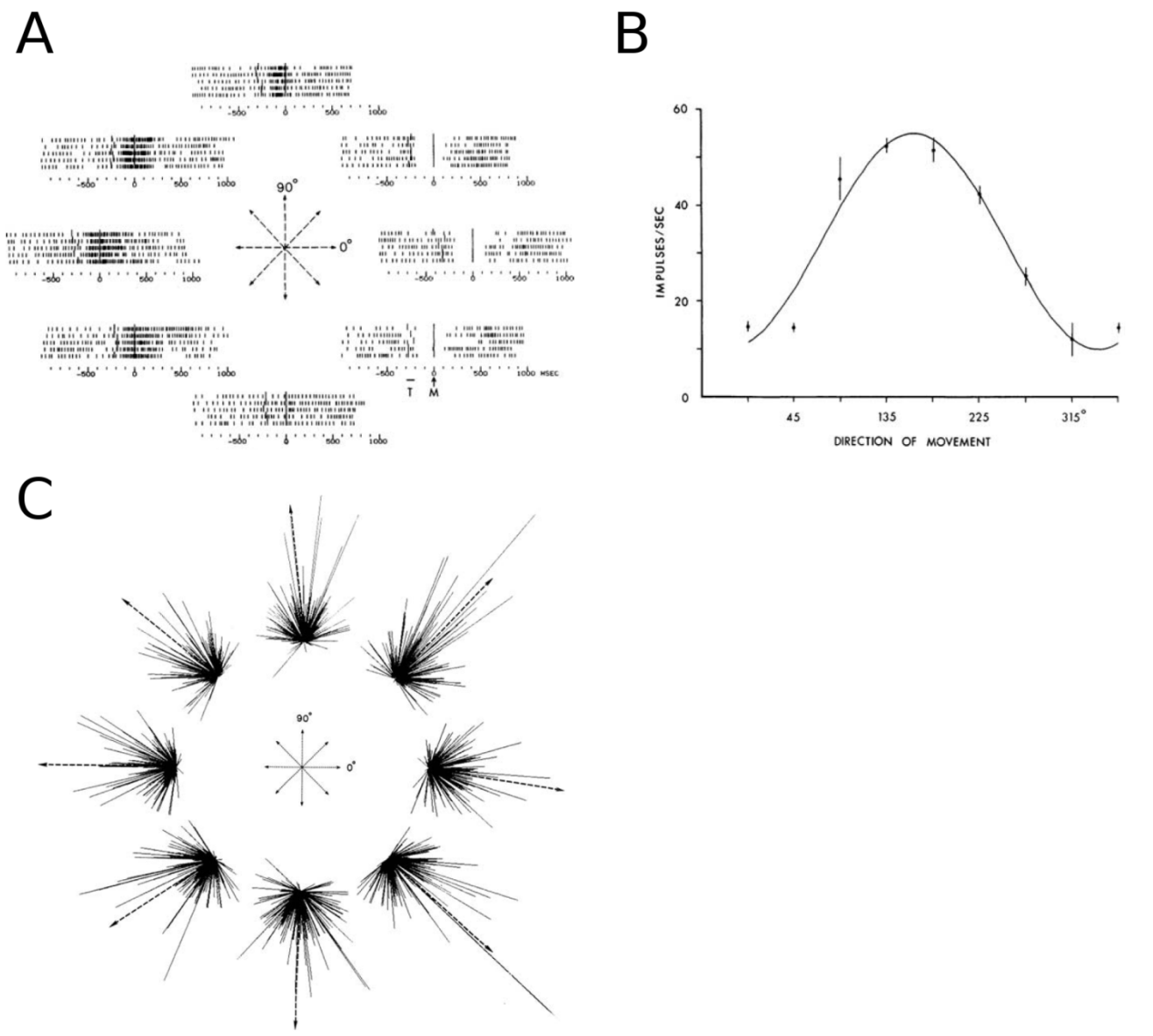


Figure 1.5: Georgopoulos' seminal work on the directional selectivity in motor cortex. A) Raster plot of the spiking activity of a neuron recorded during the movements in 8 directions (represented by the dashed arrows in the center). Abscissa is in millisecond, 0 is movement onset, each dot represents an action potential, and each line a behavioral trial. Note how the number of spikes around movement onset is modulated by movement direction. B) Cosine tuning curve fitted to the mean firing rates of the neuron in each movement direction, as illustrated in A. The preferred direction of this neuron is ~160°. C) Population vector. The preferred directions of each of 241 neurons are plotted in the eight movement directions (thin solid lines), the length of the lines indicating the firing rate of the neuron in one movement direction. Computing the vector sum of each preferred direction gives the population vector (indicated by the thick dashed arrows in each of the 8 distributions of preferred directions), which points in the direction of the actual movement. Reprinted and modified from Georgopoulos et al. 1982, 1983.

However, there are some evidences that the preferred directions could be spatially organized at the microscopic level. When penetrating the cortical surface vertically, the recorded neuronal PDs tend to be similar. However, when the penetration becomes tangential, the variability of PDs increases (Georgopoulos et

al. 1984). This topic was directly tackled recently. By re-analyzing the data from Schwartz et al. (1988), recorded during a 3D reaching task, Amirikian et al. (2003) showed that the PDs tended to be spatially organized, forming columns of similar PDs, 500µm long and 50-100µm large, spaced by 150-200µm between each other. In a follow-up study, Georgopoulos et al. (2007) assessed the spatial periodicity of the PD similarity across the cortical surface of M1. They showed that similar PDs emerged every 30, 60, 86 and 240µm. They interpreted the result as reflecting the existence of mini-columns 30µm in diameter, clustered by 2 or 3, and spaced by about 240µm. In other words, the motor cortex would be organized in super-columns of 120 µm of diameter. They thus made two predictions, that these "super-columns" should contain all possible PDs spanning all possible spherical angles, and that the difference of PDs between neurons should increase with distance, but only up to 120µm. Both predictions were validated. Thus, according to this model, each super-column should contain 64 mini-columns, each of them spanning ~6 degrees of movement angle. These results would tend to show that the motor cortex has a functional columnar organization, following the general organizational principle proposed by Mountcastle (1979; 1997). Recent data suggested that the directionality of neurons (of the rat barrel cortex) could have its origin in inputs from the thalamus, showing a non-linear summation of post-synaptic potentials only when the whisker are deflected in given orientations (Lavzin et al. 2012). Inputs from the thalamus tend to penetrate vertically in the motor cortex: thus, this common innervation by the same thalamic projection on all neurons arranged vertically would lead to the same directional preference, giving an anatomical ground to Georgopoulos' mini-columns. However, anatomical data of the microstructure of the motor cortex does not completely support these results (reviewed in Hepp-Reymond 1988): the horizontal spread of Betz cells dendrites and collaterals expands up to 3mm, and low-intensity microstimulation of few neurons lead to potentials elicited monosynaptically up to 400µm from the stimulation sites. The horizontal connections of the motor cortex seem thus to spread farther than the proposed super-columns of Georgopoulos.

1.2.1.2. Movement-related potential (MRP) of the LFPs

When performing a reaching movement, the LFPs show a typical, multiphasic modulation of amplitude, made of 3-4 successive components (P for positive, N for negative). The first component P1 precedes the movement onset, the 2nd component N1 peaks at movement onset, then the P2 and, eventually, N2 components follows it (Donchin et al. 2001; Roux et al. 2006). Like firing rates of single neurons, the amplitude of the MRP was shown to be modulated by movement direction (Donchin et al. 2001; Cardoso de Oliveira et al. 2001; Mehring et al. 2003; Rickert et al. 2005; O'Leary & Hatsopoulos 2006; Asher et al. 2007), following a cosine tuning curve (Rickert et al. 2005). However, all MRP components were not equally modulated by

movement directions. Unlike the three other components, Rickert et al. (2005) found that P1 was not tuned to movement direction, even if this feature may be related to the task that the monkey was performing (see 3.4.3). One question arises: since the LFP reflects the summed activity of neurons around the electrode tip, and the distribution of the neurons' PDs is close to homogeneous, how can we explain that a LFP MRP is tuned to one particular movement direction? It would be tempting to consider that the clustering of neuronal PDs at the microscopic level (see above) lead to a tuned signal when averaged locally. However, several elements impede this interpretation. First, all PDs are supposed to be present in the hypothesized cortical super-columns (Georgopoulos et al. 2007), which have the same size (240µm in diameter) as the lower boundaries of the cortical surface supposedly determining the tuning of a LFP (250µm, see Katzner et al. 2010). If all PDs are equally represented, the averaged activity should be unbiased. Second, in parietal cortex, Asher et al. (2007) show that the tuning of the single neuron spiking activity and that of the EPs of the LFP recorded on the same electrode are not the same (even though this relationship can change for the oscillatory activity, see also Spinks et al. 2008). Third, studies have shown that both LFPs and neurons are tuned for the shape of the hand during a grasping task (Asher et al. 2007; Mollazadeh et al. 2011). However, no clustering of the preference for grip shape has been observed (Townsend et al. 2011; Brochier, personal communication). In other words, a selectivity of the MRP for a behavioral parameter can exist without an underlying clustering of the neurons selective for this parameter. In summary, the hypothetical clustering of the neuron's PD is not be sufficient to explain the tuning of the LFP MRP. Another hypothesis would be that even though local populations of neurons have homogeneously distributed PDs, some sub-populations fire more or more synchronously, biasing the averaged activity toward their own PD.

1.2.1.3. Which movement parameter is represented in motor cortex?

Movement direction has received a large attention since Georgopoulos' seminal work. However, recent studies challenge this notion and show that other parameters of the movement may be encoded better by motor cortical neurons. For example, Hatsopoulos et al. (2007) showed that decoding fragments of trajectories instead of linear movement directions lead to a better decoding of the movement trajectory, with a better accuracy and less decoding errors. The problem with the question about parameter representation is that these parameters are highly correlated with each other. For example, the acceleration of the hand movement will also provoke a change in hand position, direction and of movement velocity. Stark et al. (2009) managed to dissociate these parameters using a "scribbling" task, in which the lag of these three parameters was systematically varied. They showed that 2/3 of M1 neurons were correlated with only one parameter, in the vast majority it was velocity. Furthermore, they found that neurons recorded on the same site were likely to

represent the same movement parameter, and to share the same preferences of kinematic values (e.g. preferred direction). Their work, thus, confirm the existence of functional columns in M1. These two studies (Hatsopoulos et al. 2007; Stark et al. 2009) show that individual neurons can encode different aspects of the movement kinematics in an external, cartesian reference frame, independent of the effector. However, a review by Kalaska (2009) points out that an extrinsic reference frame alone cannot account for the activity of all motor cortical neurons. Indeed, even though some neurons encode the movement regardless of the state of the effector (e.g. Kakei et al. 1999, 2001; Wu & Hatsopoulos 2006, 2007), others are modulated by several intrinsic parameters (i.e. related to the state of the effector). Arm posture (Scott et al. 1997; Kakei et al. 1999, 2001, 2003), shoulder angle (Caminiti et al. 1990; Wu & Hatsopoulos 2006, 2007), a combination of joint angles (Wu & Hatsopoulos 2006, 2007) or force output (Evarts et al. 1983; Kalaska et al. 1989; Georgopoulos et al. 1992; Sergio et al. 2003, 2005) were all shown to modulate the activity of motor cortical neurons. However, the distribution of the neurons tuned to different movement parameters is not completely homogeneous. Most of the neurons responding to intrinsic properties of the movement are found in the sulcus of M1 (load, arm posture), whereas neurons seemingly representing the movement in an extrinsic frame of reference are more located in the rostral M1 and the premotor cortex (reviewed in Kalaska 2009; see also Wu & Hatsopoulos 2006, 2007). Finally, neurons representing extrinsic and intrinsic parameters of the movement were often simultaneously reported in the same area, supporting the idea that movements are not represented in a single reference frame (Kakei et al. 2003; Wu & Hatsopoulos 2006, 2007). Alternatively, Kalaska (2009) supports a novel approach of behavioral control, closer to engineering concepts. This new concept, described by the "optimal feedback control" theory (Todorov & Jordan 2002), postulates that the key factor is the reduction of the motor error between a noisy feedback signal and the prediction of a desired state.

1.2.2. Effect of delay and prior information

We previously saw that providing prior information about the direction of a movement strongly reduces the RT. This reduction of the "event uncertainty" (Requin et al. 1991) allows to evaluate which aspect of the movement can be prepared in advance, to save time after GO signal occurrence, i.e., during RT. In parallel to modulations of the activity observed during movement execution, two patterns of activity can be observed during the delay separating the cue from the GO. The first one is the "signal-related activity" (Weinrich et al. 1984), which is a phasic, short lasting burst of spikes, starting with a short latency (<200ms) after the informative cue (Weinrich et al. 1984; Riehle 1991). The principle of this activity pattern is illustrated in Fig. 1.6B. The second is a tonic activity (Fig. 1.6A), which can start at various latencies after the cue, and usually lasts until movement onset ("delay-activity", or "set-related activity", Wise & Mauritz 1985).

These three patterns of activity, signal-, delay-, and execution-related activity, can be found in any combination (for instance, a signal- and execution-related neuron, with a phasic activity after the cue and during movement) and in all motor cortical areas (Riehle 2005). However, these patterns are found in different proportions along a rostro-caudial gradient (Weinrich et al. 1982, 1984; Crammond & Kalaska 1996, 2000; Johnson et al. 1999; Riehle 2005). Whereas "pure execution-related" patterns are more often observed in the sulcus of M1, delay- and signal-related activity are much more common in PMd (the bank of M1 being intermediate). Additionally, the ratio of neurons whose activity during movement execution is modulated by prior information follows a caudo-rostral increasing gradient (Crammond & Kalaska 2000). I will first discuss the general effect of prior information on the delay activity, before focusing on the signal-related activity, which is important for the two main chapters of this thesis.

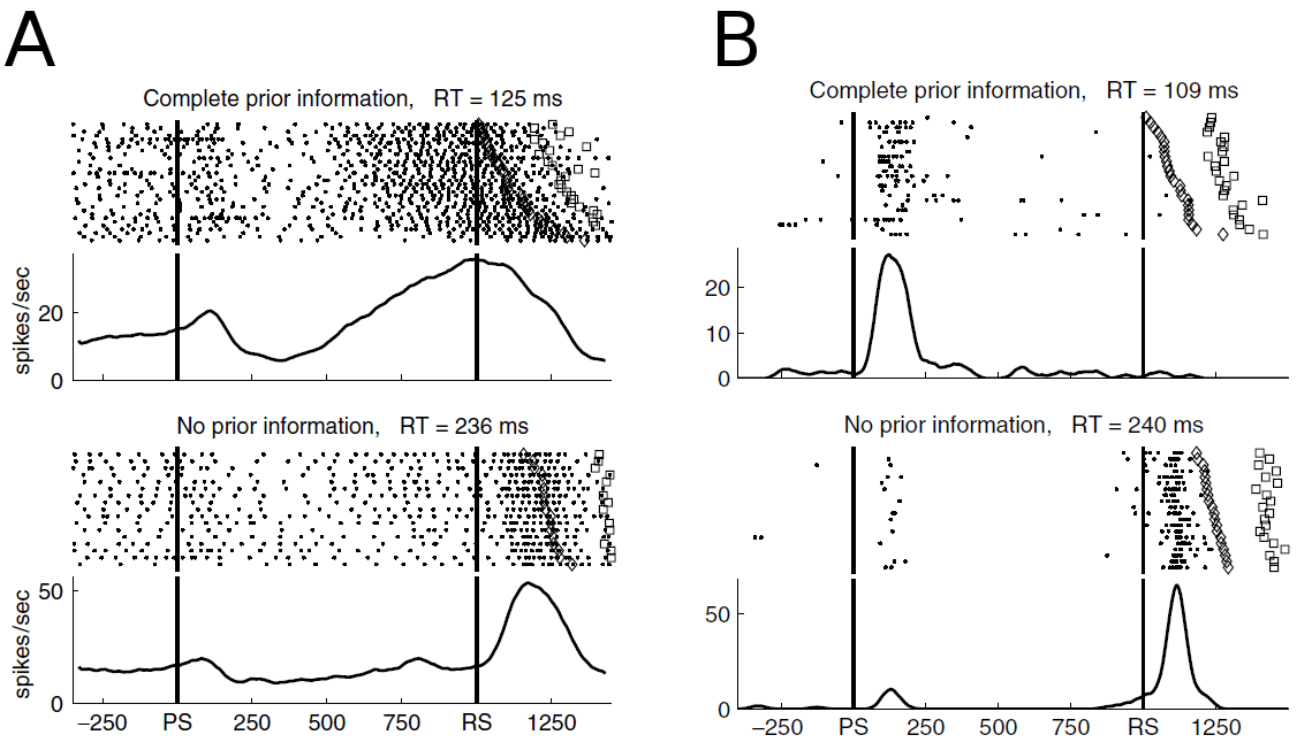


Figure 1.6. Effect of prior information about movement direction. The top of each plot represents the raster plot of the spiking activity, (each dot is a spike, each line a behavioral trial, the diamonds represent the timing of movement onset, the squares represent the end of the movement). The bottom of each plot represent the smoothed firing rate (in spikes/second). Abscissa is in millisecond, 0 is PS (preparatory signal), RS is the response (GO) signal. A and B are different neurons. Top panels: PS carries complete information about movement direction. Bottom panel: PS carries no information about movement direction. Note the difference in RT between the conditions. A) Neuron with delay- and execution-related activity. The firing rate at movement onset does not change with prior information, but a ramping activity starts to grow ~400ms before movement onset. B) Neuron with execution- and signal-related activity. Note the phasic response only after the RS in the bottom panel and only after the PS in the top panel. Reproduced from Riehle 2005.

The execution- and delay-related activity can be selective to the information provided by the cue (especially movement direction), whereas other information such as extent or frictional force does not or only weakly elicit selective activity changes (Riehle et al. 1989, 1994, 1995; Kurata 1993; Riehle 2005). In parallel, prior information about direction strongly reduces the RT, whereas knowing the force or the extent has nearly no effect on the RT (Riehle & Requin 1989, 1995; Riehle 2005). In a reach-to-grasp task, prior information about the grip type (precision or side grip) strongly reduces the RT and leads to a grip-type selectivity during the delay, whereas prior information about force only weakly influences the RT and does not induce selective changes in activity during the delay (Brochier & Riehle 2011). These two sets of data go in the same direction, whenever useful information is provided (like the end-point of a movement, or the shape of the hand), the movement can be prepared in advance, expressed both in a reduction of RT and a selective delay- and execution-related activity. In the case of movement direction or grip shape, prior information will lead to the recruitment of specific effectors. For instance, pointing leftward or rightward involves opposite muscle groups. By contrast, frictional force, extent or grip force mainly concern the level of contraction of the same effectors. For instance, to perform a forward reaching movement, a weak contraction of the triceps will lead to a small extent, whereas a strong contraction of the same muscle will lead to a large extent. In other words, prior information about the movement parameters leading to a large reduction in RT (and a deep modulation of motor cortical activity) are the ones specifying which muscles will be recruited. If this information is lacking, knowing the degree of contraction will not help to prepare the movement. This interpretation (Riehle et al. 1994) is supported by the fact that information about load or extent lead to selective activity, but only if the direction of the movement is specified in parallel (Riehle 2005). Furthermore, information about the gain of a given set of muscles (force, extent) can be mostly specified online, i.e. during movement execution. Accordingly, the advantage in RT gained by prior information about force or extent is very weak (~10ms for force, ~30ms about extent in Riehle et al. 1994, compared to ~80ms for direction in Riehle et al. 1989)

However, prior information is not represented in the same way across the whole motor cortex. When a reaching movement is performed 90 degrees away from the cue, the population vector in M1 during the delay first points in the direction of the cue, but very quickly rotates in direction of the intended movement (Georgopoulos et al. 1989; Shen & Alexander 1997a). In contrast, the neurons from PMd mainly continue to point in the direction of the target throughout the delay (Wise et al. 1996; Shen & Alexander 1997b; Schwartz et al. 2004). These differences likely reflect the different levels of visuomotor transformation. The directional preparation is not necessarily related to the preparation of one precise movement, but could instead be involved in the general reduction of the range of possible movements. Bastian et al. (2003) showed that reducing the uncertainty about the direction of a forthcoming movement (by precueing 3, 2 or 1 target(s) out

of 6) reduced the RT accordingly. In parallel, they showed that the "distribution of population activation" (a higher-order version of the population vector in which the preferred direction is replaced by the tuning curve) covered the pre-cued targets, and that its amplitude increased along the delay (Fig. 1.7). The more precise was the directional information, the narrower was the field of activation (see also Rickert et al. 2009). This pre-activation can even take place for non-adjacent targets (Cisek & Kalaska 2005), leading the authors to develop the idea that the premotor cortex is involved in a parieto-frontal network generating potential actions, the "affordance competition theory" (Cisek 2007; Cisek & Kalaska 2010).

In a large proportion of neurons (for example ~40% in Riehle 2005), the delay spiking activity recorded shortly before the GO signal is negatively correlated trial-by-trial with RT (Riehle 2005), the higher the firing rate, the shorter the RT. By contrast, when the firing rate is analyzed during RT between the GO signal and movement initiation (in a time window aligned on movement onset), the number of significantly correlated neurons drops, and the mean coefficient of correlation becomes slightly positive. Other studies have tried to decode the timing of movement initiation based on the firing rate of motor cortical neurons. For example, Lebedev et al. (2008) showed that the timing of a self-initiated movement was well correlated with the slope of the firing rate. However, it could be possible that the relationship between timing of movement and firing rate depends on the details of the task, like the presence or absence of a GO signal, or the ability to predict its timing of occurrence. This topic is described in further details in the chapter 3. Furthermore, the proportion of correlated neurons seems to depend on the ability of the animal to prepare the movement in advance. When prior information about movement direction has been provided (resulting in a large decrease in RT), the proportion of neurons showing a significant correlation with RT is large. By contrast, when a priori information about force or extent (only leading to a weak decrease in RT), or no prior information is provided, a low proportion of neurons show a correlation with RT (Riehle & Requin 1993). It is interesting to note that the correlated neurons do not necessarily modulate their activity during the instructed delay. Instead, it seems that when useful information for the preparation of a movement is available, the neuronal activity becomes more predictive of the animal's performance.

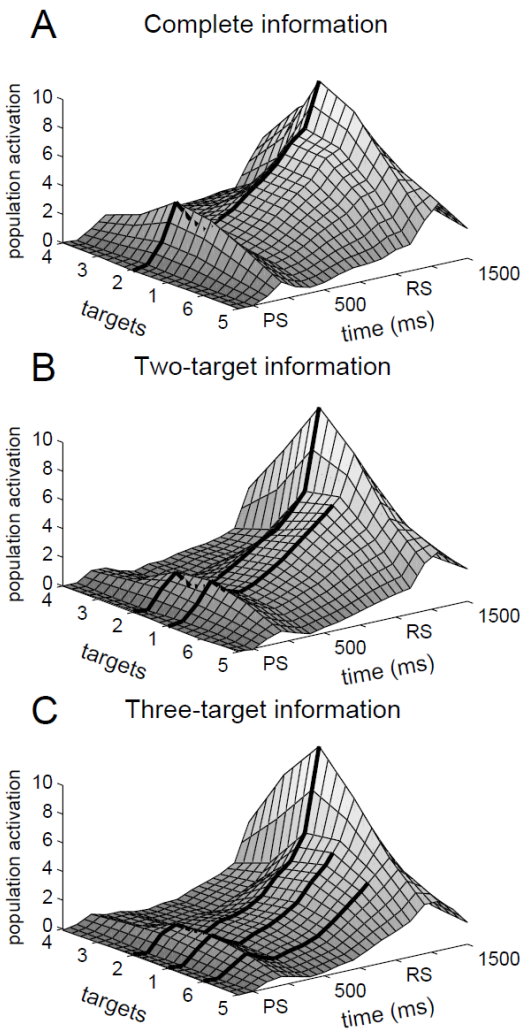


Figure 1.7: Preactivation of the motor cortex with incomplete prior information. The z-axis represents the unit-less population activation (a kind of higher-order population vector, see Bastian et al. 2003), the x-axis represents the time (in ms) and the y-axis the target numbers (from 1 to 6) arranged in a circle around the starting target. As in Fig. 1.6, PS is the preparatory signal ("cue") providing variable spatial information and RS is the response signal ("GO") requesting the motor response. Thick lines represent the cued directions indicated by the PS. A) PS carries complete directional information. B) PS provides partial directional information about 2 cues. C) PS provides partial directional information about 3 cues. Note that the population activation peaks around the cued target(s), its amplitude being function of the amount directional information. When RS provides complete information (direction 2), the population activation concentrates around it. Note also the first "bump" of activation following PS, related to the signal-related activity, followed by the gradual increase. Reproduced from Bastian et al. 2003.

However, the delay activity is not “purely” motor. As pointed out by Marc Schieber (2011), even though the activity of the motor cortex during an instructed delay may be elevated, the muscular activity can stay completely flat. In a task in which target position is dissociated from movement direction, M1 neurons can be selective for either the target position or the movement direction during the delay (Riehle et al. 1997b; Georgopoulos et al. 1989; Shen & Alexander 1997a), or have a different preferred direction than during movement execution itself (Wise et al. 1996). Furthermore, it has been shown that the delay activity of the premotor cortex (Wallis & Miller 2003; Nakayama 2008; Yamagata et al. 2009, 2012) and of the motor cortex (Riehle et al. 1997b) could be selective for complex instructions, such as the relative position of two stimuli regardless of their absolute position (Nakayama 2008, Yamagata et al. 2009, 2012), stimulus-response compatibility (Riehle et al. 1997b), or a conditional match-to-sample between two successive stimuli (Wallis & Miller, 2003). It is interesting to see that these "non-standard" (Wise et al. 1996) instructions were usually more represented and with a shorter latency in PMd than in the prefrontal areas, traditionally considered to be

involved in higher cognitive function (Wallis & Miller 2003; Yamagata et al. 2012). These results tend to comfort the idea that, at least in the context of overtrained tasks, the motor areas are part of a gradual action selection network (Cisek 2007).

As mentioned above, the onset of an informative cue about a forthcoming movement elicits a phasic, short-latency response in PMd and rostral part of M1, even if the precise differences in this repartition are not clear (Weinrich et al. 1984; Riehle 1991; Riehle & Requin 1995; Crammond & Kalaska 1996 2000; Cisek & Kalaska 2005). As a consequence, most of the studies which addressed this phenomenon focused on PMd. This phasic response is not evoked by any visual signal, as many studies reported that a directionally non-informative warning cue does not elicit any phasic response in the motor cortical neurons (e.g. chapter 4, Weinrich et al. 1984; di Pellegrino & Wise 1993a), nor that it leads to motor cortical VEPs of the LFP (see chapter 3 and Kilavik et al. 2010). The Fig.1.6B is a good example by showing that the activity elicited by the non-informative PS (bottom row) is very low. The modality of the cue does not seem to matter, since auditory and visual cues were as efficient to elicit signal-related directional responses (Vaadia et al. 1986). Roux et al. (2003) even showed that the absence of an expected cue leads to a phasic response. The cue itself does not need to be presented at particular spatial position, a central symbolic cue carrying the directional information elicits in the same way a phasic response (Hoshi & Tanji 2004, 2006; Nakayama 2008; Yamagata et al. 2009, 2012), even though with a longer latency than for a non-symbolic spatial cue (Cisek & Kalaska 2005; Yamagata et al. 2009). In summary, virtually all cues providing information about the direction or the spatial position of a movement target elicits a phasic response in a substantial proportion of motor cortical neurons.

This phasic response may be related to the movement target, or alternatively to a shift of spatial attention. Indeed, we usually pay attention to the item we want to act upon before actually starting the movement. However, a large body of evidence supports the idea that this activity is related to the movement itself. The first element is shown in Fig. 1.6B, and was described by Crammond & Kalaska (1996). Neurons with a phasic response during the RT usually also show a phasic response after the presentation of a spatial cue. Furthermore, these neurons are the ones with the strongest decrease of execution-related activity when comparing the conditions of non-instructed and instructed delay (Crammond & Kalaska 2000; "pre-processing neurons" of Riehle & Requin 1989; Riehle 2005). This phasic response even completely disappears if the monkey knows the movement target before the beginning of the trial (Crammond & Kalaska 2000). Wise and colleagues directly tackled the issue of whether the signal-related activity represents a shift of spatial attention or a movement target (Boussaoud & Wise 1993a,b; di Pellegrino & Wise 1993a,b; reviewed by Kurata 1994 and Wise et al. 1996). A very robust finding in these studies is that depending on their "meaning", two identical

cues elicit dramatically different neuronal responses. Systematically, the neurons in PMd were much more responsive (and selective) to cues indicating the target of a movement than to cues indicating the target of a shift in attentional focus. Other studies showed that, unlike other frontal areas, the signal-related activity in PMd reflects more the planned action than the characteristics of the cue itself (shape, color, location) (Hoshi & Tanji 2006; Yamagata et al. 2012). These studies tend to support the idea that the signal-related activity represents an early processing of the movement parameters.

After the presentation of a visual stimulus carrying movement information, an evoked potential of the LFP, the VEP, can be observed in many cortical areas (Ledberg et al. 2007). Its latency in motor cortical areas is less than 20ms longer than that in the early visual areas. Such a short latency "motor" visual response is not only specific to the LFPs, but can also be seen in the spiking activity. The earliest spiking response to a visual stimulus in the primary visual cortex (V1) is about 30 ms (for an average of 80ms; Raiguel et al. 1989; Nowak et al. 1995), whereas in PMd it is about 60ms (av: 140ms) in Weinrich et al. (1984) and 50ms (av: 96ms) in Riehle (1991). In M1 the latency is slightly longer, it is 60ms for the shortest, with an average of 137ms (Riehle 1991). With such short latencies, it is unlikely that all parameters of the movement have already been encoded, especially when the task requires the monkey to do complex cognitive operations (symbolic cues, GO/NoGO task, etc...). Instead, the signal-related activity could actually reflect the first, preliminary information concerning the targets of potential movements, which could simply be an alert signal. This preliminary information would then be refined, as more complex processing of the cue unfolds. First, most reaching movements that we perform are toward the location of visual information, so an automatic planning of movements toward spatial cues (which would be modified afterwards if needed) seems to be reasonable. This holds especially for monkeys, for whom it seems difficult to withhold a movement toward external stimuli presented during a delay. Several evidences support this idea. In both M1 and PMd, the vast majority of the neurons' initial phasic response to a cue is selective to the direction of the target, not the direction of the movement (Riehle et al. 1997b; Shen & Alexander 1997a,b). Additionally, the early signal-related activity seems relatively unaffected by the condition of a GO/noGO task, unlike the later delay activity (Wise et al. 1983; Miller et al. 1992). It thus seems that when a simple (peripheral) spatial cue is presented, its processing will be rather stereotypical and relatively unaffected by the behavioral context (e.g. complex instructions like GO/noGO). This automatic processing could represent the triggering of a default motor program, i.e. reaching toward the cue. By contrast, the response to symbolic, non-spatial cues will elicit a response with much longer latencies than simple spatial cues (Cisek & Kalaska 2005; Yamagata et al. 2009). Additionally, the distributions of the preferred directions of the phasic responses elicited by these two types of cues are different. The preferred directions of signal-related responses to symbolic cues are homogeneously distributed (Hoshi & Tanji 2006; Yamagata et al.

2009), whereas responses to simple spatial cues are biased toward contralateral targets (Vaadia et al. 1986; Hoshi & Tanji 2006; Yamagata et al. 2009, but see also chapter 3). This last result is congruent with the observation of Sawagushi et al. (1996), who showed that when blocking chemically the inhibition of the motor cortex (by an injection of bicuculline methiodide, a GABA-A antagonist), visual warning signals tend to trigger automatic contralateral reaching movements, shortly preceded by a phasic discharge of the motor cortical neurons. In summary, it seems that simple spatial cues are processed in a different way than symbolic, non-spatial cues. The former tend to activate an automatic response toward the cue, modified afterwards after further processing of the task instructions, whereas the latter, per nature non-directional, do not trigger this automatic response. The fact that partial information about movement direction also elicits a phasic response (although weaker than for complete directional information) supports this interpretation (Bastian et al. 2003; Cisek & Kalaska 2005).

1.3. Attention and anticipation

1.3.1. Introduction.

"Increasingly, the brain reveals itself proactive in its interface with external reality. In the past, our conception of the brain changed from that of a mirror to that of an interpreter. Several current lines of research—in fields such as memory, motivation and attentional orienting—now begin to cast the brain as a predictor. The results of experience are integrated over various timescales in order to anticipate events relevant to the current task goals and motivational state of the individual and to tune the relevant perceptual and motor machinery accordingly. In particular, research on attentional orienting has shown how signals coding predictions about the location, identity or simple features of relevant events can influence several stages of neural processing (Reynolds & Chelazzi 2004; Maunsell & Treue 2006). Recent evidence shows that these predictions are not restricted simply to the contents of events but also extend to their anticipated timing" (Nobre et al. 2007).

Indeed, movement preparation is not an isolated process, taking place separately from the rest of cognition. The performance of any movement is done under a particular behavioral or environmental context, and any context implies making predictions based on that context or on indirect consequences of our action. Thus, preparing a movement is embedded in processes related to predicting and anticipating external events (Kveraga et al. 2007), part of a complex anticipation-perception-action network, all steps tightly linked to each other (Cisek 2007). Among the several ways to anticipate an event, the most studied is spatial attention. By specifically directing our attention to one part of space, we become more efficient at processing the information provided there (Posner 1980). This spatial orientation of attention can be in the visual domain

(Reynolds & Chelazzi 2004), but also in all other modalities in which space makes sense (for example, left versus right hand in somatosensory perception, e.g., Van Ede et al. 2011, 2012a,b). Another aspect of visual attention may concern a feature of the stimulus (color, shape, etc...). By paying attention to one dimension of a stimulus, its processing will be improved in the whole visual field (Maunsell & Treue 2006).

However, attention can also be directed in time ("temporal orienting"), thus improving the ability to perceive a cue at a given moment in time or shorten the time it takes to generate a motor response (Griffin et al. 2001, 2002; Correa et al. 2004; Rolke & Hofmann 2007; Rohenkohl et al. 2012). Even though we do not always realize it, all our actions are embedded in a temporal context, which we use to time our actions and predict when useful information will be available. For example, the visual motion of a tennis ball will allow me to predict when it will enter my reach, and thus to start my backhand at the exact moment. In this case, timing is extracted from visual flow. In other situations, timing can be endogenously produced. If I am in my car, waiting for the red traffic light to turn green, I use my previous experiences of this and other traffic lights to form a prediction of when I will (finally) be allowed to go. In this case, the temporal context learned by experience ("traffic lights: more than 20 second and less than 2 minutes") shapes the distribution of the conditional probabilities ("hazard rate") that the light changes color, in turn modulating my readiness to step on the gas. In summary, we can fairly say that timing, in one form or another, influences every aspect of our behavior. However, as to now, temporal attention has received much less attention (no pun intended) than its spatial counterpart. Nevertheless, a solid body of evidences has been gathered on the behavioral and physiological effects of temporal orienting (Nobre 2001; Nobre et al. 2007; Coull & Nobre 2008), even though it is mainly restricted to the study of human participants. The next part of the introduction will be devoted to the anticipatory processes that allow us to act proactively, predicting when and where important events have to be processed or acted upon. I will first present visual spatial and feature based attention, as an example of a well-studied system of information selection. Then, I will focus on the main topic of this thesis, how timing is reflected in the brain, and specifically how it influences cue processing and movement preparation.

1.3.2. Visual attention

1.3.2.1. Spatial attention

To understand the effect of visual attention on the activity of the neurons in visual areas, one has to understand the general functional properties of these neurons. First, neurons from the visual areas have spatial receptive fields (RF), which are the zone within the visual field in which they react to stimulation. The size, shape and specificity of these RFs change somewhat according to the area (for example, neurons from V4 have receptive fields that are larger than the ones from V1). Second, they are selective to certain characteristics of

the stimulus, which varies to a certain degree with the area of recording (orientation in most of the visual areas, but also speed in MT, color in V4...). Third, they are characterized by a contrast-response function, a sigmoid function indicating the sensitivity of the neuron to a change of luminance (Fig. 1.8). Typically, low contrasts lead to very small responses, and high contrasts lead to large (saturated) responses (the two extreme flat parts in the curve of Fig. 1.8). However, for the contrasts comprised in the "dynamic range" of the neuron, a small difference of contrast will lead to a large difference in activity (the steep part of the curve). Fourth, for neurons with a large RF (as in V4), when displaying several stimuli inside the same RF, the amplitude of the initial response will correspond to the average of the responses to the two individual stimuli.

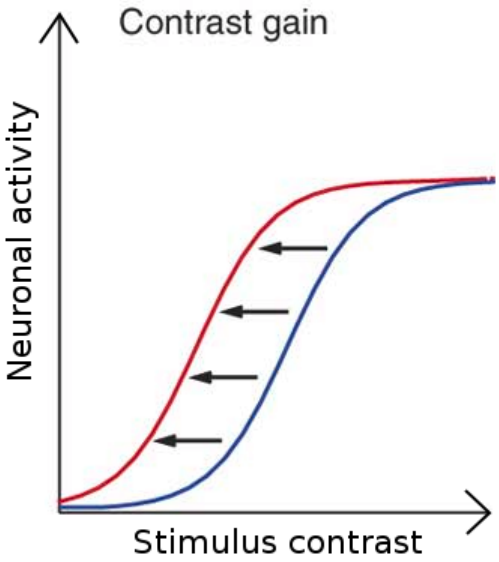


Figure 1.8: Effect of attention on the contrast-response function of a visual neuron. The blue curve represents the contrast-response function of a neuron when attention is directed outside of its RF, and the red curve the response of the same neuron when attention is directed toward its RF. Black arrows represent the effect of attention. On the abscissa, contrast increases towards the right. Modified from Herrmann et al. (2010).

Reynolds & Chelazzi (2004) reviewed studies addressing the effect of spatial attention on the single neurons of the visual areas. As Fig. 1.8 shows, the main effect of attention is to shift the contrast-response function of a neuron toward lower contrast values, for grating stimuli. The gain of its dynamic range (i.e. its slope) is unchanged, but low-contrast stimuli, which elicit very weak responses when unattended, are "shifted" inside the boundaries of the neuron's dynamic range, provoking a stronger response. In other words, the effect of spatial attention would be to increase the “effective contrast” (i.e. the perceived contrast) of the attended stimulus by 50% (Reynolds et al. 2000), without changing the gain or the directional tuning of the neuron (McAdams & Maunsell 1999). This mechanism acts by a multiplicative gain. By shifting the dynamic range of a neuron to lower values (black arrows in Fig. 1.8), small differences of contrast become magnified and the discriminability of the stimulus is improved for lower contrasts. It has been proposed that this mechanism could bias the response of a neuron toward the attended stimulus in case of two competing stimuli inside the

same RF (‘biased competition theory of attention’; Desimone & Duncan 1995). Even though the initial response of a neuron is closer to the average of the response to the two stimuli, the late component (~180ms) is often driven by the response to the attended stimulus (Luck et al. 1997; Chelazzi et al. 2001). Additionally, attending to a stimulus presented in the RF of a neuron from V2 or V4 increases its spontaneous firing rate by ~30% (Luck et al. 1997). This increase of effective contrast sensitivity is also reflected in the population activity. Bosman et al. (2012) showed that gamma oscillations (~40Hz) of LFPs recorded in V1 had a slightly higher frequency when the attended stimulus was presented in its RF. Higher frequency of gamma oscillations are also linked to presentation of stimuli with higher contrast (Ray & Maunsell 2012).

More generally, spatial attention and gamma oscillations of the LFPs in visual areas are tightly linked. The neuronal activity shows systematically an increase in the gamma power and a decrease of the beta power of the LFP when the stimulus presented in its RF is attended, whereas it is the opposite for an unattended stimulus (Fries et al. 2001). Furthermore, there is a temporal relationship between gamma oscillations and changes in spiking activity with attention (Fries et al. 2001; Womelsdorf et al. 2006), with the spikes becoming more tightly phase-locked to the gamma oscillation when the stimulus presented in its RF is attended. This phase-locking has a behavioral counterpart. In a task of visual discrimination, trials with the shortest RT were accompanied with a better phase-locking when the stimulus is attended. By contrast, if a better phase-locking is observed in the RF in which a distracter is displayed, the RTs are much slower (Womelsdorf et al. 2006). In parallel, spatial attention specifically increases the coherence of the gamma oscillations between areas. The gamma oscillations in V1 and V4 will show a peak in the gamma coherence, only if attention is directed to their common RF (Bosman et al. 2012; Grothe et al. 2012). These data are coherent with the hypothesis of "communication through coherence" (Engel et al. 2001; Fries 2005; Womelsdorf et al. 2007; but see Ray & Maunsell 2010 for a counterargument). One idea is that gamma oscillations would reflect the degree excitability of a brain area. Thus, if spatial attention increases the phase locking of spikes to the gamma oscillations and the coherence of the oscillations between areas, then the spikes emitted in a phase of an elevated excitability in one area will be more "efficient" to induce a response in the other. By tweaking the coherence of the oscillations between different areas of the brain, one could influence the efficiency of the communication between them (Womelsdorf et al. 2007). In this context, one of the effects of spatial attention would be to modulate this communication. However, according to this theory, the LFP oscillations are a by-product of the neuronal computations, and do not have a causal influence on them. Yet, as reviewed by Buzsáki et al. (2012), the LFPs could slightly modulate the excitability of the nearby neurons by an "ephaptic effect". Indeed, the synchronized activity of a large number of neurons could modulate the extracellular voltage, in a similar extent that that obtained with low-frequency magnetic transcranial stimulation. This

technique was shown to significantly modulate the firing rate of targeted areas, as well as behavioral performances. Thus, one can imagine that the spatio-temporal fluctuations of the LFPs could causally influence the excitability of the membrane of nearby neurons.

Another aspect of visual spatial attention on the neuronal population activity was recently demonstrated by Cohen & Maunsell (2009). By recording simultaneously the activity of neurons in V4 of both hemispheres, they could compare the effect of attending to one visual hemifield or the other. They showed that attending to a stimulus presented in the RFs of the neurons of one hemisphere leads to a drop in the trial-by-trial positive correlation of their firing rates (so-called "noise correlation"). This decrease of noise correlation depended on the neuron's own selectivity. Neurons with a large increase of firing rate to the stimulus presentation showed the stronger decrease of noise correlation, whereas neurons with a weak response to the stimulus showed virtually no decrease of noise correlation. The authors interpreted this result as reflecting the removal of a common inhibitory source to all neurons. Attention would decrease the influence of this common inhibition (thus decreasing correlation), especially for the neurons involved in the processing of the cue (the ones with a large response).

1.3.2.2. Feature-based attention

Feature-based attention is a mechanism by which the visual percept can be "filtered" in order to make some features (color, orientation...) more salient, while suppressing the others. This enhancement of the attended feature leads to a better processing of the stimulus (Rossi & Paradiso 1995). Maunsell & Treue (2006) reviewed the effect on the feature-based attention on the activity of the single neurons of the visual areas. In V4, most of the neurons show a stronger discharge if the stimulus that appears in their RF matches a sample stimulus presented before (Motter 1994a,b, Chelazzi et al. 2001). It is important to note that this increase of activity for a matching stimulus is not necessarily associated with a conscious recognition of the sample stimulus. Even overlooked stimuli lead to an increased response if they matched a previous sample, compared to distracters (Bichot et al. 2005). This enhanced response was accompanied by an increased phase-locking between the spikes and the LFP gamma oscillations, only if the searched stimulus corresponded to the preferred stimulus of the locus of recording (Bichot et al. 2005). This phenomenon is similar to what was shown by Fries et al. (2001) for spatial attention. Feature-based attention is not restricted to a specific part of the visual field. McAdams & Maunsell (2000) trained monkeys to attend to either the color or the orientation of a stimulus well away from the RF of the neuron they were recording (in V4), while displaying a distracter inside its RF. The attended feature modulated the response to the distracter in the vast majority of the neurons, even though the stimulus itself did not fall inside their RF. This result was also found in area MT, with the activity of neurons differently

modulated according to the direction of movement of an attended stimulus outside of the RF (Treue & Martinez-Trujillo 1999). Furthermore, this modulation of activity depends on the directional tuning of the neuron, with a further enhancement of the firing rate in its preferred direction, leading to a multiplicative gain. In this aspect again, feature-based and spatial attention share similarities. Cohen & Maunsell (2011) showed another similarity. In a task of feature-based attention, in which the monkey had to attend to either the orientation or spatial frequency of a stimulus, they observed that the drop of noise correlation with attention was larger for neurons having a preference matching the attended stimulus (large response) than for the other (low response). This result is similar to the one they observed in spatial attention (Cohen & Maunsell 2009). Furthermore, they developed a technique allowing estimating "attentional fluctuations" of the monkey based on its neuronal activity (Cohen & Maunsell 2010, 2011), allowing predicting the RT of the animal on a single trial basis. They showed that both spatial- and feature-based attentional fluctuations could be predicted in the same way using this technique. All these similarities between the two types of attention tend to support a unified model of attention. In this perspective, Treue & Martinez-Trujillo (1999) developed the "feature-similarity gain model", proposing that the gain of activity of each neuron would depend on the similarity between the attended stimulus and its own "preference". This preference can be a color or orientation (like in V4) or the direction of a movement (as in MT), leading to the effect seen with feature-based attention. However, the similarity can also be spatial, leading to spatial attention. In this theoretical frame, spatial attention would just be a type of feature attention based on location.

In summary, visual attention seems to increase the discriminability of stimuli by means of a multiplicative gain. This influence is specifically strong in the neurons potentially useful to the processing of the stimulus (either because they share the same RF or the same preference for a specific feature). This phenomenon is accompanied by the decrease of a common inhibitory influence specifically for this sub-population of neurons. Additionally, attention could affect the efficiency of inter-areal communication by modulating the phase-locking of spikes and the coherence of LFP oscillations across areas.

However, feature-based visual attention is another example of how our conceptions of cognitive processes are biased by the experimental paradigms used to study them. Indeed, most of the studies described above used visual search task. In a study of Mirabella et al. (2007), a monkey had to pay attention to either the color or the shape of a single object in order to give a motor directional response with its hand, while the activity of area V4 was recorded. They showed that paying attention to e.g. the color of the object did not enhance the selectivity to color of V4 neurons. Instead, the late part of the response to the stimuli depended on the movement direction that was associated with the stimulus color. So it seems that the influence of feature

selective attention really depends on the behavioral requirement: in a visual search task it will enhance processing of the attended feature, but in a conditional motor task, the motor response itself will influence the visual processing.

1.3.2.3. Possible physiological control of visual attention: FEF and LIP

Two areas are considered to play a key-role in the modulation of spatial attention: the frontal eye field (FEF, immediately rostral to the arcuate sulcus, see Fig. 1.1) and the lateral intraparietal cortex (LIP, located in the lateral posterior part of the intraparietal sulcus). Both have strong reciprocal connections to visual areas known to be influenced by attention (V4 for FEF, MT for LIP). The role of these two structures in spatial attention was reviewed by Gregoriou et al. (2009a).

FEF was traditionally considered to be part of a saccadic control network, the stimulation of FEF neurons leads to saccades to a specific location, the "saccade field". The idea that spatial attention is related to the saccadic control is not new; the "premotor hypothesis of attention" (Rizzolatti et al. 1987) states that the attentional focus would in fact be the target of a potential saccade. This idea received recent support by Moore & Fallah (2004), when they applied sub-threshold stimulations in FEF (so, unable to trigger a saccade), while displaying low-contrast stimuli in the corresponding saccade field. The stimuli were too weak to be consistently detected by the monkey if spatial attention was not directed to their location. The stimulation of FEF leads to an improvement of the perceptual ability of the monkey, akin to a shift in spatial attention toward the saccade field. Furthermore, the firing rate of V4 neurons with a RF overlapping the stimulated locus in FEF was influenced by the stimulation. Their firing rate increased during the stimulation, depending on their preferred direction (multiplicative gain), and the influence of adjacent distracters was decreased (Moore & Amstrong 2003). All these effects mimic the known influence of spatial attention on V4 neurons (see part 1.3.2). It also seems that the communication between V4 and FEF is modulated by attention. The gamma oscillations of the two areas become more coherent when the attentional focus is directed toward a common RF of V4 and FEF (Gregoriou et al. 2009b). By contrast, no change of gamma coherence can be observed for non-overlapping RFs. Additionally, attention improves the directed coherence between the spiking activity of FEF and the gamma oscillations of V4, indicating that FEF leads V4. This coherence between the two areas actually depends on the functional properties of 3 types of FEF neurons: the visual neurons (active if a target appears in the RF), the motor neurons (active during a saccade) and visuo-motor neurons (active during both). The spike-field coherence in the gamma band of the LFP was only observed for the visual neurons (Gregoriou et al. 2012), which are also the only ones to be modulated by the location of the attentional focus (Thompson et al. 2005). By contrast, the motor neurons are inhibited when the task involves the displacement of attentional focus

without saccade (Thompson et al. 2005), and their activity become more phase-locked to the local beta oscillations (Gregoriou et al. 2012). This phase-locking disappears if the monkey has to make saccades, supporting the idea that it is related to saccade inhibition. In summary, FEF could encode a "saliency map" to direct attention to potential saccade targets. However, the saccade programming and the shift of attention seem to be independent processes, mediated by a functionally distinct neuronal population in FEF.

However, FEF cannot be the only structure responsible for attentional selection. Even though lesions of FEF dramatically impair the attentional control of monkeys, they do not completely abolish the ability to attend to visual stimuli (especially if they stay rather constant from trial to trial, see Rossi et al. 2007). Other areas can be involved in attentional control, and among them LIP seems to be a likely candidate. LIP is thought to also contain a saliency map (Gottlieb 2007), influenced, for example, by the emotional saliency of the stimulus (if it is associated with a large reward or punishment, Leathers & Olson 2012), and to show a selective activity in anticipation of predictable events (Colby et al. 1996). LIP neurons are also more active if a saccade has to be made toward a cue than it the cue is fixated (Colby et al. 1996). This saliency map could serve as a spatial representation of the likelihood to make a saccade toward a given target.

The most direct evidence of an attentional control by LIP on visual areas (here, MT) was provided by Saalmann et al. (2007), who designed a task in which they combined spatial and feature-based attention. Two stimuli were successively displayed (S1 and S2) in a match-to-sample task, they could be either at the same location or not (spatial attention) and in the same orientation or not (feature-based attention). The monkeys had to match the orientation of the two stimuli. Both MT and LIP neurons were influenced by the location of spatial attention, but only LIP neurons were sensitive to feature attention (if S1 matched S2). This can be interpreted as the encoding of the "saliency" of S2 if it matched S1 (Gregoriou et al. 2009a). Furthermore, the LFP beta oscillations (20-35Hz) and the spiking activity of the two areas became coherent (spike-field coherence) when the stimulus was at the attended location, suggesting that LIP leads MT. They also found that attention caused an increase of 10% in the firing rate in MT neurons preceded by a spike in LIP. In other words, with attention, spikes from LIP became more efficient to induce a spike in MT. This result supports the "communication through coherence" hypothesis (Engel et al. 2001; Fries 2005).

In summary, FEF and LIP seem to have a top-down influence on the activity of the visual areas, which could be both mediated by monosynaptic projections (as deduced from the lag in the directed coherence analyses, Saalmann et al. 2007; Gregoriou et al. 2009b). Their influence would bias the activity of areas such as V4 or MT in order to reinforce the activity related to behaviorally salient stimuli.

1.3.3. Timing and the brain

1.3.3.1. Introduction

As introduced earlier, predicting expected events relies on our ability to estimate time, and thus to modulate our attention and motor preparedness around "relevant" points in time. However, studying the perception of time is dramatically different from studying other senses, like vision or audition. Unlike sounds or pictures, we don't have a dedicated organ to capture time-in-passing. The way it's processed by the brain has to be different, because there is no "T1" ("primary timing area") from where a hierarchal organization of brain areas could start. Indeed, the retinotopical and collumnar organization of V1, or the tonotopical organization of the primary auditory cortex give an idea of the basic processing undergone by picture or sounds, and thus of their neural representation. By contrast, we don't know what is timing, or even if it is represented somewhere in the brain.

Indeed, one can ask if, for example, producing a given duration involves different processes than perceiving it. Similarly, it is possible that different processes of time perception are devoted to a different time scale. Estimating the number of minutes before the bus arrives may be different than precisely timing the volley when I see the ball coming at me during a game. Thus, several attempts have been made to classify different "types" of timing. For example, Lewis & Miall (2003) distinguish between "cognitive" and "automatic" timing. The former would be involved in timing long (several seconds) and non-motor events, whereas the latter would be involved in sub-second motor acts. Choosing another line of distinction, Coull & Nobre (2008) separate the tasks involving "explicit" (tasks on timing *per se*) from "implicit" timing (timing as a by-product, like in a RT task). Whether these classifications reflect functional differences in the way the brain deals with timing is still under debate. However, conceptually dissociating the different contexts in which timing is used is heuristically very useful. Indeed, a wealth of data has been obtained about timing, using very different paradigms. By grouping these studies according to their experimental context, one can have a clearer view of how the literature can be organized in order to allow meaningful comparisons between studies.

In this section, I will quickly review the scientific literature about how the brain activity is modulated by timing in monkeys and humans, using the tree-like structure of Coull & Nobre (2008). I will then conclude by showing how the behavioral paradigm presented in this manuscript fit in the context of "temporal orienting".

1.3.3.2. Explicit vs. implicit timing

As mentioned above, Nobre & Coull (2008) draw a line between tasks involving "explicit" and "implicit" timing. Explicit timing means that the time-in-passing is at the center of the task, the object to which the

subject has to pay attention. It can be a duration discrimination or comparison task ("perceptual" explicit timing, e.g. Genovesio et al. 2009), or a duration production task like a self-initiated reaching ("motor" explicit timing, e.g. Roux et al. 2003; Mita et al. 2009). Using fMRI in human participants, several areas of the brain are consistently activated in explicit timing tasks (e.g. Coull & Nobre 1998; Coull et al. 2004). The basal ganglia seem to systematically be activated, both in perceptual and in motor tasks (reviewed in Coull & Nobre 2008). Other areas seem to be recruited differently depending on the task parameters. The SMA has shown to be active when timing is specified externally (Coull et al. 2004; Macar et al. 2006) and the cerebellum is active during tasks involving durations in the subsecond range (e.g., Lee et al. 2007).

Implicit timing is used in a simple RT task with fixed delay duration, where the participant will learn to estimate the delay duration in order to optimize his performance, even if he/she could perform the task without doing so. Implicit timing tasks usually involve the expectation of a signal, the timing of which can be predicted by several means: a symbolic cue (e.g. Miniussi et al. 1999), a rhythmic stimulus with a violation of the tempo from time to time (e.g. Praamstra et al. 2006), or an interception task, the timing of a cue being determined by its past trajectory (e.g. Doherty et al. 2005; Correa & Nobre 2008). Additionally, there are also tasks in which time-dependant variables are manipulated, like the conditional probability of occurrence of a cue (hazard rate, e.g. Riehle et al. 1997a; Ghose & Maunsell 2002). In fMRI, these types of task tend to activate sensorimotor areas, and specifically the parietal-premotor network (Coull & Nobre 1998; Coull et al. 2000, 2001; Davranche et al. 2011), even during purely perceptual tasks (Schubotz et al. 2001, 2002; Davranche et al. 2011). The fact that these areas are consistently involved in movement preparation (Kurata 1994; Bueno & Andersen 2006) led Coull & Nobre (2008) to formulate the hypothesis that the primary purpose of temporal expectation would be to optimize prospective motor behaviors. Similarly to spatial attention that may be mediated by a pre-saccadic mechanism (see section 1.3.2.3), temporal expectation may be based on a movement preparation mechanism.

Furthermore, it appears that in humans, the highest amount of brain activation seen during explicit timing tasks is located in the right hemisphere, whereas it is more concentrated in the left hemisphere during implicit timing tasks (Coull & Nobre 2008; Coull et al. 2012). This shows that even though both processes rely on a previously estimated duration, the fact that it is used either to produce a duration or to optimize a sensorimotor behavior involves different cerebral networks. However, these results cannot straightforwardly be transferred to non-human primates, because of the differences in the brain organization of the two species. For example, the monkey brain seems to be much less lateralized than the human brain (Oleksiak et al. 2010).

1.3.3.3. Explicit timing

1.3.3.3.A. Time perception

Tasks studying explicit timing focused on two aspects: duration perception and duration production. In monkeys, the former was mostly studied in the prefrontal cortex (PFC, Sakurai et al. 2004; Oshio et al. 2006, 2008; Genovesio et al. 2009), the posterior parietal cortex (Janssen & Shadlen 2003) and the basal ganglia (Chiba et al. 2008). In PFC and the basal ganglia, both parametric and categorical encoding of delay duration was recorded, often tightly linked to decision-making processes (the task usually required to report if one duration was longer than the other, or if they were similar). For example, neurons from PFC showed a phasic response following the end of a first delay, its amplitude encoding either if it was "short" or "long" (Sakurai et al. 2004; Oshio et al. 2006). Alternatively, in PFC and the basal ganglia, the amplitude of this phasic response could also depend parametrically on the delay duration (either linearly, or through a square or cubic relationship; Genovesio et al. 2009 for PFC, Chiba et al. 2006 for the basal ganglia). However, generally the neurons encoding delay durations were not "pure timing neurons", the activity of many of them also depended on the color of the timing cue (Sakurai et al. 2004; Genovesio et al. 2009). Inversely, when two durations were presented consecutively, neurons encoding the first one only rarely encoded the second one as well (Chiba et al. 2006; Oshio et al. 2006; Genovesio et al. 2009). It is also worth noting that such phasic responses depending on elapsed delay duration were also observed in tasks where no duration comparison was required and in other brain areas, like PFC, the midbrain or SEF (Genovesio et al. 2006; Fiorillo et al. 2008; Ohmae et al. 2008). The function of this phasic response, unrelated to RT, is still unclear, because its sensitivity to elapsed time is too coarse to provide a precise estimate (Genovesio et al. 2006; Fiorillo et al. 2008).

A large proportion of PFC neurons also exhibit activity modulations compatible with decision making, as was shown by Genovesio et al. (2009). In their task, two consecutive cues of different durations, one in blue, one in red, were presented to the monkey, who had to report which color (regardless of the order) lasted longer. Some neurons either reported if the first or second cue was longer, and others if the blue or red one was longer. Interestingly, the amount of "order selective" neurons was high just after the presentation of the second delay, whereas the "color selective" neurons were more numerous toward the time of response. Furthermore, the "decision" of these neurons was predictive for the behavioral response provided by the monkey. This study shows that during these temporal comparison tasks, timing and decision-making are tightly linked and almost indistinguishable. Even the neurons with a parametric relationship with the elapsed delay duration can be seen as a "probability accumulator" indicating that the previous cue is the longest. Going in this direction, the authors also showed that some neurons started to discharge during the presentation of the

second cue, at the moment when its duration surpassed that of the first cue. The activity of these neurons marks a decision point, when the second cue becomes longer than the first one. Oshio et al. (2008) and Sakurai et al. (2004) showed PFC neurons with a phasic response with a fixed onset latency in relation to stimulus onset, regardless of whether the stimulus disappeared or was still visible. Oshio et al. (2008) analyzed this population of neurons extensively and showed that the distribution of their onset latencies was centered on the moment in time that separated a "short" from a "long" duration with 50% probability. They interpreted this type of discharge as reflecting a "temporal filtering", reporting the probability that a cue had a "short" or "long" duration. In summary, temporal comparison tasks showed that the activity of PFC neurons was strongly modulated by time, but not independently of other task parameters, such as the order or the color of the cue. It seems however difficult to distinguish what would be a "pure" timing-related activity from one representing the growing probability leading to a decision.

A similar result was found by Janssen & Shadlen (2003) in the LIP. They showed that the activity of the neurons reflected the probability that the duration of cue-presentation in their receptive field was longer than a standard duration. However, Leon & Shaddlen (2005) subsequently showed that the activity of LIP neurons was much more modulated if the target of the saccade was in the receptive field than if another relevant cue was present in the RF. In this example, the time-dependent probability was not independent of any other cognitive process, it was tightly linked to movement preparation, or to the establishment of a saliency map marking the cue as a more and more probable motor target.

In human participants, the analysis of the Contingent Negative Variation (CNV) allowed to describe the mechanisms of perceptual explicit timing at a broader scale. The CNV is a slow modulation of brain activity observed in the central electrodes of an EEG recording (Walter et al. 1964), between an informative cue and a GO signal. The CNV reflects aspects of motor preparation (e.g. Zaepffel & Brochier 2012) but is also known to be modulated by non-motor parameters (see Leuthold et al. 2004). Macar & Vidal (2003) asked participants to compare the duration of a visual cue with a probe duration. They showed that the amplitude of the CNV dropped after the time of the probe was elapsed. Pfeuty et al. (2005) extended these findings by showing that this result even held if two different probe durations were used, and that the slope of the CNV was steeper for the shorter than the longer probe duration. Furthermore, they showed that a failure to report a cue longer than the probe was associated with an absence of drop of the CNV amplitude. In other words, these two studies show that the CNV peaks at the time of a memorized duration, and the slope of the CNV scales to the duration.

1.3.3.3.B. Delay production

In monkeys, studies investigating the motor side of explicit motor timing (duration production) focused mainly on LIP (Maimon & Assad 2006; Schneider & Ghose 2012), the motor cortical areas (Roux et al. 2003) and the SMA (Mita et al. 2009). As already described above, LIP may contain a saliency map of space, possibly contributing to bias the probability that certain stimuli become potential saccadic targets. Janssen & Shadlen (2003) already showed that LIP activity could correspond to the monitoring of this probability over time. Schneider & Ghose (2012) developed a task in which the monkey had to alternate saccades between two targets, self-initiating the eye movements. However, the monkey had been trained to respect a certain interval; if he produced too long or short intervals, the trial was stopped. In this sense, it was a duration production task. They showed that the integrated firing rate during the epoch preceding the saccade could account for the variations of produced durations, assuming that crossing a fixed threshold triggers the saccade. These data confirm the finding of Maimon & Assad (2006), showing the existence of LIP neurons whose activity had fixed onset latency in relation to movement initiation, but variable slope depending on the timing of the self-initiated movement. The activity of these neurons crossed a fixed threshold preceding movement onset, with a steeper slope leading to an earlier movement onset. But what is interesting in the data of Schneider & Ghose (2012) is that the correlation between firing rate and the duration of the saccadic interval changes sign between the period preceding and following the saccade, and is inversed if the target of the saccade is inside or outside of the neuron's receptive field. In other words, if the target of a cue is in the receptive field of the neuron, a high firing rate *preceding* the saccade is associated with a short saccadic interval, whereas a high firing rate *following* the saccade is associated with a long saccadic interval. It is as if the LIP neurons would play a push-pull game to determine the timing of saccades toward their own receptive field. Together, these data support the idea that the posterior parietal cortex could play a major role in determining the timing of self-initiated saccades, through a shift in the balance between the activities of neurons with different receptive fields.

Roux et al. (2003) recorded the activity of neurons in motor cortex preceding a self-initiated movement executed after a short or long delay. It appears that 40% of the neurons had a different firing rate during the delay preceding movement initiation, the vast majority of them being more active during short delays (personal communication; see the example neuron shown in Fig 1.9). By contrast, the influence of delay duration was highly reduced during the movement time, that is, between movement onset and the moment when the target was reached.

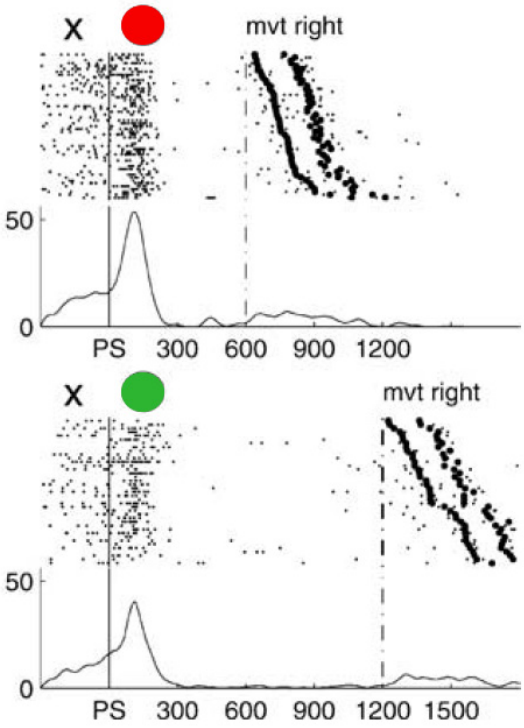


Figure 1.9: Activity of a motor cortical neuron during a self-timed movement task. On the top of each plot is the raster display, with the time of movement start and end indicated with larger dots. On the bottom part is the averaged smoothed activity, indicated in spikes/sec. In this task, the location of the cue (in this example, to the right) indicates the direction of the required movement, and its color indicates the minimal delay that the monkey has to wait (indicated by the dashed line) before self-initiating the movement. Note the difference in the short-latency phasic response to the cue according to delay duration. Reproduced and modified from Roux et al. (2003).

However, one of the most interesting pieces of work about instructed self-timed movements comes from a study of SMA activity done in the lab of Jun Tanji (Mita et al. 2009; Shinomoto et al. 2011). They trained monkeys to release a lever after 2, 4 or 8 seconds, according to a colored cue. They reversed the association between colors and durations from time to time, in order to dissociate the color sensitivity from the duration sensitivity. While the monkeys were performing the task, they recorded the activity of neurons in the rostral part of SMA (preSMA) and its caudal part (SMA proper). They found that an important proportion of neurons showed a graded response to the delay duration to be produced, either ramping during the whole delay (in both areas), or phasically following the timing cue (only in preSMA). Additionally, they showed that another class of neurons, found exclusively in preSMA, responded selectively to one of the duration, and not to the others. By contrast, many more neurons were non-selective to delay duration in SMA proper than in preSMA. To further characterize the influence of temporal sensitivity of the activity of the two areas, they fitted an exponential function to the activity of the neurons sensitive to delay duration. The exponential function $a*e^t$ has two terms, the coefficient of gain 'a', and the exponential base 'e' (t being the elapsed time). The authors showed that the fit between the activity of neurons of SMA proper and the three different durations was mediated by a change of gain (a) only. By contrast, the neurons of preSMA additionally showed a tuning of the exponential base (e) according to delay duration. In other words, neurons of these two areas show different profiles of sensitivity to elapsed time according to the temporal context (the expected duration).

Shinomoto et al. (2011) applied a decoding algorithm on these data to extract information about both delay duration and timing. They found that relative timing was better decoded from these neurons than absolute timing. In other words, timing seemed to be encoded contextually, in relation to the whole delay duration. This "scaling" of brain activity to the internal timing of the animal was already shown by Renoult et al. (2006) in motor cortex and by Brody et al. (2003) in prefrontal cortex. This means that the "brain hourglass" does not count time as an absolute value, but embedded in a given temporal context. Jazayeri & Shadlen (2010) further demonstrated this in a behavioral study. They found that a duration drawn from distribution with a long mean duration was estimated to be longer than the same duration when it was drawn from a distribution with a shorter mean.

The study of Mita et al. (2009) shows the fundamental role of the medial frontal cortex in the timing of a planned movement, and underlines the difference in this regard between the two parts of SMA. Berdyyeva & Olson (2011) indirectly confirmed this idea, by analyzing the influence of the ordinal position of a signal on the activity of several frontal areas (SMA proper, preSMA, SEF, and the dorso-lateral prefrontal cortex, DLPFC). They dissociated the effect of ordinal position from those of reward and delay duration preceding the saccade. They showed that even though the activity linked to ordinal position could not be completely explained by these other confounding variables, the area showing the greatest influence of delay duration was preSMA. By contrast, SMA proper showed a greater influence of reward size (motivation), which could be a sign of a greater involvement in movement preparation (Roesch & Olson 2005b, 2007).

The involvement of preSMA in time perception also received a strong support from the literature involving research on human participant. Macar et al. (1999) asked their participants to produce time intervals while recording their brain activity using EEG. The slow modulation of a Laplacian transform of the EEG signal recorded over SMA (similar to a CNV) had larger amplitude when subjects produced longer durations. Nobre (2001) applied a source localization technique to Miniussi et al. (1999) data, and showed that the origin of a CNV change of slope with the duration of a delay may be located in preSMA. In an fMRI study, Coull et al. (2000) showed that preSMA was one of the few areas with a significantly different activation between trials with short and long delays. Finally, Coull et al. (2004) showed that the activation of preSMA depended parametrically on the degree of attention that the participants devoted to the duration of a stimulus (versus its color).

In summary, studies on explicit timing tend to show that time estimation, either during perception and movement preparation, is generally embedded in other processes (e.g. decision-making). Furthermore, time does not seem to be encoded as an absolute value, but in a given temporal context, to which the brain activity

will scale. Finally, it seems that the posterior parietal cortex and perhaps more importantly preSMA hold a central place in the network involved in explicit timing. It would be interesting to see if neuronal activity similar to the one observed by Mita et al. (2009) can also be seen in a time perception task, that is, without movement preparation. Such a result could interestingly feed the debate of a timing process depending on the context (movement preparation vs. sensory perception) versus a general timing ability. At a broader scale, human studies tend to show that a CNV wave, likely coming from SMA, peaks at an expected duration, in perceptual as well as in motor explicit timing tasks.

1.3.3.4. Implicit timing

Implicit timing regroups all tasks in which timing plays a role but is not the center of the task. In other words, the subject does not have to specifically report duration, but would still be using time in order to optimize his/her behavior. Usually, tasks addressing implicit timing require a speeded response, or they measure the accuracy of the detection of a cue (or both), supposedly influenced by the ability of the subject to predict when the cue will appear. As described earlier, in human fMRI, most of the areas activated in such tasks involve a (leftward) parieto-premotor network, usually associated with sensorimotor processes. It intuitively makes sense that, as part of a system aiming at an efficient motor behavior, assessing the probable timing of events is a central component. Through such ability, one can regulate the readiness to respond, or the attention to certain features of the environment according to the timing. Time estimation is thus of foremost importance in sensorimotor behaviors (and as such, may be a built-in component).

The use of timing information in sensorimotor tasks can arise from two reasons: in one case, the statistics of the task events changes over time, leading the subjects to consciously or not modulate their expectancy. Such a situation is called "exogenous implicit timing" by Coull & Nobre (2008). An example would be a simple RT task with a delay of variable duration from time to time. The conditional probability that the GO appears would increase exponentially, leading to a similar change in expectancy. Accordingly, in such a situation, the RTs following long delays are shorter than after short delays. In the other situation, the subject has been trained to predict with a certain degree of reliability when a task event will happen (a cue, usually), based on a previous signal. This signal can be a symbolic cue, indicating the timing of another one to which the subject has to react. It can also be a rhythmic stimulus, inducing a prediction of the timing of the next iteration. Another way to induce implicit timing is the occluded trajectory task. A stimulus moves in space and time, following a more or less predictable trajectory. At some point, the cue is occluded, when it reappears, following or violating the prediction made from the trajectory, the subject has formed an expectation of its moment of re-appearance.

All these cases, in which the subject actively "moves" its attentional focus in the temporal dimension rely on a process labeled "endogenous implicit timing" by Coull & Nobre (2008).

The main difference between these two "types" of implicit timing is mainly methodological. In other words, there are no striking differences in the physiological markers of endogenous and exogenous implicit timing (contrary to explicit and implicit timing, Coull & Nobre 2008). However, the paradigms studying exogenous timing focus mainly on "continuous" variations of probability that a cue appears, whereas the study of endogenous timing is mainly based on "punctual" events. As such, the type of physiological signals that they analyze are usually quite different, which make these two types of study difficult to integrate. For clarity, I will thus present the literature data in the context of exogenous and endogenous timing separately.

1.3.3.4.A. Exogenous timing

Most of the studies addressing exogenous modulation of temporal attention used paradigms in which the probability of a stimulus to appear was modulated over time. It seems that this time-modulated probabilistic function influences the cortical activity at a very early stage. Ghose & Maunsell (2002) showed such a phenomenon already in the visual area V4. In their task, the neuronal activity followed the hazard rate (conditional probability) of appearance of the cue, as did the RT. The hazard rate is indeed central to the study of temporal expectancy. This variable represents the cumulative probability that something will happen, given that it did not happen yet. Janssen & Shadlen (2005) went further by converting the hazard rate in an "anticipation function". The aim was to represent the subjective expectancy, not the mathematical probability. Indeed, Gibbon (1977) has shown that the precision of our ability to estimate duration is linearly decreasing with its lenght (Weber's law). In other words, the ratio [variance of the estimate / mean of the estimate] stays fixed for all estimated durations "scalar property" of time estimation processes). Janssen & Shadlen (2005) convoluted the hazard rate function with a Gaussian function (the width of which increased with elapsed time) to represent the diminishing accuracy of the subject as time goes on. Their main finding is that the firing of LIP neurons closely reflects the subjective anticipation function and that this activity is predictive of RT. However, as already mentioned, this activity does not seems to be the "pure" growing expectancy of an upcoming event, but rather the growing readiness to perform a saccade toward the cue.

Using a different approach, Ghose & Bearl (2010) manipulated in time the probability that an event appeared in one part of the visual field or the other. In their task, the monkeys had to detect a slight change of orientation of a low-contrast stimulus. The authors showed that the selectivity to motion of MT neurons changed with the probability that the change happened in their receptive field. This manipulation was not a simple change of gain, or an unspecific increase of activity. Instead, the firing rate of the neurons increased, but

only in their preferred direction. Such an enhancement of the non-linearity of these motion-direction-selective neurons has as consequence to improve the discriminability of the stimulus. Also by modulating the probability that a visual (vs. auditory) stimulus appeared over time, Lakatos et al. (2008) showed that the dynamics of the activity in V1 was strongly modulated by this probability. Specifically, they showed that the distribution of the phases of very slow oscillations (delta band) changed with the probability. Furthermore, since the power of gamma oscillations and the population spiking activity depend on the delta phase, their amplitude were larger in the case of high expectancy. This delta phase shift seems tightly correlated with the level of attention since the RTs and the delta phase at the time of the cue were tightly correlated.

The modulation of time-varying probability was also used in human participants, associated with EEG recordings. First, in the case of a variable delay duration preceding the GO signal, the CNV continuously increases as time goes on, whereas RT becomes shorter (Trillenberg et al. 2000). In a more complex paradigm, Cravo et al. (2011) used a GO-noGO task in which the target could appear at 3 different moments in time following two possible hazard rate functions. They showed that the CNV amplitude was larger with a higher expectancy, only at the moment when the two hazard rate functions differed. Using another modality and MEG (magnetoencephalography), Van Ede et al. (2011) indicated to the participant on which hand a tactile stimulus will appear. This cue was followed by a strong decrease in the power of the alpha and beta band of the MEG signal in the contralateral somatosensory cortex. Furthermore, the probability that the stimulus appeared depended on the two possible hazard rate functions. A higher probability to appear was associated with a stronger decrease in power of the oscillations, which was also predictive of shorter RTs and higher accuracy. In a follow-up study, they showed that the time course of the decrease in power in the low-frequency oscillations might explain the increase of accuracy of the participants, but not the decrease of RT, which may only be indirectly related. (van Ede et al. 2012). Such a controlateral reduction of alpha power (over the posterior electrodes) has also been shown to be associated with spatial attention (Trachel et al. in preparation).

Most of the physiological effects that were related to this type of temporal attention are very similar to those obtained with visual or spatial attention. The increase in neuronal firing with hazard rate (Ghose & Maunsell 2002; Janssen & Shadlen 2005) is comparable with the increase of firing seen by Luck et al. (1997) in spatial attention, as is the increase in gamma power (Lakatos et al. 2008; Fries et al. 2001) depending on the delta phase (Bosman et al. 2012). Similarly, the increased non-linearity of the selectivity for motion direction of MT neurons (Ghose & Bearl 2010) was also found in visual feature attention to direction. This also holds for data from human participants. A larger CNV or a contralateral decrease of alpha and beta power are all associated with an elevated expectancy, be it in space or in time (Van Ede et al. 2011). The literature

investigating the effect of time-varying expectancy seems to show that its main impact is to potentialize or reproduce the effect of other kinds of attentional modulations, like spatial attention. This suggests that the local mechanisms underlying the behavioral effect of temporal and spatial expectancy could be similar (as Cohen & Maunsell, 2011, proposed for spatial and feature attention).

1.3.3.4.B. Endogenous timing

Tasks assessing the effect of endogenous timing first transferred Posner's paradigm from the spatial to the temporal domain. In Posner's seminal work on spatial attention (1980), a cue indicated to the participant if the target will appear to the left or the right of the screen, and the participant had to respond to the target as fast as possible. However, in 20% of the trials the cue indicated the wrong target ("invalid trials"). Since then, such paradigms show consistently that compared to a spatially non-informative cue the valid cue induces a decrease in RT, whereas an invalid cue leads to an increase of RT. To adapt such a paradigm to the temporal domain, the cue indicates duration instead of spatial location (Miniussi et al. 1999), or indicates a combination of spatial and temporal information (Coull & Nobre 1998). This procedure, and others inducing a temporal expectancy from the subject at a given moment in time, are called "temporal orienting". Temporal orienting has been shown to have a strong behavioral effect (e.g. Miniussi et al. 1999; Griffin et al. 2002; Correa et al. 2004, 2005; for a review see Correa et al. 2006; Thomaschke et al. 2011), distinct from spatial orienting when both are used in combination (Coull & Nobre 1998). Indeed, temporal orienting leads in parallel to a reduction in RT (Coull & Nobre 1998; Coull et al. 2000; Griffin et al. 2002; Correa et al. 2004; Davranche et al. 2011; Doherty et al. 2005; Rohenkohl & Nobre 2011; Cravo et al. 2011; Van Ede et al. 2011; for a review, see Nobre 2001) and to an increase of the participant's accuracy in perceptual tasks (Correa et al. 2005; Rolke & Hofmann 2007; Sanders & Astheimer 2008; Davranche et al. 2011; Rohenkohl et al. 2012). Most of the early studies on temporal orienting showed a decrease in RT with valid cues. This was associated with a modulation of relatively late EEG components, usually linked to movement preparation (see below). This led to the belief that most of the effect of temporal orienting was a modulation of movement preparedness. However, later studies showed that temporal orienting had also an effect on the perceptual level of the response process (Thomaschke et al. 2011). For example, the spatial (Rolke & Hoffmann 2007) and temporal (Bausenhart et al. 2008) resolution of visual perception was found to be degraded by temporal uncertainty (i.e. increasing with delay duration). In the auditory modality, Sanders & Astheimer 2008 showed that the probability to detect a deviant sound was increased if expected at the correct timing. Correa et al. (2004) showed that temporal orienting decreased RT even in a choice-RT discrimination task. Thereby, they demonstrated that temporal orienting was not merely the preparation of a stereotypical motor response. Correa et al. (2005) and Davranche et al. (2011) showed

that temporal orienting greatly improved the participant's performance to discriminate a stimulus in a RSVP task (Rapid Serial Visual Presentation). Using signal detection theory, they both showed that temporal orienting leads to an increase of the mean *d' (d-prime)*, meaning that the participant's sensitivity improved. Finally, Rohenkohl et al. (2012) used rhythmic or arrhythmic stimuli in a difficult visual detection task, in order to induce, or not, (implicit) temporal expectation. By modeling their data, they showed that the effect of temporal orienting was a leftward shift of the behavioral psychometric function, interpretable as an increase of effective contrast of the stimulus. In summary, spatial or temporal orienting tasks involve a modulation of both perceptual processes and motor readiness, and the emphasis will be put on one component or the other depending on the experimental paradigm.

Here are three examples of paradigms putting more or less importance on the visual or motor aspect of temporal orienting: In a "purely perceptual" task during which a visual target is expected at one moment in time, but no motor response is required until another delay is elapsed (e.g. Mauritz & Wise 1986), neuronal activity in relation to the target presentation cannot be attributed to motor processes. In the "mixed motor and perceptual" task of Doherty et al. (2005), the participant has to discriminate a small visual feature in order to determine whether to respond or not (perceptual processing), but also has to give a potential response as fast as possible (movement preparation). This example is illustrative of most temporal orienting tasks in human participants (I will, thus, refer to it as "classical temporal orienting"). In a "purely motor" task, the temporal cue is always valid, and the GO signal is very easy to process. Most of the behavioral effect is due to the ability of the participant to accurately time the preparation of his/her movement. This case corresponds to a movement preparation during which the "temporal uncertainty" would be reduced by prior information (Requin et al. 1991).

I will first summarize the literature on "classical" temporal orienting, in which presumably both perceptual and motor aspects are influenced by time estimation. Subsequently, I will review the (small) literature emphasizing the influence of temporal orienting on movement preparation, and finally, the data related to the anticipation of cues without concurrent movement preparation ("cue anticipation").

1.3.3.4.B.1. "Classical" temporal orienting

In this paragraph, the term "target" will refer to the sensory cue (usually visual) that the participant has to detect and to respond to as quickly as possible. As such, it is both an instructional cue and a GO signal. The term "temporal cue" will refer to the cue indicating to the participant when the target will appear. The studies addressing the effect of temporal orienting report on three epochs of the task: i) an epoch following the temporal cue, assessing the effect of temporal information on the neuronal activity, ii) an epoch located at

target onset, assessing the effect of the validity of temporal cueing, iii) an epoch preceding the expected timing of the target, that is during the delay between the temporal cue and the target.

First, to my knowledge only three studies addressed the physiological consequences of the presentation of a temporal cue associated with a specific duration, Miniussi et al. (1999) and Correa et al. (2006) in human EEG and Anderson & Sheinberg (2008) in monkeys intra-cortical recordings. All studies used a classical temporal orienting paradigm with a temporal cue to indicate to which delay duration to pay attention (either "short" or "long"). In 80% of the trials, the target appeared after the instructed delay duration and required a motor response. In the remaining 20%, the target appeared after the other delay, presumably unattended. Miniussi et al. (1999) showed that the early evoked potential following the temporal cue was not modulated according to the cued duration. By contrast, Correa et al. (2006) showed a larger P1 component in "attend short" than in "attend long" trials over the occipital electrodes. However, this result is hard to interpret: first, in their task design, the temporal information was blocked (i.e. did not change between trials of the same block). It is thus possible that the overall readiness of the subject was modulated from one block to the other. Second and more problematic, the cues for short and long intervals were not visually identical (respectively the spanish words "presto" and "tarde"). The modulation of P1 being occipital, it is possible that is was caused by the difference in visual stimulation. In monkeys, Anderson & Sheinberg (2008) recorded both single neuron spiking activities and LFPs in area IT, in the inferior lobule of the temporal lobe, which is thought to be involved in object recognition. They used identical pictorial cues to indicate to the monkey either the direction of the required movement (when the cues were targets) or the duration to be attended (when they were temporal cues). Thus, the only difference between the stimuli was their "meaning" (temporal vs spatial cue). Both types of cues elicited a phasic response of spiking activity and of the LFP (visual evoked potential, or VEP). However, if the stimulus was used as a spatial cue, the neuronal response was strongly reduced, and less reliable than if this same stimulus was used as a temporal cue. Since IT is supposed to be involved in object recognition, it is reasonable to ask if the response to the temporal cue was actually due to its temporal meaning, or just the default processing of a pictorial stimulus. In summary, as for now, there is no definitive evidence of an early difference in the processing of temporal cues according to their associated durations.

Second, many studies (mostly coming from human EEG) addressed the effect of temporal cueing on the response to a target. A point has to be specified in order to properly understand these studies: in most of the temporal orienting studies, two delay durations are usually tested, and the attention of the subject is directed to one duration according to the temporal cue. During trials in which the short interval is indicated by the temporal cue, the target sometimes appears instead at the end of the long interval. In these trials, the

participant notices the absence of a target at the cued (short) interval, and endogenously redirect his/her attention to the long interval. The consequence of this "attentional redirection" is that the target appearing at the long intervals always benefits from an optimal attention: either because the temporal cue pointed to the valid, long duration, or because the participant endogenously redirected his/her attention to the long duration. Accordingly, most studies on temporal orienting show a behavioral effect (RT) of temporal cueing only for the short interval (Coull and Nobre 1998; Griffin et al. 2001; Anderson and Sheinberg 2008; Correa and Nobre 2008; Rohenkohl & Nobre 2011; Lampar & Lange 2011). The effect of "redirection of temporal attention" was directly addressed by Correa et al. (2004) in a behavioral study, showing that the redirection effect depended on the certainty that the target would appear. Thus, in this paragraph, I present only data comparing valid vs. invalid temporal cueing for the short duration.

The first result is that in case of relatively easy visual tasks, temporal cueing alone does not seem to influence the early part of the VEP evoked by a visual target (Miniussi et al. 1999; Griffin et al. 2002; Doherty et al. 2005; Correa & Nobre 2008; review by Correa et al. 2006). This has to be contrasted with spatial attention, usually modulating the early components of the VEPs recorded on occipital electrodes (Griffin et al. 2002; Doherty et al. 2005). However, in perceptually demanding visual tasks (Correa et al. 2006) temporal orienting leads to a modulation of the early components (P1) evoked over the occipital areas. Interestingly, when spatial and temporal information are given in parallel, the influence of spatial attention is potentiated by temporal orienting (Doherty et al. 2005; Rohenkohl & Nobre 2011). However, the most robust result from this literature is that the late P300 component of the VEP, usually originating from the centro-parietal electrodes, peaks earlier in case of valid temporal cueing (Miniussi et al. 1999; Griffin et al. 2002; Doherty et al. 2005; Correa et al. 2006; Correa & Nobre 2008; Rohenkohl & Nobre 2011), whereas only its amplitude is modulated by spatial orienting (Griffin et al. 2002; Doherty et al. 2005). The P300 is thought to be related to motor or decision-making processes (Doherty et al. 2005; Sanders & Astheimer 2008), indicating that temporal orienting has a specific effect on the late phase of the sensorimotor transformation, distinct from the one of spatial orienting. By contrast, its influence on early visual processing is less clear and seem to depend on several task parameters, like the difficulty of the perceptual task. These results are to be contrasted with the auditory modality, in which the early component of the evoked potential elicited by a sound is generally modulated by the validity of temporal cueing (Lange et al. 2003, 2006a,b; Sanders & Astheimer 2008). In monkeys, only one study addressed the effect of the validity of temporal cueing on intracortical activity (Anderson & Sheinberg 2008). They showed that the initial phasic response to the target was decreased by invalid temporal cueing. The validity effect was very precocious, 100ms after stimulus onset for spiking activity, and 150-200ms for the VEP. It is interesting to see that such an early modulation is usually not seen in EEG. Studies reporting an early

modulation of the EEG signal always locate it over the most occipital electrodes (Griffin et al. 2002; Doherty et al. 2005). It would therefore be interesting to record the intracortical signal from visual areas such as V4 or V1 in monkeys to assess if temporal orienting influences the discharge of these neurons.

Third, the modulations of brain activity which occur before the expected timing of the target have been described in slow ERPs and oscillatory activity. As for time production (Macar et al. 1999), comparison of durations (Macar et al. 1999; Macar & Vidal 2003; Pfeuty et al. 2005), or even movement preparation (Müller-Gethmann et al. 2003), the CNV following a temporal cue has a steeper slope when it is associated with a shorter than a longer duration (Miniussi et al. 1999; Praamstra et al. 2006; Correa et al. 2006 for the visual modality. Sanders & Astheimer 2008 for the auditory modality). Furthermore, in an implicit timing task, Praamstra et al. (2006) report an interesting phenomenon, similar to what was found in an explicit timing task described by Macar & Vidal (2003) and Pfeuty et al. (2005). In their task, a rhythmic stimulus was displayed visually, to which the participant had to react by pressing a button. In some instances, the stimulus deviated from the pace, leading to an interval either too short or too long. When it was too long, the CNV amplitude grew until the expected timing and then started to decrease. This similarity with the data of Macar & Vidal (2003) suggests a unified mechanism of time prediction, when time is directly measured and when it is used as a mean to shorten RT. However, the source of the CNV might be different in the two tasks. As Macar et al. (1999) and Nobre (2001) showed, a likely source for the CNV in explicit timing tasks may be SMA. By contrast, Praamstra et al. (2006) tend to localize the source for the CNV in PMd. Whereas SMA may be central for sensing time per se, PMd may be more involved in using it to optimize a motor behavior (see Coull & Nobre 2008). Rohenkohl & Nobre (2011) studied the modulation by temporal orienting of the Lateralized Readiness Potential (LRP), which is an ERP originating over the motor cortical areas contralateral to the moving arm. This potential is thought to be linked to movement preparation (reviewed by Leuthold et al. 2004). They showed that the LRP was larger when the timing of the target was predictable (i.e. when a dot moved along a trajectory in rhythmic vs. arrhythmic way reappeared after being occluded). In summary, the slope and amplitude of both the CNV (general anticipation) and the LRP (movement preparation) are modulated by the expected delay duration preceding the apparition of a target.

Several studies also looked at the modulation of oscillatory activity preceding a predictable target. On occipital sites, the power of alpha oscillations (EEG) decreases if a target is presented later than expected (Praamstra et al. 2006), or during the epoch preceding the expected occurrence of a target (Rohenkohl & Nobre 2011). Intracortical recordings in area IT showed an increase of beta power at the expected timing of appearance of a target (Anderson & Sheinberg 2008). Finally, the same authors found that in area IT, the phase

locking between the spikes and the gamma oscillations is higher before an expected than an unexpected cue. This result is similar to that described by Fries et al. (2001), showing a higher phase-locking of the spikes to the LFP gamma band in an attended vs. unattended receptive field. However, as Kilavik et al. (2012b) described in their review about beta oscillations in sensorimotor areas, the anticipation of a cue (not requiring an immediate response) is usually associated with an elevated beta power (e.g. Saleh et al. 2010; Fujioka et al. 2012), whereas beta power decreases during movement preparation. Since the paradigm of temporal orienting triggers both processes because of the dual nature of the target (both an informative and a GO signal), the interpretation of the modulation of beta oscillations is in this context complicated.

In summary, valid temporal cues lead to a modulation of the VEP evoked by the target, an increase of the amplitude of the early components, but only in specific conditions (usually in combination with spatial information) and a shortening of the latency of the late component P300. Additionally, the slow wave reflecting movement preparation is larger when the GO signal can be predicted, and the slope of the CNV depends on its timing. Finally, it seems that occipital alpha oscillations are reduced in anticipation of a target (similar to Van Ede et al. 2011 for a tactile stimulus).

1.3.3.4.B.2. Movement preparation ("motor temporal orienting")

This section will address the effect of timing in the context of movement preparation. In this specific case, all parameters of the movement are known in advance, including – and this is the important aspect – the moment in time when it has to be executed. In fact, very few studies analyzed the effect of delay duration on motor preparation outside of the context of probabilities. For example, Roux et al. (2003) used two tasks: in the first one, the color of the GO indicated the direction of the movement, but the association between movement direction and color depended on the timing of the GO. Thus, the monkey had to estimate the timing of the GO in order to reach to the correct target. The GO signal could appear after an either short or long delay, with a 50% probability. As a consequence, the monkey could not precisely anticipate its timing. The second task involved a self-timed movement, with the temporal window for movement initiation and the direction of the movement being indicated at trial start. Even though both tasks studied the modulation of movement preparation by time estimation, the authors did not specifically look at the effect of the expected timing of the GO signal.

First, in human EEG studies, it has been shown that the CNV is steeper and has a larger amplitude when the participants know in advance that the delay preceding the movement is shorter (Müller-Gethmann et al. 2003; Praamstra et al. 2006).

Second, the power of beta oscillations usually decreases before movement initiation (reviewed in Kilavik et al. 2012b). Indeed, Praamstra et al. (2006) showed in EEG that the beta power over the motor areas contralateral to a movement decreased with different slopes according to the interval between two movements. Additionally, Kilavik and colleagues (2013, in press) showed recently that the frequency of beta oscillations decreased slightly (but systematically) prior to movement execution (2-4 Hz). Specifically, they showed that the slope of this decrease in beta frequency depended on the duration of the instructed delay, reaching the same frequency at the end of a short or long delay.Third, regarding spiking activity, Lucchetti & Bon (2001) used a reaching task in which the delay duration before the GO signal was variable but kept constant within blocks, and as such predictable after a few trials. They found that the PMd neurons with a ramping activity (like the example shown in Fig. 1.6A) showed a gradual shift of the activity onset, getting closer to GO from trial to trial. Thus, after a few trials of a predictable delay, the activity had a fixed onset before GO, whatever the delay duration. They confirmed this result in another study (Lucchetti & Bon 2005), also showing another type of neurons which were only active for delay durations shorter than 2 seconds. However, the fact that in this study, they did not precise the area of recording more specifically than "frontal lobe" limits the interpretation. But as for now, the most extensive study of the influence of temporal aspects in movement preparation on the activity of cortical neurons comes from Roesch & Olson (2005a,b). They recorded the activity of single neurons in six frontal areas (DLPFC, orbito-frontal cortex (OFC), SMA, SEF, FEF and PMd), while the monkey was performing a center-out saccade task. In each trial, a symbolic cue instructed the monkey about the delay duration between the occurrence of a visual target and the GO signal (either 500 or 2500ms). The delay duration had a strong influence on the neuronal firing rate, but not identical in all frontal areas. In DLPFC, SEF and FEF, the strongest influence of delay duration was seen during saccade execution itself. The firing rate was higher after a long duration had elapsed. In addition to this pattern, neurons from SMA and OFC also showed a higher firing rate during the delay preceding the GO when a short duration was expected. Finally, PMd neurons discharged more during the delay preceding the GO when a short duration was expected, but showed virtually no difference during the saccade itself. The pattern observed in PMd in a saccade task is relatively similar to the result reported by Roux et al. (2003) in a hand reaching task in motor cortex. In their self-timed task with two different delays, short delay duration was associated with a higher firing rate during the delay, and less difference in discharge during movement. Roesch & Olson (2005a) linked their result to the subjective expectancy of reward value. Indeed, it was shown that the value of a reward was discounted by the delay duration that one has to wait before getting it ("temporal discounting", Lowenstein & Elster 1992). Roesch & Olson (2005a) tested the same neurons as described before, but with a symbolic cue indicating the size of the reward. They showed that the same neurons were strongly influenced by delay

duration and reward size, and that this relationship was stronger for PMd than for the other areas. This study shows that the influence of timing and of motivation is hard to separate. This topic will be discussed further in chapter 3 of the results.

In summary, brain activity prior to the execution of a movement is strongly influenced by the delay duration, in implicit as well as explicit timing tasks. Typically, the delay activity is larger in short delays, and the movement-evoked activity is larger in long delays, even though this relationship varies as a function of the area of recording (Roux et al. 2003; Roesch & Olson 2005a,b). Additionally, a subpopulation of neurons tends to change their activity starting at a fixed delay preceding GO signal, when the duration can be predicted (Lucchetti & Bon 2001, 2005), which resembles the activity seen before a self-timed movement (Lebedev et al. 2008, in motor cortex; Maimon & Assad 2006, in the parietal cortex). In EEG, the CNV scales to the expected delay duration, leading to steeper slopes in short than in long delays in implicit (Müller-Gethmann et al. 2003) as well as in explicit timing (Macar et al. 1999).

However, the (few) studies addressing the influence of delay duration on motor preparation during an implicit timing tasks analyzed mainly the spiking activity, and usually focused on the movement-related activity (tonic or phasic). In chapter 3 of this manuscript, I will describe how the temporal aspects of movement preparation influence both the spiking activity and the LFP evoked by the main events of the task. Specifically, I will focus my analysis on the activity evoked by the visual target and by movement execution in motor cortex (M1 and PMd). Additionally, I will discuss the relationship between time estimation, preparatory activity and RT in the context of the task.

1.3.3.4.B.3. Pre-cue anticipatory activity

In typical sensorimotor tasks, three main events are usually present (Fig. 1.10): the trial start, the cue, and the GO. The trial start can be initiated by the subject (e.g. a button press), or be externally indicated by a warning signal. The cue can provide information about various aspects of the forthcoming movement (movement direction, extent, delay duration, etc...). Then the GO requires the subject to perform the cued action, or the subject self-times it. In part 1.2 of the introduction, I presented the modulation of activity during movement preparation, that is, during the "preparatory delay" of Fig. 1.10. However, in many tasks, the delay between "Trial start" and "Cue" is predictable. The fact that its duration is variable but limited to a certain range, is kept constant, or presented in blocks allows the subject to predict its timing. The anticipation of the cue can then be considered as an elementary form of temporal orienting without movement preparation. By anticipating the onset of the cue, the subject may improve its detection, or prepare further processes to retrieve the information carried by the cue (which may also be predictable).

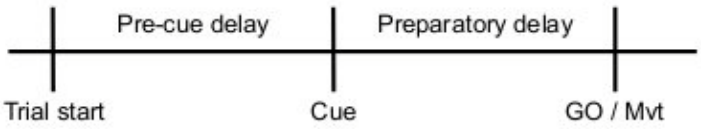


Figure 1.10: A generic sensorimotor delay task.

Since the movement preparation paradigm was first implemented, it was regularly reported that a fraction of motor cortical neurons modulate their firing rate well before the presentation of the cue. This was observed even if the delay between the cue and the subsequent GO signal was several seconds. Several surveys reported on this phenomenon in motor cortical areas as a side note (Wise & Mauritz 1985; Vaadia et al. 1986; di Pellegrino & Wise 1993; Crammond & Kalaska 1996; Shen & Alexander 1997a; Cisek & Kalaska 2005; Hoshi & Tanji 2006) or showed examples of neurons with such an activity (Weinrich et al. 1984; Roux et al. 2003), but only a few of them studied it directly (Mauritz & Wise 1986; Vaadia et al. 1988). Furthermore, this type of anticipatory activity has been described in a wide range of brain structures, such as the caudate nucleus (Hikosaka et al. 1989; Takikawa et al. 2002; Lauwereyns et al. 2002), the prefrontal cortex (Niki & Watanabe 1979; Sakagami & Niki 1994) or the somatosensory cortex (Meftah et al. 2009). In this part I will review the main characteristics of this "cue-anticipatory activity", only from monkey literature. By convenience, I will call the neurons showing such an activity "cue-anticipatory neurons", regardless of how they were defined originally by the authors. Most of the emphasis will be put on the motor cortex, but I will nonetheless describe some studies presenting recordings from other areas. One of the main problems I encountered when looking through the literature concerning the "pre-cue anticipatory" activity is that it seems to be half-forgotten. Most of the studies reporting on it date back from the eighties, and the different papers do not seem to know about each other. In consequence, this short review is likely not to be exhaustive. Additionally, a recent review by Kilavik et al. (2012b) shows that the beta activity in the motor cortex is modulated before the onset of a cue. I will therefore start by a quick summary of this topic. Another detail that should be specified is that I will restrict this short review to rather "simple" sensorimotor tasks. For example, anticipatory activity before a cue was shown in visual match-to-sample tasks in area IT (Reutimann et al. 2004) or in the prefrontal cortex in a tactile comparison task (Brody et al. 2003), both influenced by the timing of the task. But diving into this literature would require 50 more pages of introduction.

Beta band oscillations in motor cortical LFPs in anticipation of a cue

In a recent study using rhythmic streams of auditory stimuli, Fujioka et al. (2012) recorded MEG in human participants and found beta power in motor cortical areas (as well as in several other areas including auditory

cortex) to peak just before each sound event, even though participants were only required to passively listen to the rhythmic streams. The authors proposed that this distributed pre-cue increase in beta power provides a mechanism for maintaining predictive timing. Arnal (2012) proposed that this motor cortical sensory prediction may rely on the simulation of movements via an internal model, allowing the prediction of the timing of a stimulus and of its sensory consequences. However, it is also possible that this activity is a reflection of an automatic, covert movement preparation entrained by the rhythmic stimuli. For example, an automatic facilitation of the gait by rhythmic stimuli has already been shown in Parkinsonian patients (McIntosh et al. 1997). Saleh et al. (2010) recorded LFPs in primary motor cortex of a human patient who had to point to a spatial target with his chin. Five spatial cues were displayed successively, and he had to select either the 2nd or the 4th cue, in different trials. The power of beta oscillations peaked transiently before each spatial cue, with the highest pre-cue power for the correct cue. Furthermore, the beta power was phase-locked to slower delta oscillations (0.5-1.5Hz) that matched the duration of the inter-cue intervals, and the entrainment was maximal for the correct cue. More generally, a handful of papers show that whenever the timing of a cue is predictable, the beta power during the pre-cue epoch is elevated (van Wijk et al. 2009; Saleh et al. 2010; Haegens et al. 2011; Takahashi et al. 2011; Kilavik et al. 2012a; reviewed in Kilavik et al. 2012b). This elevated beta power preceding the cue in motor cortical areas may reflect the anticipation of a forthcoming event, even though further studies are needed to rule out other hypotheses, like the general involvement in a sensorimotor task (Kilavik et al. 2012b). Finally, in the context of cue anticipation, beta power seems to show epochs of transient coherence with slow oscillations (Saleh et al. 2010; Cravo et al. 2011), leading to the hypothesis that these slow waves could play a role of regulating the activity over large networks.

Pre-cue activity in single neurons

In cingulate and prefrontal cortex, Niki & Watanabe (1979) were the first to show an anticipatory activity preceding the onset of a cue, even though it was only present in 3% of the neurons they recorded. In the ventral prefrontal cortex, Moody & Wise (2000) also recorded from neurons whose firing rate was much higher when the timing of the cues was predictable. In a GO/noGO task, Sakagami & Niki (1994) used either the spatial location of the cue, its color or its shape to instruct the behavioral response (Fig. 1.11). All aspect of the cue were present at the same time, but the monkey was instructed to pay attention to only one of the three, different in each trial. Some neurons showed an anticipatory activity preceding the cue, which were selective for the type of instruction. Interestingly they were located in different areas of the prefrontal cortex according to their selectivity. On the one hand, the neurons selective to the shape or color were located in the lateral prefrontal cortex, where the projections from the inferotemporal cortex are concentrated (area involved in

shape and object recognition). On the other hand, the neurons selective for location (like the example on Fig. 1.11) were located near the principal sulcus, which receives projections from the posterior parietal cortex, a part of the brain thought to be strongly involved in spatial processing. All together, the neurons from the prefrontal cortex exhibit an anticipatory activity preceding a predictable cue, but their selectivity seems to be determined by the dimension of the cue which is behaviorally relevant. We will see now that a similar type of activity was also found in the basal ganglia (Lauwereyns et al. 2002).

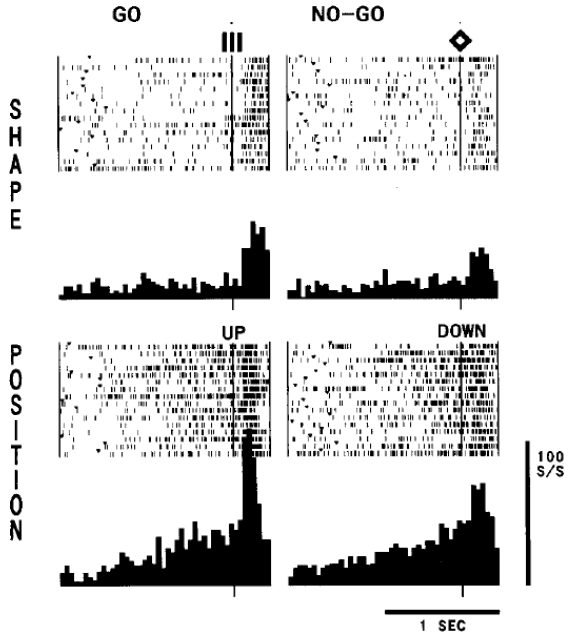


Figure 1.11: Prefrontal cortical neuron showing an anticipatory activity before the cue. Convention of the raster plot and PETH are as in Fig.1.6. On the left are the "GO" trials, in which the monkey has to respond, and on the right the "noGO" trials. On the top, the instruction is given about the shape of the cue, and on the bottom about the location of the cue. This neuron fires more for the GO than for the noGO trials (left vs right plots), but its main selectivity is in respect to the nature of the cue (top vs bottom). Reproduced and modified from Sakagami & Niki (1994).

A pre-cue anticipatory activity was also recorded in the caudate nucleus preceding a cue, but only if this cue was the target for a future eye movement. Indeed, a visually identical cue used as distracter was not preceded by anticipatory activity (Hikosaka et al. 1989). This pre-cue anticipatory activity was later found to depend on the expected reward (Takikawa et al. 2002). In their task, the monkey had to perform eye movements in 4 possible directions. However, only one direction was rewarded, changing from block to block, but kept constant within one block. The pre-cue activity depended on the rewarded direction. The vast majority of the neurons fired the most when the contralateral direction (to the recorded caudate nucleus) was rewarded, and fired the least when the ipsilateral one was rewarded. Surprisingly, in blocks in which all directions were rewarded equally, hardly any pre-cue activity was observed. The relation between reward and movement direction seems to be even more complex, because the pre-cue activity also depended on the eccentricity of the target on the horizontal axis. Going further, Lauwereyns et al. (2002) added a condition in which the presence or absence of reward was indicated by a colored cue. The population of neurons sensitive to the

directional reward mapping was different than the population of neurons which were selective for the color reward mapping. Furthermore, the cue indicated the direction of a required eye movement, allowing the authors to assess the influence of the pre-cue activity (depending on reward) on the directional selectivity following the cue. They found that the directional selectivity following the cue was completely unchanged by the pre-cue activity: the only difference was the overall level of activity. In contrast to spatial or temporal attention increasing the discriminability of a stimulus (Reynolds & Chelazzi 2004; Ghose & Bearl 2010), the pre-cue activity only acts through an additive gain on the response to the cue. This interesting pre-cue activity may participate at a network setting stimulus-reward contingencies, or, as the authors put forward: "the pre-cue bias would serve to incorporate the reward value of sensory features in the signal-detection process" (Lauwereyns et al. 2002). In other words, a stimulus would have an increased "neuronal representation" even before it appears, because it was previously associated with a reward.

One of the few studies that specifically addressed the pre-cue activity in non-visual areas looked at the activity of the sensorimotor areas (S1 and S2) during a visuo-tactile discrimination task (Meftah et al. 2009). In their task, a visual cue indicated to the monkey which modality he had to attend to (visual or tactile). After a fixed delay during which the modality instruction stayed on, a “standard stimulus”, composed of both a visual and a tactile array, was presented to the animal. The animal had to detect a subtle change of the standard stimulus in the cued modality, in order to release a lever and get a reward. Indeed, in some trials, both modalities of the standard stimulus changed at different latencies, and the monkey had to respond only to the cued one. 40% of the neurons from S1 and S2 (same proportion in the two areas) exhibited an anticipatory activity change during the delay preceding the standard stimulus, with either a gradual increase (80% of those, example Fig.1.12 A) or decrease (the remaining 20%, example Fig.1.12 B) of activity preceding the onset of the visuo-tactile cue. The mean modulation of activity was of about 5Hz, and the pre-cue activity was not correlated trial-by-trial with the performance (RT and error rate). Neurons with an increasing pre-cue activity tended to be associated with a post-cue phasic response, whereas those with a decreasing pre-cue activity were associated with a post-cue inhibition of similar latency (compare Fig. 1.12 A & B, in the epoch just following the onset of the Standard cue). In chapter 4 it becomes clear that their results are remarkably similar to the ones we obtained in the motor cortex.

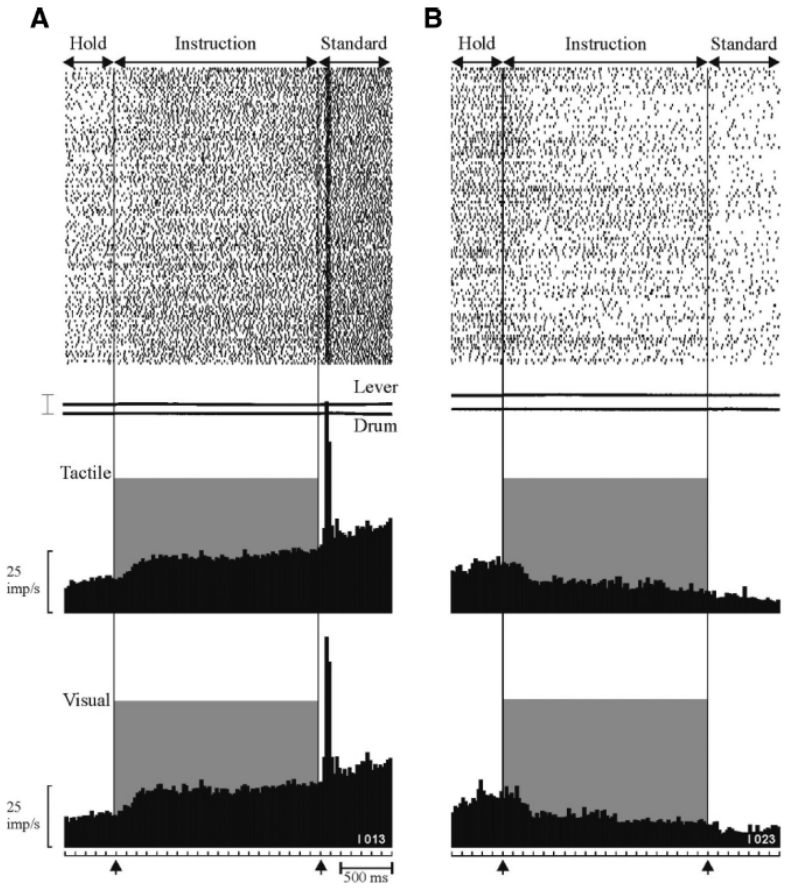


Figure 1.12: two neurons from S1 with a pre-cue anticipatory activity. The monkey had to hold a lever ("hold"), then an instruction cue indicated to pay attention to the tactile (top PETH) or the visual (bottom PETH) part of the stimulus. During the delay marked by a gray rectangle, the monkey waits for a fixed delay with the instruction cue on. At the end, the visual/tactile stimulus is switched on ("standard"), indicating that the monkey has to discriminate in order to give a behavioral response (not shown in the figure). Note that none of the two neurons are sensitive to the attended modality indicated by the instruction cue (compare top and bottom PETHs). A) Neuron with an increasing pre-cue activity. Note the phasic response to the standard cue, even in the visual task. B) Neurons with a decreasing pre-cue activity. Note the small transient inhibition following the cue. Reproduced from Meftah et al. 2009.

In a previous study, the same authors showed that 20% of the neurons from S2 (and none from S1) were influenced by the instruction cue, directing the attention to the tactile or visual stimulus (Meftah et al. 2002). It is interesting to note that the activity of 30% of the neurons with a pre-cue activity in S2 was modulated by the attended modality (indicated by the instruction cue), but no neurons showed such a selectivity in S1. This result would tend to show that the pre-cue activity emerges from a different mechanism than those directing attention. In a companion study, the authors tested human participants in a psychophysics task, to see if the amplitude of the pre-cue activity (increasing with time) improved tactile detection. To this end, they trained human participants to perform exactly the same task of “cross-modal attention”. An instruction signal oriented their attention to the visual or tactile modality, and then they had to wait for a fixed delay before the onset of the “standard stimulus”. During this delay, the participants received very weak electric shocks on the fingers, occurring at different latencies. The probability to detect these shocks was assessed as a function of the latency of the shock during the delay. If the cue-anticipatory activity enhances tactile discrimination, the probability to detect a shock should increase during the delay. The authors showed a strong effect of the instructed modality (more detection of the shocks when the tactile modality was cued), but the sensitivity to electric shocks stayed constant during the whole delay (Meftah et al. 2009). This negative result suggests that the level of pre-cue

activity does not improve basic perceptual abilities (even if the authors support this interpretation). In summary, in S1 and S2, almost half of the neurons enter in a pre-cue activity linked to the response of the neuron to the cue (excitation vs. inhibition), but seemingly orthogonal to attentional modulation, and without any behavioral correlate (RT and error rate).

In the last part, I will describe the studies addressing the pre-cue activity in motor cortical areas. Two studies directly addressed the pre-cue activity (Mauritz & Wise 1986; Vaadia et al. 1988), while the remaining only reported on it as a side-note (e.g. di Pellegrino & Wise 1993a; Crammond & Kalaska 1996; Hoshi & Tanji 2006). All works describing an anticipatory activity used a fixed or highly predictable pre-cue delay. In the task of Vaadia et al. (1988), monkeys were extensively trained in a task including a 3 seconds delay preceding the cue. During this delay, different patterns of firing rate modulations were observed in motor cortex. Some neurons show an increase of firing rate preceding the cue, some others show a decrease, and the remaining neurons do not change their activity. Even though the proportions vary from study to study, most works report more neurons with an increasing than a decreasing firing rate (Vaadia et al. 1988; Crammond & Kalaska 1996). However, the proportion of neurons with anticipatory activity seems to depend on the location of recording. In most studies including recordings from several frontal areas, PMd shows the strongest representation of anticipatory activity. Crammond & Kalaska (1996) showed a higher percentage of anticipatory neurons in PMd than in M1. Additionally, they demonstrated that the proportion of anticipatory neurons with increasing/decreasing activity changed with the distance to the central sulcus. In PMd and in the rostral part of M1, the majority of the anticipatory neurons had an increasing activity. In contrast, all anticipatory neurons recorded in the caudal (sulcal) part of M1 had a decreasing activity. Interestingly, this zone of M1 has the highest density of direct cortico-motoneuronal projections (Rathelot & Strick 2009). Hoshi & Tanji (2006) showed many more anticipatory neurons in PMd than in PMv. They also showed that the anticipatory neurons of PMd were more sensitive to the expected information contained in the cue than those of PMv (see below). di Pellegrino et al. (1993) showed similar differences between PMd and the prefrontal cortex (PF, between the principal sulcus and the ventral part of the arcuate sulcus), with more neurons showing an anticipatory activity in PMd. Furthermore, the PMd activity was more sensitive to the expected information carried by the cue. Similarly, Vaadia et al. (1988) observed more anticipatory neurons in PMd than in PF (in this study between the dorsal part of the arcuate sulcus and the principal sulcus).

As mentioned above, some studies described an effect of different aspects of the cue on the anticipatory activity. In particular, three studies showed an influence of the expected information carried by the cue (albeit to a different extent). Vaadia et al. (1988) used a block-wise presented task, in which the cue in one block

either indicated the target of the movement or was non-informative. They showed that a small fraction of the recorded neurons changed selectively their activity preceding a cue depending on the block. Similarly, in di Pellegrino & Wise (1993), the cue could indicate either the target of the movement or a shift in attentional focus, irrespective of the movement target. Two thirds of the anticipatory neurons modulated their firing rate depending on the information carried by the cue, being largely more active in the "movement" than the "attention only" blocks. Finally, Hoshi & Tanji (2006) showed that the activity of more than 10% of the anticipatory PMd neurons significantly changed their activity depending on whether the cue was expected to be informative about the movement target or about which arm to use. However, expected reward can also modulate the cue anticipatory activity. Vaadia et al. (1988) added a condition in which the trials were rewarded at random. They showed that a subsample of anticipatory neurons stopped responding after several unrewarded trials in a row. Finally, Mauritz & Wise (1986) reported that the vast majority of the neurons with an increasing activity also displayed a phasic response to the cue (similar to Meftah et al. 2009).

In summary, during sensorimotor tasks, a seemingly ubiquitous pre-cue anticipatory activity emerges in various areas, with different properties according to the site of recording (see Takikawa et al. 2002). The pre-cue activity seems to be influenced by the rewarding "value" of the cue, the information it carries, the relevant dimension to be considered... But its functional purpose is still elusive.

1.3.4. Summary

In our everyday life, we optimize our actions by predicting the timing or the location of relevant events. Doing so leads to both an enhancement of the perceptual abilities and to the preparation of potential actions, improving motor performance. Some neuronal mechanisms seem to be common to spatial and temporal orientation, like the phase-locking of the spikes in gamma oscillation, or a modulation of beta power over the somatosensory cortex. Others are specific to one mechanism, because of its very nature: the modulation of the slow components of the EEG signal tends to have different slope and amplitude according to the level of expectancy. The fact that spatial and temporal attention are deeply embedded in a sensorimotor context leads to the idea that both mechanisms may be mediated by premotor mechanisms, involving a parieto-premotor network (LIP-FEF for spatial attention, and posterior parietal-premotor for temporal orienting). This points to an integrated model of "event anticipation" spanning the whole spatio-temporal aspect, modulating attentional ressources in order to improve motor behaviors.

The major problem is that timing is always associated with other processes. Even when the subject explicitly reports durations, timing and decision-making are deeply embedded. During sensorimotor tasks, timing is associated with the anticipation of cues and the preparation of movements. This leads to various modulations

of the brain activity, both of the slow LFP potentials evoked by the targets and of the population (spiking) activity preceding it. Regarding movement preparation, the delay activity seems larger in short delays, perhaps the result of an overall scaling of the activity to a shorter duration or due to motivational reasons. Additionally, predictable cues lead to a ramping anticipatory activity in various areas of the brain, not necessarily linked to attentional processes.

In the two Results chapters of this manuscript, I will present our work on the influence of temporal orienting in motor cortex. The task we designed had two important features: 1) the timing of the main events of the task (cue and GO) changes trial by trial but is indicated to the monkey in advance, allowing a precise temporal expectation. 2) The spatial cue is temporally distinct from the movement execution, allowing to assess the influence of temporal orienting on these two events separately. In other words, this task allows studying the influence of endogenous temporal orienting on the processing of a spatial cue and on the preparation of a movement. Chapter 3 will address the influence of temporal orienting during movement preparation, on the spiking activity and on the evoked potentials of the LFPs. Chapter 4 then addresses the spiking activity starting in anticipation of the cue, and relates it to the activity during movement preparation described in the preceding chapter.

2. Material & Methods

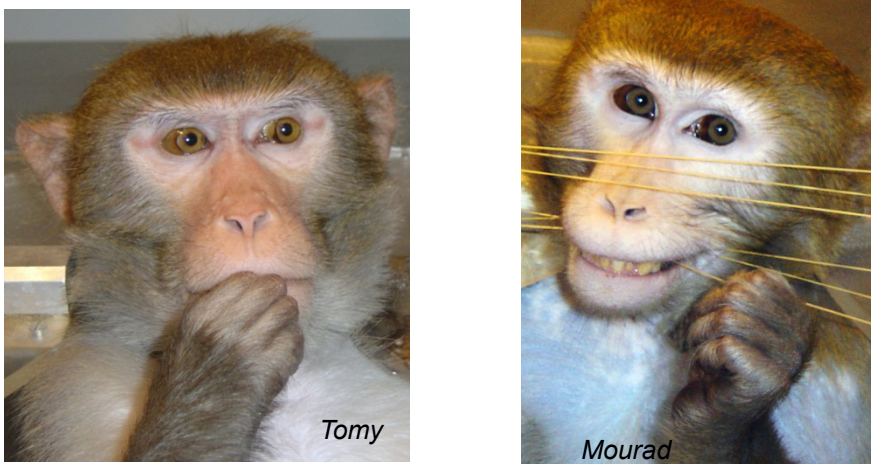
2.1. Introduction

This section will introduce the technical aspects of the experimentation conducted during my PhD. I will start by presenting the animal housing and training methods. Then, I will introduce the experimental methods that are common to the two studies. Since the two chapters exploits different aspects of the same behavioral task and the same apparatus, the main conceptual and technical features of the task will be presented here.

2.2. Animal housing and training

Two adult male Rhesus monkeys (*macaca mulatta*) participated in this study: Tomy, ('monkey T') and Mourad, ('monkey M'), both 9kg (see fig. 2.1). Care and treatment of the animals during all stages of the experiments conformed to the European and French Government Regulations. They were housed in one Allentown modular cage of about 4m³, which could be divided in 4 independent volumes. If possible (i.e. if the behavior of the animal was not dangerous for himself or the others), the animals were pair (or trio)-housed to promote social interactions between them and ensure their well-being. Perceptual enrichment was provided by mean of a television, showing movies and documentaries about 2 hours per day. Additionally, it was meant to habituate the monkeys to human noise, so that they would feel less stressed or distracted during the experimentations.

Figure 2.1: our two monkeys.



The training of both monkeys lasted about one year (5 days a week), followed by 6 months of recordings in monkey T and one year in monkey M. The recording period of monkey T is shorter because he lost his head implant, so we had to interrupt the experiments. After the experiment of (0.5-1 year), the head implants were removed and the scalp sewed back, then the monkeys were transferred back to an independent animal facility

located on the same campus. In this place, they lived in cages of several tens of cube meters with an indoor and outdoor part, where they were housed with other individuals. During this period, monkey T could mate freely and had a baby (Nikos).

The training started with a period of habituation with the experimental apparatus (chair, experimental room, joystick/screen). To do so, the animals were conducted to the primate chair with a stick attached to their collar, in which they would receive water and pieces of fruits, in order to associate this new (stressing) environment to a rewarding stimulus. During the first attempts, if the animal was so anxious that he could hurt himself, a light anesthetics was administered. After they got used to the primate chair, they were trained using a step-by-step procedure, during which they would learn sequentially different aspects of the task. For example, they would start by just manipulating the joystick, then learn to reach to a large target, then learn to wait for a GO signal, and so on. Each correct trial was rewarded by a drop of liquid (water or diluted juice, depending on the animal preference). Additionally, pieces of fruits were given to the animal while they were working, to reinforce particularly appropriate behaviors. To motivate the animals to learn and perform the task, they were kept on water restriction during the week, and had free access to water only during the week-ends. To make sure that this restriction did not impact their health, they were weighted one a week. Twice a year, they usually got 2-weeks "vacation" periods, during which they would receive *ad libidum* food and water.

2.3. Behavioral task (conceptual aspects)

In the introduction, I discussed the fact that temporal constraints influence motor cortical activity during the preparation of movements. Alexa Riehle and her student Sebastien Roux specifically studied the influence of delay duration before a self-paced movement on motor cortical activity (Roux et al., 2003). They demonstrated that about 40% of the neurons had a different activity during the delay preceding movement initiation depending on delay duration.

Nevertheless, a major drawback of the task they used is that it did not allow to separate the influence of the timing itself from the influence of movement preparation. For example, this task did not allow to study a potential influence of timing on the motor cortical activity when no movement was planned. It was to address this issue that a new task was developed (Fig. 2.2). I will first describe it conceptually and explain why particular features were implemented, before presenting the technical details.

As explained above, the main point of this task was to dissociate time estimation from movement preparation. To do so, we used a delayed center-out pointing task (Georgopoulos et al., 1982), that we

modified by using two successive delays instead of one. In each trial, the informative spatial cue (SC) was displayed at the end of the first delay (D1), but the movement could only be performed at the end of the second delay (D2). At the beginning of the trial (thus starting D1), the animal was provided with a cue indicating the duration of the two delays (Time Cue, TC). The TC was a tone, that could have two different pitches, to which corresponded two possible delay durations ('short' or 'long'). Thus, the animal did not have to choose between a multitude of possible delay durations, but had instead a binary choice between only two possible durations. At the end of D1, the spatial cue was presented very briefly, and immediately followed by a visual "mask" (all the other possible target being switched on): perceiving it was very difficult, and lead to a high ratio of errors, even after one year of training (see part 3.2.2). This was meant to encourage the monkey to precisely estimate the duration of the first delay, in order to know precisely when the SC would appear and thus ease its detection. Rolke & Hoffman showed in 2007 that the perceptual (visual) abilities decreased with increasing temporal uncertainty. The perceptual task was thus supposed to be difficult enough to force the monkeys to take into account the timing information provided by TC, in order to make the detection of SC easier. By separating the delay during which the monkey had to estimate the time before the cue (D1), and the one during which he had to estimate the delay duration and at the same time prepare a movement (D2), we could segregate the influence of timing and motor preparation on the motor cortical activity.

Importantly, one thing has to be kept in mind while reading the results presented later in this thesis. The two delays of the task involve two conceptually different tasks.

During D1, the monkey cannot prepare any movement, since he does not know which target to go to yet. He is engaged in a visual detection task, which may be eased by time estimation.

During D2, the monkey possesses all the possible information about the movement: he knows where to go, and when the GO signal will appear. He is then engaged in a motor preparation task with complete prior information.

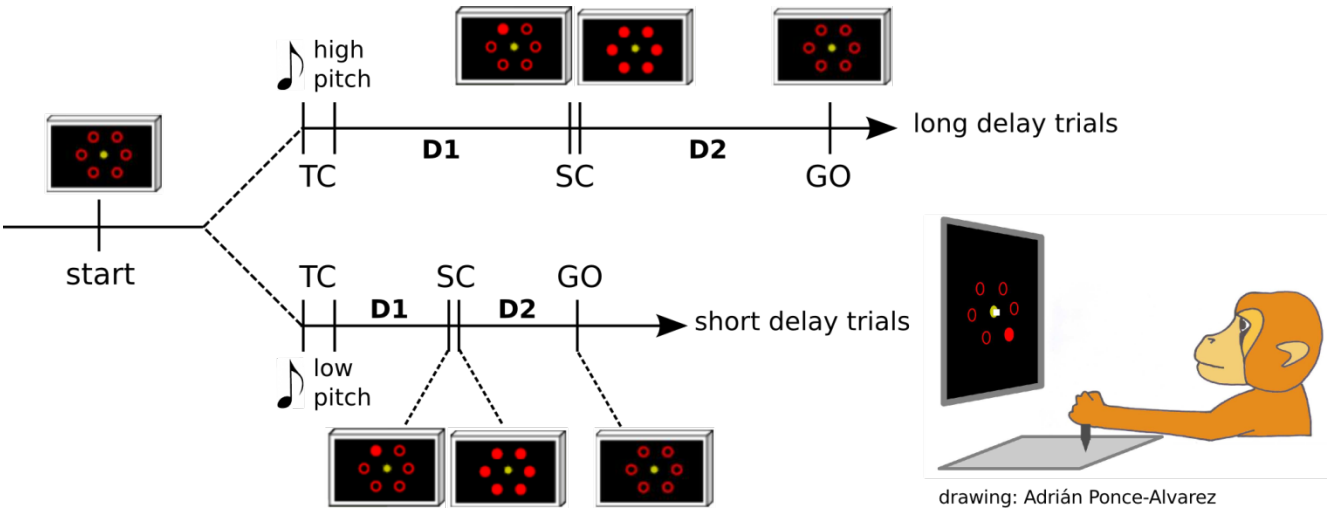


Figure 2.2: Experimental task. Lower right: drawing of the experimental apparatus, showing the SC epoch (note the cursor on the central fixation dot). Left part, sequence of task events (not to temporal scale). Start indicates the moment when the monkey brings the cursor to the center of the screen to initiate a new trial. The note indicates the presentation of a tone, of different pitch according to delay duration. All screen-shots shown in the cartoon stay on until the next one appears (the cursor is not shown). TC: Time cue, 200ms; SC: Spatial Cue, 55ms; D1/D2: delay 1 and 2, respectively (both either 700 or 1500 ms in monkey T, 1000 or 2000ms in monkey M). There is also a 700ms delay between start and TC.

2.4. Behavioral task (technical aspects)

We trained the monkeys in a delayed center-out task to make arm movements in 6 directions in the horizontal plane, from a common center position. The movement was executed by holding a handle, freely movable in the 2D plane, which contained a pen transmitting a signal to a digital tablet (WACOM Intuos2). The monkey had continuous monitor feedback about pen and target positions, the center target in yellow and the six peripheral target locations as red outlines.

The monkey started each trial by moving the handle to the center and holding it there for 700ms before a temporal cue (TC) was presented (Fig. 2.2). TC consisted of an auditory signal of 200ms duration, its pitch indicating the delay duration to be estimated (low pitch for short and high pitch for long delay duration). In monkey M, a visual cue was additionally presented centrally on the monitor, simultaneously with the sound cue, a blue square for short and pink triangle for long delay duration (not shown on the figure), in an attempt to ease the discrimination of TC and help time estimation. Two delays of equal duration were presented successively in each trial, the first (D1), starting with TC offset, demanded attention to perceive the briefly (55 ms) presented visual spatial cue (SC) at the end of it. During the second delay (D2) the movement indicated by SC could be prepared. Apart from the initial TC, short and long trials were identical in terms of visual input and movements required, only the task timing was different. The short and long delay durations were fixed to 700 and 1500ms for monkey T, 1000 and 2000ms for monkey M. Delay duration was changed from trial to trial, in a

pseudo-random fashion. The delay durations are different in the two monkeys, because the data of monkey M were used in a complex analysis method (Ponce-Alvarez et al. 2012) requiring long delay durations.

For an exact temporal precision of SC we used LEDs as cues, placed over the target positions on the screen. After illumination of the target LED as SC, the target was masked by the additional illumination of the 5 remaining LEDs, marking the start of the preparatory delay (delay 2). At its end all the LEDs were turned off, providing the GO signal to swiftly execute the movement to the cued SC location. The distance between the central and peripheral targets (center to center) was 6.75 cm in the movement plane. Their diameters (required touch precision) were 0.91 and 2.24 cm, respectively. In some sessions we reduced the number of conditions by only presenting two opposite targets (4 conditions instead of 12, or 2 directions instead of 6). These sessions were included in the analysis, apart from studies of directional selectivity in which it is stated otherwise. Trials were considered to be correct when the movement was initiated within 500ms of GO signal occurrence and completed to the correct target within another 500ms, plus a final period of holding the handle at the target position of 300ms. Only correct trials were rewarded by a drop of water (about 0.3ml). When quantifying the percent of errors we only considered those occurring after the GO signal.

Here is a little lexicon of the terms I will employ in the next chapters: a "session" means an experimental session during which the monkey performed the task once, which lasted between 45 minutes and one hour. Typically, 1 to 3 sessions per day were recorded. A "6 directions session" is a session in which all movement directions were used, whereas a "2 directions session" is a session in which only 2 opposite movement directions were used. A "condition" means all trials recorded in a given movement direction with a given delay duration: a 6 directions session has thus 12 conditions, and a 2 directions session 4 conditions. About 20 trials per condition were recorded, leading to 240 trials per session in the 6 directions session, and more than 80 trials in the 2 directions session.

2.5. Experimental procedures

After learning the behavioral task the monkeys were prepared for multi-electrode recordings in the right hemisphere of motor cortex, contra-lateral to the trained arm. A cylindrical stainless steel recording chamber (inner diameter: 15mm in monkey T and 19mm in monkey M) was implanted under aseptic conditions and general isoflurane anesthesia (<2.5% in air). A T-bar was cemented to the skull to fixate the animal's head during recording sessions. Before and after surgery, antibiotics and analgesics were administered. During recordings, biological samples were taken from the recording chamber regularly to detect possible infections.

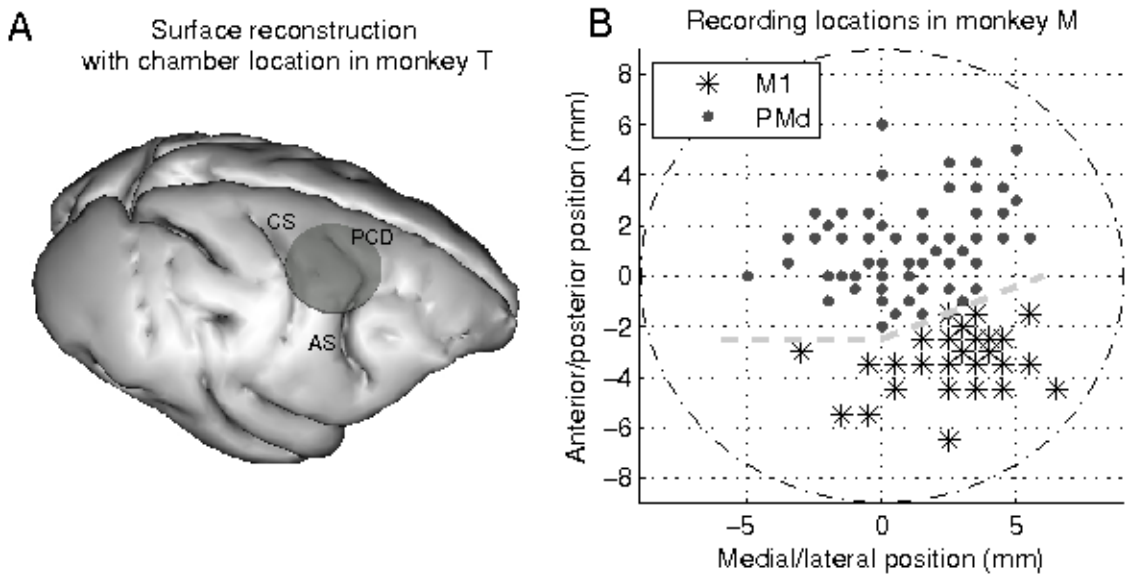


Figure 2.3: Recordings location. A) Reconstruction of recording location in monkey T, based on T1-weighted MR-scans. The circle indicates the position of the recording chamber (inner diameter: 15mm diameter), CS: central sulcus, AS: arcuate sulcus, PCD: pre-central dimple. Anterior is towards the right. B) Recording positions within the chamber (inner diameter: 19mm diameter) in monkey M. Locations are assigned to M1 or PMd based on micro-stimulation controls. The gray dashed lines indicate the estimated location of the border between M1 and PMd. Zero is chamber center. Anterior is up, medial is left (chamber in right hemisphere). Reproduced from Kilavik et al. (2010) (Fig. 1).

The chamber locations above motor cortex were verified with T1-weighted MRI scans in both monkeys (see Fig. 2.3 A for monkey T) and additionally with intra-cortical micro-stimulation in monkey M. Micro-stimulation allowed further sub-division into M1 and PMd in monkey M (Fig. 2.3 B), based on observed micro-stimulation thresholds for inducing muscle twitches (micro-stimulation protocol as in Asanuma and Rosén 1972). Locations in which micro-stimulation below 20μA current induced muscle twitches were defined as M1. Other locations, where higher current was needed to induce twitches were denoted as PMd. Twitches could also be evoked at the most anterior recording locations, indicating we remained within the caudal part of PMd (F2), where there are direct projections to the spinal cord (Dum and Strick 1991; Boudrias et al. 2010). In monkey M, only 37/490 of the analyzed LFPs and 80 of the 740 neurons were recorded from 'non-arm' regions (i.e. the evoked muscle twitches at threshold were not in hand/arm/shoulder muscles; note that in several of these so-called 'non-arm regions', arm movements were also evoked when applying currents slightly above threshold). These LFPs were included in the analysis, as their evoked potentials contained similar effects (notably modulations in evoked potential size in relation to delay duration) as LFPs recorded from arm-related regions. This also holds for the neurons: the average modulation of firing rate of the neurons recorded in non-arm areas did not differ qualitatively with the one of the neurons recorded in arm-related area. Thus, we decided to pool them together. It is also clear that even if the monkeys were seated in a primate chair, with the head fixated and the

non-active hand restrained, and performed an arm-movement task, we cannot exclude systematic task-related small shifts in body posture etc, possibly involving other muscle groups.

A multi-electrode, computer-controlled microdrive (MT-EPS, AlphaOmega, Nazareth, Israel) was used to transdurally insert up to four or eight (in monkey T and M, respectively) microelectrodes (glass or epoxy insulated tungsten electrodes, Frederick Haer, 0.5-1.2MΩ at 1,000Hz). In monkey T the electrodes were organized in a bundle in one common guide tube holding the individual electrode guides, with an inter-electrode distance less than 400µm (MT, AlphaOmega). However, since the electrodes were driven independently, their position in depth varied for each electrode. In monkey M, on some days electrodes were organized in a bundle as for monkey T and on others the electrodes were positioned independently within the chamber (19mm diameter) with separate guide tubes (Flex-MT, AlphaOmega). From each electrode, signals were amplified with a gain of 5,000 to 10,000 (MCP+, AlphaOmega) and separated into two channels with different band-pass filtering by using in-house hardware (active filtering): from 0.3 to 10kHz for spiking activity, and from 1 to 250Hz for LFPs. The LFPs were stored with a temporal resolution of 1kHz (AlphaMap, AlphaOmega). An on-line spike shape detection method was applied on the high-frequency signal (MSD, AlphaOmega, Nazareth Illith, Israel), allowing isolation of up to three spike shapes per electrode. The timing of each spike was then stored as TTLs at a temporal resolution of 32kHz. In parallel, the raw, unfiltered signal was digitized and stored at 32kHz, and used to carry out an off-line spike sorting when the on-line spike sorting was unsure. The off-line spike sorting was made using Mclust (http://www.stat.washington.edu/mclust/), a software allowing the clustering of the data along several dimensions (e.g. the three first component of the signal), to help separate the different spike shapes. The behavior was transmitted online to AlphaMap from the CORTEX software (NIMH, http://www.cortex.salk.edu), which was used to control the task. Timed x and y hand position information was recorded with CORTEX and aligned with the neuronal signals offline (see Fig. 2.4 A).

2.6. EMG activity

Electromyographic (EMG) activity was recorded from 10 muscles of the active (left) arm, shoulder and chest of monkey M in multiple representative and dedicated sessions by using differential surface electrodes on the skin. Raw EMG signals were recorded at 1kHz resolution and subsequently full-wave rectified and integrated offline (see Fig. 2.4 B). We find that the main arm and upper body muscles that were activated during movement execution (see Fig. 2.4 B right panel, data aligned to movement onset) showed no systematic activity modulation during the delay periods (left panel). These behavioral controls confirm that neither of the

two animals made systematic overt movements during periods when they should not move, i.e. during delays and following SC.

2.7. Reaction times and movement trajectories

In order to control behavior, reaction times (RTs) were defined on-line by CORTEX as the time between extinguishing the target LEDs (GO) and leaving the center target. For analysis, however, correct RTs were determined off-line from the movement trajectories. Single trial movement trajectories were measured (in mm) in x and y coordinates by acquiring two vectors in time. The final time resolution was 1ms. Missing values (because of the temporal imprecision of MS Win XP) were extrapolated from the neighboring values. The mean of each x and y position in each correct trial was calculated during the 500ms before GO, in order to determine the movement's starting position. The moment when reaching a 2mm deviation from the starting position, minus a fixed latency of 35ms (average movement duration from the starting position to the threshold) was then determined as movement onset. From each of the two vectors (x and y), the shortest time was defined as RT. These values were controlled by visual inspection of single trial trajectories. Fig. 2.4A shows representative single-trial movement trajectories during the delays (separated in simultaneous left/right (X) and towards/away from the body (Y) pen-positions on the digital tablet) in one movement direction, recorded in monkey T in one session. Clearly no systematic movements were made after the hand position stabilized at the center position before TC and until after the GO signal. Filled circles after GO indicate movement onset. In monkey M, we recorded EMG activity in several sessions. As a result, we find that the main arm and upper body muscles that were activated during movement execution (see Fig. 2.4B right panel, data aligned to movement onset) showed no systematic activity modulation during the delay periods (left panel). These behavioral controls confirm that neither of the two animals made systematic overt movements during periods when they should not move, i.e. during delays and following SC.

All analyses presented later were conducted using the software Matlab (The MathWorks Inc., USA) version 7.10 and 7.12 on a Linux, Macintosh or Windows XP platform.

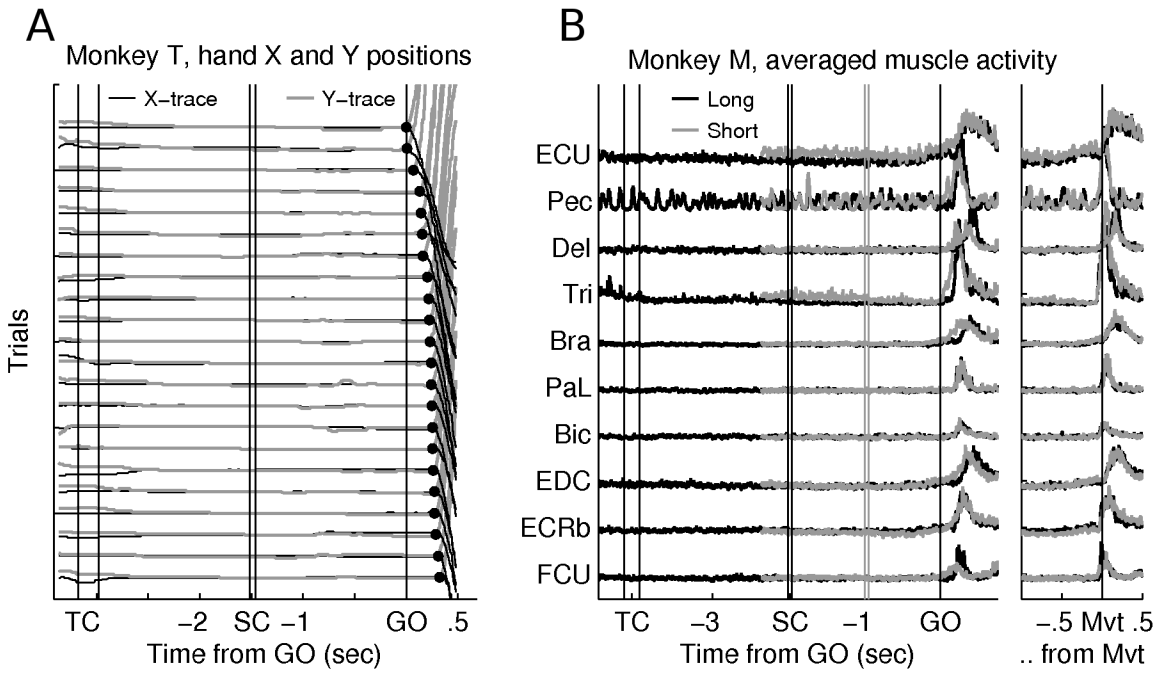


Figure 2.4: Movement trajectories and arm muscles activity. A) Example of hand X and Y positions for monkey T, from multiple correct repetitions of a long trial-type. The X-axis indicates the timing of events. Each pair of black (x-direction of movement) and gray (y-direction of movement) lines corresponds to 1 trial (trials are displaced in steps of 0.5cm on the Y-axis for clarity). Filled circles after GO indicate movement onset. Trials are sorted offline for increasing RT. B) Averaged muscle activity from 10 arm and upper body muscles on the active side, recorded in representative and dedicated sessions in monkey M. Activity is aligned to the signals, at the left, and to movement onset (Mvt), at the right. Short trials are shown in gray, long trials in black, both for movements to the lower right (T2). The time of SC onset and offset in long trials is indicated by two vertical black lines. The two vertical gray lines indicate SC in short trials. The selected muscles were from top to bottom: the extensor carpi ulnaris (ECU), the pectoralis (Pec), the deltoideus (Del), the triceps brachii (Tri), the brachioradialis (Bra), the palmaris longus (PaL), the biceps brachii (Bic), the extensor digitalis communis (EDC), the extsensor carpi radialis brevis (ECRb), and the flexor carpi ulnaris (FCU). Artifacts due to the heartbeat can be observed on the EMG taken from the pectoralis muscle. Reproduced from Kilavik et al. (2010) (Fig. 2).

2.8. Analysis methods for the LFP EPs

2.8.1. Analysis of the LFPs

Since there is no DC offset in the LFP signal, the 0 Volt baseline is the overall mean of the LFP over time. Each LFP was z-scored prior to further analysis. This normalization allowed comparison of EP sizes across LFPs. In order to measure single-trial EP sizes (see below), the LFPs were first band-pass filtered between 1-15 Hz (4th order Butterworth filter; example of single-trial filtered LFPs are shown in Fig. 3.2A). This frequency range corresponds to the main frequency band of these signals as shown in the spectrogram in Fig. 3.2B. Furthermore, it excludes the prominent beta oscillations (20-25Hz), which were neither phase-locked to the SC nor to movement onset, but partly overlapping in time (not in frequency) with the EPs. The exclusion of the beta band activity in the present study does not imply that we consider it to be non-informative regarding for

instance the task timing (e.g. Kilavik and Riehle 2010; Kilavik et al. 2012a), but we here focus on the evoked activity. The spectrogram in Fig. 3.2B was calculated using Welch's method, including all short trials in a single representative LFP recorded in monkey T. The data were analyzed in 300ms sliding windows, with 50ms offsets.

2.8.2. Evoked potentials

EPs were analyzed in epochs that were selected based on visual inspection of trial-averaged LFPs (prior to filtering). The main MRP epoch was -150ms to +250ms around movement onset for both monkeys, selected to capture the three main components P1, N1 and P2 (see Figs. 3.2D). The main VEP epoch was +60ms to +200 (monkey T) and +60ms to +190ms (monkey M) after SC onset, selected to capture the two main components N1 and P1, which were the most frequent VEP components (see Fig. 3.2C). The size of EPs was determined in single-trials by calculating the area in the selected epochs using the root-mean-square (rms). The areas were subsequently averaged across trials for each condition. To test the significance of the EPs, the sizes of VEPs and MRPs were compared with a control epoch, the rms area during the 200ms prior to TC, by using a one-sided t-test ($p<0.05$) including all single-trial values, separately for short and long delay trials. If a particular EP was significant, all single-trial values were included in the further analysis.

2.8.3. Single EP components

We also quantified individual VEP and MRP components separately. The MRP N1 quantification was done as in Roux et al. (2006) similar to the method used by Donchin et al. (2001). For each LFP and each condition, N1 was determined by its negative peak (see Fig. 3.2D) and the preceding and subsequent zero-line crossings (using trial-averaged LFP traces). The epoch between these two zero-crossings was then defined as N1 (typically of about 100ms duration). In the preceding 100ms and subsequent 200ms we also quantified the MRP P1 and P2 components, respectively.

The individual VEP components were analyzed in a similar way, but because of the more variable amplitude of the components in different conditions and LFPs (making the method described above less reliable), we used fixed epochs, selected for each animal based on visual inspection. For monkey T, three components could be distinguished, N1 (60-120ms after SC onset), P1 (120-200ms), and N2 (200-310ms; less frequent and thus not included in the main VEP analysis epoch). In monkey M, four VEP components could be distinguished, N1 (60-110ms), P1 (110-190ms), N2 (190-290ms) and P2 (290-460), the latter two not being part of the main VEP analysis epoch, because of their less frequent occurrence across LFPs. We mainly report findings obtained from analyses of the complete VEPs and MRPs, as results were very similar for the individual components (mentioned in the results).

2.8.4. Peak amplitudes of single components

We also calculated the peak amplitudes on single trials for the largest and most frequent VEP and MRP components, VEP P1 and MRP N1. Peak amplitude was defined as the positive/negative deviation from the zero-baseline. The VEP P1 peak amplitude was measured in the epoch 120-200 or 110-190ms after SC onset, in monkey T and M, respectively. Similarly, peak amplitude for the MRP N1 component was selected in the epochs used to measure the N1 rms areas (see above). In the results we mainly report findings obtained from rms measurements. However, very similar results were obtained when observing the peak amplitudes, as mentioned on several occasions in the results.

2.8.5. Directional selectivity

We determined directional selectivity of RTs and EP sizes. For the EPs we used the P1 component of VEPs and the N1 component of MRPs, as they were the largest and most frequent of the EP components. The EP 'preferred direction' was defined as the direction of the vector sum of the average single-trial rms values in the 6 directions. The 'preferred direction' of the average RTs was defined as the opposite direction of the vector sum, to indicate the direction with the fastest RTs.

We also estimated the significance of the directional selectivity using a re-sampling procedure (similar to what was employed by Asher et al., 2007). The length of the vector sum was taken as the strength of directional selectivity. For each EP (or session for RTs), single trial rms (or RT) values were then randomly reassigned to one of the six movement directions and a new vector sum was calculated. This procedure was repeated 1000 times, and the length of the vector sum of the original data was then compared to the vector sums of the re-sampled data. If the original vector sum was larger than 950 of the 1000 re-sampled vector sums ($p<0.05$), the EP (or the RTs in this session) was deemed directionally selective.

As we were interested in comparing preferred directions in short and long delay trials, we subsequently calculated the circular distance (angle) between preferred directions in short and long delay trials of RTs for all recorded sessions (Fig. 3.1C-D) and for all EPs that were significantly directionally selective in short and in long trials (Fig. 3.8).

Circular statistics were done using the circular statistics toolbox for Matlab (http://www.mathworks.com/matlabcentral/fileexchange/10676).

2.9. Analysis methods of the spiking activity

2.9.1. Averaged activity

The averaged Peri-Event Time Histogram (PETH) of each neuron category was computed. The mean spike count of each neuron was computed with a temporal resolution of 1ms across all movement directions, then smoothed with a Gaussian filter (length 50ms, standard deviation 15ms) and converted into spikes per second. Then, the smoothed firing rates were averaged separately for each neuron category (see Fig. 4.4). To assess the difference between firing rates of different categories of neurons at the population level, we typically used a Mann-Whitney U test on fixed time windows, as described in the Results part.

2.9.2. Firing rate peak detection

We analyzed the phasic spiking activity following SC and around movement onset by determining its peak amplitude and frequency. Two analyses were performed, one based on the averaged firing rate across trials, and the other on the single trial spike count. The first method allowed peak detection based on a smoothed, less noisy signal, since the spike count of several trials (typically 20) was averaged. The second method provided information about the trial-by-trial fluctuations of the characteristics of the phasic response. The median of the peak latencies detected in single trials (2nd method) and the latency based on the averaged activity (1st method) were compared. The two analyses yielded similar results.

2.9.2.1. Method 1: Peak detection based on the averaged firing rate

In this analysis, only the preferred movement direction of the neuron was considered (the one with the highest spike count), computed during a 200ms window following SC onset for the "cue" epoch, and during 200ms around movement onset for the "movement" epoch. The epochs were chosen to comprise the peaks of the averaged activity shown in Fig. 4.4. Only long delay trials were considered, in order to better dissociate in time the activities related to SC and to movement execution.

For each neuron, the PETH was computed with a 1ms resolution in a 700ms time window starting 200 before the onset of SC. The moment of the peak firing rate (see Figs. 4.5C) was located using the Matlab function *findpeaks*, which compares each data point against its two neighbors under the following set of parameters: the peak amplitude (difference between the peak frequency and the minimum frequency in the window) must be at least 5 spikes/sec, and the peak latency 40ms or more after SC onset. If several peaks were detected, the one with the highest firing rate was selected. All analyses were subsequently controlled by visual inspection to avoid any spurious peak detection. The same method was used to detect the moment of the peak firing rate during movement execution, except that the data of all trials in one movement direction were

aligned to movement onset before computing the PETH. The peak was then searched in a 600ms window centered on movement onset, with the constraint 5 spikes/s minimally.

2.9.2.2. Method 2: Peak detection based on single-trial firing rate

This analysis was restricted to the cue epoch, since the broad peaks in the movement epoch makes single-trial peak detection difficult. As in the first analysis, only long delay trials were considered. Since this method was designed for single-trial analyses, we tried to maximize the number of usable trials. If the firing rate in the respective analysis window was not significantly different in respect to movement direction (Kruskall-Wallis test, $p>0.05$), the trials from all movement directions were pooled. Otherwise, only the preferred direction was considered (the one with the highest spike count). To compute the peak frequency and latency of the response in each individual trial, each spike train was convoluted with a triangular kernel in order to compute a "single-trial estimated firing rate function" (Nawrot et al. 1999), in a 400ms epoch starting at SC. The peak of the estimated firing rate function was then searched using the *findpeaks* function (see above), with the constraint that the peak frequency had to be higher than 80% of the maximum value of the analysis window (for example, if the maximum firing rate of the time window is 50Hz, the peak has to be 40Hz or more. This threshold avoid detecting several peaks of firing rate, including local maxima). As a control, an alternative method (Miller et al. 1992) was used, yielding similar results. In this control method, the spike count was computed in a sliding window of 100ms, shifted by steps of 10ms in the same 400ms epoch. For each trial, the peak was considered to be located at the center of the window with the highest spike count. If several windows had the same spike count, the central window was selected.

2.9.3. Directional selectivity

The time-resolved directional selectivity of the firing rate during D2 was computed separately for each neuron (see Fig. 4.9). The same method than the one used for the EPs was used (see 2.8.5). Only the sessions including 6 directions were used for this analysis, to avoid misclassifying as non-selective neurons that may have a preferred direction orthogonal to the two presented in a 2-direction session. For each neuron, in long delay trials the mean firing rate in each direction was computed in a sliding window of 200ms, which was shifted by 25ms along the data, from 400ms before SC to 900ms after GO. To avoid false positives due to a very low firing rate, a minimum 5Hz criterion in the preferred direction of the neuron was added. Additionally, to control for local firing rate fluctuations, only a series of at least three consecutive significant windows were considered. Fig. 4.9 shows that the amount of directionally selective neurons before SC is virtually 0, indicating that our method to control for false positives is efficient.

To assess the potential directional tuning of a neuronal population as a whole, a Hodges-Ajne test (Circular statistics toolbox, Berens 2009) was used. If the test reached significance ($p<0.05$), the neuronal population deviated from uniformity, and its preferred direction was calculated by a mean resultant vector. Otherwise, the preferred directions of the neurons were too evenly spread, and the population as a whole was considered not to be tuned. Consequently, the angular difference between the preferred directions of two populations could only be computed if both of them were significantly tuned. In this the case, the angular difference between the mean vectors of the two populations was computed. Finally, to test for the angular difference in the preferred directions of the two populations in a non-parametric way, the individual vectors of the two populations were shuffled and dispatched at random in two groups, and a new angular distance was computed. This procedure was repeated 1000 times. The angular difference between the two populations was deemed significant if it was larger than 95% of the random ones.

2.9.4. Comparison of firing rates in short and long delay trials during D1

To evaluate if the activity of a neuron has an early selectivity for the duration of the delay, the firing rate in the epoch following TC was compared between short and long delay trials (see Fig. 4.13). For each neuron, the spike count during D1 in the short delay trials was compared with the corresponding epoch in the long delay trials (corresponding to the period comprised between TC and the dashed lines in Fig. 4.4). A sliding window of 100ms was shifted in steps of 25ms, starting 300ms before TC and ending at the onset of SC in short delay trials (and at the same moment in long delay trials). In each sliding window, the spike counts of the short delay and the long delay trials were compared (Mann-Whitney U test), independently for each neuron. Then, the proportion of neurons with a significant difference ($p<0.05$) was computed during each time window.

3. Influence of timing on movement preparation: evidence from spiking activity and event-related potentials (EPs) in motor cortex

3.1. Introduction

The temporal constraint of any behavioral context helps us to choose the most appropriate way to plan a movement. If a given context makes me understanding that I have plenty of time to prepare a movement, or alternatively that it has to be executed immediately, the process of motor planning is likely to be different. Supporting this idea, I reviewed some studies in the general introduction (section 1.3.3), showing a strong influence of the temporal constraint on the spiking activity during motor preparation (reviewed in Kilavik & Riehle 2010; Kilavik, Confais & Riehle 2013). However, whether the task requires an *explicit* or *implicit timing* of the task events, the influence of time on the brain activity could be completely different (see Coull & Nobre 2008; Roux et al. 2003, for a discussion). *Explicit timing* means that the subject will have to keep track of the time passing to complete the trial: for example, if he or she has to respond "after 2 seconds but before than 3 seconds", or if he or she has to compare two durations and report which one is the longest. By contrast, *implicit timing* means that the task can be accomplished without counting time, but that for example, estimating the duration of a delay will help improve performance by decreasing the reaction time (RT). In everyday life, as in experimental psychology, virtually all tasks involve implicit timing to different degrees. For example, simple RT tasks with a fixed delay duration before GO will show an increase of mean RT with delay duration due to the increasing difficulty to precisely estimate longer durations. Alternatively, tasks using a finite number of delay durations before GO, presented at random with equal probability, will show a decrease of RT with increasing delay duration, due to the increasing conditional probability for the GO signal to appear, given that it has not appeared yet (both phenomena are reviewed in Niemi & Näätänen, 1981). In summary, few behaviors escape the influence of timing, be it explicit or implicit.

Alexa Riehle and her collaborators have studied the influence of timing on motor preparation for many years. Roux et al. (2003) showed that 40% of the motor cortical neurons modulated their activity as a function of the delay duration to be estimated before a self-timed movement. However, they did not distinguish between the epochs of the task when these modulations of firing rate appeared. Additionally, Renoult et al. (2006) showed that the variability of the behavioral response (i.e., RT) in a sensorimotor task depended on the "internal timing" of the animal. However, these two studies focused either on the influence of a complete temporal information on the generation of a self-timed movement (Roux et al. 2003), or on the temporal uncertainty about the occurrence of the GO signal (Roux et al. 2003, 2006; Renoult et al. 2006). Thus, one

aspect of the temporal constraint on motor preparation was left aside: the "countdown" situation, in which the subject knows in advance (complete temporal information) when the GO signal will appear. Indeed, before the kick-off of a Grand Prix, all pilots' eyes are dead-set on the traffic lights overhanging the track: the countdown of the red lights tell them exactly when the green one will be lit... This is the event they have to anticipate in order to take the lead in the first run. This "temporal orienting" or "expectancy" situation has already been extensively studied in human participants (reviewed by Nobre 2001; Coull 2004; Coull & Nobre 2008), but scarcely in non-human primates (Roesch & Olson 2005a,b). Roux et al. (2003) used two tasks, one in which the monkey did not know in advance when the movement would be required (temporal uncertainty), and one in which he was instructed to self-time a movement after a specific delay. Even though the self-timed task of Roux et al. (2003) seems close to temporal orienting, the fact that it involves a self-timed movement makes it completely different. While temporal orienting is an implicit timing task, a self-timed movement relies on explicit timing. Several fMRI studies in human participants showed that the brain areas preferentially activated by implicit or explicit timing tasks are only partially overlapping (Coull & Nobre 2008; Coull et al. 2012). So, even though transferring results from fMRI in humans to monkeys is not straightforward (especially because of the weaker lateralization of monkeys, see Oleksiak et al. 2011), they indicate that temporal orienting seems to activate different networks than a self-timed movement.

This chapter will focus on the influence of temporal orienting on motor preparation: how is the motor preparatory activity influenced by knowledge of the duration between an informative cue (and thus knowing which motor act to execute) and the GO signal. We will analyze both the spiking activity of single neurons and local field potentials (LFPs) recorded in the primary motor cortex (M1) and the dorsal premotor cortex (PMd). We focused our analysis on the evoked potentials (EPs) of the LFPs evoked by the two main events of the task (the cue onset and the execution of movement), and compared them directly with the spiking activity recorded in parallel. The data concerning the LFPs were published in Kilavik, Confais et al. (2010) and a part of this chapter is based on this article. Additionally, the spiking activity data were published in part in an invited book chapter (Kilavik, Confais & Riehle, 2013).

Whereas the spiking activity is considered to reflect the output of the neuron, the LFP is thought to mainly reflect the synaptic input activity to large populations of neurons around the electrode tip, with additional contributions from spike after-potentials and intrinsic trans-membrane current changes (Mitzdorf 1985; Logothetis et al. 2007). Accordingly, the LFP in motor cortex should be a sensitive indicator of ongoing motor (preparatory) processes. A prominent feature of the LFP in motor cortex is the movement-related potential (MRP). Typically it starts with a positive component (P1) prior to movement onset followed by a negative

component (N1) around onset, and slower positive and negative (P2, N2) components during and immediately after the movement (Donchin et al. 2001). Visual evoked potentials (VEPs) were also described in motor cortex, induced by visual cues that are related to the upcoming movement (O'Leary & Hatsopoulos 2006; Ledberg et al. 2007; Asher et al. 2010; see also Gemba et al. 1981,1990). During the same epochs, the spiking activity of the motor areas is strongly modulated, during movement execution (Evarts 1968), but also during the early epoch following an informative cue, the so-called "signal-related activity" (Weinrich et al. 1984). Analyzing both types of neuronal activity in parallel will allow us to evaluate which aspects of the modulations in the EPs are actually shared by the spiking activity, as O'Leary & Hastopoulos (2006) did for the directional selectivity.

Motor cortical MRPs modulate their amplitude in a directionally selective way (Cardoso de Oliveira et al. 2001; Mehring et al. 2003, 2004; Rickert et al. 2005; O'Leary & Hastopoulos 2006). In addition, Roux et al. (2006) found recently that the MRP pre-movement (P1) component also modulates in size with the level of expectancy and pre-processing, suggesting that at least certain components of motor cortical EPs are influenced by the behavioral context. If motor cortical EPs reflect ongoing processing during movement preparation and execution, one would indeed expect their modulations to be correlated with changes in behavioral performance, as well as with changes in the context in which the movement is performed. To study the influence of different temporal constraints on motor cortical EPs and spiking activity, we recorded the cortical activity from two macaque monkeys during the execution of a pre-cued arm-reaching task in 6 directions (described in detail in section 2.3-4). The task included two successive delays, which varied in duration from trial to trial in a pre-cued fashion, allowing the monkey to anticipate the time of occurrence of the visual spatial cue at the end of the first delay and the GO signal at the end of the second delay (i.e. temporal expectancy; Coull & Nobre 2008). The available time for movement preparation and the overall pace of the task thereby varied from trial to trial, while the visual spatial cues and required movements were the same in short and long delay trials. The anticipation of the cue (during the 1st delay) and how it influences motor preparation is detailed in the next chapter. In this chapter, we focus on the 2nd delay, during which the monkey knows since its beginning which target to reach to, as well as when the GO signal will appear.

We found significant modulations in VEP and MRP sizes in relation to the pre-cued delay duration, whereas the accompanying modulation of spiking activity was comparatively weak (although significant and in the same direction). The size of the EPs also varied according to the animal's behavioral performance (reaction times). Most of the RT variability may be explained by a shift in the onset of the preparatory spiking activity, rather than by other models of movement preparation such as a change of slope. Our data show that apart from reflecting the specific parameters of the movement being prepared/executed (e.g. movement direction), the

different measures of neuronal activity are strongly influenced by the temporal context in which the movement is made. Our results show an inverse modulation of the LFP activity by expected (VEP) and elapsed (MRP) delay duration.

3.2. Results

3.2.1. LFP database

We recorded 287 LFPs in motor cortex from monkey T in 90 sessions across 37 days and 759 LFPs in monkey M in 151 sessions across 73 days. Of those, 183 LFPs (71 sessions; 59 of those recorded with all 6 movement directions) in monkey T and 490 LFPs (132 from M1 and 358 from PMd; in a total of 123 sessions; 74 of the sessions recorded with all 6 directions) in monkey M were selected for further analysis. The remaining LFPs were excluded because of too many artifacts, or due to the lack of sufficient correct trials (if less than 10 correct trials per condition; typically 20 correct trials per condition were obtained). In the selected LFPs, some trials with obvious artifacts detected by visual inspection were further excluded from analysis. The database concerning spiking activity is reported at the beginning of the next chapter (section 4.2.1).

3.2.2. Behavioral performance in relation to delay duration

Recordings started when the monkeys were proficient in the task, performing with a success rate of 75% and 69% on short and long delay trials, respectively, for monkey T and 84% and 75%, respectively, for monkey M, averaged across all included recording sessions. Both monkeys performed the task with significantly fewer errors in short delay trials (paired t-tests across sessions, $p<<0.001$ for both). Additionally, the percentage of errors decreased across sessions in short and long delay trials in both monkeys ($p<0.01$ for all). Although the majority of the behavioral errors was directional (more than 67% of errors in both monkeys), it was mainly the percentage of other types of errors that decreased across sessions (e.g. responding or completing the movement too late, $p<0.001$ in both monkeys in short and long delay trials).

Averaged RTs across all trials in short and long delay trials, respectively, were 173ms and 225ms in monkey T, and 238ms and 262ms in monkey M. RTs were significantly different ($p<<0.001$) in short and long delay trials, (see Figs. 3.1A-B). This numbers differ slightly from the ones reported in Kilavik et al. (2010), since here all sessions with behavioral data are included, not only the ones with LFPs selected for analysis. The mean inter-quartile ranges (the difference between the upper limit for the lower quartile and the lower limit for the upper quartile of single-trial values) of RTs in short and long delay trials, respectively, were 88ms and 58ms in monkey T (significantly different, paired t-test, $p<<0.001$), 77ms and 62ms in monkey M ($p<0.001$). This measure was

chosen instead of the classical standard deviation, because it represents better the spread of a non-normal distribution, and it does not take outliers into account.

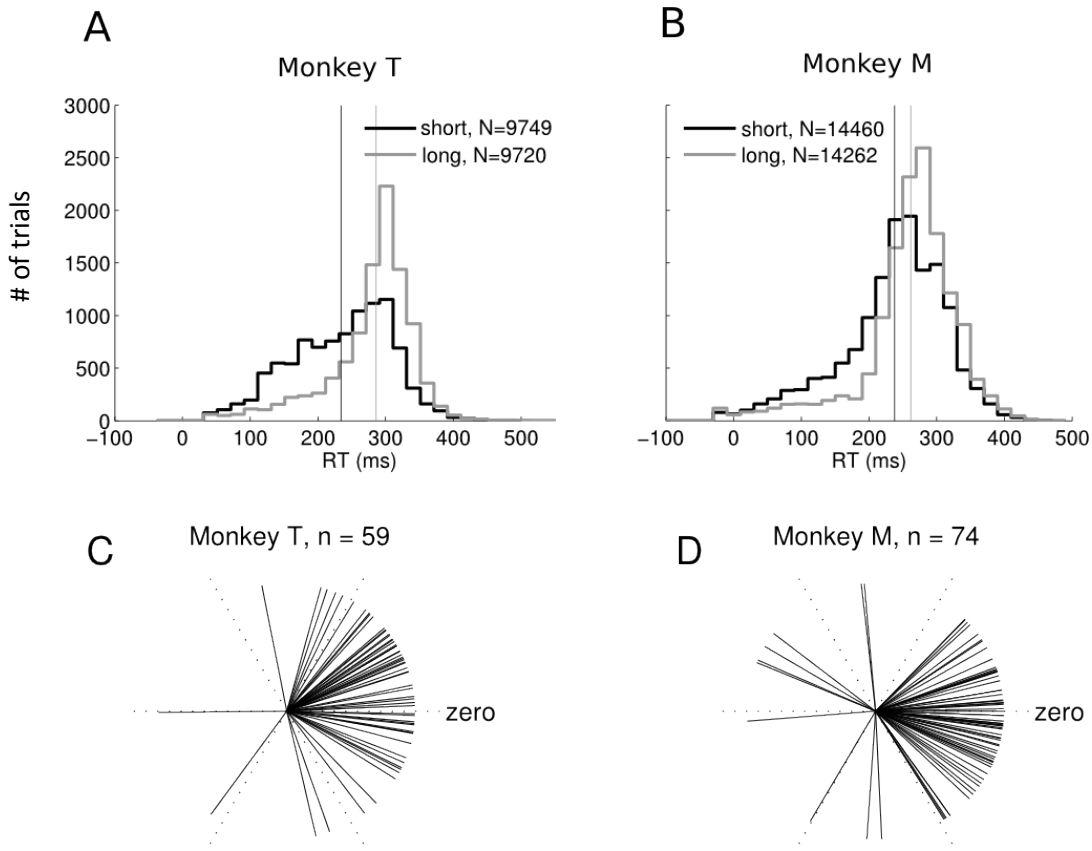


Figure 3.1: Behavioral performance. A-B) Distributions of RTs for monkey T and M. The short delay trials are in black, the long delay trials are in gray. The 0 indicates the onset of the GO signal (negative RTs are due to the different way we computed RT online and offline, see section 2.7 for details). The thin black and gray lines represent the median RT for short and long delay trials, respectively. The number of trials in short and long delay trials are indicated in the figure. C-D) Difference between short and long delay trials (circular distance) of preferred directions (direction with the fastest RTs in average) for monkey T (C) and monkey M (D) for all recording sessions in which all six movement directions were included. RTs in 57/59 and 59/74 of the sessions were significantly directionally selective in either short or long delay trials for monkey T and M, respectively. The closer the lines to zero, the smaller the circular difference in behavioral preference in short and long delay trials. C-D are reproduced from Kilavik et al. 2010 (Fig.2).

Note that for simplicity we here employ the term "reaction time", although we are entirely aware of its questionable use in instructed delay tasks. It is evident that subjects do not necessarily react to the GO signal, but most often anticipate its occurrence. Monkey T, in particular, had relatively fast RTs in short delay trials, combined with a higher trial-by-trial RT variability, indicating a stronger tendency to anticipate the occurrence of the GO signal. This higher inter-trial variability in short delay trials reflects the fact the animals reacted to the GO signal in some trials, but initiated the movement before the Go signal appeared in others. However, the

distributions of RTs across all trials were not bimodal in short or long delay trials for either of the two animals (see Figs. 3.1 A-B). In the following analysis we therefore did not attempt to select only those trials with sufficiently long RTs to be qualified as 'reactions' to the GO signal. In summary, although both animals performed the task sufficiently well in short and long delay trials, their performance was significantly different depending on the delay duration. In short delay trials, RTs were faster but more variable and less errors were made than in long delay trials.

RTs were different in the six movement directions, but the preferred directions for each animal (the direction with the fastest average RTs) tended to be similar in different sessions (Rayleigh's test, $p<0.05$). The preferred direction was calculated using the vector sum (see Methods). Figure 3.1C-D shows the circular distance (angle) between the behaviorally preferred movement directions in short and long delay trials for both monkeys. Each line corresponds to one session. In both monkeys the preferred directions were similar in short and long delay trials (circular mean +15 and -5 degrees in monkey T and M, respectively), with the mean circular distance only significantly different from zero for monkey T ($p<0.01$). Thus, even though the overall RTs were strongly modulated by delay duration (see above), the directional selectivity of RTs was similar in short and long delay trials.

3.2.3. Motor cortical visual evoked potentials as response to the spatial cue

Fig. 3.2A shows filtered (1-15Hz) single trial LFPs from multiple repetitions of a long delay trial-type during one session. Trials are aligned to the signals. MRPs are clearly visible in single trials around movement onset (marked with filled circles; trials sorted offline according to increasing RT), and VEPs are visible after SC. In the example LFP shown in Fig. 3.2A, the magnitude of the VEPs and MRPs were indeed similar (trials aligned to signals). However this was not always the case, it was more typical to observe MRPs than VEPs in single trial traces.

We analyzed VEPs in an epoch following SC onset, with single-trial LFPs aligned to the signals, whereas MRPs were analyzed in an epoch around movement onset, with single-trial LFPs aligned in each trial to movement onset (see Methods). Figure 3.2B shows the spectrogram for all short trials of a representative LFP from monkey T. Apart from the clear increases in power below 10Hz after SC and GO, reflecting the EPs, there was an increased power in the beta frequency range (20-25Hz) during the delays. The band-pass filtering between 1-15Hz minimally compromised the size of EPs on single trials while excluding beta oscillations that were partly overlapping in time with the EPs, but not phase-locked to SC or movement onset. Figs. 3.2C-F show trial-averaged VEP and MRP waveforms from filtered and unfiltered LFPs recorded in short and long delay trials (same LFP as in Fig. 3.2A).

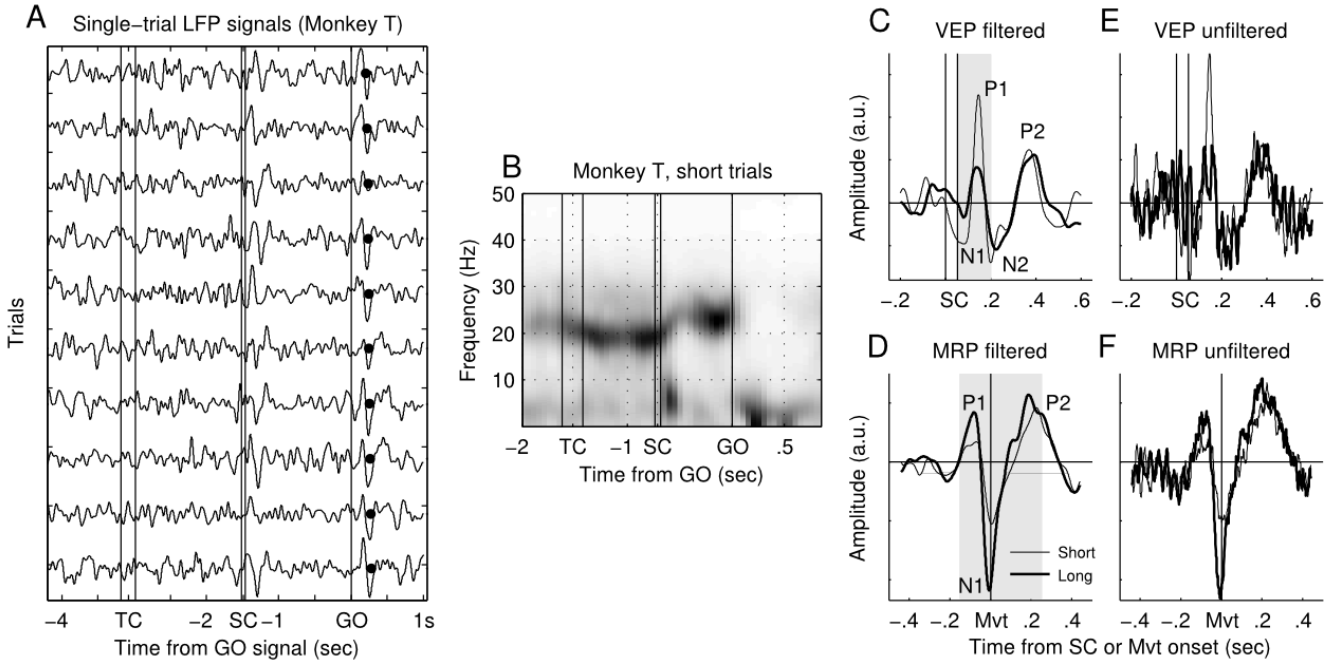


Figure 3.2: Example of a representative LFP. A) LFP recorded from one electrode during one recording session in monkey T. Each line corresponds to a single trial in the same condition, band-pass filtered between 1-15Hz and sorted offline according to increasing RT (filled circles after GO). Data are aligned to the signals. B) Spectrogram of a representative LFP recorded in monkey T. All short delay trials were included in the analysis. The color code indicates the beta power, the darker the gray-value the higher the power. Beta oscillations (around 20-25Hz) were strong during long periods of the task. The EPs after SC and around movement onset (after GO) are also visible as short-lasting increases in power below 10Hz. C-D) average VEP and MRP of a single LFP for short and long delay trials in the same movement direction (same LFP as in A). The LFP was bandpass filtered offline between 1-15Hz before trial-averaged. Components are tagged. Note, that the P2 component of VEPs was rarely observed in monkey T. The gray zones indicate the trial epochs in which the rms was calculated. E-F) The same data as shown in C-D, but without offline filtering (only online filter during recording between 1-250 Hz). The main trial-averaged EP components that are tagged in C-D can also be found in the raw data. The Y-axis scales are the same in E and F as in C and D, respectively. (Fig. 3 in Kilavik et al. 2010)

VEPs and MRPs were frequently observed in both monkeys, in both M1 and PMd. For monkey M, LFPs recorded in M1 and PMd are quantified separately. Fig. 3.3 shows the percentage of LFPs that contained significantly identified VEPs and MRPs in short and long delay trials in both monkeys. In monkey T, MRPs occurred more often than VEPs, a difference which was significant in long delay trials (Chi-square, $p<0.01$). In monkey M, VEPs occurred significantly more often than MRPs in short delay trials (Chi-square, $p<0.05$; data pooled across M1 and PMd, not shown). There was a decrease in the percentage of significant EPs across sessions. Comparing the first and last third of recorded LFPs, the percentage decreased from 98% to 72% in monkey T and from 75% to 40% in monkey M (summing across VEPs and MRPs in short and long trials). This suggests that there was a decrease in average EP size compared to the baseline epoch for later sessions.

Although VEPs and MRPs were frequent in M1 and PMd, MRPs occurred significantly more often in M1 than in PMd (60% vs. 41% in short and 72% vs. 43% in long delay trials, Chi-square, $p<0.01$ for both comparisons). VEPs were more often present in PMd than in M1, in short delay trials (66% vs. 53%, however not significant, $p=0.09$). In summary, the most frequently occurring EP in M1 was the MRP in long delay trials, whereas in PMd it was the VEP in short delay trials.

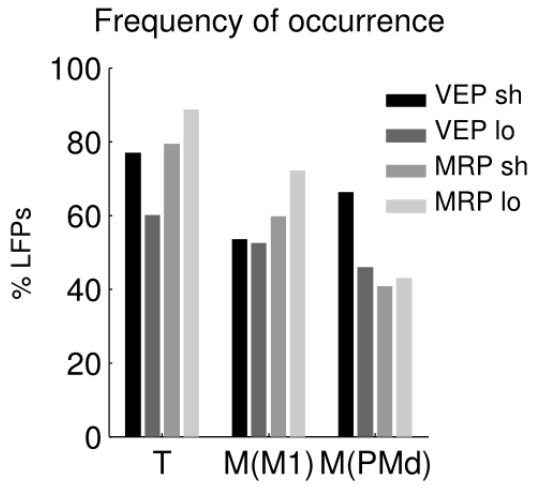


Figure 3.3: Percentages of detected EPs. Overall percentage of LFPs containing statistically significant VEPs and MRPs, in short and long delay trials, in the two animals (T and M), shown separately for M1 and PMd in monkey M. (Figure 4A in Kilavik et al. 2010)

3.2.4. Delay duration modulates the size of the VEP and MRP

The different numbers of significant EPs in short and long delay trials suggest a difference in their size in short and long delay trials. Figs. 3.2C-F shows a LFP where the VEP was significantly larger in short delay trials, whereas the MRP was larger in long delay trials. We find that this was indeed a general effect in the majority of LFPs in both animals, by computing the normalized difference (by using a contrast measure) of the EP size between short and long delay trials. The contrast is the difference between the EP amplitude in short and long delay trials, divided by its sum. In other words, it gives an index of the ratio between two values, irrespective of their magnitude and is comprised between -1 and 1. For example, a positive contrast value means that an EP in short delay trials is larger than in long delay trials, whereas a negative contrast value means that an EP is larger in long than in short delay trials. For comparison, a ratio of 0.33 would mean that the EP is twice as large in short than in long delay trials. Fig. 3.4A shows the distribution of the contrast values for the VEP and MRP, for all detected EPs (non-filled histogram) and for the ones with a significant difference between short and long delay trials (filled histogram, two-way ANOVA with delay duration and movement direction as the 2 factors, $p<0.05$). In monkey T, out of the 137 VEPs, 108 (79%) have a significant difference between short and long delay trials (filled blue area), whereas 91 of the 144 detected MRP (63%) have significantly different amplitude in short and long delay trials (filled pink area). In Monkey M, 222 of the 299 VEPs (74%) and 126 of the 275

MRPs (46%) have a significant difference according to delay duration. As can be seen in Fig. 3.4, all significant differences in monkey T and almost all significant differences in monkey M go in the same direction: the VEP is larger in short than in long delay trials, whereas the MRP in larger in long than in short delay trials (all distributions are shifted away from 0, Wilcoxon sign-rank test, $p<0.01$). A significant delay duration effect was more frequently observed for the VEP in PMd, and for the MRP in M1 (Chi-square, $p<0.01$ for both comparisons). The results were very similar for the individual VEP and MRP components. In fact, only N2 component of the VEP in monkey T, which was the least frequent VEP component in this monkey, was more often significantly larger in long than in short delay trials. All other VEP components in both monkeys were more often significantly larger in short than in long delay trials. All MRP components in both monkeys were more often significantly larger in long trials.

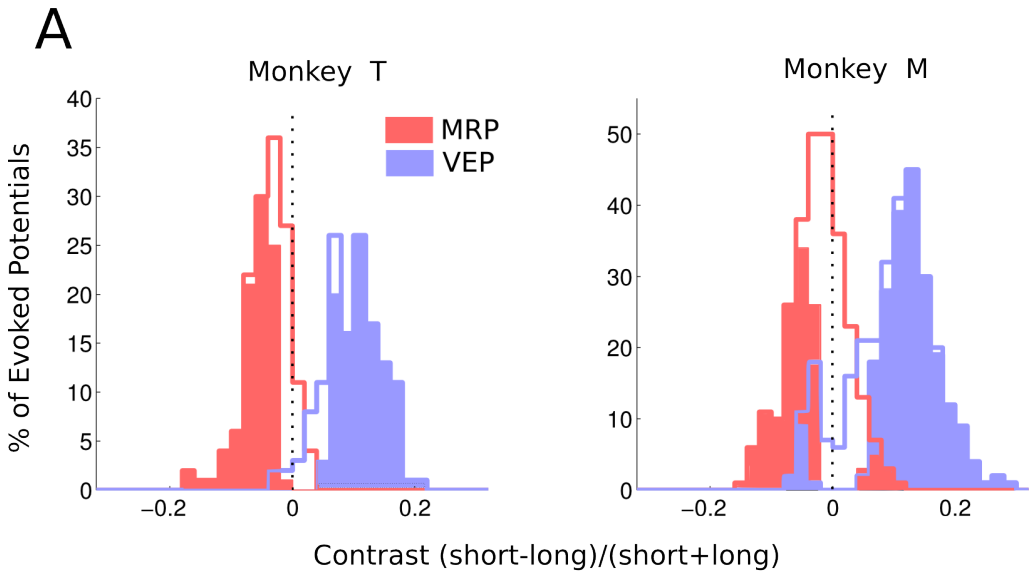


Figure 3.4: Influence of delay duration on the EP amplitude. A) The histograms represented by thick outlines represent the distributions of the contrast values for all EPs, whereas the filled parts represent only the fractions with a significant difference in rms between short and long delay trials. Data points close to the 0 dashed line represent the EPs with almost identical rms in short and long delay trials, the positive values correspond to larger EPs in short than in long delay trials, whereas the negative values correspond to larger EPs in long than in short delay trials. B) Percentages of significantly different EP sizes according to delay duration for monkey M, separately for M1 (left) and PMd (right). VEPs are in blue, MRPs in red. The short>long differences are in darker colors, and the long>short differences in light. B) is modified from Kilavik et al. 2010 (Fig. 4).

As a control, we also measured peak amplitudes of the VEP P1 and the MRP N1 components (see 2.8.3 for details). The same conclusions as described above were reached when comparing peak amplitudes in short and long delay trials. In particular, all VEP P1 components with significantly different peak amplitudes in short and long delay trials had larger peak amplitudes in short delay trials in both monkeys. Correspondingly, a large majority of the MRP N1 components with a significant delay-duration effect had larger peak amplitudes in long delay trials in both monkeys.

In summary, VEPs were more often present and were larger in short delay trials, whereas MRPs were more often present and were larger in long delay trials. The effect was strong for both monkeys and in both M1 and PMd.

3.2.5. Spiking activity in relation to delay duration

In parallel to the LFPs, we recorded the spiking activity of 470 and 740 single neurons in monkey T and M, respectively. The results concerning the spiking activities are described to a higher extent in the next chapter, along with most of the data analysis methods used in both chapters. This section will mainly deal with the single neuron spiking activity recorded during the same period as the LFP EPs in order to compare the two types of neuronal activity. When averaging the spiking activity of all neurons across all trials (of all movement directions) for each monkey, we observed an activity modulation during the same epoch as the occurrence of the VEPs and the MRPs (Fig. 3.5), which is illustrated in the next chapter (Fig. 4.4).

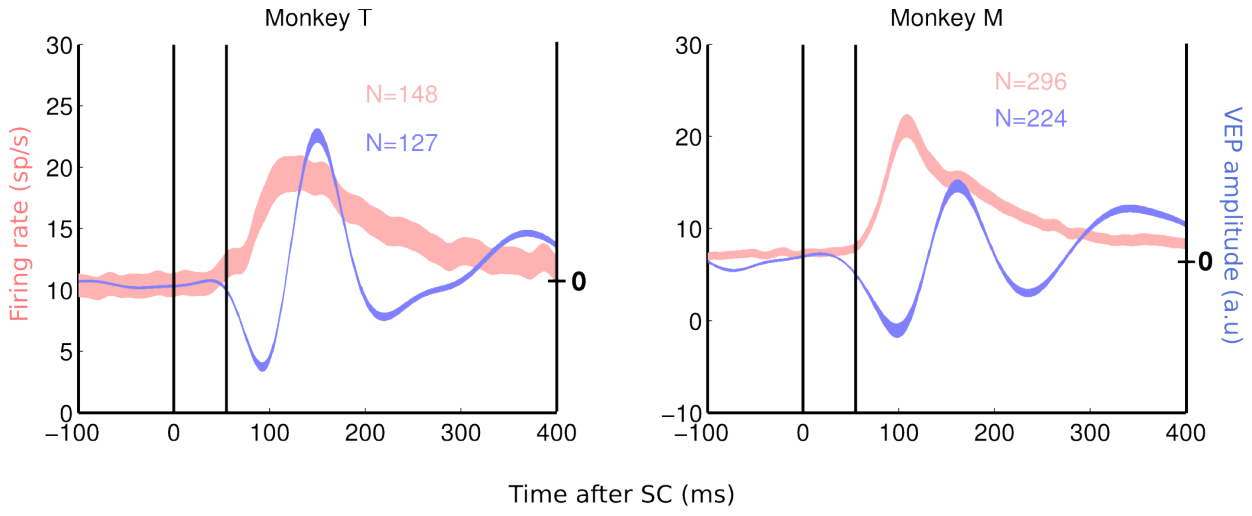


Figure 3.5: VEP and spiking activity in response to the SC. The mean firing rate of all neurons (±sem) is shown in pink and the average VEP across all LFPs (±sem) in blue. Note that whereas the amplitude of the firing rate is in spikes/second, the one of the VEP is purely arbitrary (due to normalization before averaging). The numbers indicate how many neurons and VEPs matched the selection criteria for this figure (see section 4.2.2 and 4.3.5). The two vertical black lines indicate the onset and offset of SC, respectively.

In Fig. 3.5, we plot the mean firing rate of a subset of neurons (smoothed with a gaussian kernel of 15ms and a standard deviation of 5ms), overlaid with the averaged EPs of the LFPs, both signals recorded during the epoch following SC. Neurons were selected as a function of their phasic response during the first 250 ms following the SC. The next chapter will explain in detail the selection method used to determine the neurons with a phasic response, please refer to section 2.9.2 and 4.2.5 for further details. Here, the VEP epoch is taken from the short delay delay trials (i.e. the condition in which they are larger, see above section 3.2.4). The averaged MRPs are not shown, because their latencies are much more variable across sessions than those of the VEPs. Averaging them is thus meaningless. For example, across electrodes and sessions, the standard deviations of the latencies in respect to movement onset of the N1 components of the MRPs are 8.7 and 16.5ms in monkey T and M, respectively (for a mean latency of 8 and 15ms, respectively). For comparison, the standard deviations of the latency of the P1 component of the VEP (shown in Fig. 3.5) are 2.8 and 2.9ms in monkey T and M, respectively (for a mean latency of 157 and 158ms, respectively). Note that, even though the VEPs were averaged across all movement directions and all electrodes, and across recording sessions spanning up to one year of recordings, with the only criteria of having a detected VEP in a trial, the mean VEP is almost identical to the individual example shown in Fig. 3.2C. In other words, the latencies of the two first components of the VEP in motor cortex, triggered by the SC, are very stable across sessions and cortical localization. Such a stable latency of the two VEP components suggests a highly stereotypical activity modulation, time-locked to the signal. The second interesting point of this figure, even though qualitative, is that in both animals the averaged phasic response of single neuron firing rate starts to develop less than 50ms after the onset of SC, and peaks shortly after the peak of the N1 component of the VEP (during the rising slope). It is very difficult to discuss precise latencies on averaged data, but the close temporal overlap between the two signals suggests that they reflect (at least partly) common sensorimotor processes.

If this is the case, the single neuron spiking activity following SC should be modulated by the delay duration in the same way as the VEPs. To test this hypothesis, we compared the spike counts of the neurons shown in Fig. 3.5 in a 200ms time window starting 50ms following SC in short and long delay trials. As comparison, the same was done for the spiking activity around movement onset (from 150 before to 250ms afterward, similar as for the MRP) for all neurons modulating their activity during the movement epoch (1Hz minimum). Fig. 3.6 show the contrast values obtained from the data recorded during short and long delay trials, in the epochs in which the VEPs and MRPs were analyzed. As explained before, the contrast value is the difference between the spike counts in short and in long delay trials, divided by their sum. To compute if the difference of firing rate due to delay duration was significant, a two-way ANOVA (movement direction & delay duration) was applied.

Neurons with a p-value of 0.05 or less were considered significantly modulated (by delay duration) (filled histogram in Fig. 3.5).

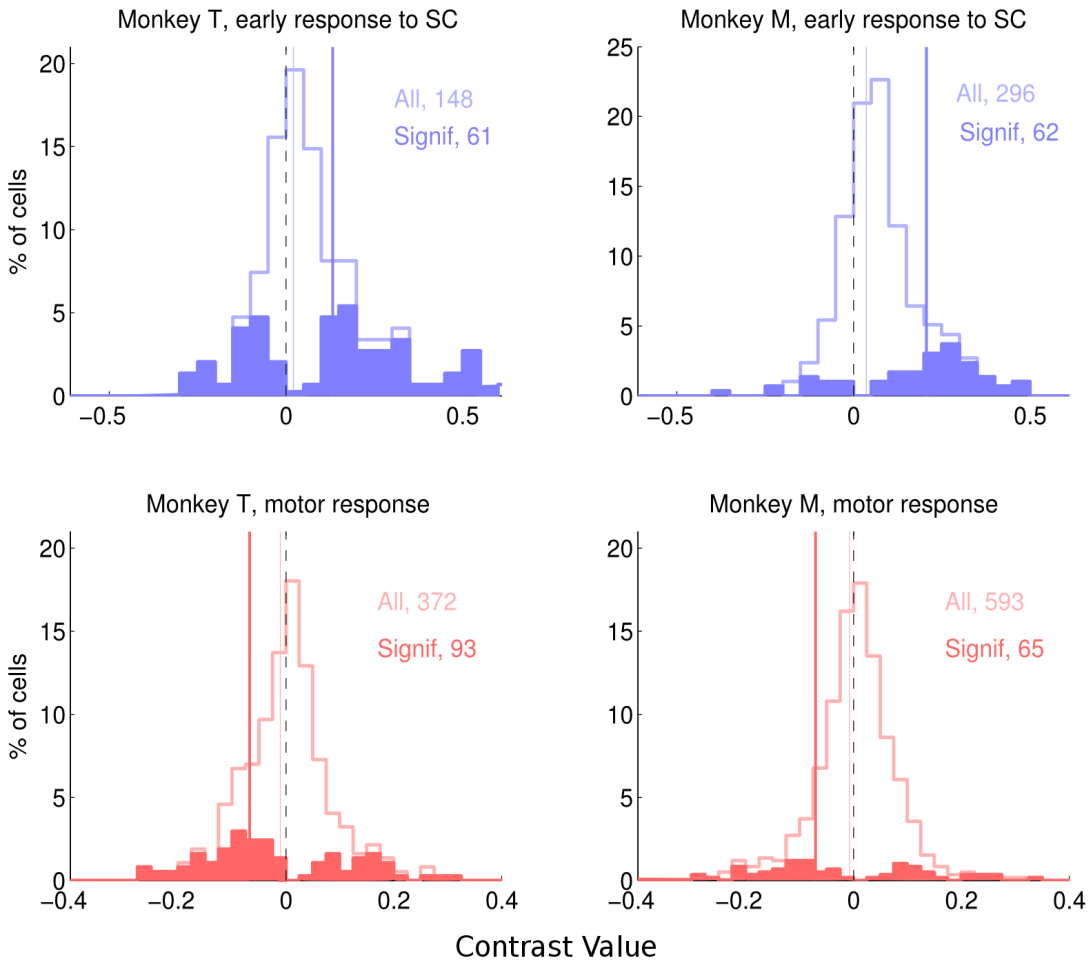


Figure 3.6: influence of delay duration on spiking activity during D2. This figure shows the contrast values of the spike count of individual neurons according to delay duration, in the epoch following SC (top row) and around movement onset (bottom row). The empty histograms represent the distributions of contrast values for all selected neurons (see text for the selection criteria), and the filled histograms only the contrast values of neurons with a significant difference according to delay duration. The sample sizes are reported in the top right corner of each plot. The thick vertical lines represent the median contrast value for significant neurons, and the thin one is the median contrast value of the whole population. All distributions (significant and whole population) are significantly shifted away from 0 (Wilcoxon sign-rank test, $p<0.05$). In all plots, a rightward shift of the population compared to the 0 means that the firing rate is larger in short than in long delay trials. Note the difference of scale on the x-axis between the upper and lower plots.

The modulation of spiking activity by delay duration goes in the same direction as that of the EPs (Fig. 3.4A). The phasic response to SC tends to be larger in short than in long delay trials, and the movement-evoked activity tends to be larger in long than in short delay trials. This confirms that EPs and spiking activity are partly related. However, the "temporal tuning" of the spiking activity is much weaker than that of the EPs. First, the proportion of significantly modulated elements is lower for the spiking activity than for the EPs. Second,

virtually all VEPs and MRPs with a significant difference between short and long duration went in the same direction, whereas it was much more balanced for the spiking activity. Third, when taking the whole population of neurons and not only the ones responding phasically to SC, the distributions became centered on 0 (Wilcoxon sign-rank test, $p>0.05$ even though N increased). In other words, the tendency seen in VEPs disappeared: only the neurons which respond phasically to SC are more active (as a population) in short than in long delay trials.

In summary, both the EPs of the LFPs and the spiking activity are modulated in amplitude by delay duration. The response to SC and the activity recorded during movement are inversely modulated.

3.2.6. Do delay duration-related modulations of EP size affect directional selectivity?

Motor cortical MRPs were shown to be directionally selective (e.g. Cardoso de Oliveira et al. 2001; Mehring et al. 2003; Rickert et al. 2005) as confirmed by our data. Also directional selectivity of stimulus-locked oscillations in different frequency ranges was recently reported in motor cortex (O'Leary & Hatsopoulos 2006). They may partly reflect the directionally selective VEPs we are reporting on. We estimated separately directional selectivity in VEPs and MRPs in short and long delay trials. We calculated the vector sum of the average VEP P1 and MRP N1 components in the six movement directions (for all LFPs which were recorded in sessions with 6 movement directions and contained statistically significant EPs). Only the VEP P1 and MRP N1 components were taken into account, as they were the most frequently occurring components. Preferred direction was defined as the direction of the vector sum. The significance of directional selectivity was estimated with a re-sampling procedure (see 2.8.5).

Many VEPs and MRPs were significantly directionally selective (in monkey T between 42-58% of VEPs and MRPs in short and long trials, similar for VEPs and MRPs; in monkey M, 25% of VEPs in short trials and 13% in long trials and about 40% of MRPs). As can be seen in the Fig. 3.7 for Monkey T for monkey M, the preferred directions were significantly non-homogeneously distributed around the circle (Rayleigh's test, $p<0.05$), but rather clustered in one or two main directions (a result also found by O'Leary & Hatsopoulos 2006).

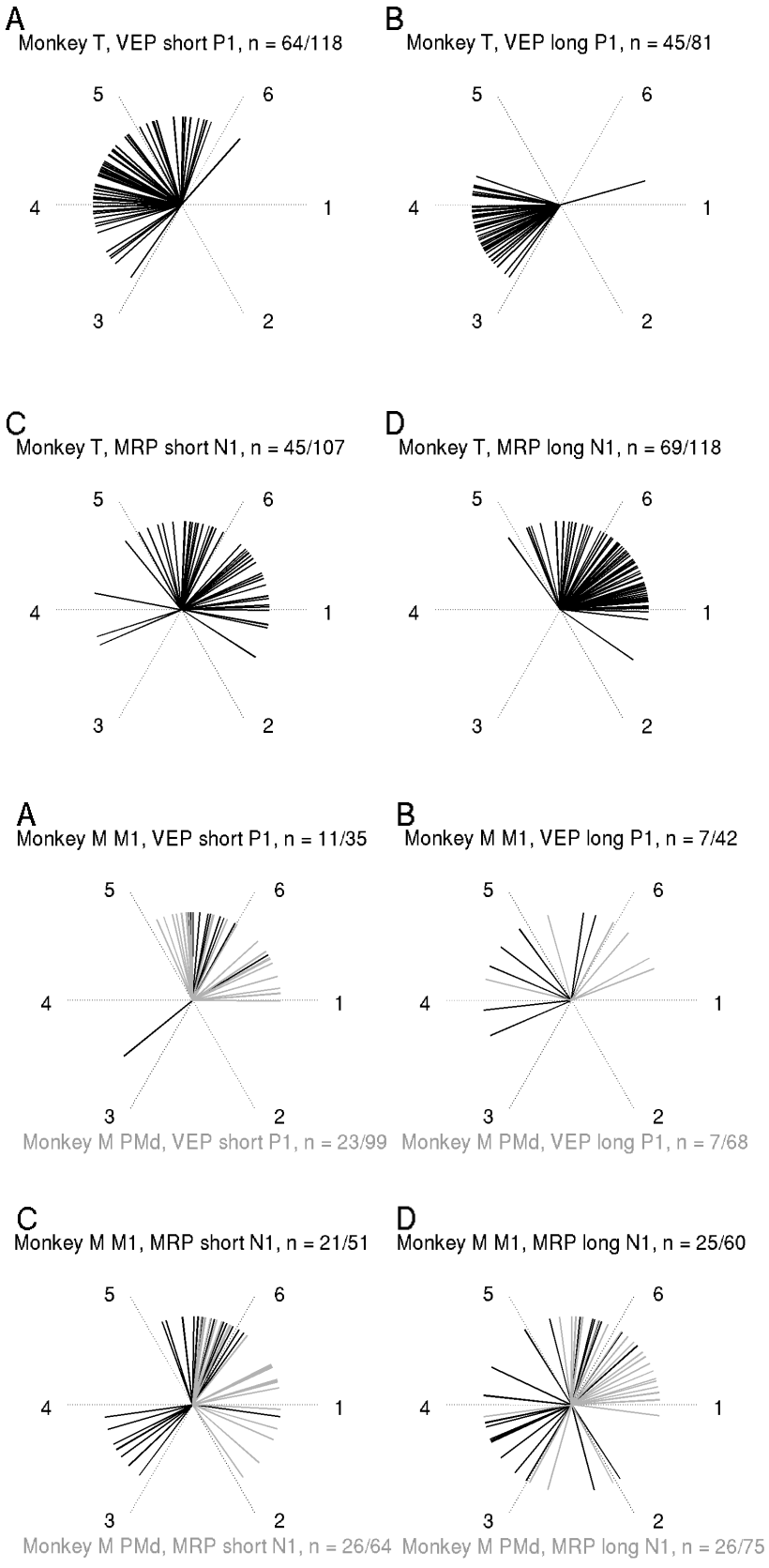


Figure 3.7: Preferred directions of EPs of monkey T. Each thick line represents the preferred direction of the main component of an EP. The thin dashed lines represent the direction of the 6 targets. The number of both the EPs significantly tuned for movement direction (numerator) and the detected EPs (denominator) are indicated in the title. A) VEP in short delay trials; B) VEP in long delay trials; C) MRP in short delay trials; D) MRP in long delay trials.

Figure 3.7 (continued): Preferred direction of EPs of monkey M. All conventions are the same than Fig. 3.7. As in title, black lines are EPs recorded in M1, gray lines EPs recorded in PMd.

The quantifications of directional selectivity of EPs in our and other studies were based on different measures of size or 'amplitude' of the signal (e.g. rms area as used here and in Asher et al. 2007; rms of band-pass filtered data in different frequency bands in O'Leary & Hatsopoulos 2006; waveform-amplitude in Rickert et al. 2005; peak-to-peak size in Cardoso de Oliveira et al. 2001). In the previous section, we described how delay duration strongly modulated the EP size in a majority of LFPs. The behavioral results show that even if RTs were strongly modulated by delay duration, the animal's preferred direction remained the same, as the direction with the shortest RTs was similar in short and long delay trials (see Fig. 3.1C-D). We were therefore interested in whether the EP modulation induced by delay duration interacted with the modulation related to movement direction. We calculated the circular distance (angle) between the preferred directions in short and long delay trials, for all significantly detected EPs that were directionally selective in both short and long delay trials. In Fig. 3.8 we show the circular distance for VEPs (A and C) and MRPs (B and D) for the two monkeys. Solid lines mark EPs that were directionally selective in short and long delay trials and additionally significantly modulated by delay duration, whereas the dashed lines indicate EPs that were only directionally selective. Note that in monkey M only a few VEPs were directionally selective in both short and long delay trials (cf Fig. 3.8C). In general, the circular distances of all EP signals were clustered within less than +/- 1 target (neighboring target distances were 60 degrees). The mean angles of circular difference between short and long delay trials in monkey T were -35 degrees (significantly different from zero, $p<0.0001$) and 2 degrees (not significant), for VEPs and MRPs, respectively. In monkey M the mean circular difference of VEPs was +8 degrees (not significantly different from zero, but only 4 data-points) and that of MRPs +10 degrees (not significant).

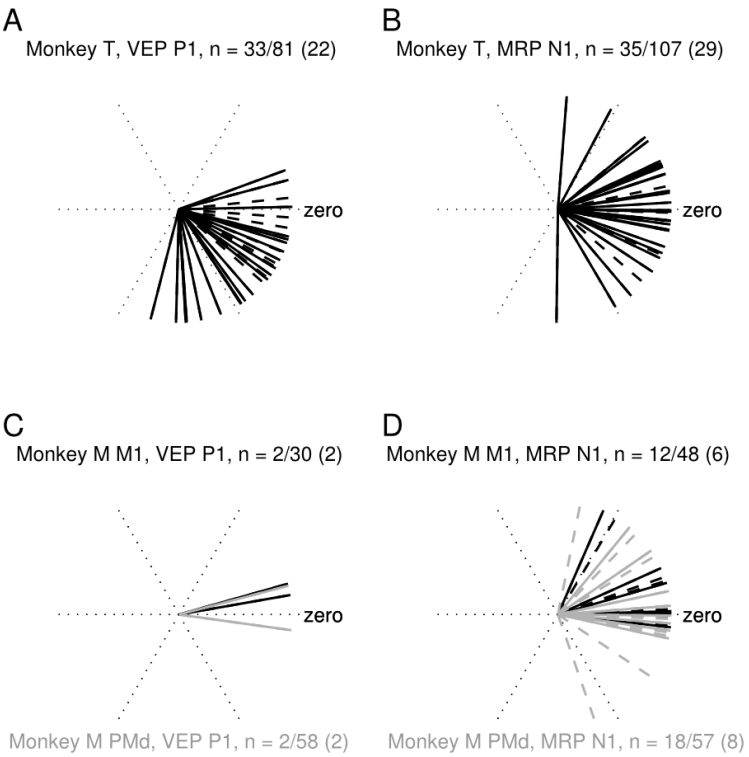


Figure 3.8: Directional selectivity of the EPs and delay duration. The circular distance (angle) between preferred directions in short and long delay trials for all statistically significant EPs (denominator in heading) and selective (numerator in heading) in short and long trials. Each line represents the angle of the circular difference between short and long delay trials of one EP. Zero indicates no difference in preferred directions in short and long delay trials. Dashed lines indicate all EPs which were selective in short and long delay trials, whereas overlying solid lines indicate the subset (numbers in parentheses in heading) that are additionally significantly modulated by delay duration. M1 in black and PMd in gray (monkey M). (Fig. 5 in Kilavik et al. 2010)

There was no evident difference between the subsets that were modulated by delay duration (solid lines) and the non-delay-sensitive populations of directionally selective EPs (underlying dashed lines). Similarly, there was no systematic difference between M1 and PMd in monkey M (black vs. gray lines). Another interesting point is that the proportions of the directionally selective EPs with delay duration effects were similar to the proportions found in all LFPs (Fig. 3.4). Overall, these results indicate that the preferred direction remained stable in short and in long delay trials, even when the size of the EP was significantly modulated by delay duration.

3.2.7. Do delay duration-related modulations of spiking activity affect directional selectivity?

The directional preference of LFPs EPs does not seem to be influenced by delay duration. The tuning curve of individual neurons has been shown to change under several conditions, like adapting a reaching movement to the application of an external force field (Li et al. 2001). However, in the contest of the same task performed over and over again, the neuron's tuning curve seems to be relatively stable, on the course of hours (Chestek et al. 2007) up to several days (Dickey et al. 2009). We thus tested if the tuning of the spiking activity recorded during the same epoch as the EPs was unchanged by delay duration. We performed the same analysis than in the last paragraph, using the spike count instead of the EP amplitude. The same database than the one used for Fig. 3.6 was used, since we showed in this figure that some of these neurons were tuned to delay duration. Thus, for the SC epoch, only the early phasic neurons were used, whereas all neurons were used for the movement epoch. Here again, we only selected the 6 directions sessions and applied the same vector sum and randomization method to compute the preferred direction (see section 2.8.5 and 2.9.3 for the details). Additionally, the neurons were required to fire at least at 5Hz in their preferred direction to be considered reliable for the analysis. The database used for this analysis, along with the percentage of significantly tuned neurons are shown in table 3.1. To have a sufficiently large sample size, we pooled the data from M1 and PMd in monkey M. The preferred directions computed during the SC epochs of short and long delay trials of both monkeys were homogeneously distributed (Rayleigh's test, $p<0.05$). During the movement epoch, the preferred directions were distributed homogeneously only in short delay trials of Monkey T, whereas long delay trials of monkey T and short and long delay trials of monkey M were non-homogeneously distributed ($p<0.05$). However, when looking at the circular distributions of the preferred directions during the movement epochs, no clear tuning of the whole population can be seen (Fig. 3.9). When comparing the non-homogeneous distributions of the neurons' preferred directions to their respecitve EPs, no clear relationship could be seen. This result confirms the study of O'Leary & Hatsopoulos (2006), in which they did not find any relationship between the preferred directions of the LFPs (VEP & MRP) and those of the single neurons. Similarly, they also

reported that the preferred directions of single neurons where more or less homogeneously distributed, whereas those of the LFPs tended to be clustered in few compact groups.

	SC short	SC long	SC both	Mvt short	Mvt long	Mvt both
Monkey T	51/115 (44%)	33/115 (29%)	24/115 (21%)	177/346 (51%)	181/346 (52%)	156/346 (45%)
Monkey M	98/193 (51%)	84/193 (44%)	67/193 (35%)	191/476 (40%)	190/476 (40%)	149/476 (31%)

Table 3.1: Directional tuning of the spiking activity. In each case, the first number is the number of directionally tuned neurons, and the second the sample size. The sample size changes between the SC epoch and the movement epoch, because only neurons with a phasic response to the SC are selected. The two columns in blue are the ones used to compute the angular direction between the preferred directions of neurons in short and long delay trials.

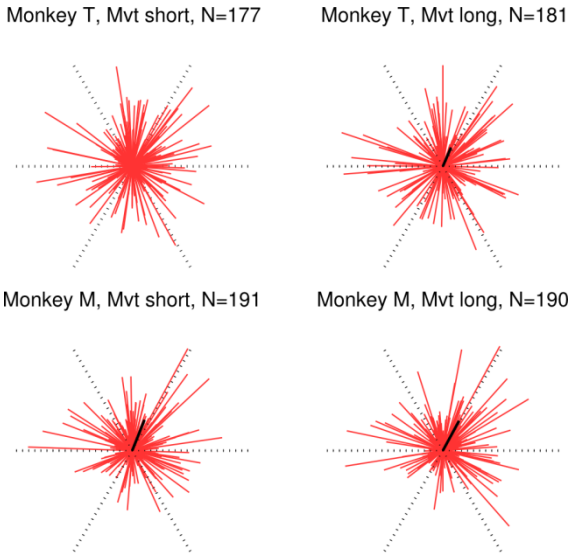


Figure 3.9: Circular distributions of the neurons' preferred directions during the movement epoch. The length of each red line indicates the strength of the tuning, and its direction the preferred direction of an individual neuron. The dotted lines indicate the 6 movement directions. The thick black lines represent the angular mean of the non-homogeneous distributions. Mvt: movement. Compare monkey T, short delay condition, (homogeneously distributed) to the other distributions: even the non-homogeneously distributed distributions lack a clear mode.

We then selected only neurons which were significantly tuned to movement direction in both short and long delay trials during the same epoch (columns in blue in table 3.1), and measured the angular difference between their preferred directions in short and long delay trials. The results are shown in Fig. 3.10. Indeed, the results follow closely the ones obtained with the EPs, there is no systematic shift of preferred directions linked to delay duration, and the difference between short and long delay trials is always comprised between ±1 targets. Furthermore the distributions of the angular differences between the preferred directions in short and long delay trials of the delay-selective and delay-non-selective neurons are completely overlapping. Altogether,

we conclude that neither in the EPs nor in the single neuron spiking activity the selectivity for delay duration influences the selectivity for movement direction. This result goes in the same direction as those of Roesch & Olson 2005a, where they showed that the proportion of neurons with a significant interaction between selectivity to delay duration and movement direction was very low. Their result and ours support the idea of a relative independence of the two selectivity processes. Additionally, we did not find any correspondence between the directional tuning of the spiking activity and of the LFPs (as did O'Leary & Hatsopoulos 2006).

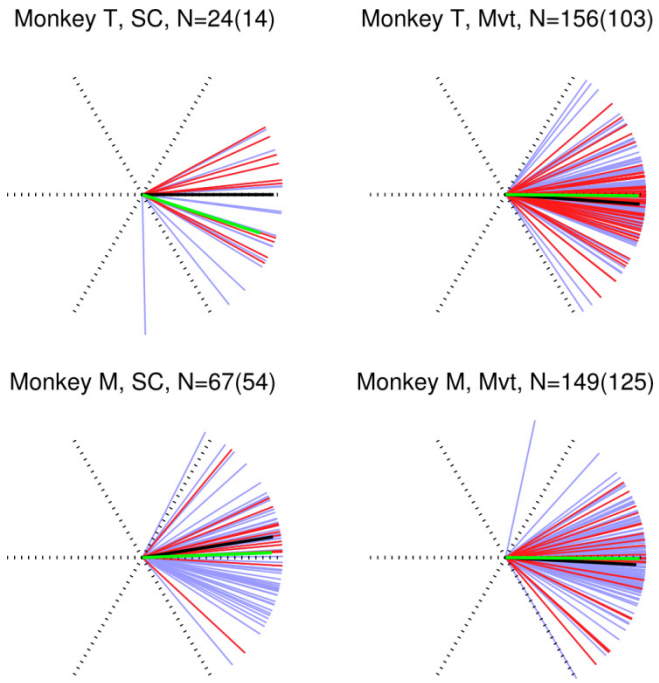


Figure 3.10: Directional selectivity of the spiking activity and delay duration. Each line represents the angular difference between the preferred directions of a neuron in short and long delay trials. The lines in light blue represents the data from delay-duration non-selective neurons (with the angular mean in green), whereas the dark red ones represents delay-duration selective neurons (with the angular mean in black). The numbers indicate the sample size, and the one in parentheses the size of the subsample of delay-duration selective neurons.

3.2.8. Selectivity of individual EP components to delay duration and movement direction

Individual EP components may reflect different aspects of the task, and hence have different degrees of selectivity to different variables (i.e. delay duration and movement direction). For example, Roux et al. (2006) showed that uncertainty about movement timing modulated specifically the P1 component of the MRP. To assess this possibility, we computed the selectivity for delay duration and movement direction for each EP component individually (N1 and P1 for VEPs, and P1, N1 and P2 for MRPs), using the method described in the previous sections 2.8.2 and 2.8.4. We selected all LFPs to study the delay duration selectivity (Figs 3.9 A-B), but only the ones recorded in the 6 directions sessions to study direction selectivity (Figs 3.9 C-D), leading to different sample sizes (indicated in the subtitles of Fig. 3.11). Fig. 3.11 shows the percentages of detected individual components (light colors), and among them the proportion of selective ones (dark colors). To detect the existence of individual EP components, we applied the same method as the one used to detect the presence of an entire EP (described in section 2.8.2), but in time windows covering each individual component

(described in section 2.8.3). Then, all EPs detected either in short or in long delays were included as "detected EPs", which explains why the percentages are higher than those reported in Fig. 3.3. Subsequently the selectivity analyses were performed on the rms value of each single component. An ERP was considered "directionally selective" if it was significantly tuned (see section 2.8.5) either in short or in long delay trials.

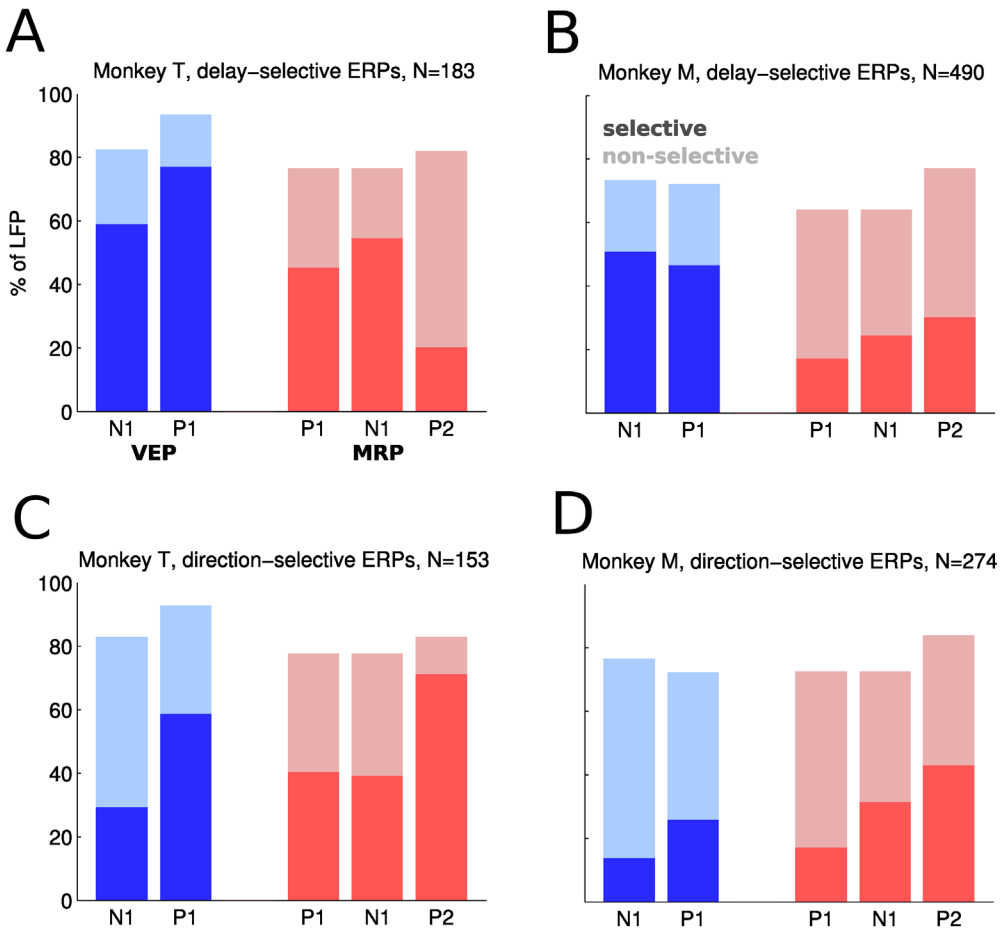


Figure 3.11: Selectivity to delay duration and movement direction of individual EP components. Bar plots are shown as percentage of the sample sizes indicated in the titles. Blue colors are for the VEP components, red colors for the MRP components, as indicated in A. To see the shape of the components, see examples in Figs. 3.2 C-F. Light colors indicate the percentage of the detected components which were not tuned, dark colors indicate the percentage of detected components which were tuned. All y-axes have the same scale. A-B) selectivity to delay duration (ANOVA, $p<0.05$, see section 3.2.4). C-D) selectivity to movement direction (vector sum randomization method, see section 2.8.5 & 2.8.6). A-C) Monkey T. B-D) Monkey M.

Several important observations have to be made: first, even the early component of the VEP (N1) shows a significant selectivity to movement direction, since Fig. 3.11C-D shows that more than 5% (chance level) of the EPs show such a directional selectivity. Second, the late component of the MRP in monkey T shows a strong directionality (Fig. 3.11C) and less delay duration selectivity (Fig. 3.11A) compared to the other MRP components. A tendency of having a stronger direction than delay selectivity for P2 can also be seen in monkey

M (see Fig. 3.12B), but much weaker. However, when plotting separately the data from M1 and PMd in monkey M (data not shown), the only difference is that the P2 component is much more directionally selective in M1 than in PMd. Third, the two components of the VEP seem more influenced by delay duration than by movement direction, especially N1. This aspect can be better seen in Fig. 3.12, where we plotted for each component the contrast value between the percentage of delay selective and directionally selective EPs (0 being the same percentage for both).

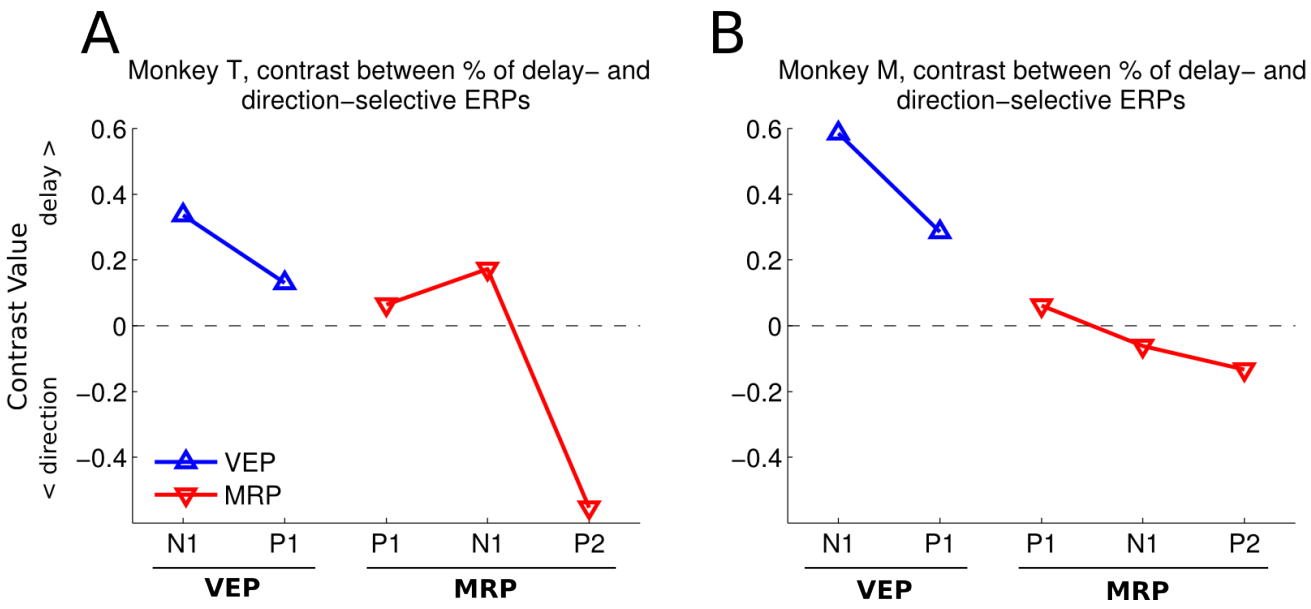


Figure 3.12: Contrast value between the percentages of delay- and direction-selective neurons. Positive data points indicate a higher percentage of delay than direction selective EPs. In other words, the more positive the contrast value, the more the percentage of delay selective EPs is superior to the percentages of directionally selective EPs. Blue lines indicate VEPs, red ones MRPs. A) Monkey T, B) monkey M. For reference, a value of 0.33 means that there are twice as many EPs with delay than direction selectivity.

Fig. 3.12 shows the difference between the percentages of delay and directionally selective neurons, divided by their sum, so positive values indicate a higher percentage of delay- than direction-selective EPs. The N1 component of the VEP is much more often selective for delay duration than for movement direction. The same tendency can be seen for the VEP P1, although weaker. Both dimensions are represented in the MRP, but the direction is more represented in the late component, especially in monkey T. Additionally when looking at M1 and PMd separately, no qualitative difference can be seen (monkey M).

This shift of dominance from selectivity to delay duration to selectivity to movement direction as the trial progresses was also observed in the spiking activity. Indeed, from an early to a late epoch in the trial, the proportion of neurons with a significant directional selectivity increases whereas neurons with a significant temporal selectivity decreases (Fig. 3.13). Here, we used a simple method to calculate the percentage of neurons being selective either to delay duration, to movement direction or to both, by applying a 2-way ANOVA on the spike count of each neuron, separately in a 200ms time window following the SC and around the GO signal, without restricting the analysis to any specific type of neuron. In other words, regardless of the pattern of activity of the neurons, the influence of delay duration on the neuronal population seems much stronger at the beginning of the delay, whereas movement direction is more represented as the GO signal approaches. As was shown in Fig 3.10, these two types of selectivity do not seem to interact strongly, even if often co-present in the same neurons. This result goes in the same direction as the gradual "preference" for directional selectivity over delay duration selectivity that we observed in the EPs as the trial progresses (Figs. 3.11 & 3.12).

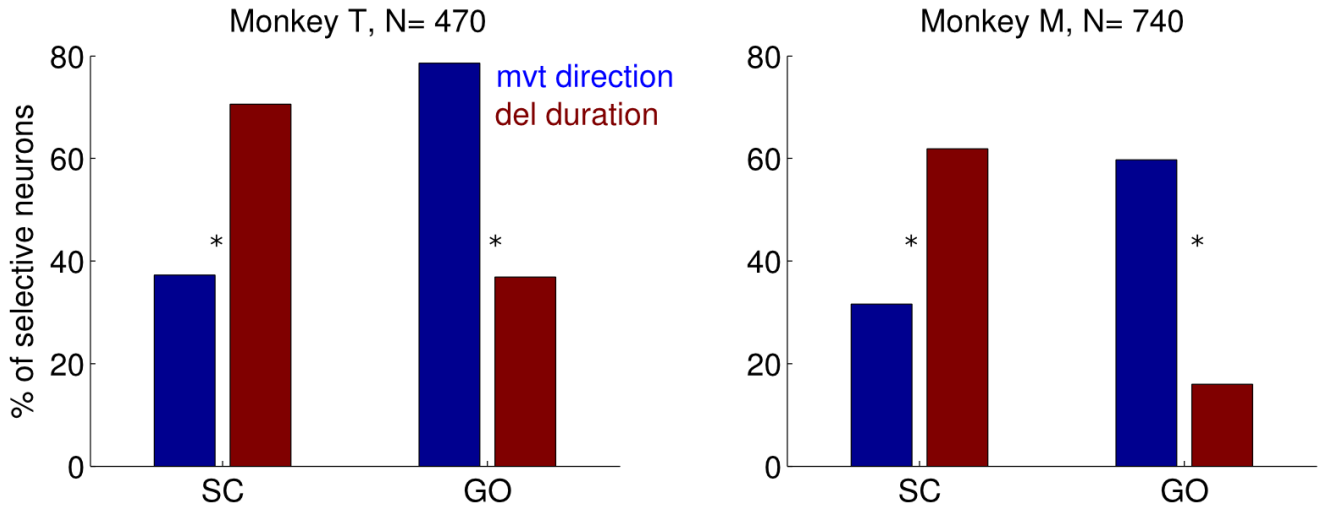


Figure 3.13: Selectivity to movement direction and delay duration at the beginning and end of D2. The percentages of neurons with a significant selectivity to movement direction (in blue) and delay duration (in red) are plotted (2-way ANOVA, $p<0.05$). 'SC' and 'GO' indicate the two time windows in which the spike counts were analyzed. 'SC' indicates a 200ms time window following SC, and 'GO' indicates a 200ms time window around GO. Stars indicate significant differences (chi-square, $p<0.05$). mvt: movement, del: delay.

In summary, both delay duration and movement direction are represented in all components of the EPs, even the very early VEP components following SC and the late MRP components during movement execution. However, VEPs, and especially the N1 component, are relatively more selective to delay duration, whereas the late component of the MRP is more influenced by movement direction. Following the same trend, the spiking

activity is more selective to delay duration in the early epoch of the delay, whereas it is more selective to movement direction around movement onset.

3.2.9. Trial-by-trial relationship between evoked potentials and RTs

We have shown that the size of motor cortical EPs is strongly modulated by the temporal context. If these modulations reflect the amount of ongoing motor processing, then EP size should also correlate with RT, which varied considerably from trial to trial (see the section 3.2.2). We therefore correlated EP size (measured in single trials) with RT, for all movement directions together, for each LFP. Fig. 3.14A shows an example LFP with a significant negative correlation between VEP and RT, in short trials. Fig. 3.14B shows another LFP with a significant positive correlation between MRP size and RT, in long trials. In Figs. 3.14C-D we show the average waveforms of the same EPs as in Fig. 3.14A-B, separated for the 50% of trials with faster vs. slower RTs in each direction, plotted together for all directions. The amplitude is different in fast and slow RT trials (mainly P1 for the VEP), whereas the latency of the component peaks remains similar. This indicates that the significant trial-by-trial correlations between EP size and RT was mainly due to modulations in amplitude of the EP component, not due to a systematic shift in latency, which could have led to a misalignment of the rms analysis epochs with respect to the EP components. The same conclusion was reached when inspecting component latency and amplitude of other LFPs with significant EP-RT correlations (see also results of more detailed analysis of peak amplitudes below).

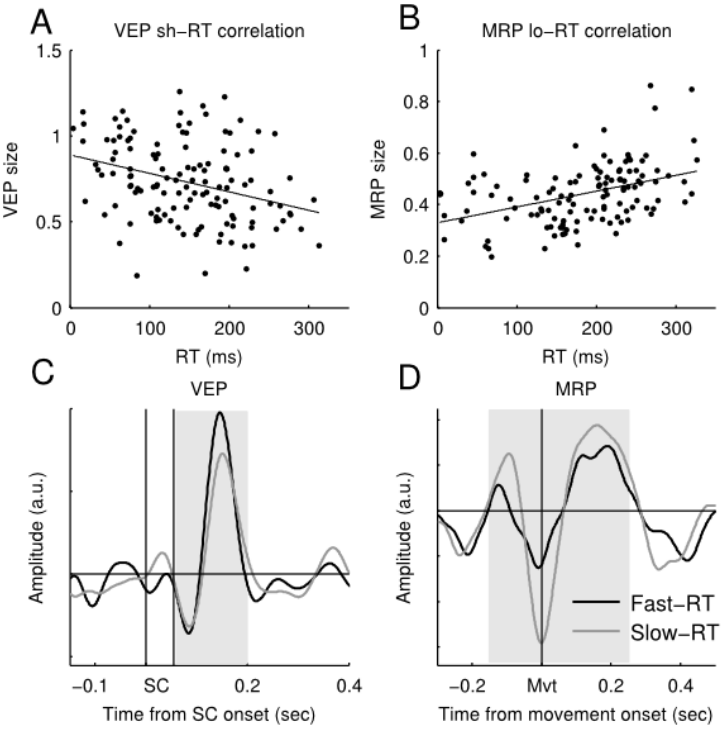


Figure 3.14: Example of correlation between RT and EP amplitude. A) Example of a significant negative trial-by-trial correlation between VEP size and RT for one LFP (short delay trials; r=-0.33, p<0.001). B) Example of another LFP with significant positive trial-by-trial correlation between MRP size and RT for one LFP (long delay trials; r=+0.41, p<<0.001). C and D) Comparison of the average VEP-short (C) and MRP-long (D) waveforms in the 50% of trials with fast (black) and slow (gray) RTs, for the same LFPs as shown in A and B. The gray zones indicate the trial epochs in which the rms was calculated. (Fig. 6 in Kilavik et al. 2010).

Figs. 3.15A-B show all correlation coefficients from correlation analyses between the EP size and RT, for all EPs in both monkeys. In the plots, data from short and long delay trials are grouped together, as well as data from M1 and PMd in monkey M, for clarity. All LFPs are included, regardless of whether the correlations were significant. In both monkeys, a majority of the MRP-RT correlation coefficients was positive (monkey T: 94% and 94%, and monkey M: 90% and 79%, in short and long delay trials, respectively, significantly more positive than negative, Chi-square $p<<0.001$ for all). Correlations between VEP and RT were weaker, as could be expected because of the long separation in time between SC and movement onset (700ms to 2s delay duration). Still, there was a small majority of negative correlation coefficients (monkey T: 80% and 50% and monkey M: 57% and 55% in short and long delay trials, respectively, significantly more negative than positive for short delay trials in monkey T, Chi-square $p<<0.001$), reflecting a slight shift of the VEP population to the left of zero in Fig. 3.15A, for monkey T. In Fig. 3.15 C-D, only the significant ($p<0.05$) correlation coefficients are shown. Almost all significant MRP-RT correlations were positive (41-77% significant, 95% or more of these were positive correlations in short and long delay trials for both monkeys; short and long delay trials grouped in the plots). Only few individual VEPs were significantly correlated with RT, but most of the significant correlations were negative [monkey T: 12% (all 16 cases negative) in short, but only 3% (2/3 negative) in long delay trials; monkey M: only 4% (6/12 negative) in short, but 13% (22/28 negative) in long delay trials]. LFPs in which RT was significantly correlated with both VEP and MRP size were rare (11/118 in short and 3/101 in long delay trials in monkey T, 4/170 in short and 8/144 in long delay trials in monkey M). Significant MRP-RT correlations were much more frequent in M1 (83% and 66%, in short and long delay trials, respectively) than in PMd (33% and 26%, respectively; significant difference between M1 and PMd in both short and long delay trials, Chi-square, $p<<0.001$).

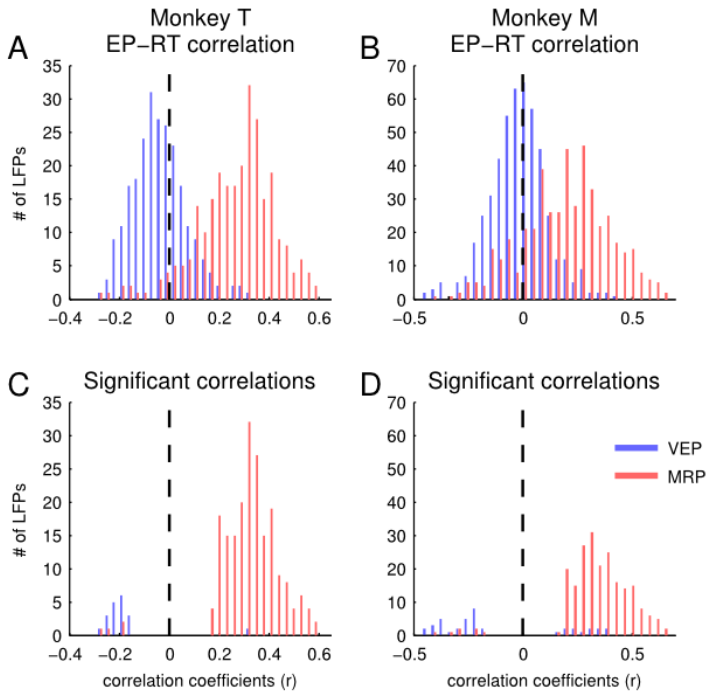


Figure 3.15: Correlations between EP amplitude and RT: population data. A and B) Distributions of correlation coefficients (r) from trial-by-trial EP-RT correlations for monkey T (A) and monkey M (B). C and D) Same as A-B, but only the correlation coefficients of significant correlations are shown. (Modified from Fig. 7 in Kilavik et al. 2010)

The number of significantly correlated VEPs was quite low. However, the correlation may be influenced by the directional tuning of the EPs, so we performed another calculation, less prone to noise. For the entire population of LFPs we calculated the average EP size in each session, separately for the two halves of trials with the fastest and slowest RTs (selected separately for each movement direction). The comparison between the two distributions revealed that VEPs were larger in fast RT trials (significant for short delay trials in monkey T and for short and long delay trials in monkey M, paired t-tests, $p<<0.001$), whereas MRPs were larger in slow RT trials ($p<<0.001$ for all comparisons). When looking separately at EPs recorded in M1 and in PMd in monkey M, only VEPs in short delay trials in M1 were not significantly different in fast and slow RT trials ($p<0.05$ VEP in long delay trials in M1, $p<<0.001$ for the other comparisons). To illustrate this additional analysis, Fig. 3.16 shows the distributions of the normalized differences of the mean amplitudes (contrast value, see section 3.3.4) according to RT (50% slower vs. 50% faster) for each LFP, across directions and delay durations (as explained above, the RTs being selected separately in each condition). All distributions are significantly shifted away from 0, even if the proportion of significant differences (especially for the VEPs) is very low. In other words, even though the influence of RT on individual VEPs is relatively weak, a clear effect can be seen at the population level.

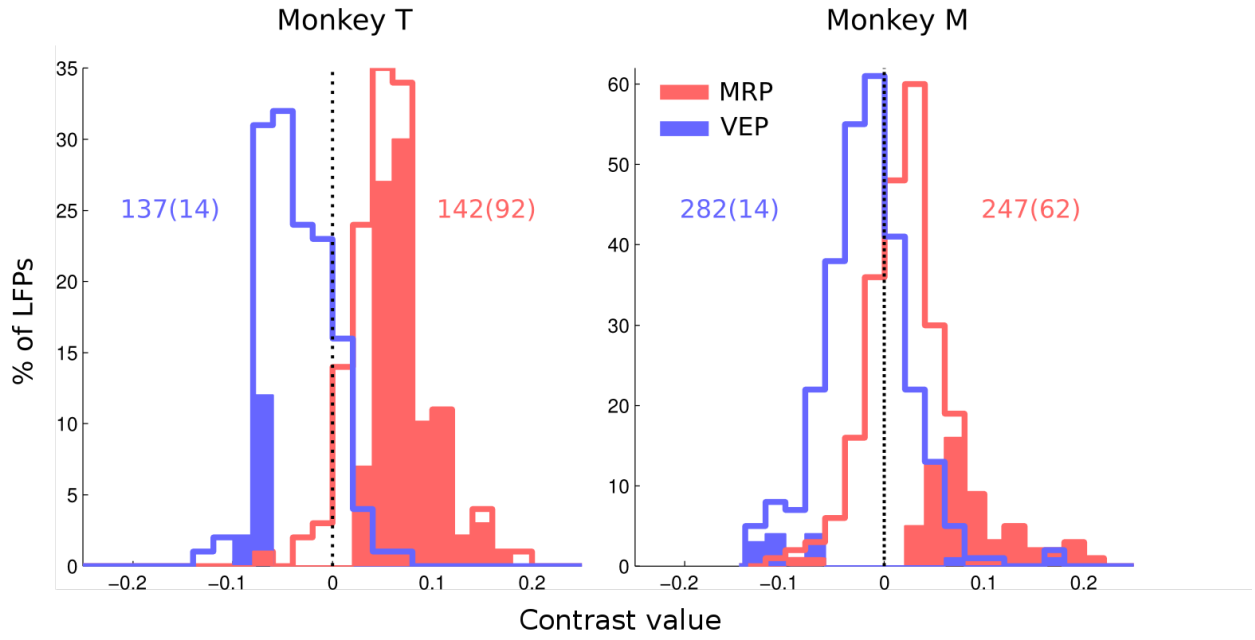


Figure 3.16: Difference of EP amplitudes in trials with long and short RTs. The outlines correspond to all detected EPs, the VEP in blue and the MRP in red. The filled histograms represent subsets of EPs with a significant difference of amplitude between the trials with the 50% longest and shortest RTs in each movement direction (t-test across all movement directions grouped, $p<0.05$). A distribution shifted to the right signifies mean amplitude larger for slower RTs, and a distribution shifted to the left means that the EPs are larger for faster RTs. Note that all distributions are significantly shifted away from 0 (Wilcoxon signed-rank test, $p<0.05$).

We also calculated the correlation between the individual EP components and RT. There were only a few significant VEP-RT correlations, with a weak majority of negative significant correlations. For the MRP components, the results were very similar to those described above, with a large majority of positive correlations. In addition, we analyzed the peak amplitudes of the components (see Methods) for fast and slow RT trials. In particular, we compared peak amplitudes in the 50% of trials with fast and slow RTs, for individual LFPs (e.g. Fig. 3.14C-D). For the VEP P1 component, the majority of significant cases had larger peak amplitudes in trials with fast RTs, compared to trials with slow RTs. In monkey T, 34% and 23% of the VEP P1 component showed a significant difference between fast and slow RTs in short and long trials, respectively. Of these, 83% and 48% were larger in fast than in slow RTs. In monkey M, 21 & 29% of the VEP P1 component changed in amplitude with RT (in short and long delay trials, respectively), with 90 & 70% of these being larger for the faster RTs. This is consistent with the negative VEP-RT correlations described above. The MRP N1 component, whenever significantly different in fast and slow RT trials, was larger in trials with slower RTs, consistent with the positive MRP-RT correlations described above.

To assess whether correlations between the MRP and RT could be due to an underlying visual evoked activity in response to the GO signal (all 6 LEDs turned off), we measured the trial-by-trial variance of the peak latency of the MRP P1 component with respect to GO signal occurrence and movement onset (see Roux et al. 2006). This component is more likely to reflect GO signal-related activity than any other MRP component. For each LFP with a significantly detectable MRP P1 component, we defined an index Q=(varX-varY)/(varX+varY), in which varX is the variance of the P1 peak latency with respect to GO signal occurrence and varY the variance of the P1 peak latency with respect to movement onset. A positive Q indicates that the component is more related to movement onset than to GO signal occurrence. The means of Q for all LFPs were 0.69 +/- 0.10 and 0.54 +/-0.18 in short and long delay trials for monkey T and 0.54 +/-0.17 and 0.64 +/-0.16 for monkey M. Only 2 individual Q values were negative across all four data sets (both individual Q values were less negative than -0.05; total N=256). Thus, MRP P1 peak latency varied much less with respect to movement onset than to GO signal onset, indicating that it reflects movement-related activity to a much stronger degree than any GO signal-related activity. This confirms the conclusion of Roux et al. (2006) who found P1 to be movement-related in the context of complete information concerning time and direction of movement.

Finally, as RT varied with movement direction, we also analyzed the correlation between the EP rms size and RT in each movement direction separately. Again, the majority of the relatively few significant correlations between VEP size and RT were negative. The large majority of the significant MRP-RT correlations were positive, confirming the results from the analysis done across all directions together, described above. In

summary, we found that RT was weakly negatively correlated with VEP size, but strongly positively correlated with MRP size.

If the VEP and the MRP in the same LFP was correlated with RT, one may also expect a direct correlation between VEP and MRP size in some LFPs. In particular, as most VEP-RT correlations were negative and most MRP-RT correlations were positive, we may expect VEP-MRP correlations to be negative. We therefore quantified VEP-MRP trial-by-trial size correlations in all LFPs with significant VEPs and MRPs, separately for short and long delay trials. It turned out that a small subset of LFPs in monkey T indeed had significant VEP-MRP correlations (17/118, 14%, in short delay trials; 10/102, 11%, in long delay trials), and a majority of the significant correlations were negative (13/17 and 8/10, in short and long delay trials, respectively). However, only very few correlations were significant in monkey M, with a small majority of positive correlations [9% (15/170, 4 negative) in short delay trials, 6% (9/144, 3 negative) in long delay trials]. It is noteworthy that we found a higher proportion of significant correlations in conditions where the delay durations were shorter; i.e. in both monkeys more often in short than in long delay trials, and more often in monkey T, who had shorter delay durations than monkey M (indeed in monkey M only a few correlations were significant that it possibly reflects the significance level of 5%). In monkey T, the majority of significant VEP-MRP correlations was negative, in agreement with the inverse VEP-RT and MRP-RT correlations described above.

3.2.10. Delay duration and pre-movement ramping activity

We saw in the previous sections that the amplitude of the phasic response of neurons as well as that of the movement-related activity was modulated by the delay duration. This section will now address the modulation due to the delay duration on the delay activity. In M1 and PMd, some neurons show a ramping-up activity preceding the GO signal or movement onset. Examples of such an activity were already shown in the Introduction (Fig. 1.6 A). This ramping pattern of activity was found to be strongly predictive of the RT (Lebedev et al. 2008), and thus likely related to the preparation of movement. Previous studies showed that the activity of these "ramping" neurons was modulated by delay duration, but in several ways. In the motor or frontal cortex, the onset of activity of some neurons shifted with a predictable delay duration, leading to a more or less constant lag before the GO signal (Lucchetti & Bon 2001, 2005). On the other hand, neurons from the thalamus (Komura et al. 2001) or the area IT showed a perfect scaling of their slope with delay duration (Reutimann et al. 2004). Additionally, the firing rate of neurons from LIP increases with different slopes according to the duration of probe signal, to which a second duration has to be compared (Leon & Shadlen 2003). This suggests, that in the context of motor preparation with a predictable delay, the activity of

ramping neurons may be modulated either by a shift of the onset of the ramping activity ("shifted onset"), a tilting of its slope ("tilted slope"), or both at the same time ("mixed").

We selected neurons with a steady increasing activity during the last 600 ms before GO in long delay trials and a minimum firing rate of 10 Hz around the GO signal (these criteria were chosen to restrain the analysis to neurons with a very "clean" activity, enabling visual inspection. However, choosing less restrictive criteria does not change the observed trend). This selection led to a sample of 58 neurons by pooling both monkeys, which we classified visually as "tilted slope", "shifted onset" or "mixed" according to delay duration. We inspected the PETH and raster plots of individual neurons, then classified the neurons according to two criteria: the onset of the change of firing rate in short and long delay trials when the activity was aligned on SC (Figs 3.17-18 A), and the slope of the firing rate when aligned on movement onset (Figs 3.17-18 B). Three experimenters classified the neurons independently, yielding similar classifications. Fig. 3.17 and 3.18 show two examples of these neurons, one "shifted onset", and one "mixed". Altogether, only 3 neurons were classified as "tilted slope", 41 as "pure shifted onset" and 13 as "mixed". These preliminary results tend to support the idea that the monkey estimates the duration of the expected delay, and starts to prepare his movement at a relatively fixed delay before movement onset (which depends on his subjective time estimation of delay duration).

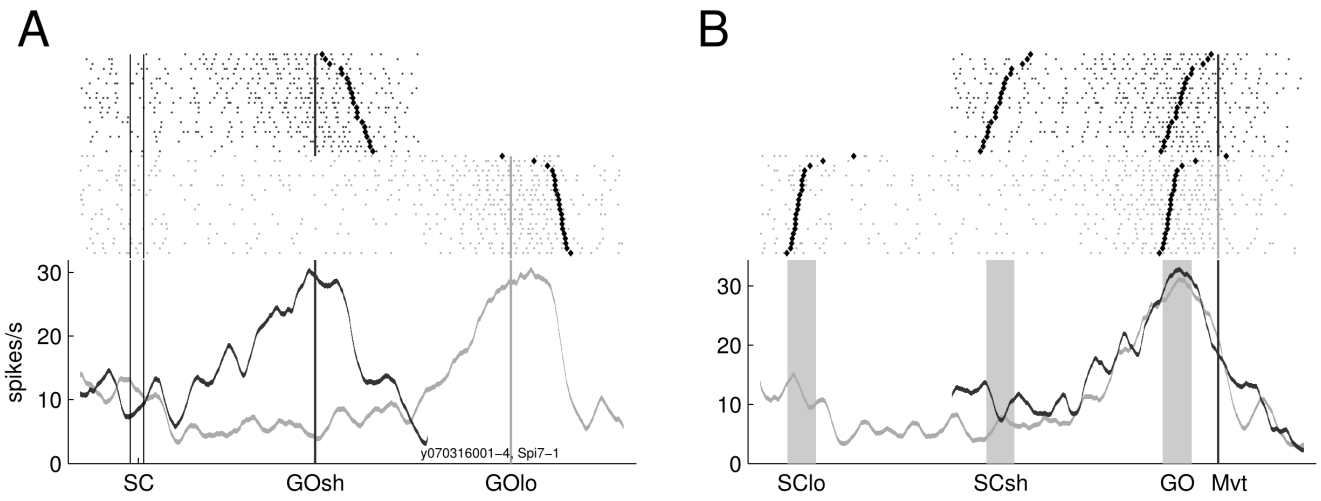


Figure 3.17: Example neuron with a shift in the onset of preparatory activity according to delay duration before the GO signal. A-B) represents the same neuron, aligned on different events of the task. The upper part of each plot represents the raster of the neuron's activity in its preferred direction, with each dot representing a spike. Diamonds represent the RT of each trial. Trials are ordered per increasing RTs. Lower parts of the plots represent the smoothed firing rate. Long delay trials are represented in light gray, short delay trials in dark gray. SC: spatial cue, GO: go signal, Mvt: movement, sh: short delay, lo: long delay. A) Data aligned on SC, B) data aligned on movement onset. Since the latency of movement onset is variable from trial to trial, aligning trials on it make the timing of external events variable from trial to trial. Thus, the timing of SCsh, SClo and GO and represented by thick rectangles indicating the timing of the cues regarding that of the GO, plus the mean RT and ±sd of RTs. For convenience, only one GO signal is represented for both delay durations because the timing of a GOsh and GOlo would overlap too much.

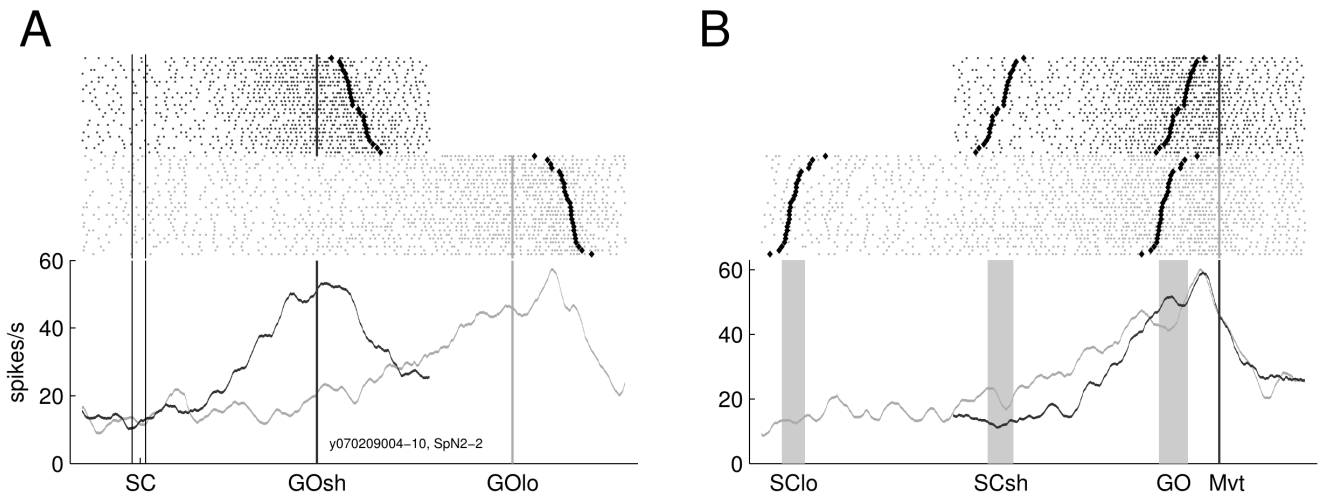


Figure 3.18: Example 'mixed' neuron with tilted slope and shifted onset according to delay duration before the GO signal. Same conventions as in Fig. 3.17.

3.2.11. Relation between firing rate and RT

As exemplified in Fig.3.17 and 3.18, the RT (marked as a black diamond) varies strongly from trial to trial. Numerous studies looked at the relationship between the firing rate preceding the movement and the timing of its onset, usually relating it to the slope of the firing rate (e.g. Hanes & Schall 1996; Lebedev et al. 2008). However, the context of the task could be a critical criterion to determine the relationship between neuronal activity and RT. For example, in the task used by Hanes & Schall (1996), the spatial information was provided by the GO signal, that is both RT and the slope of the firing rate likely reflect the speed of processing of this information However, in our task the context is completely different. The monkey already received the information about all movement parameters long time before, and the timing of the GO is predictable. Accordingly, the relationship between the RT and firing rate could also be different than described in the literature (e.g. Hanes & Schall 1996). We saw in section 3.2.2 that the average RT of our monkeys was slower in long than in short delay trials, a phenomenon related to time estimation (Niemi & Näätänen 1981). In long delay trials, the estimation of the timing of the GO signal was less precise (scalar property of time estimation; Gibbon 1977), leading to a lower readiness than in short delay trials. We then make the hypothesis that the trial-by-trial variability in RT in the condition when the delay duration is kept constant may also be due to the trial-by-trial fluctuation of the subjective estimation of delay duration. In the previous paragraph, we saw that the main consequence of delay duration on the spiking activity is a shift of the onset of the ramping activity. If the fluctuations of RT are also due to time estimation, then the same change of firing rate should be observed

during fast and slow RTs. To test this hypothesis, we looked in detail at the relationship between firing rate and RT. More specifically, we assessed which of three models linking preparatory activity to RT could describe the best our data. Hanes & Schall (1996) already described two models, i.e., "variable slope" and "variable threshold". The first postulates that a fixed threshold of firing rate can be reached with different slopes of activity, a steeper slope leading to a shorter RT. The second assumes that the slope of the activity is fixed, but that fluctuations in the threshold to be reached will determine the RT (lower threshold leading to faster RT). We added a third model, "variable onset", in which both slope and threshold are fixed, but the activity starts at different times, leading to a straightforward relationship with RT, the earlier the onset, the shorter the RT. The next paragraph and Fig. 3.19 illustrate these three models, and describe the relationship between firing rate and RT that could be expected according to each of them:

Variable slope of firing rate: if a fixed threshold of firing rate has to be reached at movement onset, and the onset of the increase of firing rate modulation is fixed relative to the GO signal, the main factor which would influence the RT would be the slope of the firing rate modulation. In this case, the mean firing rate in a fixed blue window in the left upper panel of Fig. 3.18) preceding the GO signal would be negatively correlated with RT, whereas the mean firing rate around movement onset would be uncorrelated with RT (see green window in the right panel). Additionally, the mean firing rate during a fixed window (see pink window in the right panel) recorded during movement preparation should be positively correlated with RT when the trials are aligned to movement onset.

Variable onset of increase in firing rate: on the other hand, if a fixed threshold of firing rate has to be reached at movement onset, and the slope of the increase of activity is fixed, the main factor to influence the RT would be the timing of the onset of firing rate modulation. As in the "changing slope" model, the mean firing rate in a fixed window preceding the GO signal would be negatively correlated with RT, whereas the mean firing rate around movement onset would be uncorrelated with RT. However, the mean firing rate in a fixed window recorded during movement preparation should not be correlated with RT when the trials are aligned to movement onset.

Variable spiking threshold at movement onset: if the RT depends on a variable threshold of firing rate needed to trigger the movement, long RTs should be associated with a high threshold and a high firing rate, and short RTs with a low threshold and a low firing rate. In other words, the mean firing rate around movement onset should be positively correlated with RT. However, in a fixed window during movement preparation, no correlation would exist between the mean firing rate and RT.

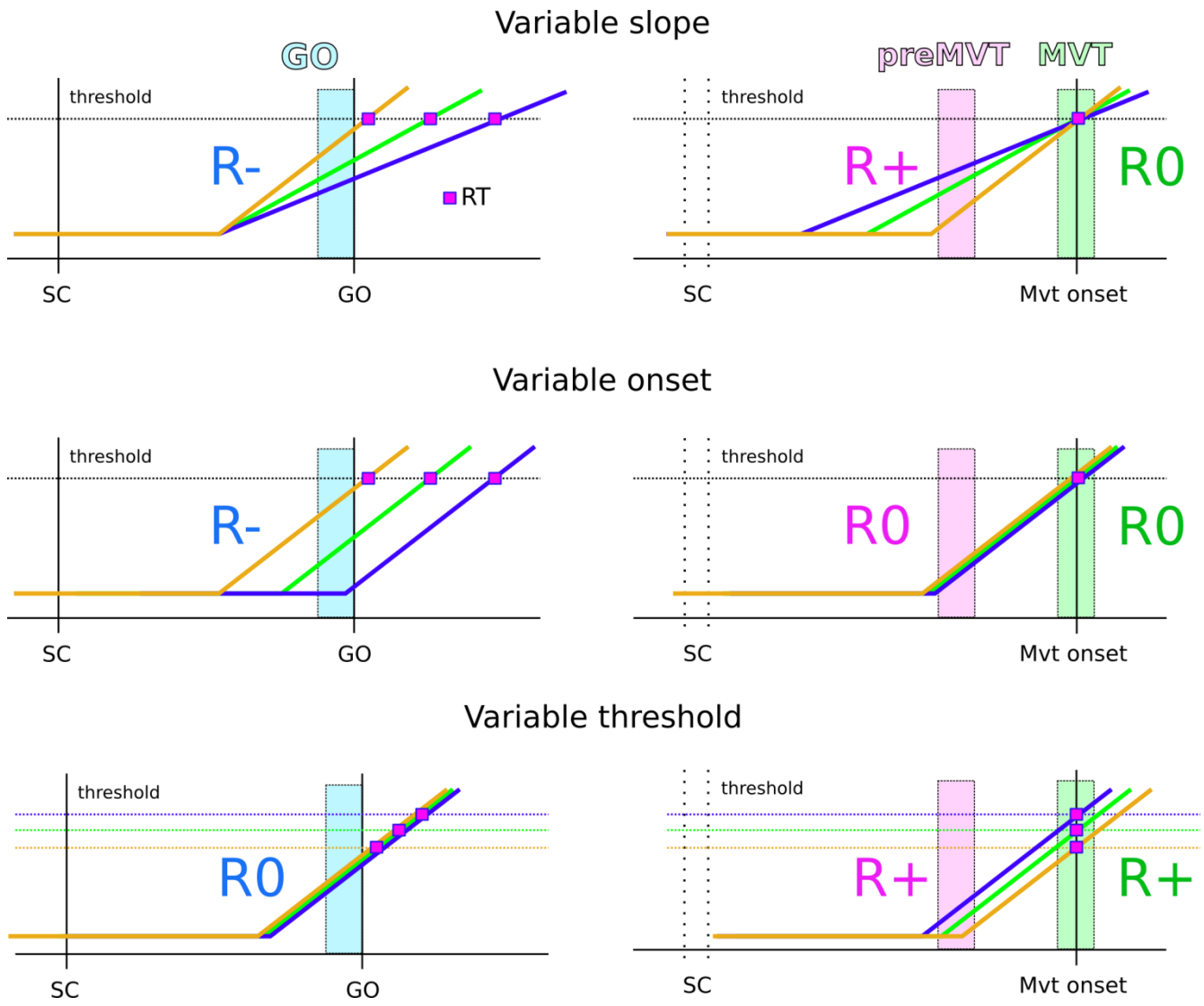


Figure 3.19: Three models of movement preparation and prediction of the correlation with RT. The thick colored lines represent the firing rates of a neuron in three trials with different RTs, marked by a pink square. The trials with shortest RTs are in orange, the medium in green and the slowest in blue. In the three schemata of the left column, the "firing rates" are aligned to external signals (GO), and on the right they are aligned to movement onset. The three pale-colored rectangles indicate the three analysis windows described in the text: during movement preparation and aligned to GO ('GO', in blue), during movement preparation and aligned to movement onset ('preMVT', in pink) and around movement onset ('MVT', in green). The colored 'R' indicates the expected relationship between firing rate in the corresponding window and RT: R- for a negative correlation, R0 for no correlation and R+ for positive correlation. The horizontal dotted line indicates the threshold that has to be crossed to trigger movement onset, either fixed or variable according to the model. The vertical solid line indicates the timing of events when fixed across trials, whereas the dotted vertical lines indicate the timing of events when variable across trials (i.e. when aligned on movement onset).

We determined the spike count in three 300ms time windows, illustrated in Fig. 3.19: 300ms before GO for the spiking data aligned to signal occurrence ('GO', in blue in the figure), from 600 to 300 ms before movement onset ('preMVT', in pink), and 300ms centered on movement onset ('MVT', in green). As described above, the relation between spike count and RT should change according to the analysis window. We thus had to compute

this relation in the three different time windows for each neuron. Unfortunately, the low number of trials per condition (typically 20) precluded a trial-by-trial correlation analysis (the noisy data combined with the small sample size would hardly ever reach significance). Instead, we applied a similar method as the one described in section 3.2.9. For each neuron, we grouped the trials as a function of RTs, taking the 50% fastest and slowest trials of each condition. Theoretically, doing so would compensate for any possible effect of movement direction or delay duration, since the fast and slow trials were selected inside each condition and grouped afterward. To take into account a possible residual effect of the experimental conditions (direction and delay duration), we assessed the effect of RT with a 3-way ANOVA. The 3 factors were movement direction (5 or 1 degrees of freedom, depending on the session type) delay duration (1 degree of freedom, short vs long delay duration) and RT (1 degree of freedom, fast vs. slow). In case of a main effect of RT ($p<0.05$), the difference between the mean firing rate of slow and fast trials was computed (contrast value). A negative difference indicates that short RTs are accompanied by high firing rate and slow RTs by low firing rate (negative correlation, R-), whereas a positive contrast value means that short RTs are accompanied by low firing rate (positive correlation, R+). If no main effect of RT was found, the firing rate was considered as being not related to RT (R0). In other words, the significance of the difference of firing rate due to RT was assessed by an ANOVA, and the sign of this difference was computed via the contrast value of the mean firing rates in fast and slow RT trials.

Using this method, we cannot speak of a proper "correlation", but rather of a "co-variation", or simply a "relation". By assessing the relation between firing rate and RT in the three time windows described in Fig. 3.18, we can assess if a neuron shows a pattern compatible with one of the three models of movement preparation. For example, a neuron whose firing rate has a negative relation with RT during the GO epoch, a positive relation during the preMVT epoch, and no significant relation with RT during the MVT epoch would match the "variable slope" model of movement preparation. Such a pattern of relation between firing rate and RT could be described as [GO- preMVT+ MVT0]. Conversely, a neuron with the pattern [GO- preMVT0 MVT0] would be classified as "variable onset".

We analyzed the neurons already presented in Fig. 3.6 (372 neurons in monkey T, 593 in monkey M), and classified them according to their match to one of the three models. The number of neurons with a significant relationship between firing rate and RT was surprisingly low. However, a previous analysis had hinted that less than 20% of the neurons showed a significant correlation (Spearman rank correlation, $p<0.05$) between firing rate and RT (data not shown). The low number of neurons with a co-variation between firing rate and RT does not seem to be due to the method of calculation, but rather to a feature of the task (too long delays preceding

the GO?). In monkey T and M, 3 and 1 neurons, respectively, matched the "variable slope" pattern, 81 and 60 neurons matched the "variable onset" model, and 2 and 3 neurons matched the "variable threshold" pattern. A shift of onset of the preparatory activity between fast and slow RTs seems to be the most common pattern. However, this method of classification is not completely fair. In the "variable slope" and "variable threshold" patterns, the ANOVA has to reach significance in two different analysis windows (in the 'GO' and 'preMVT' windows for the "variable slope" pattern, and in the preMVT and MVT in "variable threshold"). By contrast, the "variable onset" only requires significance in one analysis window (the 'GO' window). This means that by chance, a neuron is more likely to match the "variable onset" than the two other patterns. To compensate for this bias, we selected only two windows per pattern, with only one of the two needing to be significant. Each pattern is unique and matches a given model, and since significance has to be reached in only one analysis window for all three models, this analysis is not biased toward a specific model. This control confirms the previous result. In monkeys T and M, we found 15 and 17 neurons with the pattern [preMVT+ MVT0], respectively, compatible with the "variable slope", 91 and 78 with the pattern [GO- preMVT0] ("variable onset") and 18 and 25 neurons with the pattern [GO0 MVT+] ("variable threshold"). In other words, the activity of more neurons can be described by the model of "variable onset", compared to the two other models.

However, the "variable onset" model cannot explain alone all the performance variability. Fig. 3.18B shows the distributions of the contrast values between the firing rates in trials with slow and fast RTs during the MVT epoch (i.e. aligned to movement onset) for each neuron. As explained above, for each neuron, the firing rate in the trials with the 50% slowest and the 50% fastest RTs were averaged, and a contrast value was computed ([slow-fast] / [slow+fast]). The significance of this difference was assessed by performing a 3-way ANOVA on the single-trial spike counts (movement direction x delay duration x RT). It appears that on average the contrast values are shifted in the positive direction, compatible with the "variable threshold" model. However, compared with the same analysis performed in the GO epoch (aligned on external signals, Fig. 3.20A) the median contrast values (shown by a vertical thin line) are closer to 0 when aligned on movement onset (3.20B) than when aligned on the external signal (3.20A). Additionally, less neurons showed a significant difference of firing rate due to RT when aligned on movement onset than when aligned on GO. Taken together, even though the firing rate at movement onset is slightly higher in trials with slow RTs, the difference is much weaker than when aligned on external events. This result is at odds with the correlation seen in the EPs: the MRP, by definition aligned on movement onset, is strongly positively correlated with RT.

Altogether, even though the final mechanism of motor preparation may involve a mixture of the three models we reviewed, the "variable onset" model seems to be prevalent in the task we used. Indeed, we can

fairly assume that with such long delays, the movement preparation was not strongly constrained, ruling out the use of a "variable slope" model. By contrast, being able to accurately time the duration of the delay, and by doing so starting to prepare the movement "right on time" would be the major factor to influence performance. These results confirm the ability of the animal to anticipate the GO signal where the correct anticipation seems to be the best predictor of the RT.

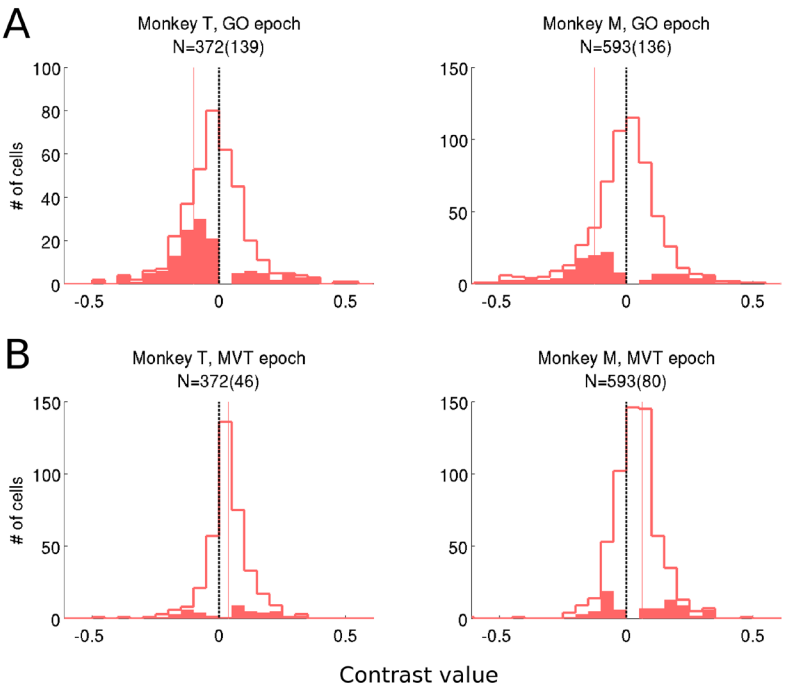


Figure 3.20: Contrast values of mean spike count in trials with slow and fast RTs. Negative values indicate a higher spike count in short RT trials, whereas positive values means a higher spike count in slow RT trials. The outlines represent all contrast values of mean spike count in trials with the 50% slowest and fastest RTs, across all movement conditions and delay durations. The filled histograms are the subsets of the neurons showing a significant main effect of RT (3-way ANOVA, see text). The vertical red line is the median of the filled histogram. The numbers in the titles indicate the sample size and the number of neurons with a significant difference. A) GO epoch (300ms centered on GO signal). All histograms are shifted toward negative values (Wilcoxon sign-rank test, $p<<0.001$). B) MVT epoch (300ms centered on movement onset). Both outlines are shifted toward positive values ($p<0.05$), filled histogram is significantly different from zero only in monkey M.

Additionally, in monkey M we looked for a difference between the neurons recorded in M1 and those in PMd, in the epoch during which most of the neurons showed a significant difference (the GO epoch). The neurons recorded in M1 showed more often a significant main effect of RT than those in PMd, and the median contrast value is more negative in M1 than in PMd. Thirty-one percent of M1 neurons have a significant main effect of RT, whereas only 21% of the PMd neurons have a significant effect (Chi-square test, $p<0.05$). Additionally, in PMd the distribution of the contrast values of the neurons showing a significant difference is centered on 0 (Wilcoxon sign-rank test, $p>0.05$), whereas it is shifted toward negative values in M1 ($p<0.05$). Thus, variations of RT are more reflected in the firing rate of M1 than PMd neurons, suggesting a stronger involvement of M1 in movement execution (see also Lebedev et al. 2008). This result goes in the same direction as the LFP data where a higher percentage of MRPs were detected in M1 than in PMd (see Fig. 3.3).

We previously showed that the size of the LFP VEP was negatively correlated with RT. We thus wanted to see if a similar relationship could be seen between RT and the phasic response of spiking activity following the SC. Indeed, it would be interesting to see if the firing rate modulation long before movement execution correlates with the modulation of RT. A similar analysis as described before (3-way ANOVA for movement direction, delay duration and RT) was performed on the spiking activity following the spatial cue, in a 200ms time window starting 50ms after SC (only phasic neurons were selected). The proportion of neurons with a significant ($p<0.05$) main effect of RT for the SC epoch did not differ from chance level, showing virtually no relationship between RT and the activity following the cue at the single neuron level. Furthermore, the distributions of contrast values between the activity in trials with short and long RT (as in Fig. 3.20A) was centered on 0 (Wilcoxon sign-rank test, $p>0.05$). We performed the same analysis on the peak firing rate detected trial-by-trial (see section 2.9.2 for the method), which yielded exactly the same results. In summary, the spiking activity in the epoch following the SC does not seem to influence RT, neither at the individual nor at the population level.

3.2.12. Co-variation between EPs and spiking activity

As we saw earlier in this chapter, the spiking activity and the EPs of the LFP have some characteristics in common and some differences. On the one hand, in both signals the activity evoked by SC and by movement onset tends to be inversely modulated by delay duration, even though the magnitude of this difference is much larger in the LFP. Delay duration does not influence the directional selectivity, neither of the EPs nor of the spiking activity. Furthermore, both signals recorded close to movement onset are correlated with RT. On the other hand, the directional selectivities of the two signals have completely different distributions, and whereas the VEP is marginally modulated by RT, the phasic response to SC of the spiking activity is not. So it seems that, as other studies have shown before (O'Leary & Hatsopoulos 2006), spiking activity and LFPs share some aspects, but not all. We thus wanted to assess if the variability of one signal, regardless of experimental conditions, can predict the variability of the other. To evaluate if the variations of VEP amplitude and spiking activity were related, we performed the same kind of analysis as the one described in section 3.2.11, but using the EP amplitude as the source of variation instead of RT. We first selected pairs of LFPs and neurons recorded simultaneously (on any electrode), and analyzed separately the VEP and MRP epochs. In other words, some neurons and LFPs were analyzed several times, each time in association with a different neuron/LFP. However, we failed to show any meaningful relationship between the two signals, as will be described below.

Co-variation of VEP and phasic spiking activity following SC

We classified the trials according to the size of the VEP and computed the spike count in each of them, in order to assess a possible co-variation of the two signals. To be selected, a LFP-neuron pair needed to fulfill a set of criteria: a VEP had to be significantly detected in the analysis window (see Methods), and the neuron had to show an early phasic response to SC (see section 3.2.5 and 4.2.4). These criteria lead to a database of 126 and 256 pairs in monkey T and M, respectively. For each pair, the VEP amplitude (rms) was sorted in two groups ("large" and "small"), independently for each experimental condition. These 3 factors (delay duration x movement direction x VEP amplitude) were then used in a 3-way ANOVA to determine if the spike count in a 200ms time window starting 50ms after SC was influenced by the VEP amplitude. The results are mainly negative: in monkey T, 12 neurons (10%) have a significant effect of VEP amplitude, which is not different from what would be expected by chance (Chi-square, $p>0.05$). In monkey M, 28 neurons (11%) showed a significant effect of VEP amplitude, which is different from chance level ($p<0.05$). For each pair, we computed the contrast value of the mean spike count between the trials with the large and the small VEPs. The distributions of the significant differences were not significantly shifted away from 0 (Wilcoxon sign-rank test, $p>0.05$). The same analysis was performed using the amplitude of the different components of the VEP instead of the rms, and yielded the same results. Additionally, we extended the analysis to all neurons (not only the phasic ones), to no avail. In summary, we failed to show any co-variation between VEP amplitude and spike count in the epoch following the SC.

Co-variation of MRP and spiking activity aligned to GO signal

The same analysis was repeated for the MRP and the firing rate recorded around movement execution. To be selected, a MRP had to be detected in the LFP, and the associated neuron had to fire at least 5Hz during a 300ms time window centered on GO, leading to a database of 194 and 320 neuron-LFP pairs in monkey T and M, respectively. Then, a 3-way ANOVA (delay duration x movement direction x MRP amplitude) was applied to the spike counts during the GO epoch. A small number of neurons, significantly above the chance level, showed a main effect of MRP amplitude (chi-square, $p<0.05$): 36(18%) and 34(11%) in the two monkeys. The distributions of (significant) contrast values of the spike count in trials with large vs. small MRPs were computed, and their medians were significantly different from 0: -0.08 and -0.09 in the two monkeys (Wilcoxon signrank test, $p<0.05$). This negative contrast value indicates that in this small subset of neurons, large MRPs are associated with a small spike count. However, both spiking activity and MRPs are modulated by RT, which could explain this negative relation between the two signals. When adding a parameter "fast vs slow RT" (see section 3.3.9) to the ANOVA, the number of significant neurons decreases in both monkeys. In monkey T, there

are still 22 (11%) neurons with a main effect of MRP amplitude, which is more than would be expected by chance ($p<0.05$), and the distribution of these contrast values is still shifted toward negative values ($p<0.05$). However, monkey M is at chance level with 22 neurons (7%). Additionally, when the spike count is taken around movement onset and the same analysis is performed, the number of neurons with a main effect of RT is not different from chance level. In summary, the relationship between spike count and MRP amplitude, if any, is very weak, and can most probably be accounted for by the modulation of both signals by RT (high firing rate and small MRPs are both associated with short RTs). In conclusion, we cannot show any co-modulation of the spike count and LFP EPs independent of the experimental conditions.

3.3. Discussion

Like the modulations of firing rate in single neurons, the amplitude of motor cortical EPs in the LFP is related to several aspects of motor performance, observed for both MRPs around movement onset (Donchin et al. 2001; Cardoso de Oliveira et al. 2001; Roux et al. 2006) and VEPs as response to visual cues (O'Leary & Hatsopoulos 2006; Ledberg et al. 2007; Asher et al. 2010). Here we show for the first time that both types of EPs are strongly influenced by the pre-cued delay duration, and are correlated with the behavioral performance on a trial-by-trial basis. Some of these aspects are shared with the spiking activity but in different extent (like the influence of delay duration), and some are not at all (like the correlation between the signal-related activity and RT). These results show that it is not possible to directly infer the modulation of single neuron spiking activity by analyzing the EPs of the LFP.

3.3.1. Spiking activity and EPs

Some aspects of our spiking activities and LFPs replicate previous findings. As described in O'Leary & Hatsopoulos (2006) for PMd and Asher et al. (2007) for the posterior parietal cortex, we found that, unlike the preferred directions of single neurons, the distributions of the preferred directions of the EPs are far from being uniform and appear to be clustered. Additionally, both the spiking activity and the EP amplitude were modulated by delay duration (Figs. 3.4 & 3.6) and were correlated with RT (Figs. 3.16 & 3.20). However, much more LFPs than neurons showed such task-related modulations. For instance, up to 80% of the MRP but only 40% of the neurons were significantly correlated with RT. Thus, even though they seem to reflect similar processes, the EPs and spiking activity recorded in the same areas are differently modulated by the parameters of the task.

We did not find any direct relationship between modulations of LFPs and spike count in a related time window. The most evident explanation for this lack of result is that the single neuron activity is an extremely

local signal, which can be "heard" in maximally 140µm distance from the electrode tip for the large pyramidal neurons (Henze et al. 2000). By contrast, the LFP is supposed to reflect the combined averaged activity of many neurons in a volume of a few hundreds of µm (Katzner et al. 2009 to a few mm (Logothetis 2003; Kreiman et al. 2006; Lindén et al. 2011). In these conditions, many factors can influence the LFP signals and "drown" the signal of the single neurons, including the activity of other areas. Another explanation, not exclusive from the first one, is based on the interpretation that the LFP reflects mainly the post-synaptic potentials of a large population of neurons (Mitzdorf 1985), as well as the influence of other slow potentials like post-spike potentials or other voltage-dependent membrane currents (see Logothetis 2003 or Buszáki et al. 2012 for a review), whereas the spiking activity reflects mainly the output of pyramidal neurons (Goense & Logothetis 2006). In other words, whereas the spiking activity is informative about the net output of a given neuronal population, the modulations of the LFP supposedly reflect local computations, like local excitation-inhibition loops (Goense & Logothetis 2006). Thus, it is possible that, first, the LFP modulations that we recorded reflect sub-threshold activity, which does not necessarily lead to the emission of a spike. Second, we have to keep in mind that the spikes we recorded may have a distant rather than a local influence, for example on the spinal cord. And third, in case of a recurrent inhibition, the net emission of spikes would be very weak, whereas the summed local activity of all interneurons could produce large LFP modulations. Mathiesen et al. (1999) directly showed the existence of such a dissociation between LFPs and spikes by stimulating a Purkinje neuron, monosynaptically projecting to a recurrent inhibiting neuron. Logothetis et al. (2001) and Goense & Logothetis (2008) also showed a direct example of different time course of the spiking activity and LFPs in V1 after presentation of a picture. The relative independence between spikes and LFPs is also supported by the fact that on the same electrode, several neurons can have different preferred directions and the LFP still another one (O'Leary & Hatsopoulos 2006), which may even be different for different frequency bands (Asher et al. 2007). Donchin et al. (2001) showed that components of the MRP were likely modulated by input from other areas (see also section 3.3.3). In motor cortex, most inputs project to layer 3 (see Hepp-Reymond 1988), whereas most individual neurons recorded in extracellular recordings are pyramidal neurons from layer 5 (Logothetis 2003). It is thus possible that the spiking activity we recorded is "blind" to a part of the EPs generated by inter-areal communication.

However, even though it seems hard to find direct, trial-by-trial correlations between the modulations of single neurons and those of LFPs, general co-modulations have been observed. For example, in M1 and PMv, Bansal et al. (2011) could predict the simultaneous spiking activity summed over 96 electrodes based on the low-frequency LFPs (equivalent to MRPs), during a natural reach-to-grasp task. In the same areas, Spinks et al. (2008) showed that on the same electrode, the "preferred" grip types of neurons and of LFP power were

correlated, even though the sign of the correlation differed according to the frequency band. A similar example comes from a study where spiking multi-unit activity (MUA) and LFPs were recorded in the inferotemporal cortex, during the passive viewing of a series of pictures (Kreiman et al. 2006). The authors found a weak but significant correlation between the preferred picture of the LFPs and that of the MUAs recorded on the same electrode. These studies show that even if fine-graded variations of the spiking activity cannot be seen in the LFPs, coarse aspects like the local tuning of a neuronal population or the averaged activity of multi-channel MUAs can sometimes be decoded from the LFP. The fact that the correlation between spiking activity and LFP power could change according to the frequency band might indicate that local neuronal populations with different tuning would be synchronized at different frequencies. Alternatively, different clusters at different distances from the electrode may have different tuning, the lower frequency having a more spread influence due to the low-pass filtering properties of dendrites (reviewed in Buszáki et al. 2012). In summary, the LFP can reflect coarse properties of the local spiking activity, and different aspects can be represented in different frequency bands (Asher et al. 2007; Spinks et al. 2008).

Another aspect of the relationship between spiking activity and LFP is the spike phase-locking of spikes on the LFP oscillations (for example, Murthy & Fetz 1996; Destexhe et al. 1999; Fries et al. 2001; Womelsdorf et al. 2006; Denker et al. 2010, 2011). Even though these studies focused on the beta or gamma oscillations and not on the EPs, they all report that the neurons tend to fire more during the downward phase of the LFP. Our result tends to go in the same direction. During the epoch following the cue, the abrupt peak of mean firing rate occurs during or just after the VEP N1 peak (see Fig. 3.5). As presented in the Introduction, this phasic modulation of the spiking activity as response to an informative cue in motor cortex is a well-described pattern of neuronal activity, called "signal-related activity" (Weinrich & Wise 1982). It can be observed shortly (less than 200 ms) after information necessary to produce a movement has been made available. Our result supports the idea that the signal-related activity contributes to the N1 component of the VEP, or more likely that the N1 reflects the cortical or sub-cortical input leading to the signal-related peak of firing rate. Supporting this last view, a recent paper of Ito et al. (2011) showed that the slope of the VEP in V1 of macaques performing a freely viewing task of natural pictures strongly influenced the phase-locking of the spikes. More specifically, they showed that the spikes emitted following a visual fixation (especially the first one) are phase locked to the first negative component (N1) of the VEP. A comparison of their data and ours is shown in Fig. 3.21.

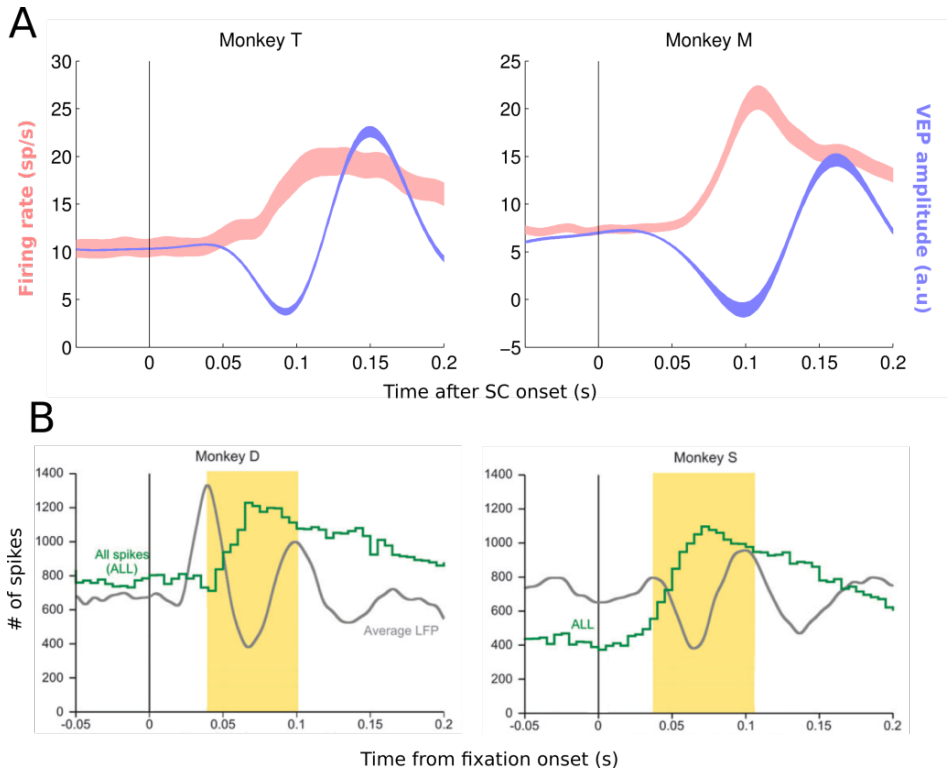


Figure 3.21: VEP and spikes recorded in motor and visual cortex. A & B show the VEP and firing activity from our dataset (A) and from Ito et al. (2011), with the same time scale. A) reproduction of Fig. 3.5 with a smaller time scale. The pink and blue curves represent the firing rate and VEP induced by SC onset (at 0). B) modified version of figure 5 of Ito et al (2011). V1 activity recorded in two macaques in a freely viewing task of natural pictures. 0 indicates the end of the visual saccade (i.e., fixation onset). The gray curve is the mean VEP. The green curve is the histogram (5ms bins) of the spike count of all neurons recorded during the same period.

Several observations can be made when comparing A and B of Fig. 3.21. First, the first component of the VEP in motor cortex peaks about 60ms later than the first component of the VEP in the visual cortex. Furthermore, the motor cortical VEP is much more stretched in time that the visual cortical VEP, and starts to show a modulation only ~30ms after the beginning of the visual P1 component (a latency matching the results of Ledberg et al. (2007), see below). However, the most interesting result regarding the matter at hand is that the relationship between the timing of the firing rate modulations and that of the VEP is very similar in the two areas. In both A and B, the peak of firing rate is reached shortly after the negative peak of N1. Indeed, Ito et al. (2011) showed that the spikes were more phase-locked to the VEP during its negative component. The authors interpret this result as an interaction between the activation due the visual input and the VEP produced by the onset of the saccade. The excitability of the neurons' membrane would be modulated differently according to the slope of the EPs, thus biasing the timing of the spikes: a steeper slope leading to a stronger phase-locking. It would be interesting to see if such a relationship between spikes and VEP can be extended to motor areas.

3.3.2. Motor cortical VEPs: visual processing or movement preparation?

Interestingly, whereas a majority of motor cortical LFPs recorded in our study contained VEPs in response to SC, only a handful of them showed an evoked response to TC, even in monkey M, where TC consisted of simultaneously presented auditory and visual stimuli. The lack of evoked responses to TC suggests that

stimulus-induced evoked activity in motor cortex is tightly linked to movement preparation, not merely reflecting sensory inputs. Similarly, Asher et al. (2010) observed a VEP following a spatial cue, but not following a cue indicating the beginning of the trial. This is not to say that single neurons in motor cortex cannot be selective for visual stimuli (Wannier et al. 1989; Riehle 1991; Zach et al. 2008), modulating their activity even when, using a GO/noGO task, a movement has not to be executed (Miller et al. 1992). In a similar experimental context (temporal cue, then spatial cue) but in another area (inferotemporal cortex), the modulation of spiking activity and LFP VEPs were induced by both cues (Anderson & Sheinberg 2008), but the response to the temporal cue was much weaker than the response to the spatial cue, and more variable from trial to trial. However, one must be careful to avoid an experimental bias: in all studies addressing the VEP in motor areas (O'Leary & Hatsopoulos 2006; Ledberg et al. 2007; Asher et al. 2010; this study), the eye movements of the monkeys were not controlled. Yet, Ito et al. (2011) showed that the VEP in V1 are more time-locked to the onset of the visual saccade than to the onset of the fixation. Since the onset of the visual cue could trigger an automatic saccade, saccadic activity might influence the motor VEP. Accordingly, further studies addressing VEPs should control for this parameter.

Ledberg et al. (2007) showed that even if motor cortical VEPs could be observed in the first tens of milliseconds following the VEP in the visual areas (typically, the VEP latency was 55ms in striate cortex, and less than 70ms in the motor/premotor cortex), it was only modulated by movement decision (GO/noGO) after 150ms or more. By contrast, we noticed a directional selectivity very early following the SC. Up to 30% of the detected N1 components (between 60 and 120ms following SC onset) were directionally selective, as well as twice as many P1 components (between 120 and 200ms). In the same direction, even though they do not comment on it, the VEP recorded by O'Leary & Hatsopoulos (2006, their Figure 7C) seems to be directionally selective early after the cue. The main difference between the task of Ledberg & al. (2007), on one side, and the one of O'Leary & Hastopoulos (2006) and ours, on the other side, is the complexity of the visuo-motor mapping. In our task and in the one of O'Leary & Hastopoulos (2006), the visual instruction and the motor target are identical, whereas in Ledberg et al. (2007), a complex visual pattern has to be deciphered to decide whether or not to release a lever. It is possible that the early selective activity that we recorded represents a response to a salient (potential) motor target, as has been shown in the parietal cortex (in PRR for reaching: Calton et al. 2002, and LIP for visual saccades: Campos et al. 2009). By contrast, the stimulus of Ledberg et al. (2007) did not allow such a simple spatio-motor mapping, leading to a late selectivity of the response. Data from the spiking activity seem to confirm this idea. The peak latency of the phasic response to a symbolic cue (decoding needed) is much longer than that after a simple spatial cue (Cisek & Kalaska 2005; Yamagata et al. 2009).

In other words, the early VEP (or "first sweep" of Ledberg et al. 2007) may reflect a first general activation of the sensorimotor chain, containing the available "potentially interesting" information for the generation of a movement. Thus, in the case of Ledberg et al. (2007), even though the motor cortex would be activated, the useful information would only be available later on. This pre-activation of the sensorimotor chain, even in absence of the final information, would render the movement preparation more efficient when enough information is gathered to execute the motor act. The progressive representation of the movement parameters in the first hundreds of milliseconds following SC is illustrated by the increased selectivity for movement direction in the second component of the VEP compared to the first one (Figs. 3.11-12). This idea is derived from the "affordance competition theory" of Paul Cisek (2007), postulating that potential actions ("affordances") are being planned continuously, even as sensory information is still being processed. Such a framework would explain the fact that, in a reaching task with mental rotation, the initial population response of the motor cortical neurons is in the direction of the cue, and only shifting afterwards towards the direction of the movement (Georgopoulos et al. 1989; Shen & Alexander 1997a,b). It would also explain the fact that in motor cortex, the neurons responding with the shortest latency to SC tend to be less selective to the information carried by the cue compared to the neurons with longer response latencies (Riehle 1991). More generally, several studies have shown that movement preparation starts even if only partial spatial information is available (e.g. Bastian et al. 2003; Cisek & Kalaska 2005).

In summary, the VEP observed in motor cortex might reflect early preparatory activity. This may involve the planning of a movement based on the immediately available information, which will be refined as more information is extracted from the environment. The VEP would then reflect a mixture of progressively incoming spatial information and the associated local processing.

3.3.3. Motor cortical MRPs

MRPs have been studied extensively, but their origin is still controversial and might depend on the behavioral context. The component preceding movement onset (epidural negativity; probably corresponding to our intra-cortical MRP P1) was described to originate in motor cortex, whereas the component following movement onset (epidural positivity; probably corresponding to our MRP N1) was found to be generated in both pre- and post-central cortices (Arezzo & Vaughan 1975, 1980; Pieper et al. 1980). The pre-movement component (MRP P1) was mainly interpreted as reflecting input to motor cortex to launch the movement, or more directly the descending motor volley itself (Vaughan et al. 1970; Gemba et al. 1981; Donchin et al. 2001), but it was found to be modulated with expectancy and prior information, indicating that it partly also reflects preparatory processes (Roux et al. 2006). In motor cortex the component MRP N1, occurring around

movement onset, was found to be unaffected by deafferentiation (Vaughan et al. 1970; Rothwell et al. 1982), but it was absent in somatosensory cortex of a deafferented patient (Kristeva et al. 2006). Our motor cortical MRP N1 might therefore reflect central feedback to motor cortex, whereas the MRP N1 in somatosensory cortex probably reflects feedback from the periphery (Kristeva et al. 2006). After cerebellar hemispherectomy, the phasic motor cortical MRP components preceding and following movement-onset disappeared in a simple visually triggered RT task (Sasaki et al. 1981), but they remained largely unchanged if a warning signal preceded the visual GO signal with a fixed delay (Sasaki et al. 1990). Thus, motor cortical MRPs might reflect cerebellar input only to a minor degree in delay tasks such as ours. Finally, the late component, probably corresponding to our MRP P2, seems to originate in motor cortex and is interpreted as being evoked by sensory feedback from the periphery and from lower motor centers (Cui and Deecke 1999, their MEP1 component). This is supported by the strong dominance of directional over temporal selectivity of the P2 component (see Figs. 3.9 & 3.10). Donchin et al. (2001) suggested that MRP P2 (and the preceding N1) reflect recurrent synaptic activation within motor cortex. The composite nature of the MRP is also supported by studies decoding movement parameters from the LFP signal. A good decoding could be achieved in a wide range of time windows before and after movement onset (Mehring et al. 2003; Rickert et al. 2005; Bansal et al. 2011), showing that both movement preparation and sensory feedback are represented in the MRP.

It seems likely that the phasic pre-movement MRP component reflects preparatory and movement-launching processes, whereas post-movement components reflect a variety of sub-cortical and peripheral feedback and recurrent activity in motor cortex, to optimize the ongoing movement. In our task the monkey used visual feedback during movement execution (i.e. feedback of hand trajectory and the target position presented on the monitor) more than during the simple wrist or finger extension movements that were performed in most of the studies mentioned above. The modulations in size of the different MRP components in relation to delay duration and RT might therefore reflect the degree to which the animal relies on visual feedback during execution.

3.3.4. Task timing modulates evoked potentials: strategies of motor preparation?

In our task, we found that larger VEPs were elicited in response to the cue in short delay trials, whereas movement execution was accompanied by larger MRPs in long delay trials. The firing rate was modulated in a similar way, even though the percentage of significantly different neurons is one order of magnitude smaller than that of LFPs.

A few studies with human participants explored electroencephalographic (EEG) activity using temporal orienting paradigms (e.g. Miniussi et al. 1999; Correa et al. 2006; Praamstra et al. 2006). These studies mainly

reported on EPs over occipital areas to validly (presented at the pre-cued time) and invalidly (presented earlier than indicated by the pre-cue) cued targets. VEPs were larger to validly cued targets (Correa et al. 2006; Praamstra et al. 2006). But these studies did not directly compare (Miniussi et al. 1999; Correa et al. 2006) or failed to observe (Praamstra et al. 2006) differences between EPs to targets presented after short and long intervals (validly cued). It is therefore questionable whether modulations in the neuronal responses to the targets after the delay in relation to pre-cue validity can be directly compared with effects of delay duration.

Correa et al. (2006) found in the EEG larger P1 responses to cues predicting short than predicting long delays before target onset. When comparing their cue to our SC and their target presentation to our GO signal, even though the paradigms are not completely comparable, their results correspond to ours, with larger VEPs in trials with shorter delay duration between the cue and the upcoming movement.

Why are VEPs and MRPs modulated by the delay duration? One can speculate that the effect of delay duration on VEPs is partly related to time estimation, whose variability increases with increasing delay duration (scalar property of time estimation processes, Gibbon 1977). The smaller VEP and the higher fraction of (directional) errors in long delay trials could partly reflect less optimal estimation of the time of SC occurrence (Rolke & Hoffmann 2007). However, as mentioned above, an effect of delay duration prior to the onset of a visual target was not observed in human EPs (Praamstra et al. 2006). In contrast, in two studies reporting a higher firing rate in motor cortical areas during expected shorter delays, the delay preceding the cue was fixed (Roux et al. 2003; Roesch & Olsen 2005a). These two elements indicate that the modulation of the VEP size and of firing rate might rather reflect the following than the preceding delay duration.

The fact that also MRPs are modulated by delay duration could reflect a behavioral strategy related to the pace of the trial. In long delay trials, the animal has more time available to prepare the movement than in short delay trials. The long interval between SC and GO might indeed make it less beneficial to prepare the movement early after SC (1500/2000ms before movement onset), but the information about the movement must be kept in memory for up to 2sec before actually being executed. Note, in our task, the GO signal does not provide any information about the movement, but only the moment when to execute it. Additionally, it might be more difficult to estimate the exact time of GO occurrence in long delay trials (Niemi & Näätänen 1981). It might therefore be beneficial, in long delay trials, to wait for the GO signal before finalizing movement preparation, whereas in short delay trials the movement preparation may start shortly after SC, resulting in anticipating the GO signal. This is supported by the fact that average RTs were longer in long delay trials and only a few trials with very short RTs were observed, suggesting little anticipation. Thus, the pattern of RTs, RT variability and errors suggest an anticipatory response mode in short and a reactive response mode in long

delay trials. This implies a complementarity between early preparatory processes (partly reflected in the VEP) and late movement preparation/execution-related processes (partly reflected in the MRP), adapted to the temporal constraints of the task.

3.3.5. Alternative explanation: motivation and readiness?

However, the selectivity to delay duration of the two EPs does not necessarily have the same cause. An alternative explanation stems from the study of reward expectation, even though all data reported so far concern single neuron spiking activities and not LFP EPs (Roesh & Olson 2003, 2005a,b, 2007; Fiorillo et al. 2008). Indeed, in our task, the delay duration influences equally the time before movement onset and the timing of the reward. This may be of importance, because the subjective value of the reward is supposed to be higher if it is available after a short than after a long delay (*temporal discounting*, Löwenstein & Elster 1992). Motivational factors were already shown to influence the activity of the premotor cortex (Roesch & Olson 2003, 2005a) during a task similar to ours, as well as influencing behavior and EMG activity (Roesch & Olson 2003, see also Tsujimoto & Sawaguchi 2005). Having these results in mind, reward expectation might have also influenced our results. It is unlikely that the reward value by itself is processed in motor cortex. However, the animal may be more motivated to perform an action if its outcome is rewarded earlier, thus influencing movement preparation (Roesch & Olson 2007). The fact that the effect of anticipated reward size was greater in the most "motor-related" areas supports this interpretation (Roesch & Olson 2003, 2005a).

Fiorillo et al. (2008) have shown that the cue indicating the delay duration preceding a reward strongly influences the activity of midbrain dopaminergic neurons. Their response to the cue is larger for shorter duration, and their response to the reward is larger for longer duration. During a task in which monkeys were informed at the beginning of the trial about the duration of a delay preceding a saccade toward an eccentric target, Roesch & Olson (2005b) showed a similar pattern of activity in orbito-frontal cortical neurons. They extended their analysis to several areas of the frontal cortex (Roesch & Olson 2005a) and showed that in PMd, a similar effect was observed around cue onset, that is, the single neuron firing rate was higher in response to a cue indicating a short than a long delay. Furthermore, the neurons sensitive to the expected delay duration were also modulated by the expected reward size, and this correlation was strongest in PMd compared to all other frontal areas. Our data, as well as those from Roux et al. (2003), go in the same direction than those of Roesch & Olson (2005a); there was a higher response to the cue during short than long expected delays. However, we have to be careful when comparing their results and ours, since their monkeys were performing saccades and ours reaching movements. Furthermore, their recordings in PMd were localized in the rostral part of the area (on the bank of the arcuate sulcus), whereas ours were much more caudal. Most importantly, the

effect they observed concerned the spiking activity, whereas the our data showed a strong effect in the LFPs and a weaker one in the spikes. However, the strong influence of motivation on movement preparation that they observe can be used to formulate a hypothesis, defeating the principle of parsimony but worth considering. It is indeed possible that in our task, the activities related to the cue (*expected* delay) and to movement execution (*elapsed* delay) are both modulated by delay duration, but for different reasons. On the one hand, the modulation of the neuronal response to the cue would partly be due to different levels of motivation, related to the subjective value of the reward discounted by the *expected* delay duration. Or simply the perspective of a shorter expected delay would lead to a higher arousal or preparedness. On the other hand, the activity during movement execution would be more modulated by motor factors, e.g. the ability to time the movement preparation after a delay of various *elapsed* duration. We discussed earlier (section 3.3.2) the possibility that the early visual response to the cue would be a general response signaling an involvement in the sensorimotor chain, which, at this point, would be more influenced by internal (motivation, arousal...) factors than by external information concerning the movement to be made (not completely processed at this point). This 'draft' of movement (see part 3.3.2 of the discussion), weighted by the motivation of the animal could be reflected in the early response to the cue. As the trial progresses, the influence of movement preparation-related factors (like time estimation) would become greater, and the influence of motivation would proportionally decrease. The larger MRP observed during movement execution after long delay trials, as explained above, would then reflect a less optimal movement preparation due to temporal imprecision. In a study using transcranial magnetic stimulation, Davranche et al. (2007) indeed showed that the degree of movement preparation (reflected by pre-movement cortico-spinal inhibition) was lower for longer delays. This interpretation would explain why the percentage of neurons influenced by delay duration is so high during the period following the cue, compared to the percentage of neurons being selective to movement direction (Fig. 3.13), a result also found by Roesch & Olson (2005a). Similarly, we showed that whereas the percentage of N1 (early) components of the LFP VEPs being sensitive to delay duration is already elevated, the percentage of directionally selective N1 components is quite low. However, this dichotomy decreases for the later component (VEP P1) (Figs. 3.11-12). When one reaches the movement execution epoch, the tendency is then reversed, that is, more neurons are selective to movement direction than to delay duration (Fig. 3.13), as are some components of the MRP (Figs. 3.11-12). The influence of motor parameters would at this point be stronger, “diluting” the effect of expected subjective reward. A paradoxical result of Roesch & Olson (2005b) supports this interpretation. They found that the selectivity of orbitofrontal neurons for *expected* delay duration and reward size was correlated after the spatial cue. However, no correlation was found between the selectivity to *elapsed* delay duration (at saccade onset) and reward size. Similarly, the same authors showed

that the selectivity to *expected* delay duration of PMd neurons vanished at saccade onset (Roesch & Olson 2005a), which correspond to us to the late components of the MRPs.

The existence of two processes, one “internal”, the other “motor” is also supported by the relative independence of the influence of movement direction and delay duration on neuronal activity and behavior. We found many EPs of the LFP and neurons to be directionally selective with preferred directions being similar in short and long delay trials (see also Roesch & Olson 2005a). This was the same for the directional preferences of the behavior, observed in RTs. This stability in relation to delay duration might not be surprising by itself, as the visual spatial cues and the required movements were the same in short and long delay trials. However, it becomes interesting by the fact that the delay duration modified strongly both RTs and EP sizes. Task timing (trial pace) and directional selectivity seem to influence the neuronal activity in a rather independent way, suggesting that they may be mediated by independent processes. This suggests that the modulation of neuronal activity induced by directional information may ‘ride’ on top of a more general modulation induced by delay duration. These two types of modulation may thus come from different origins, one “internal” (or motivational), and the other regarding movement preparation.

In summary, one possible hypothesis to explain our results would be the dual influence of motivational and motor preparatory (temporal) factors. The first one being stronger at the beginning and diminishing with time, whereas the second would grow as movement execution approaches.

Furthermore, the possible influence of reward expectation on movement preparation has some further epistemological consequences. When comparing results obtained in human participants (for example, recordings in implanted patients prior to surgery, EEG studies...) and monkeys performing a sensorimotor task, we assume that they are vaguely doing the same thing. However, monkeys are rewarded in each trial and the reward is supposedly the main aspect motivating them to be part of the experiment. By contrast, human participants are not rewarded in each trial, and the incentive to participate in the task is more of a middle-term reward (e.g., a fixed amount of money, or merely to help out a colleague, see Donchin et al. 1971, 1991). The attention and motivation of the monkey and the human subject would then be differently modulated as they perform the same task, inducing (yet another) difficulty in comparing their data (for example, it may explain the lack of difference in the VEPs following a cue indicating a forthcoming short or long delay in the EEG study of Miniussi et al., 1999).

To conclude, an interpretation *a minima* of our results would be that in short delay trials, the monkey would be more "ready" to respond compared to long delay trials, either because he knows that he must move soon or because he is more motivated, or a combination of both. This would lead to a strong delay-selective VEP, but

not necessarily a directionally selective one.-d As movement is prepared and executed, longer delays would lead to a lack of preparation and a stronger on-line processing of the movement, leading to a large, directionally selective MRP.

3.3.6. Models of movement preparation

The method we used to assess the relationship between single neuron firing rate and RT was quite coarse, because of the low number of trials per condition. For example, the elegant method used by Maimon & Assad (2006) based on a response threshold could not be used here because it requires a large number of trials in comparable experimental conditions in order to assess the behavioral variability due to "internal" factors only.

Our results show that during our task, the motor cortical activity during movement preparation tends to be negatively correlated with RT when aligned to an external signal, and almost not correlated with RT when aligned to movement onset. This pattern, supporting the interpretation that the onset of the preparatory activity is the main factor determining the RT, was also found by Riehle (2005). Additionally, Chen et al. (2010) found such a correlation in SMA when looking at the MUA. By contrast, many studies found that the slope of the activity modulation, and not its onset, was the best predictor for the animal's performance (e.g., Hanes & Schall 1996; Maimon & Assad 2006; Kalensher et al. 2006; Lebedev et al. 2008). As explained in the result part, we assume that the differences in the task design may explain this discrepancy. In our task and in the ones described by Riehle (2005) and used by Chen et al. (2010), the monkey knew in advance when and where to go, and had a substantial delay to prepare his movement. As explained in section 3.2.1, we observed that the RTs were actually a mixture of anticipations of GO signal occurrence and of actual reactions to it. In these conditions, it makes sense that the best predictor of the monkey's performance is his ability to accurately anticipate the timing of the GO signal, rather than speeding up the movement preparation itself. The fact that longer fixed delays are accompanied by longer RTs supports this interpretation (Niemi & Näätänen 1981; this study).

By contrast, in Hanes & Schall (1996), the timing of the GO signal was randomly chosen, and the monkey could not prepare the movement before perceiving the GO signal because the cue was also informative about the target of the saccade to be performed. In other words, the entire movement preparation had to be done during the RT, and anticipating the timing of the GO was of little advantage to improve the performance. In their task, the time it takes to complete the preparation of the movement explains most of the variability. In M1 and PMd of macaque monkeys (Lebedev et al. 2008) or in the prefrontal cortex of pigeons (Kalenscher et al. 2006), the slope of the activity was negatively correlated with the RTs during the generation of self-timed movements. Here, no time estimation comes into play, and the "duration of movement preparation" is the

main factor determining the RT. The example of Maimon & Assad (2006) is more complex. They recorded in LIP and area 5 (two areas of the posterior parietal cortex) during a visual interception task. The monkey had to release a lever when he estimated a moving bar would hit a target. They showed that some neurons of LIP showed a variable slope of firing rate according to RT, reaching a constant firing threshold shortly before movement onset. However, the neurons of area 5 had exactly the same pattern as our neurons. Hence, in this hybrid task, in which the monkey had to time the generation of the movement himself, but based on external events, both patterns can been seen. According to the authors, the LIP activity would be the "internal trigger" of the motor activity (in area 5) and of movement initiation.

In summary, in a temporally predictable context, with complete information about the movement to be performed, the efficiency of movement preparation (shorter RT) would be mainly explained by time estimation. This idea is supported by our data. A shift of onset of the preparatory activity was observed both between fast and slow RTs (Fig. 3.20), and between short and long delay trials (Figs. 3.17-18). Similarly, the EPs (especially the MRP) show the same modulation of amplitude according to delay duration (Fig. 3.4) and RT (Figs. 3.14-15-16). Going in the same direction, Lucchetti & Bon (2001, 2005) showed that when a monkey learned a predictable delay preceding a GO signal, the onset of the preparatory activity adjusted to the new duration. However, if other parameters come into play, like the impossibility to predict either the timing of the GO signal or the target of the movement to be executed, the delay taken to process the spatial information and to actually prepare the movement will strongly influence the performance. One way to test this hypothesis would be to compare the correlation between RT and firing rate in a delayed pointing task, with or without prior spatial or temporal information (e.g. Riehle & Requin 1989, 1993; Crammond & Kalaska 2000), or with or without temporal predictability of the GO . Thus, even though one of the tasks of Roux et al. (2003) and ours seem very similar (in both tasks, the monkey knows when and where to go from the beginning of the trial), the fact that theirs is self-timed and ours rely on a GO signal make them completely different regarding movement preparation.

3.3.7. Trial-by-trial correlations between EP size and RT

The size of many MRPs of the LFP was significantly positively correlated with RT on a trial-by-trial basis, whereas VEPs were mainly negatively correlated with RT, albeit very seldom significantly for single LFPs. The weaker correlation between VEP and RT, mainly visible when pooling across all LFPs, could be due to the long time interval between SC and movement onset. Trial-by-trial modulations in RT and neuronal responses might be related to fluctuations in attention or with the monkey's readiness to respond, which could easily vary during the time course of a single trial. Likewise, this might partly be the reason why we found only few

significant correlations between VEP and MRP sizes in individual LFPs, with more significant correlations in the conditions with short delays. Monkey T who had a larger proportion of significant correlations had mainly negative VEP-MRP correlations, corroborating the inverse pattern of VEP-RT and MRP-RT correlations. However, VEPs and MRPs (and task-related modulations thereof) were not necessarily observed in LFPs recorded on the same electrodes. One reason for this might be that MRP effects were more often observed in M1 whereas VEP effects were more often observed in PMd (discussed below). It is therefore not evident that one should expect to observe significant VEP-MRP correlations in LFPs recorded on the same electrode. Asher at al. (2010) showed a strong positive correlation between VEP and MRP amplitude recorded on the same electrode, as well as a negative relationship between VEP and RTs. However, they do not mention a correlation between MRP and RTs. The discrepancies between their results and ours may be due to the delay duration, which was fixed and predictable in our task, and unpredictable in theirs. As explained above, the main source of RT variability in our task seem to be the ability to estimate the delay duration, thus diluting the influence of the "readiness to respond" potentially reflected in the VEP. It would make sense that in the case of a variable delay, the readiness to respond is the main factor determining the RT, which would explain the strong correlation that they observed between VEP and RT (even with a delay of 1.5 seconds). This interpretation would comfort the idea that the modulation of the early response to the cue would reflect more the arousal or general preparedness of the animal rather than complete movement preparation. It is indeed noteworthy that the correlation between any variable and RT does not necessarily imply the preparation of a specific movement. In the spinal cord, the delay activity is strongly correlated with RT, but poorly directionally selective, thus reflecting more a general motivation/involvement/readiness... of the monkey (Asher et al. 2010; reviewed in Cohen et al. 2010).

According to the hypothesis formulated in the previous paragraph, since the RT in our task is mostly determined by the ability to estimate the *elapsed* delay duration, it is unlikely to find a reflection of such process as early in the trial as in the VEP. This would explain why our VEP is not as modulated by RT as the one observed by Asher et al. (2010). By contrast, the amplitude of the MRP would reflect the degree at which the monkey relies on external events and on-line processing: temporal preparation would thus be degraded as temporal uncertainty increases, leading to longer RT.

3.3.8. Differences between M1 and PMd

There were several quantitative differences between M1 and PMd. In particular, MRPs were observed more often in M1 than in PMd, were more often modulated by delay duration and were more often correlated with RT in M1 than in PMd. Additionally, the late component of the MRP was strongly directionally selective in M1

and spiking activity toward movement execution more correlated with RT. On the other hand, VEPs were more frequently observed, were more often modulated by delay duration and were more strongly correlated with RT in PMd than in M1. However, the fact that all results described above were indeed observed in both areas indicate only a gradual difference in neuronal representations of visual information and movement preparation in the two areas (e.g. O'Leary & Hatsopoulos 2006), as also suggested for single neuron activity (Crammond & Kalaska 1996, 2000; Lebedev et al. 2008; reviewed in Riehle 2005).

3.3.9. Conclusion

In conclusion, we have shown that motor cortical EPs and spiking activities are strongly modulated in relation to an *expected* (following the cue) and *elapsed* (at movement execution) delay duration. These two aspects of timing modulate the neuronal activity in an opposite way, with *expected* shorter delay and *elapsed* longer delay leading to larger EPs and firing rate responses. The two modulations could be due to a motor strategy, i.e. preparing the movement at different point in times according to the temporal constraint. Alternatively, it would involve rather independent processes, one related to internal factors (readiness, motivation), and the other to the temporal aspects of movement preparation. The relative independence of the influence of directional and temporal aspects of the task supports this idea. In this temporally predictable context, the main factor determining the behavioral efficiency (RT) seems to be the ability to time the delay duration. Accordingly, most of the modulation of neuronal activity was similar according to RT and to delay duration. This result shows that regarding movement preparation, motor tasks involving *implicit* or *explicit* timing are completely different.

4. On the pre-cue anticipatory activity in motor cortex

4.1. Introduction

In the first chapter, we saw that depending on the temporal context (i.e. when the animal knows how much time is available to initiate an action), the evoked potentials of the LFPs, as well as the spiking activity are strongly modulated. These modulations may reflect the readiness to respond depending on the forthcoming duration, and/or the ability to properly time the initiation of the movement. However, the temporal context also means that the animal knows when meaningful events are supposed to happen, like the occurrence of a relevant cue. In everyday life, the timing of these cues is often extracted from the context. This can be a ball flying toward us with a given dynamics, or a stereotypical duration before the red traffic light switches to green. In other words, most of our actions are embedded in an implicit temporal context that we use to predict and anticipate important information and actions. In this second chapter, I will evaluate the influence of the temporal context on the cue anticipation, asking the main question: how does the motor cortex anticipate the processing of a cue when its timing is known in advance?

In the movement preparation paradigm, a delay is used to temporally separate the moment when the subject receives information about the action to be done (for instance, a spatial cue indicating the target of a movement), and the moment when this action can be performed (i.e., the moment of GO signal occurrence). However, in some paradigms, like ours, the subject can estimate the duration preceding the onset of the informative cue. Indeed, the pre-cue duration is sometimes explicitly indicated to the subject, because the delay between a first event and the cue is kept constant. The first event can be produced by the monkey (e.g., reaching to the central point), or externally provided (e.g., a warning signal). Since the movement preparation paradigm was first implemented, it has been regularly reported that when an animal can estimate the duration preceding an informative cue, neurons throughout the entire brain show a modulation of their firing rate well before the onset of the cue. This phenomenon could be observed even when the subsequent GO signal appeared several seconds after the cue. This was reported, among others, in the prefrontal and cingulate cortex (Niki & Watanabe 1979), the somatosensory cortex (Meftah et al. 2009), the basal ganglia (Hikosaka et al. 1989) and the motor cortex, which will be the central theme of this chapter.

Even though several surveys reported on an anticipatory activity in motor cortex as a side note (Wise & Mauritz 1985; Vaadia et al. 1986; Wise et al. 1986; di Pellegrino & Wise 1993; Crammond & Kalaska 1996; Shen & Alexander 1997; Cisek & Kalaska 2005; Oshi & Tanji 2006) or showed examples of neurons with such an activity (Weinrich et al. 1984; Roux et al. 2003), only a few addressed it directly (Mauritz & Wise 1986; Vaadia

et al. 1988; Confais et al. 2012). As summarized in the introduction (part 1.3.3.4E), this so-called cue anticipatory activity in motor cortical areas has several characteristics:

- It is usually more prominent in PMd compared to all the other areas of the frontal lobe recorded in parallel.
- Even though both decreasing and increasing patterns of activity changes are described, there are always more neurons with increasing than decreasing activity changes.
- Only the activity of a small subset of such anticipatory neurons is modulated by some aspects of the awaited cue, like the expected information provided by the cue or the expected reward it may lead to.

However, the modulation of neuronal activity in motor cortex prior to a predictable cue does not only concern spiking activity. Numerous papers show that whenever the timing of a cue is predictable, the power of LFP oscillations in the beta band (12-35Hz) during the pre-cue epoch is elevated (van Wijk et al. 2009; Saleh et al. 2010; Haegens et al. 2011; Takahashi et al. 2011; Kilavik et al. 2012a; reviewed in Kilavik et al. 2012b). Furthermore, the pre-cue beta activity seems to be sensitive to the information carried by the cue (Saleh et al. 2010). In a task in which a human participant had to select one target out of five presented successively, Saleh et al. (2011) showed that the beta power peaked before each cue up to the correct one, and decreased afterwards. Fujioka et al. (2012) asked participants to listen passively to an isochronous sound, while recording oscillatory brain activity in the MEG. They showed that the beta power dropped after each tone, but increased afterwards until it peaked just before the next tone. The slope of the increase depended on the tempo of the tones. The modulations of beta activity during the task presented in this thesis were also addressed by our group (Kilavik et al. 2012a), even if the anticipatory activity was not the main topic of the survey. In this paper, we showed that the beta power increased at the beginning of D1 (when the temporal cue was given) and stayed elevated until SC appeared. After the cue, the beta power dropped and increased again until movement onset. Furthermore, the frequency of the beta oscillation was systematically higher during cue anticipation (D1) than during movement preparation (of about 3Hz), suggesting a change of state between the two delays.

Hence, both the modulation of spiking and LFP activity show that the motor cortex is far from being silent during cue anticipation. However, the function of this anticipatory activity (or state) is still unclear. For instance, this activity may participate in a general timing process of cue occurrence (Durstewitz 2004; Coull & Nobre 2008; Fujioka et al. 2012), or be part of a preparatory process in anticipation of a future motor event, similar to what was found before movement onset (Lucchetti & Bon 2001; Renoult et al. 2006; Lebedev et al. 2008). Indeed, when going through the literature, it is difficult to have a clear idea about what this anticipatory activity might represent. On the one side, the results concerning beta modulations are hard to compare with each other, due to major differences between the experimental paradigms (see Kilavik et al. 2012b). On the

other side, although very detailed, the studies reporting on pre-cue spiking activity of single neurons remained descriptive and focused only on neurons with increasing activity, ignoring those with a systematic decreasing firing rate preceding the spatial cue (Mauritz & Wise 1986; Vaadia et al. 1988). Furthermore, little attempts were made to link the anticipatory activity to the subsequent movement preparation.

This chapter will exclusively address the spiking activity of motor cortical neurons during the same task as reported on in chapter 3, and described in parts 2.3 and 2.4. Whereas the topic of chapter 3 was the neuronal activity recorded during movement preparation, this chapter will focus on neuronal activity preceding the cue (that is, during D1). In this chapter, mostly based on a recent paper (Confais et al. 2012), we will address both patterns of pre-cue activity (increasing and decreasing), linking them to the subsequent phases of visuomotor transformation and movement preparation. Furthermore, our analysis will include neurons recorded from PMd (as was usually the case in most studies regarding anticipatory activity), but also from M1.

Our main finding is that almost half of the neurons recorded in both M1 and PMd are involved in cue-anticipation, with 60% of those showing an increasing and the remaining a decreasing pre-cue activity (referred to as two "categories" in the remainder of this chapter). The subsequent preparatory activity depends on the category to which the neuron belongs, especially in the epoch immediately following the cue and during late movement preparation. Despite marked differences in firing rate modulations, the prevalence of directional selectivity in the two categories of anticipatory neurons is comparable, suggesting a similar degree of involvement in early and late movement preparation. Furthermore, two evidences point to a complementarity of the two categories of anticipatory neurons. First, their activities tend to be inversely modulated by delay duration. Second, the trial-by-trial fluctuations of firing rate are positively correlated between pairs of neurons of the same category, and negatively correlated between pairs of neurons belonging to different categories. We propose that this anticipatory activity reflects a presetting mechanism to allow an optimal processing of the visual information, while prohibiting a premature response.

4.2. Results

4.2.1. Neuronal database

The activity of 470 and 740 single neurons was recorded in monkey T and M, in 85 and 147 recording sessions, respectively. In monkey M, in which the location of recordings was controlled by ICMS (see 2.5, in the Materials & Method section), 251 neurons were assigned to M1 and 489 to PMd. Typically, the activity of each neuron was recorded during 20 correct trials per trial type (direction x delay duration). The activity of 350 and 477 neurons was recorded in the 6 direction sessions in monkey T and M, respectively, and of 120 and 263

neurons in the 2 direction sessions. The raster plot of each neuron was visually inspected, and trials with obvious artifacts were removed from analysis (on average, 9% of the trials in 6% of the neurons were removed in monkey T, and 12% of the trials in 14% of the neurons in monkey M).

4.2.2. Anticipatory activity during D1

During the first delay (D1), between the time cue (TC) and the spatial cue (SC), the monkey knew in advance the moment of SC appearance, thus enabling its anticipation (see Fig. 2.3.1). A large number of neurons systematically modulated their firing rate before SC. We labeled such an activity pattern as anticipatory. Note that here "anticipatory activity" refers to the activity preceding SC, and does not concern the preparatory activity in anticipation of movement execution during the second delay (D2, between SC and the GO signal). These anticipatory neurons were defined as "D1up" and "D1down" neurons if they showed a significant upward or downward modulation of activity, respectively (see below).

Fig. 4.1 shows the mean activities and spike trains (raster displays) of two representative neurons. The D1up neuron illustrated in Fig. 4.1 A has a low firing rate at the beginning of the trial and a systematic increase of activity starting at about 400 ms after TC in short and long delay trials. It is worth noting that the onset of the increase in firing rate is variable from trial to trial (see raster displays). As a response to SC, it exhibits a short-latency burst of activity. This pattern of activity is typical, as it will be described later. In contrast, the D1down neuron illustrated in Fig. 4.1 B shows a continuous decrease in firing rate throughout D1, reaching a minimum just before SC, and no burst after SC.

To quantify how many neurons displayed one of these two prominent activity patterns, a systematic analysis of the firing rate during D1 was conducted. A neuron is defined as anticipatory if its spike count measured in two separate epochs, following TC and preceding SC (measured in two 150ms time windows, marked by black thick bars in the raster displays in Fig. 4.1), differed significantly within each trial (Wilcoxon signed-rank test, $p<0.05$). For this analysis, all movement directions of the short delay trials were pooled, since no spatial information had been provided to the monkey at this moment of the task.

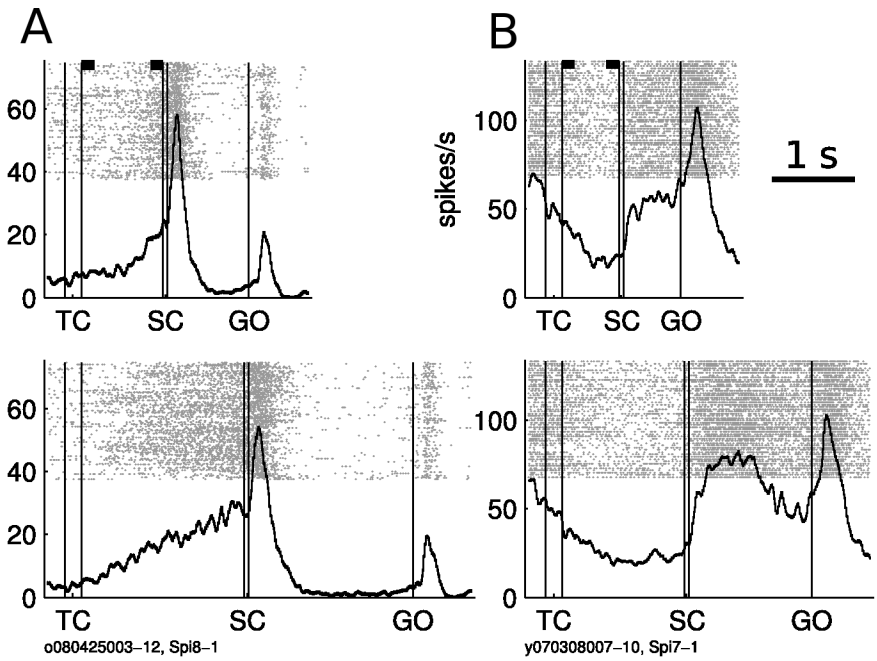


Figure 4.1: Raster plots and mean firing rates of two neurons. The short delay trials are on top, the long delay trials on the bottom, all movement directions included. In the raster plots, each dot is an action potential, and each row a trial, ordered in chronological order of recording from bottom to top. The thick black line represents the neuronal activity averaged across all trials and movement directions (PETH). The black rectangles on top indicate the analysis windows used to classify anticipatory neurons (see section 4.3.2). A) D1up neuron; B) D1down neuron. Figure reproduced from Fig. 1 of Confais et al. (2012).

In monkeys T and M, respectively, 49 and 38% of the neurons were classified as anticipatory. Among them, 57 and 60% were classified as D1up in monkeys T and M, and the remaining neurons as D1down (43 and 40%, respectively). There are significantly more D1up than D1down neurons in both animals ($p<0.01$, chi-square test). Fig. 4.2 shows these results, but expressed here as a fraction of the total neuronal population: 28 and 23% of the neurons are D1up in monkey T and M, respectively, and 21 and 15% are D1down neurons. The D1flat category contains neurons whose activity is not significantly modulated when comparing the beginning and end of D1. In monkey M, the same percentages of D1up and D1down neurons were recorded in M1 and PMd (D1up: 25 and 22%, respectively, $p=0.85$, chi-square test; D1down: 12 and 16%, $p=0.55$). All subsequently described analyses were performed separately in the two areas, but yielded similar results. Therefore, the neurons from M1 and PMd were pooled in the results.

In summary, almost half of the recorded motor cortical neurons show an anticipatory activity before the occurrence of a visual cue providing prior information about movement direction. About 60% of these show an increase of activity, being equally distributed in M1 and PMd.

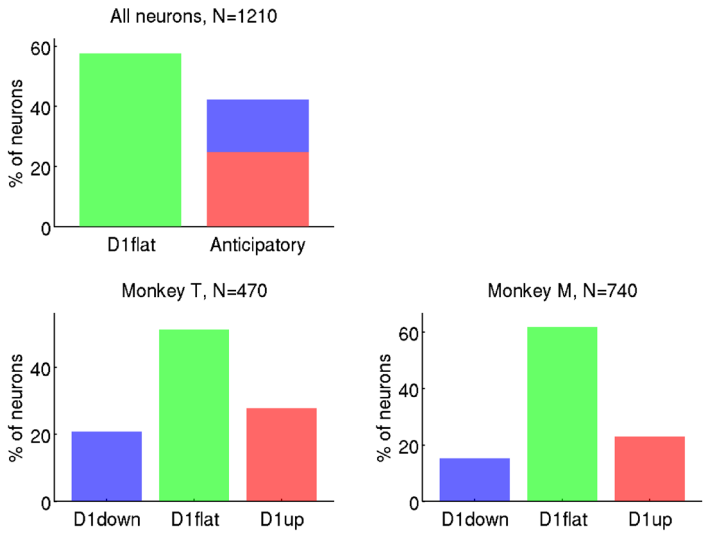


Figure 4.2: Percentages of anticipatory neurons. Top: Percentages of D1flat and anticipatory neurons (D1up in pink, D1down in blue), data were pooled for the two monkeys. Bottom: percentage of D1down (blue), D1flat (green) and D1up (red), separately for the two monkeys.

The two categories of D1up and D1down neurons may reflect two discrete patterns of activity. Alternatively, it may rather be the two extremes of a continuum of more or less decreasing and increasing activity. To address this issue, we computed the contrast values of activity (see section 3.2.4) using the two time windows marked in Fig. 4.1. It provided a distribution of values comprised between -1 and 1, indicating the changes of activity of all neurons between the beginning and the end of D1 (Fig. 4.3). One can clearly see that the distribution for all neurons (in black) is unimodal, and the subgroups of D1up and D1down are represented in pink and blue, respectively. Hence, the two categories that we defined are the two extremes of a continuum of descending and ascending activities.

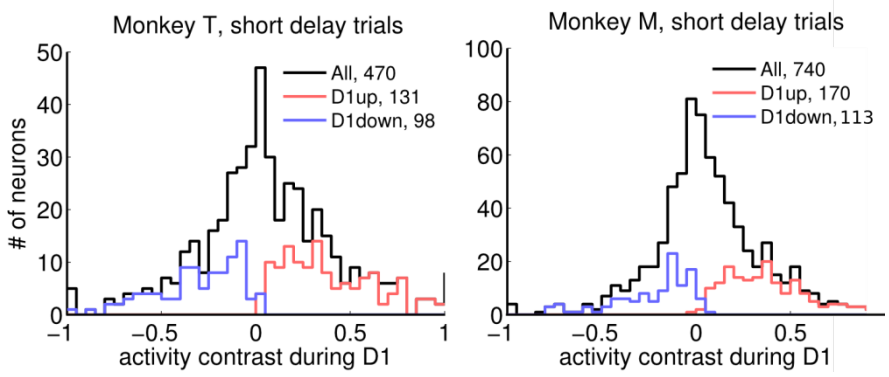


Figure 4.3: Contrast values of firing rates determined at the beginning and end of D1. The contrast is a normalized difference of activity between two values (see text above), in this case similar to a slope of activity. The number next to the legend represents the number of neurons of each population. The Y-axis represents the number of neurons.

When looking at the D1up and D1down activity in long delay trials, we saw that some of them had a different pattern of activity after the moment in time when the SC in short delay trials (SCsh) would occur. For instance, a D1up neuron would increase until the end of the short delay, and then decrease in the second half of the long delay. These kinds of patterns (up-down or down-up) were quantified by doing the same analysis

than for the D1up and D1down, but in the second half of D1 in long delay trials. In monkey T, 28% of the D1up and 23% of the D1down showed such a change of modulation, whereas in monkey M only 9% of the D1up and D1down had such a pattern. No obvious difference could be seen in their population firing rate during D2 compared to the "real" D1up or D1down. Therefore, we chose not to treat them as separate categories, to keep data analysis from turning into a collection of examples.

4.2.3. Population activity

Fig. 4.4 shows the mean firing rate of all neurons classified in the three categories, averaged across all movement directions (see section 4.2.1). It is clear that the mean firing rate of the three neuronal categories is modulated differently in time. The D1down neurons have the highest activity of all three categories of neurons before TC, the D1up neurons show a strong burst of activity shortly after SC and the D1down neurons seem more active than the other two categories during movement execution. The firing rates of the individual neurons in the three categories were measured in three epochs (200ms before TC, after SC and around movement onset) to examine these differences of activity. The results are reported in table 4.1, revealing that, first, the population activity of the D1down neurons is significantly higher than that of the D1up neurons in the epoch preceding TC ($p<<0.001$ for both monkeys, Mann-Whitney U-test across all neurons, with short and long delay trials pooled). Second, the phasic activity following SC is significantly higher in the D1up neurons than in the D1down neurons (virtually absent in the latter: $p<0.01$ and $p<<0.001$ for monkey T and M, respectively). Third, the firing rate during movement execution is higher in the D1down than in the D1up neurons, but this is significant only in monkey T ($p<<0.001$). The mean activity of the D1flat neurons shows less modulation than that of the other neuron categories, which may be due to the possible heterogeneous nature of this category.

Monkey T	before TC	after SC	around movement
D1up (N=129)	5.6±0.6	11.9±1	9.6±1.1
D1flat (N=228)	6.8±0.8	7.9±0.8	10.5±1
D1down (N=95)	11.6±1.1	8.4±0.9	15.9±1.8
Monkey M			
D1up (N=168)	4.9±0.4	13.1±0.8	9.2±0.8
D1flat (N=455)	6.0±0.4	8.6±0.5	8.5±0.5
D1down (N=113)	9.4±0.9	8.1±0.8	10.4±1

Table 4.1: Mean population firing rate (±sem) during three epochs of the task.

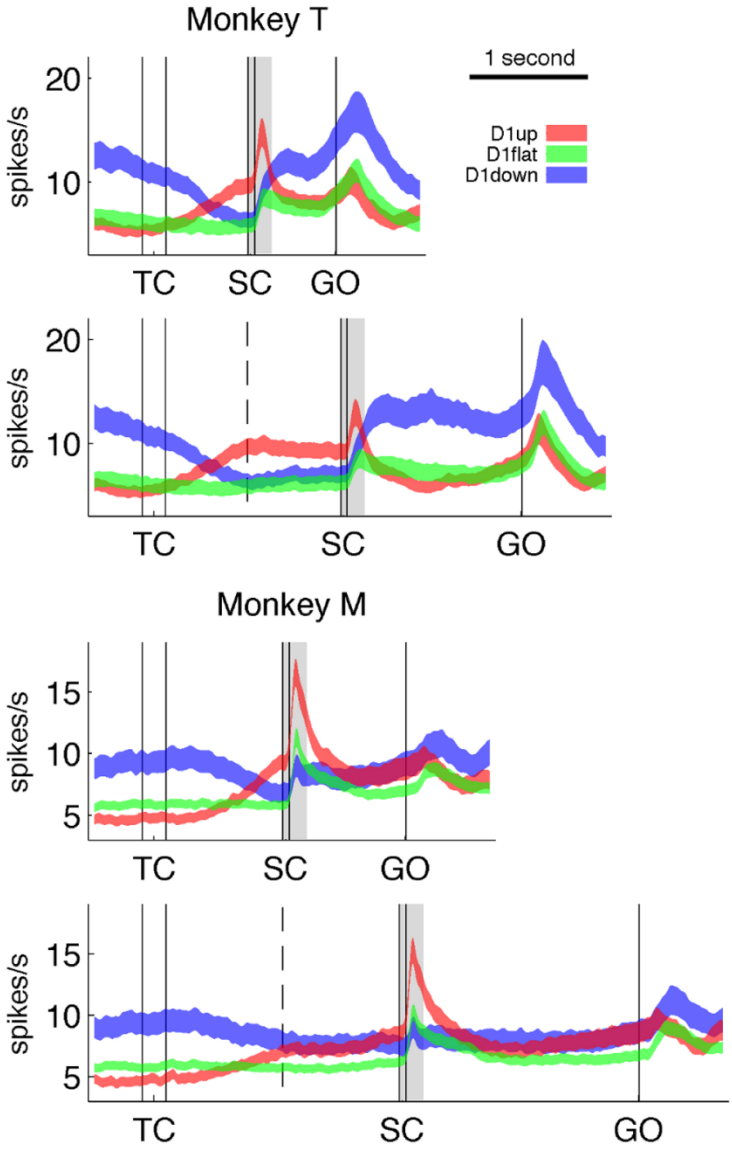


Figure 4.4: Population activity and proportion of neurons in the three categories. The curves represent the mean firing rate ± standard error of the mean, with the D1up neurons in red, D1down in blue, and D1flat in green. The dashed lines in the middle of D1 (delay between TC and SC) in long delay trials indicate the time of the SC in short delay trials. The light gray rectangles illustrate the central analysis window described in the next section of this chapter. Figure reproduced from Fig. 2 of Confais et al. (2012).

The fact that the firing rate of the D1down neurons is larger than that of the other categories before TC may be partly due to the way we classified the neurons. In the D1up and D1flat, the firing rate had to increase or stay equal during D1, allowing neurons with very low firing rate before TC to fall in these categories. By contrast, a neuron with a low firing rate before TC is less likely to show a decrease of firing rate during D1, and thus to belong to the D1down. Thus, a low proportion of neurons with a low baseline firing rate may lead to the difference of mean firing rate observed before TC between the D1down neurons and the other categories (Fig. 4.4 and table 4.1).

4.2.4. Characterization of the phasic activity following SC

By classifying the neurons on the sole basis of their increasing or decreasing firing pattern during D1, two striking differences in their mean activity during D2 emerge (see Fig. 4.4). The D1up neurons show a strong burst of activity shortly after the SC, which is virtually absent in the D1down neurons. Conversely, the D1down neurons are more active than the D1up neurons during movement execution. These two epochs will therefore be studied in more details in the following, starting by the epoch following the cue. However, the population peak amplitudes observed in Fig. 4.4 might differ between neuronal categories for two reasons. Either fewer D1down neurons display a phasic response to the cue, or their activity peaks have more variable latencies. To account for these two possibilities, two analyses (Fig. 4.5A and C) were performed to characterize the phasic activity of individual neurons following SC.

The proportion of neurons with an early phasic response to SC was first computed for each neuron category. A neuron was defined as “phasic” if its firing rate was higher in a short epoch following the onset of SC than in the two adjacent periods around it. For each neuron, the single trial spike count during the 200ms time window shown in gray in Fig. 4.4 was compared with the two adjacent ones (of the same duration), with all movement directions pooled together (two Wilcoxon signed-rank tests, $p<0.05$). If both tests reached significance, the neuron was classified as "phasic". Only long delay trials were considered, to temporally dissociate the activity related to SC from the movement-related activity. This analysis shows that the probability of having an early phasic activity burst following SC is strongly correlated with the type of activity during D1 (Fig. 4.5A). The percentage of neurons with an early phasic activity burst after SC is highest in the D1up neurons, lowest in the D1down, and intermediate in the D1flat neurons. All differences were statistically significant in both monkeys (chi-square tests, $p<0.05$), except between D1flat and D1down in monkey T.

Many motor cortical neurons are directionally selective as soon as an informative stimulus such as SC is presented (Riehle & Requin 1989; reviewed in Riehle 2005). If one of the populations of neurons contained more directionally selective neurons than the other, this could artificially increase the firing rate variability when pooling trials across all movement directions (thus decreasing the likelihood to reach significance). We therefore tested again all neurons using only the preferred direction of each neuron, calculated in the same time window (in gray in Fig. 4.4). The overall results were the same (Fig. 4.5B), but the percentage of significantly phasic neurons decreased slightly (presumably because of the smaller amount of included trials).

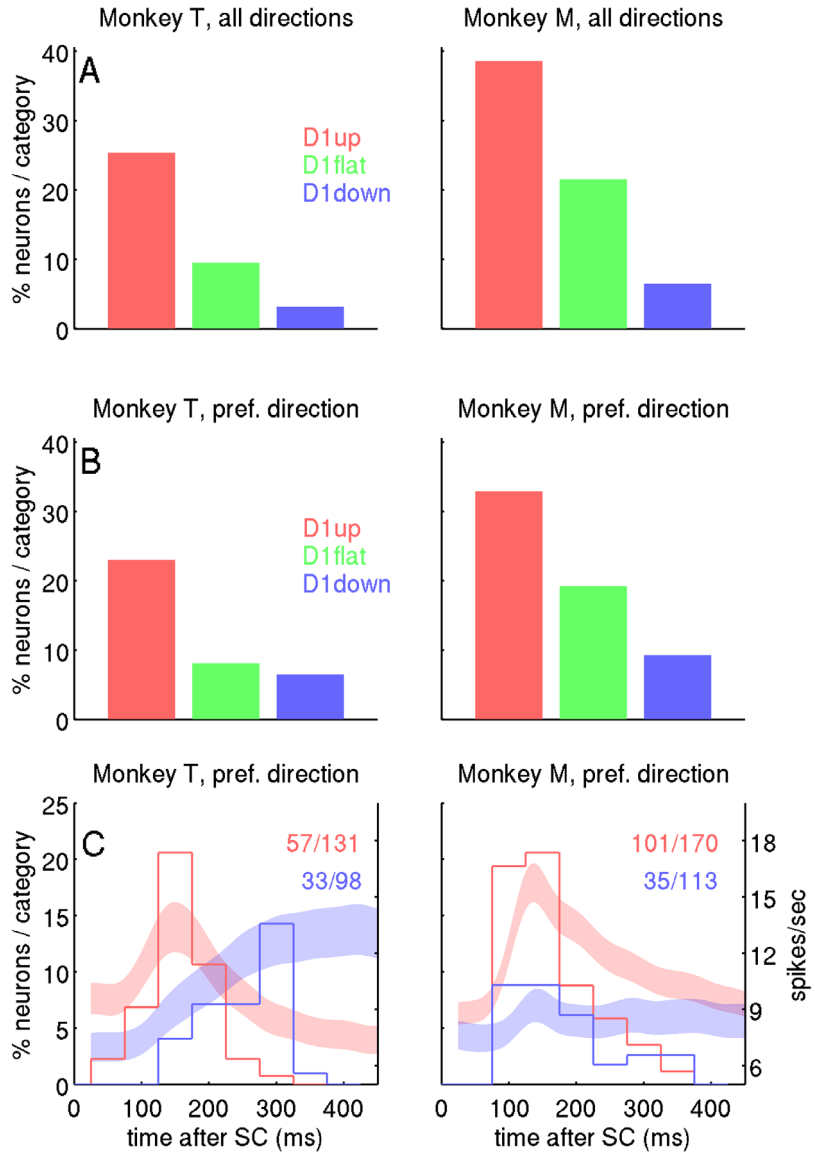


Figure 4.5: Phasic activity following SC. A) Percentages of neurons with a phasic burst of activity in a 200ms window following SC, expressed as the fraction of neurons belonging to each category. The trials of all movement directions of long delay trials are pooled for this analysis. All comparisons of differences between categories are significant for both monkeys (chi-square test, $p<0.05$), except between D1flat and D1down neurons in monkey T. B) Same as in A but using only the trials in the preferred direction of the neuron, in long delay trials. C) Histograms: distributions of the latencies of the peak firing rate, shown as percentages in each category (indicated on the left ordinate axis). D1up neurons are in red, D1down in blue. In the top right corners are the numbers of neurons with a detected peak out of the total number of neurons in each category. The abscissa represents the peak latency compared to SC onset, in milliseconds. For comparison, the PETHs of the two categories during the same epoch have been reproduced in the background, with the firing rate scale indicated on the right ordinate axis. Please note that unlike the histograms of peak latencies, the PETHs include the activity of all recorded neurons, not only the ones with a detected peak. Figure modified from Fig. 3 of Confais et al. (2012).

Second, the latency of the mean peak firing rate after SC was computed for each neuron. The peak was searched in a time window between 40 to 500ms after SC (40ms corresponding to the shortest motor cortical response to a visual stimulation, see Riehle, 1991), only in long delay trials. The distributions of all peak latencies were then plotted separately for the D1up and D1down neurons (Figs. 4.5C). The results from this second analysis confirm those from the first analysis, in the sense that more D1up than D1down neurons have an early peak (<200ms). However, there are clear differences between the two monkeys. In monkey T, we found similar proportions with a detected peak in both categories of neurons (44 and 34%, respectively, chi-

square test: p=0.15), but the median peak latencies were significantly shorter in the D1up than in the D1down neurons (151 and 250 ms, respectively, Mann-Whitney U-test: p<<0.001). In monkey M the median peak latencies were not different between D1up and D1down neurons (145 and 162ms, p=0.22), but significantly more D1up than D1down neurons showed a peak of activity (59 and 31%, p<<0.01). These differences in peak latency distributions are clearly reflected in the mean population activity shown in Fig. 4.4, which is reproduced in the background of Fig 4.5C. In monkey T, the response to SC of the D1down neurons seems delayed compared to the D1up activity, but has a similar amplitude, whereas in monkey M, all categories of neurons show a short-latency change of activity, but with a higher amplitude and a more phasic response in the D1up than in the D1down neurons. In conclusion, the second analysis confirms that the D1up neurons are more likely to have a burst of activity in the early epoch following SC than the D1down neurons.

The same analysis was carried out on the movement-related activity, using the same method as in the previous paragraph, but in a 600ms time window centered on movement onset. On average, the D1up neurons have the tendency to peak before movement onset, and the D1down neurons afterwards (latency compared to movement onset, in ms: -16.6 and -14.7 for the D1up and 9.4 and 8.6 for the D1 down neurons, for Monkey T & M, respectively.) However, the difference of peak latencies between neuronal categories is very weak compared to the variability of each distribution (standard deviation, in ms: 79.6 and 81 for the D1up, 71.6 and 76.5 for the D1down neurons). As a consequence, the distributions are only marginally significantly different from each other (p=0.06 and p=0.07 for monkey T and M, respectively). In summary, on average, the distributions of the peak latencies of both D1up and D1down are centered near movement onset, and are mostly overlapping.

4.2.5. Influence of the anticipatory activity on the phasic response

We wanted to assess to which extent the anticipatory activity influences the phasic response following the cue. We thus performed a trial-by-trial correlation analysis between the activity preceding the cue and both the peak frequency and its latency of the phasic response to the cue. Since this analysis is performed using single trials, any spurious change of activity from one trial to the other would induce a strong positive correlation between the firing rate recorded in two windows from the same trials (for example, if the electrode moved during the recording session, suddenly missing a certain percentage of spikes). To avoid this, this analysis was restricted to the neurons with a stable inter-trial stationarity and a minimum firing rate of 5Hz in the analyzed epoch, starting 300ms before to 400ms after SC. The spiking activity of a neuron was deemed stable across trials if the visual inspection of the raster plot did not show any change of spike density when the trials of a recording session were ordered chronologically. This strict selection criteria lead to a sample of 145

neurons in monkey T and 143 in monkey M. Among those, we only selected the neurons with a detected phasic response following SC (shown in Fig.4.5C), further narrowing down the samples to 80 and 85 neurons in monkey T and M, respectively.

The peak was detected using a single trial rate estimation (Nawrot et al. 1999), and controlled by another method using the calculation of the spike count in a sliding window (see 2.9.2.2). The spike count in the last 300ms of D1 was then correlated trial by trial with the peak latency and the peak frequency, using a Spearman Rank Correlation. We first addressed the correlation between the firing rate preceding SC and the peak latency: respectively 12.5 and 8.2% of the neurons showed a significant correlation ($p<0.05$) in monkey T and M (10/80 and 7/85), which is not different from chance level (5%, $p>0.05$, chi-square test). However, most of the significant coefficients of correlation were negative (10/10 in monkey T, 5/7 in monkey M). Thus, the peak latency seems to be weakly influenced by the firing rate preceding the cue.

By contrast, 39 and 15% of the neurons showed a significant correlation between the activity preceding the cue and the peak frequency, which is more than would be expected by chance (chi-square, $p<0.05$). The relatively low number of correlated neurons in monkey M could be due to the difficulty of measuring reliably the peak of firing rate. Therefore, we performed the same analysis but replaced the peak frequency by the spike count in a time window stretching 50 to 300ms after SC. With this simpler and more robust analysis, a significant correlation was found in 51 and 24% of the neurons in the two monkeys (the numbers shown in the titles of Fig. 4.7).

However, a correlation between two signals means merely that they co-vary, but does not indicate the nature of their relationship. The example shown in Fig. 4.1 shows that the activity following SC is about twice as large as the one preceding it. The D1 activity can influence the response to SC in two ways: either by a multiplicative or additive gain. In the case of a multiplicative gain, the firing rate in the pre-cue epoch is multiplied by a large coefficient and lead to the peak amplitude. The pre-cue activity would thus have a large influence on the phasic response. Alternatively, an additive gain would mean that the phasic response is added on top of the activity in D1, leading to a weak influence of the pre-cue activity on the phasic response. If the activity before the cue had a multiplicative influence on the response to the cue, then the slope of the linear regression between the two signals would be steep (>1.5). In other words, whenever the firing rate during D1 increases a certain amount, the response to the cue would increase of more than this amount. On the other hand, if the slope is rather flat (between 0 and 1), this would indicate the response to the cue simply "rides on top" of the baseline shift which occurred during D1. To assess the relationship between the firing rate preceding and following SC, we performed a first-degree polynomial fit (least-square difference), similar to a

linear regression, on all neurons which reached significance to the correlation test. The linear regression (y= a*x+b, with y being the firing rate following the cue and x the firing rate preceding the cue) provides us with two parameters, a and b. The Fig. 4.6 plots an example of such a linear regression. Here, the slope (parameter a) is 0.4, and the y-intercept (parameter b) is 24.1. These values mean that an increase of 1Hz before the cue leads to an increase of 0.4Hz after the cue, and that the firing rate after the cue is on average 24.1Hz higher than before.

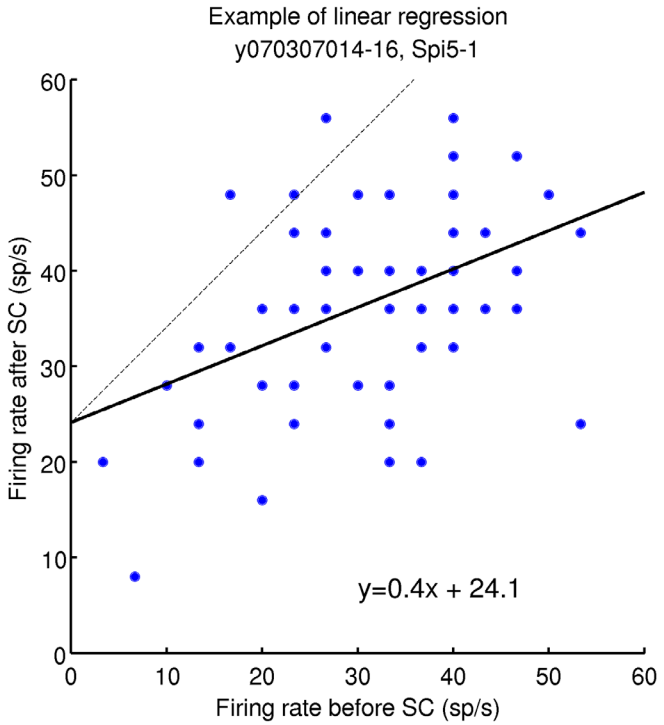


Figure 4.6: Linear regression between the firing rate preceding and following SC: individual example. The blue dots represent the firing rates in the two analyzed epochs in different trials. The thick black line is the regression line, defined by the formula indicated in the right bottom corner. For comparison, the dashed thin line represents a line defined y=x+24.1, to illustrate a slope of 1 with same y-intercept. Note that the dots are aligned on a grid, since the number of spikes in a given time window is finite. In this example, the correlation is significant (Spearman's rank correlation, r=0.39, p<<0.01)

The regression slopes of all neurons showing a significant correlation are plotted in Fig. 4.7. Unsurprisingly, the slopes are virtually all positive, meaning that an increase of firing rate before the cue leads to an increase afterwards. However, the slopes are usually weak, with a mean slope of 0.45 in the two animals. On average, an increase of 1Hz before the cue leads to an increase of less than half this amount after the cue, and most of the increase of activity is due to an offset of activity. By comparison, the offset of the slope (defined by parameter b) was large, with a mean of 16.3 (sd 10.3) and 19.1 (sd 14.4) Hz in monkey T and M, respectively. These values are of the same order of magnitude as the mean difference between the firing rates preceding and following SC, as can be seen in Fig. 4.4. In summary, the firing rate during D1 has a moderate influence on the response to SC. The phasic response will mainly be "added on top" of the level of firing rate reached just before the SC appears. In other words, the anticipatory activity influences mainly the response to the cue by an additive, rather than multiplicative gain.

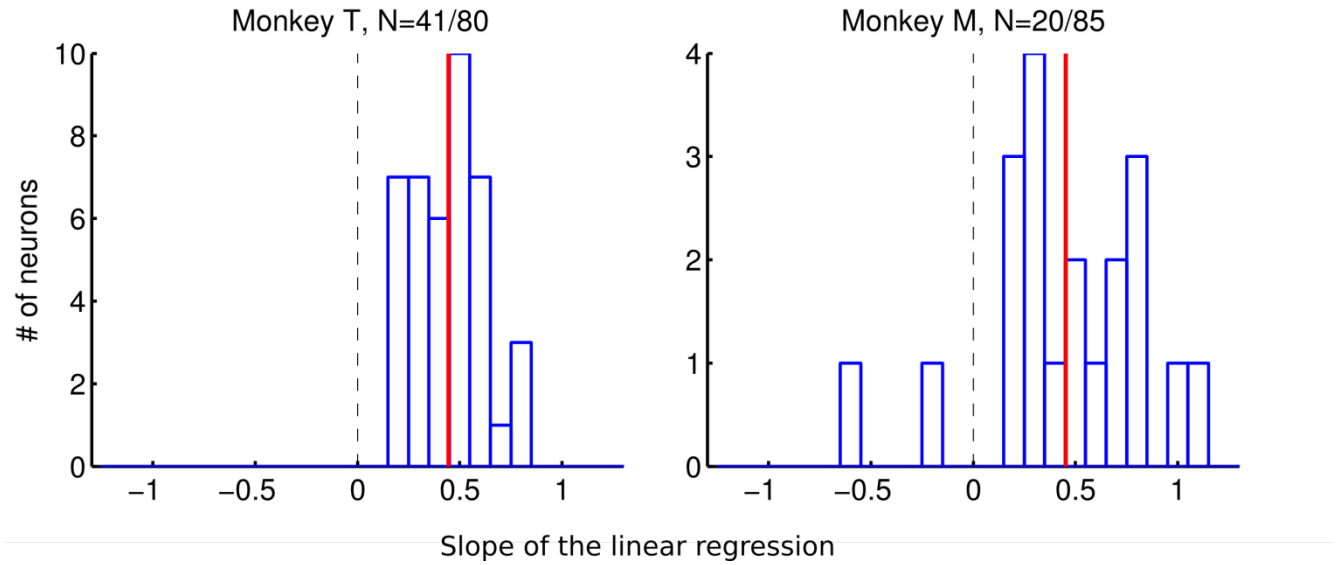


Figure 4.7: Slopes of the linear regression between the activity preceding and following SC. The abscissas represent the slope of a first-degree polynomial fitting the relationship between the trial-by-trial spike count before and after SC. Only neurons with a phasic response to SC were selected (denominator in the titles), and only the neurons with a significant Spearman rank correlation are plotted ($p<0.05$, numerator in the titles). The vertical red line corresponds to the median of each distribution.

4.2.6. Comparison of visual- and movement-related activity

As described above, the D1up neurons discharge, on average, with a higher firing rate during the epoch following SC than during movement execution, whereas the D1down neurons exhibit the opposite firing pattern (see Fig. 4.4). However, this difference in mean activity may be due to a high firing rate of a small number of dominant neurons. To rule out this possibility, the discharge evoked by SC and that related to movement execution were compared for each neuron (Fig. 4.8). The single-trial spike counts in two 200ms time windows were compared, the first one following SC and the second around movement onset. Fig. 4.8 shows for each monkey the proportion of D1up, D1flat and D1down with a significant (Wilcoxon signed-rank test, $p<0.05$) higher spike count in the visual than in the motor epoch (left part), and with a significant higher spike count in the motor than in the visual epoch (right part). The proportion of neurons with a higher firing rate during the visual than during the movement epoch is highest in the D1up and lowest in the D1down neurons. Conversely, the D1down category has the highest proportion of neurons with a preference for the movement over the visual epoch, followed by the D1flat and then the D1up categories. Note that the proportion of neurons with a higher firing rate in the visual epoch cannot be accounted for solely by the presence of an early phasic activity. For example, this phasic activity is only present in 30% of the D1up neurons of monkey M (Fig. 4.5A-B), and Fig. 4.8 shows that twice as many D1up neurons are more active in the visual than in the movement epoch.

In summary, neurons that increase their activity before the spatial cue have a tendency to exhibit a short-latency burst following it, and to have in general a higher firing rate during the first part of D2 than during movement execution. In contrast, neurons that decrease their firing rate before the spatial cue tend to be more silent in the early epoch following it, and are more active during movement execution than during the early processing of the visual cue. This suggests that the two populations of neurons are sequentially involved in movement preparation: the D1up could be more involved in the early steps of movement preparation, whereas the D1down would be more involved in its execution.

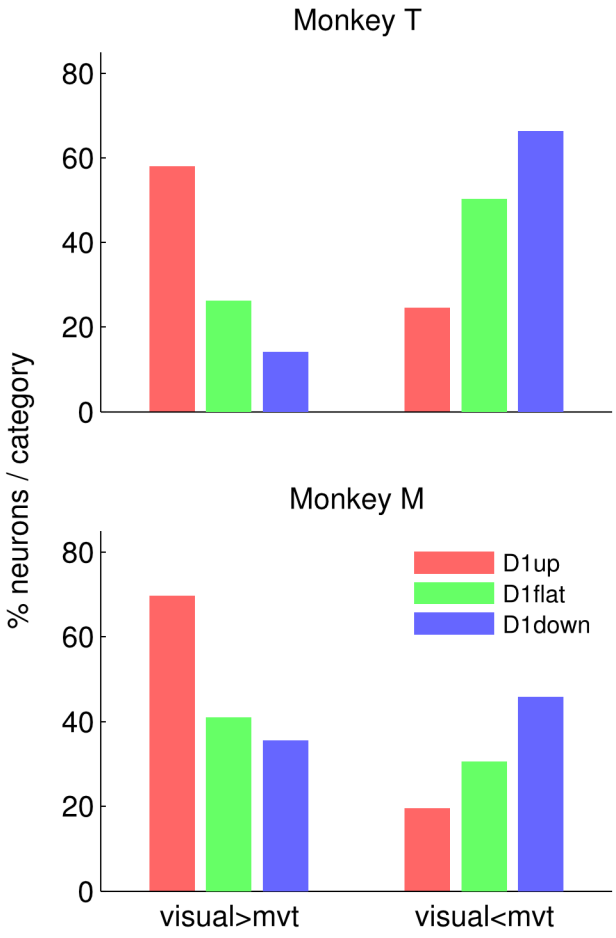


Figure 4.8: Differences in firing rates between the visual and movement epochs. Proportions of neurons in each category with a significantly higher activity in the "visual" than in the "movement" epoch (on the left in each panel), and with a significant higher activity in the "movement" than in the "visual" epoch (on the right in each panel). Only long delay trials are used, and all movement directions are considered. Note that the total percentage within each neuron category does not necessarily add up to 100, since only the neurons with significant differences are shown here. All comparisons of differences between categories are significant for both monkeys (chi-square test, $p<0.05$), except the difference between D1flat and D1down for visual>mvt in monkey M. Figure modified from Fig. 4 of Confais et al. (2012).

4.2.7. Directional selectivity

If neurons are involved in the preparation of a motor act, their activity should reflect some movement parameters, such as its direction (Riehle & Requin 1993). In other words, if the D1up and D1down neurons are involved in different phases of movement preparation, one might expect the activity to be differentially selective to movement direction, depending on the task epoch. The percentage of neurons being selective for

movement direction was therefore computed in a time-resolved manner, separately for the three categories, to evaluate if the evolution of the directionality differed across categories. We assessed the directionality of the neurons with a method based on the vector sum, in a 200ms sliding window shifted by 25ms (see Materials and Methods).

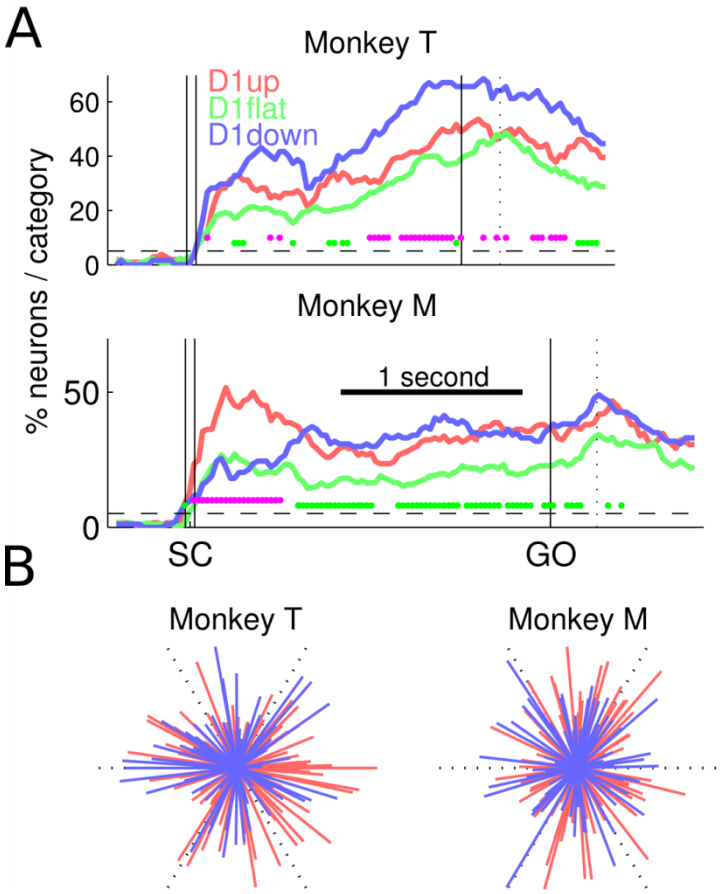


Figure 4.9: Directional selectivity. A) Percentages of neurons (fractions of the sample size of each neuron category) with significant directional selectivity, calculated in a 200ms sliding window, shifted by 25ms. Each magenta dot indicates a window with a significant difference between the D1up and D1down categories (chi-square test, $p<0.05$), and each green dot indicates a significant difference between the D1flat category and the lowest of the D1up and D1down categories. The dashed lines indicate the 5% level expected by chance. The dotted vertical lines indicate mean RTs. Only neurons recorded in sessions with 6 directions are used. B) Distributions of the directional vectors of the D1up (in red) and D1down neurons (in blue), selected in the bin of its maximum (significant) magnitude for each neuron. Each vector indicates the preferred direction of a neuron and its magnitude of selectivity. The black dashed lines indicate the directions of the 6 targets, and their length indicates the unit vector (maximum vector length). All distributions are homogeneous. All neurons that were significantly tuned at least once during D2 are considered: 92/106 for D1up and 65/67 for D1down in monkey T, and 109/124 and 72/94 in monkey M. Figure modified from Fig. 5 of Confais et al. (2012).

The results are shown in Fig. 4.9A for long delay trials. Only the sessions with 6 target directions were included. These data are the same than the ones presented in the previous chapters, only separated according to their activity during D1. First, two epochs of increased directional selectivity emerge, the epochs following SC and around movement onset. In addition, all three neuron categories have significant ($p<0.05$) proportions of tuned neurons during preparation and execution. Second, the two categories of anticipatory neurons (D1up and D1down) generally contain higher proportions of significantly directionally selective neurons than the D1flat category. This might be due to the fact that the D1flat neurons did not have to pass any statistical test to be classified as such, and therefore could include noisier or less task-related neurons. Third and most important here, the overall levels of directional selectivity of the two categories of anticipatory neurons have

comparable magnitudes, albeit with more D1down neurons tuned than D1up neurons in monkey T and more D1up neurons tuned after SC in monkey M. Indeed, no systematic differences of directional selectivity between the D1up and D1down populations can be seen for the two monkeys related to the different task epochs. The overall differences in tuning observed between monkeys could be due to the localization of the electrodes. We mainly recorded in PMd in monkey M, but we do not have this information for monkey T. Additionally, the relatively low directional selectivity of D1up neurons in the early epoch following SC, even though they displayed a strong phasic response to the SC (virtually absent in the D1down neurons) is not so surprising. Shen & Alexander (1997) showed that about 75% of the motor cortical neurons with a phasic response to the cue were not directionally selective. In summary, the sequentiality observed in the firing rate patterns of the two categories of neurons (Figs. 4.4 & 4.8) is not reflected in their directional selectivity.

Mauritz & Wise (1986) interpreted their anticipatory activity as the anticipation of a rightward movement instruction. In their task, the monkey could reach in only two directions. In some conditions, the spatial information was not provided by the cue but by the GO. They noticed that in the trials in which the monkey performed a rightward movement, no difference of RT was observed between the conditions in which the spatial information was provided by the cue or only at the GO signal. By contrast, the leftward movements showed a much shorter RT when the spatial information was provided by the cue than by the GO. Based on this behavioral result, they concluded that the monkey always chose to reach to the right target by default, and modified its choice afterward if the cue indicated to reach to the left one. They called this strategy "guess and switch" (see also Cisek & Kalaska 2005). In other words, the anticipatory activity would merely be a reflection of the preparation of a default movement direction. Even though there is a major difference between their paradigm and ours (they used only 2 directions, and we mainly used 6 directions), their interpretation could also apply to our data. In our case, the mean RTs of the monkeys in the different movement directions seem to indicate that the behavioral preferred direction (the one with the shortest mean RT) remained stable throughout the recordings (not shown). The assumption that this would also be the case for the putative default direction that the animals may expect/prepare during D1 allows us to directly test the interpretation proposed by Mauritz and Wise (1986). According to this hypothesis, neurons with a preferred direction close to the preferred behavioral direction would be the D1up, and the ones with a preferred direction close to the opposite direction would be the D1down. Thus, as populations, the distributions of the preferred directions of the D1up and D1down neurons should then be unimodal, and have close to opposite selectivity during D2 (see schematic description in Fig. 4.10).

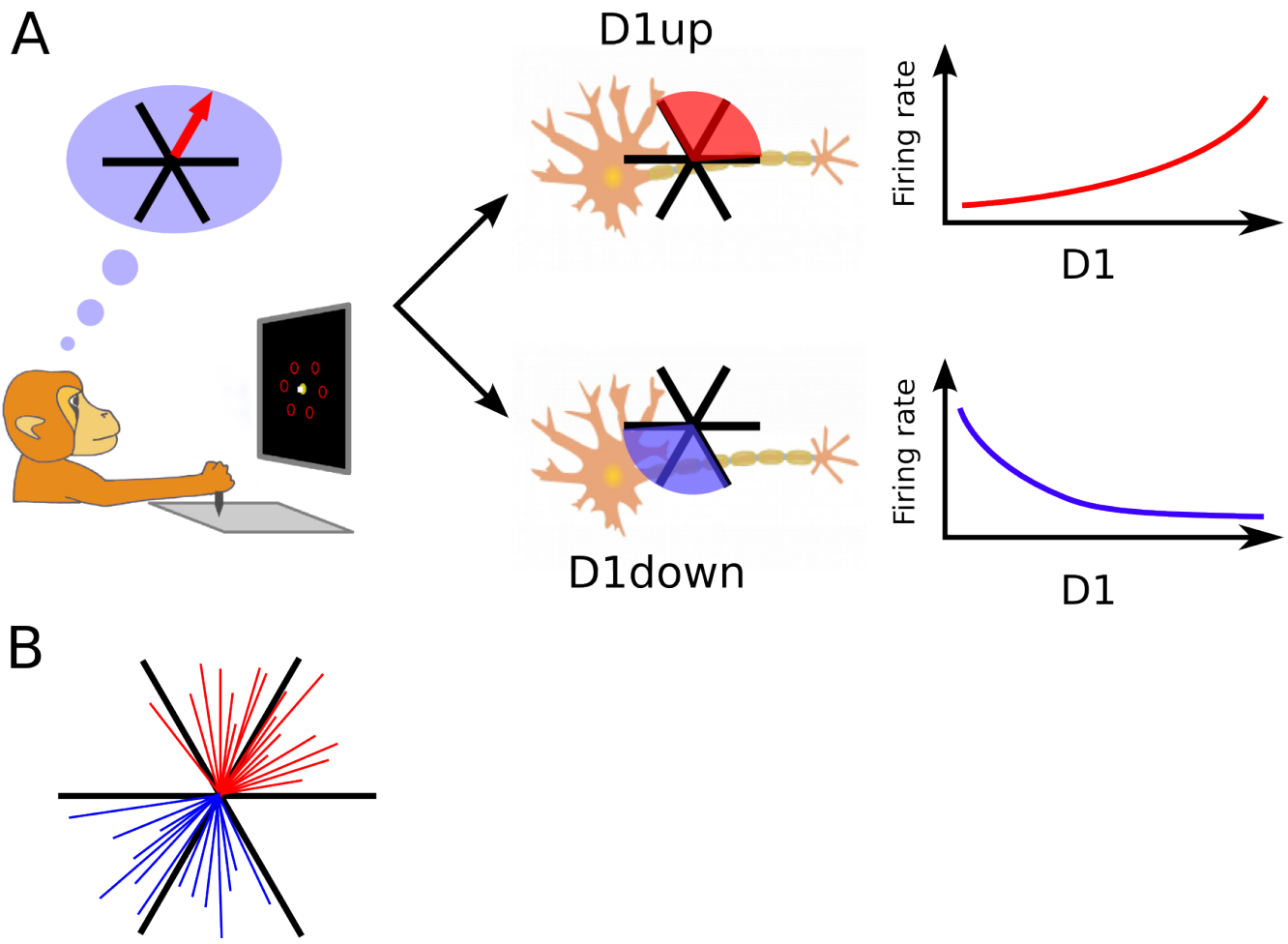


Fig 4.10: Schema of the D1 "preparatory hypothesis". A) During D1, the monkey presumably prepares a movement in his preferred direction, indicated in the blue bubble above his head. The neurons with an intrinsic preferred direction (represented by a colored arc of a circle) close to this one are the D1up (in red), the ones with an opposite preferred direction are the D1down (in blue). B) Prediction of this hypothesis. Same convention as in Fig 4.9B. As a population, the D1up and D1down neurons should have an opposite preferred direction during D2. Please compare with experimental results shown in Fig. 4.9B.

With the aim to test this, we first compared the distributions of the preferred directions of the significantly directionally selective D1up and D1down neurons in the two task-relevant epochs mentioned above (see Fig. 4.8), 200ms following SC and around movement onset (trials aligned to movement onset). But, in none of these two epochs were the two populations tuned (data not shown). However, it is possible that the neurons are tuned at different moments during D2. Thus, restricting the measure of directional selectivity to these two epochs may not give an accurate picture of the directional selectivity of the two neuronal populations. To compensate for this possible bias, we selected for each neuron the preferred direction in the epoch with its strongest directional selectivity (the epoch with the longest significant mean vector). The distributions of these preferred directions are shown in Fig. 4.9B, all distributions are homogeneous (Hodges-Ajne test, p=0.5 and

0.95 for D1up and D1down in monkey T, and p=0.72 and 0.73 in monkey M, respectively). These results suggest that either by taking meaningful epochs of the task, or by pooling together all the possible selective epochs during D2, no striking difference in preferred directions can be detected between D1up and D1down neurons. It is therefore unlikely that the anticipatory activity is explained by anticipatory directional selectivity.

4.2.8. Comparison of correct and error trials

We postulated that the anticipatory activity in D1 may influence the detection and processing of SC. Monkeys T and M made 21 and 15% directional errors, respectively (i.e. reaching to the wrong target), indicating that although they were over-trained, the task was still difficult. These directional errors could be (in part) due to the inability of the monkey to correctly identify SC in some trials. If the activity of the anticipatory neurons in D1 influences the probability of detecting SC, it should be different when the monkey selected the correct target than when he made an error. The firing rates of the 200ms preceding SC of correct and error trials were compared in two different ways. First, the spike counts in the window were compared across single trials for each neuron (Mann-Whitney U-test), and the percentage of significant neurons calculated for the three categories. Second, the activity in all correct and error trials was averaged separately for each neuron, and a Wilcoxon signed-rank test was computed across all neurons for each of the three categories. Both analyses yielded the same results. There was no significant difference in activity preceding the SC between correct and error trials, in any of the neuron categories. The same analysis was conducted in a similar task by Meftah et al. (2009), on the activity of neurons from the primary and secondary somatosensory cortex. They did not find any relationship between the outcome of the trial and the anticipatory activity (nor between the anticipatory activity and RT).

4.2.9. Behavioral strategies

Fig. 4.4 shows that the mean activity during D1 in long delay trials is different between the two monkeys. This difference could be due to the variety of strategies that the monkey may have used to solve the task, illustrated by Fig. 4.11. One strategy may be to use the temporal information contained in TC to expect SC at the correct moment ("exact timing" strategy). A second strategy would be to expect SC at the end of the short delay duration in all trials. If then it does not appear, the monkey would know that it is a long delay trial ("wait and see" strategy). The expected modulations of firing rate profiles for the use of one strategy or the other are completely different, as schematized in Fig. 4.11. If the animal used the "exact timing" strategy, the firing rate in short and long delay trials would start to diverge early during D1. In the other case, if the animal used the "wait and see" strategy, the activity in short and long delay trials would not be significantly different during the whole duration of the short delay.

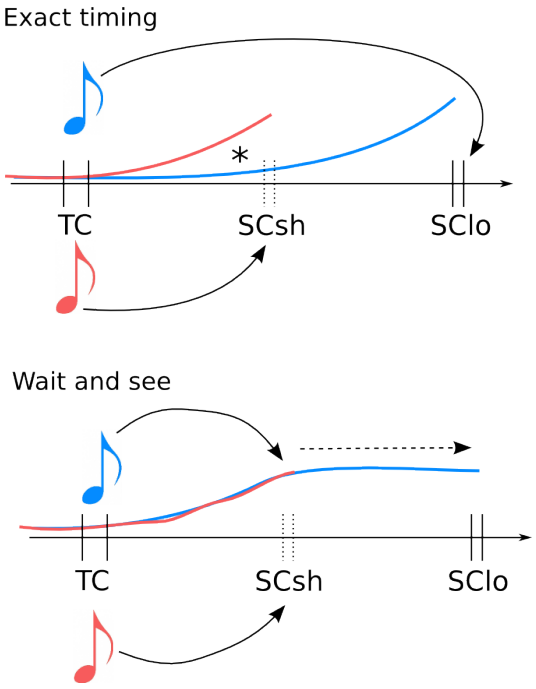


Figure 4.11: Schema of the possible strategies used by the monkeys and their electrophysiological correlates. The blue and red notes indicate the TC corresponding to the long and short delay trials, respectively. The black arrows represent the moment when the monkey putatively expected the cue to appear, depending on the information given by the TC. SCsh indicates the timing of the SC in short delay trials, SClo is the timing of SC in long delay trials. Here the spiking activities are aligned to TC. The thick blue and red lines represent the expected firing rate profiles of a D1up neuron in long and short delay trials, respectively, depending on the strategy used by the monkey. The asterisk indicates a significant difference of firing rate. For more information, see part 4.2.9.

While looking for differences in firing rate that may validate the one strategy or the other, we noted that in monkey M, the firing rate profile during D1 changed along the recording sessions. To quantify these differences we compared the firing rate during D1 in short delay trials and the corresponding first half of the long delay trials (that is, between the beginning of the trial and SCsh indicated in Fig. 4.11), separately for the first and second halves of the recording sessions (that is, for two periods of 3 months in monkey T, and of 5 months in monkey M). Fig. 4.12 shows the proportion of anticipatory neurons with a significantly different firing rate in short and long delay trials (Mann-Whitney U-test, $p<0.05$, see Method), separately for each recording period. In monkey T, the proportion of neurons with a different firing rate in short and long delay trials never deviates from chance level (dashed line), neither in the first nor in the last recording sessions. This result suggests that he used the "wait and see" strategy during the 6 month of recordings. Monkey M shows the same pattern as monkey T in the first period of recordings, but a completely different one in the second half of the recording sessions. In the late sessions, the activity of up to 40% of the anticipatory neurons differed in short and long delay trials already shortly after TC, as a function of the temporal information provided by TC. These results suggest that monkey M initially adopted the same strategy as monkey T (always expecting the SC at the end of the short duration delay), but then switched to the "exact timing" strategy (using the temporal information of TC).

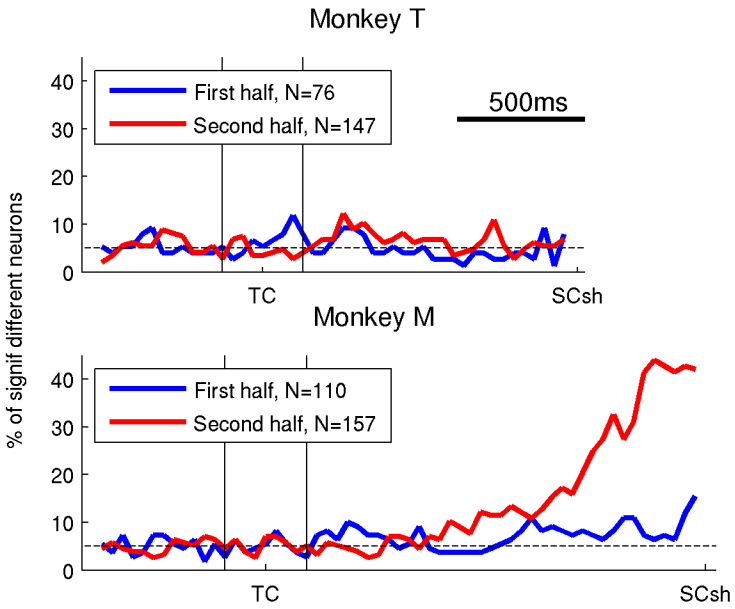


Figure 4.12: Selectivity to delay duration during D1. Proportions of anticipatory neurons with an early selectivity to delay duration during D1, according to the period of recording. TC: time cue, SCsh: onset of the spatial cue in short delay trials, and equivalent timing in long delay trials.

Nevertheless, it seems that this possible change of strategy in monkey M did not have a striking influence on the behavior, since the error rate does not change between the two halves of the recording sessions (chi-square test, p=0.75). Additionally, even though the mean reaction time of monkey M decreased by 21% between early and late sessions, monkey T showed a decrease of 43%, without a noticeable change in behavioral strategy. To see if the possible change in behavioral strategy by monkey M influenced the neuronal activity patterns in D2, all analyses presented above were repeated in monkey M by separating the data according to the date of recording (first versus second half of the sessions). No qualitative difference was observed. In particular, we did not observe any significant change in the proportion of anticipatory neurons, nor in the phasic response to SC or in the temporal profile of the directional selectivity. In conclusion, these results suggest that the possible switch of strategy adopted by monkey M did not change the observed neuronal activity in second part of the trial.

4.2.10. Effect of delay duration on anticipatory activity

We were interested in the effect that the delay duration could have on the neuronal activity during D1, especially in the last epoch preceding the SC. If one of the function of the anticipatory activity is to bring the cortical activity to a level at which it can optimally process the spatial cue, it should be equivalent in short and long delay trials. Figure 4.13 plots the firing rate of short and long delay trials, superimposed and aligned to the timing of the SC.

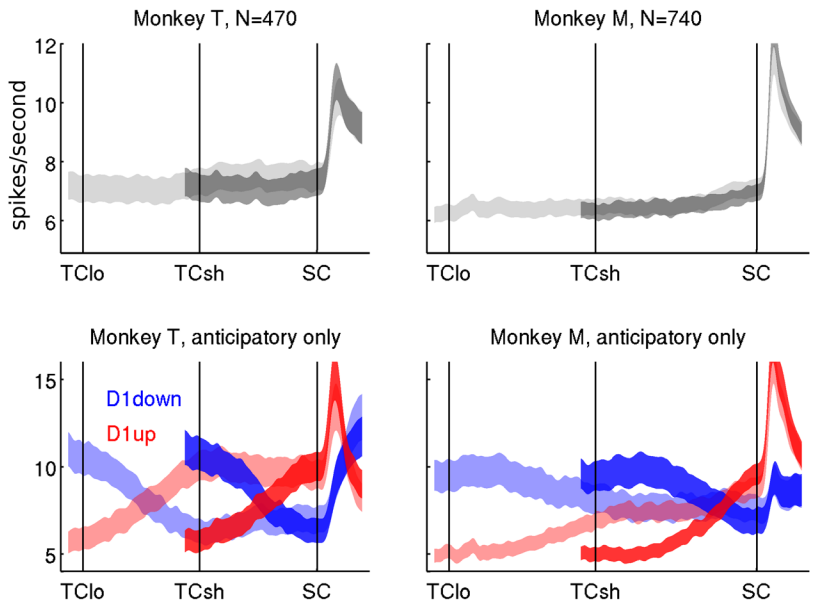


Figure 4.13: Influence of delay duration on the anticipatory activity. Monkey T is on the left, monkey M on the right. Top row: mean firing rate of all neurons during D1, with short and long delay trials aligned to SC. TClo and TCsh mark the timing of the time cue relative to the SC, in long and short delay trials, respectively. Bottom row: only anticipatory neurons, with D1up and D1down were plotted separately. In both rows, light colors are long delay trials, dark colors short delay trials. The Y axis is the same scale in left and right plots.

The top row of Fig. 4.13 shows that the firing rates in short and long delay trials largely overlap, even during the last 200ms window preceding the cue. The difference in firing rates at the population level is not significant, neither by taking short and long delay trials as independent samples (Mann-Whitney U-test, $p>0.05$), nor by comparing each neuron against itself (Wilcoxon Sign-rank test, $p>0.05$), in both monkeys. When analyzing each neuron individually, 33 and 22% of the neurons have significantly different firing rates in this window in monkey T and M, respectively (Wilcoxon rank-sum test, $p<0.05$). However, there are as many neurons with a higher firing rate in short than in long delay trials as neurons showing the opposite pattern. In monkey T, 14% of neurons are more active in short than in long delay trials, and 19% show the opposite pattern (chi-square test, $p=0.06$). In monkey M, 10% of the neurons are more active in short delay trials and 12% in long delay trials, which is also not significantly different ($p=0.4$). In summary, the changes in firing rate of all individual neurons seem to balance each other out, leading to the same mean population firing rate before the onset of SC, regardless the duration of D1.

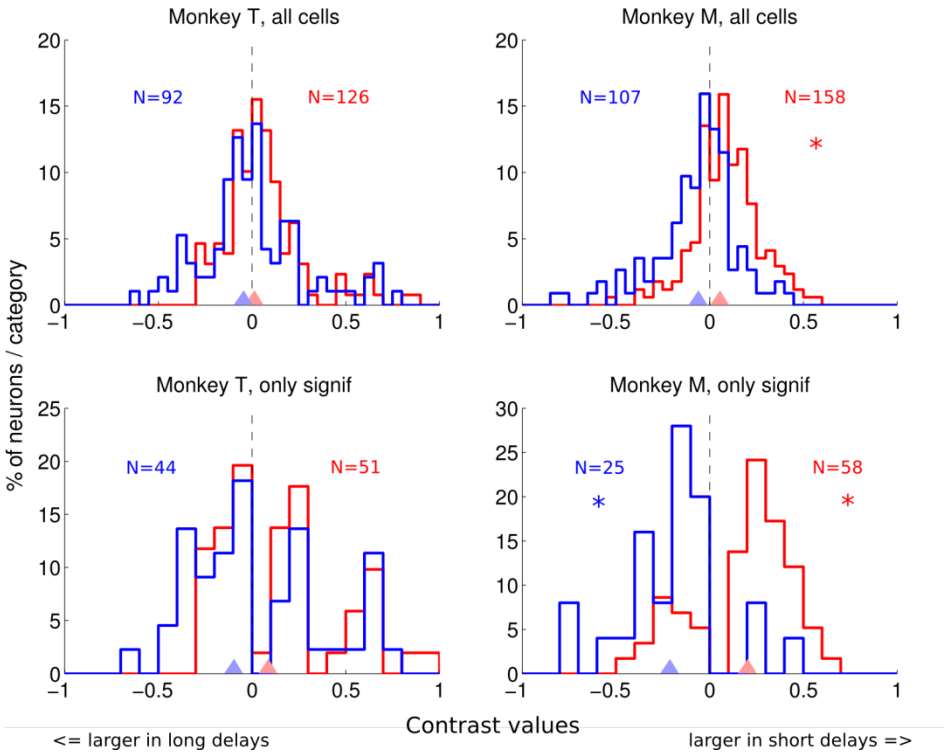


Figure 4.14: Distribution of the contrast values of firing rates in short vs. long delay trials in the last 200ms of D1. As usual, the D1up neurons are in red, and the D1down in blue. The colored numbers indicate the sample sizes of the data plotted. The little triangles at the bottom of each plot indicate the medians of the distributions. An asterisk indicates that the median of the distribution of the corresponding color is significantly different from 0. The vertical dashed line indicates a contrast value of 0: same firing rate in short and long delay trials. Positive values indicate a higher firing rate in short than in long delay trials. Top row: distributions of the contrast values of all anticipatory neurons. Bottom row: only neurons with a significantly different firing rate in short and long delay trials (Wilcoxon sign-rank test, $p<0.05$).

However when the mean firing rates are plotted separately for D1up and D1down neurons, a more detailed picture emerges (Fig. 4.13, bottom row). Especially in monkey M, the anticipatory neurons tend to modulate their activity much more in short delay trials (the D1up firing more and the D1down firing less). To assess the influence of delay duration on the activity of anticipatory neurons, we plotted the contrast values of the firing rates in short versus long delay trials, recorded during the last 200ms of D1. In Fig. 4.14, top and bottom rows show the same type of data, but the top rows show all neurons, and bottom row shows only the ones with a significant difference in firing rate. In these plots, a distribution shifted to the right means that on average, neurons fire more in short than in long trials, and a distributions shifted to the left means the opposite. It can clearly be seen that the results in the two monkeys are different. In monkey M, the larger modulation of firing rate in short delay trials, as seen in Fig. 4.13 (bottom row), is confirmed. Both anticipatory categories of neurons are shifted away from 0, the D1down exhibiting on average a higher firing rate in long and the D1up in short delay trials. In monkey T, the distributions of contrast values of both anticipatory categories are centered on 0 (Wilcoxon sign-rank test, $p>0.05$), even when only significantly different neurons are considered (bottom row). However the non-significant tendency goes in the same direction than in monkey M (median of D1up and D1down to the right and left of 0, respectively).

The different results in the two monkeys can be related to the different strategies that they might have used. In monkey T, who we believe used the "wait and see" strategy, no difference can be seen in the first half of D1 (during D1sh) between short and long delays (Fig. 4.12). That means that any difference in activity between short and long delay trials is not due to a difference of slope before SCsh, but rather to the stationary activity of long delay trials, occurring between SCsh and SClo (Figs. 4.4 and 4.13). The activity of anticipatory neurons, when it had reached a "plateau", could then fluctuate, slightly increasing or decreasing, regardless of their pattern of anticipatory activity. This is reflected in the high proportion of Chinese hat neurons, compared to monkey M (see section 4.3.3). Whereas in monkey M (who adopted the "exact timing" strategy), Fig. 4.12 shows that many neurons have a significantly different firing rate already from the beginning of the trial on. Additionally, Fig. 4.13 (bottom row) shows that after SCsh, the population firing rate keeps increasing/decreasing (for D1up/D1down neurons, respectively), until they reach SClo, but with a much shallower slope. The difference in firing rate in monkey M occurs then already during the first part of D1 (D1sh), i.e. during the "rising" part of the anticipatory activity. In summary, a large number of neurons have a different firing rate as a function of delay duration, and the distribution and timing of this difference depends on the strategy adopted by the monkey to anticipate SC. However, at the population level, these modulations cancel each other out and the mean firing rate stays the same just before SC.

4.2.11. Pairwise correlation during D1

The different patterns of activity of the D1up and D1down neurons during movement preparation, as well as their "mirrored" modulation of activity during D1 with delay duration lead us to formulate an hypothesis about the functional relevance of the anticipatory activity (extensively discussed in 4.4). The D1up and D1down neurons may reflect different processes during motor preparation: the D1up neurons would mainly be involved in processing the cue and in the early phase of motor preparation, whereas the D1down would be more involved in movement execution processes, and as such should be suppressed during D1 to prevent an early triggering of the movement when SC appears. In order to implement an optimal movement preparation, these two complementary processes should be coupled: the facilitation of the cue processing and the inhibition of the motor output should be co-modulated. In other words, when the D1up neurons fire more, the D1down neurons should fire less. An example of this "equilibrium" was provided in the previous section. The D1up and D1down neurons tend to be inversely modulated by the delay duration (Figs. 4.13-14), leading to the same mean firing rate when the whole population is considered. If one considers that facilitation of the cue processing and movement suppression are necessarily coupled, a prediction can be made: trial by trial, the

activity of the same category of neurons should be positively correlated, while neurons from different categories should be negatively correlated.

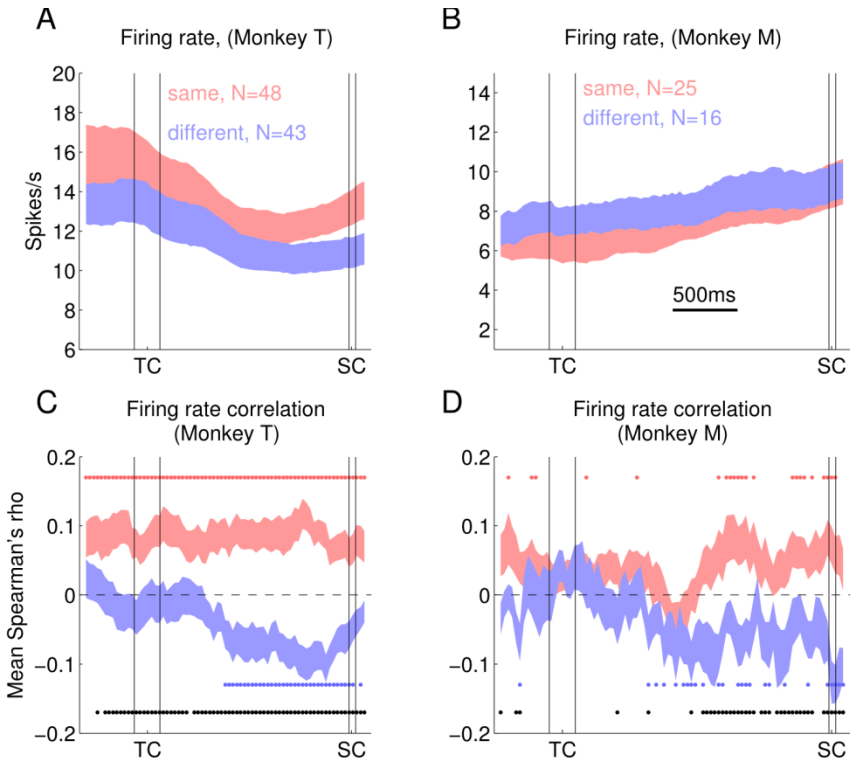


Figure 4.15: Trial-by-trial correlation of anticipatory activity. Only long delay trials are shown. Pairs of neurons of the same category (see text) are in red and pairs of different categories are in blue. Their sample sizes are indicated in the top graphs. A-B: mean firing rate (±SEM) of all pairs in each combination, as control. The firing rates are not significantly different (Mann-Whitney U test, $p<0.05$) C-D: mean coefficients of correlation (Spearman's rho) ±SEM, converted in Fisher z before averaging, and then retransformed in r. Red dots: median of the "red distribution" (pairs of the same category) is significantly different from 0 (Wilcoxon sign-rank test, $p<0.05$); blue dots: same for blue distribution (different categories); black dots: red and blue distributions diverge significantly from each other (Mann-Whitney U test, $p<0.05$). The size of the sliding window is 250ms, shifted by 40ms.

To test this prediction, we performed a pair-wise correlation between the instantaneous firing rates of the neurons recorded simultaneously. We first selected the neurons with a stable firing rate across trials (as explained in section 4.2.6), with a minimum firing rate of 2Hz throughout D1. Among these neurons, only pairs of neurons recorded on different electrodes were selected. An error of spike sorting may result in a positive correlation between the spike counts of the ill-sorted neurons, if the spike emitted by one neuron "spill" in the other neuron's spike count. This leads to a total of 219 pairs in monkey T and 166 in monkey M. In each pair, the spike count of each neuron was computed in 250ms time windows (in order to assure a sufficient amount of spikes per window), shifted by steps of 40ms throughout D1. The Spearman rank correlation was then computed between the single-trial spike counts of the two neurons, separately in each time window (the so-called "noise correlation", see Cohen & Maunsell 2009). Averaging the coefficients of correlation of all pairs in each time window gave us an overview of the correlation of the trial-by-trial fluctuations of the activity in a given neuronal population. Since the coefficient of correlation (R) is a non-linear measure, it needs to be converted into Fisher's z before averaging. The averaged z is then reconverted back into R and shown in Fig. 4.15C-D. Only long delay trials were considered. Because of the long time windows we were using, a large part of D1 would be affected by a spill-over of D2 activity (for example, in monkey T, the short delay trial is 700ms

long, so only the first two thirds of D1 would be "pure D1 activity"). The mean correlation was then computed separately for pairs of neurons of the same category (D1up/D1up and D1down/D1down, in red in Fig. 4.15) and of different categories (D1up/D1down, in blue). On average, 5.5 and 5 neurons were recorded simultaneously in monkey T and M, respectively, these numbers dropping to 1.7 and 0.9 when only neurons matching the above-described criteria are concerned. On top of this, analyzed neurons had to be recorded on different electrodes, further decreasing the odds. Thus, the probability to record anticipatory neurons at the same time was quite low, and only few pairs could finally be analyzed (see numbers in Fig. 4.15).

Before presenting the results, caution must be taken. It is problematic to perform second-order statistics (like a correlation) with low spike count, because the distributions of values become discrete and less Gaussian, artificially drawing the correlation towards 0. To ascertain that this phenomenon was not the main cause of the differences between the groups, Fig. 4.15 (top row) shows the mean firing rate for the two groups: there is no significant difference in mean firing rate between the groups. Furthermore, the mean firing rate in blue decreases in monkey T and increases in monkey M, whereas the correlation in blue (bottom row) becomes stronger in both animals. In summary, it is unlikely that the modulations or differences in the mean correlation are due to low firing rates.

The bottom part of Fig. 4.15 shows the mean correlation between the firing rates of the pairs of neurons showing the same pattern of modulations ("same" group, in red), as well as between pairs of neurons with different pattern of pre-cue modulations ("different" group, in blue). First, the mean correlation of the "same" group is indeed positive, at least in the period preceding the SC. In monkey M, the average correlation is weak at first and then becomes stronger, whereas in monkey T, the correlation stays positive during the whole delay period. Second, in the "different" group, the mean correlation tends to be weak at first, but becomes much stronger (negatively) as D1 progresses. Even though it is noisy (especially in monkey M), the mean correlation of both groups clearly departs from 0, but in opposite directions. Both populations are significantly shifted away from 0, which is surprising, given the low number of pairs in the final sample (for example 16 pairs in monkey M for the "different categories" population). In summary, if the D1up neurons fire more, the D1down neurons will fire less (and vice-versa), whereas the neurons with the same pattern co-vary in the same direction.

4.3. Discussion

4.3.1. Summary

We here show that almost half of the neurons in M1 and PMd exhibit anticipatory activity preceding the expected presentation of a task-relevant spatial cue, well before movement parameters can be processed and a movement prepared. The firing rate increases in 60% of these neurons, whereas it decreases in the remaining 40%. This anticipatory activity during the first, non-motor delay is predictive for the temporal profile of the activity during the second, preparatory delay. The difference in activity between neuron categories is especially prominent during the early epoch following the cue, where the probability of having a phasic burst of activity depends upon the type of anticipatory activity. However, both types of anticipatory neurons seem to be similarly involved in movement preparation, as reflected by comparable proportions of directionally selective neurons. In one monkey who presumably used the information about the duration of the delays from the beginning of D1, both populations of anticipatory neurons tend to modulate their firing rate more in short delay than long delay trials, but in opposite directions, leading to the same mean population firing rate. Finally, the firing rate of pairs of neurons showing the same pattern of anticipatory activity tend to be positively correlated trial by trial, whereas pairs of neurons with opposite pre-cue modulations show a negative correlation.

4.3.2. Anticipation and processing of task-related information

Three studies by Wise and collaborators (Mauritz & Wise, 1986; Vaadia et al. 1988; di Pellegrino & Wise, 1993a) addressed anticipatory activity as defined here, i.e. neuronal activity changes in motor cortex occurring before the presentation of task-relevant information. Our results confirm theirs, even though they mainly focused on PMd and on neurons that we call “D1up”: 1) the proportion of anticipatory neurons is close to ours (between 30 to 50% of the neuronal population) and 2) Mauritz & Wise (1986) show that most of their "D1up" neurons exhibit phasic activity following the SC. Thus, it seems that the anticipatory activity we describe is not simply related to our specific task, but a pattern of activity consistently present in motor cortical areas when the monkey can predict the timing of relevant (spatial) information. For the first time, our study includes the analysis of the activity of anticipatory neurons with decreasing activity preceding an informative cue (D1down). Their modulation in time during the processing of the spatial information and during movement preparation clearly differs from that of the D1up neurons. Furthermore, even though their activity profiles are different, their involvement in the task seems similar, as reflected in the proportions of directionally selective neurons during D2. Finally, this anticipatory activity seems similar in M1 and PMd, even though this could be explained by the fact that we recorded rostrally in M1 and caudally in PMd. MacKay & Crammond (1987) reported an

anticipatory activity preceding visual stimuli in parietal area 5 of the monkey. The posterior part of this area (PEc) projects directly to PMd, and the anterior part (PE) to M1 (see Geyer et al. 2000). The authors interpreted this activity in terms of predictions of upcoming events based on experience. Our data suggest that these predictions might then be fed to motor cortex to facilitate the future processing of the information provided by an instruction signal.

As presented in the introduction, most motor tasks involve the presentation of a cue (in our case, it is called SC), providing information about the forthcoming movement (in our case, movement direction). Several studies have shown that the information about movement parameters (such as direction) is processed as soon as it is presented ("pre-processing", Requin 1985; Riehle & Requin 1989; reviewed in Riehle, 2005). This early processing may be reflected in motor cortical areas by the so-called "signal-related" activity (Weinrich & Wise 1982; Riehle & Requin 1989; Crammond & Kalaska 2000. See also Cisek & Kalaska 2005), a short-latency (less than 200ms) burst of spiking activity following any cue carrying relevant information about the forthcoming movement. We find that the proportion of neurons discharging with a peak latency of less than 200ms following SC drops dramatically from the D1up to the D1down category (Fig. 4.5). This suggests that the signal-related neurons are much more likely to display an increasing activity preceding the spatial cue than the others. Furthermore, signal-related activity (Wise et al. 1992; Boussaoud & Wise, 1993a,b; di Pellegrino & Wise, 1993a,b; Crammond & Kalaska, 2000) and pre-cue anticipatory activity (Vaadia et al. 1988; di Pellegrino & Wise, 1993a) are both modulated by the motor meaning of the cue, that is, whether or not the cue is informative regarding the movement to be executed. This suggests a functional link between the two patterns of activity. Altogether this supports the idea that when the timing of an informative cue is known in advance, motor cortex will enter into a preparatory state, enhancing the response to the cue by increasing the baseline activity.

4.3.3. Interpretations of the anticipatory activity

4.3.3.1. Attention

Our task is attentionally very demanding, as it requires waiting for the very brief presentation of the SC at a precise moment. Rolke & Hofmann (2007) showed that temporal uncertainty (which increases with delay duration; see Gibbon 1977) degrades perceptual processing. This could in part explain the higher rate of directional errors in long compared with short trials (even though other causes could intervene, like the monkey being more easily distracted during the longer D2). The activity seen during D1 may therefore reflect attentional processes, as it has been shown in PMd in other tasks (Boussaoud 2001; Lebedev & Wise 2001). Yet, in the trials during which the monkey reached to a wrong target, the firing rate preceding SC was not

different from that in the correct trials. Thus, even though the anticipatory activity of the motor cortical areas during D1 relies on temporal attention, we could not find that it influences directly the ability to perceive SC or to choose the correct target.

Additionally, it has been shown in several cortical areas that attentional processes increase the firing rate of neurons whose receptive field overlap with the attentional focus. For example Luck et al. (1997) showed an increase in the spontaneous firing rate of 30-40% in neurons of the areas V2 and V4, when the attention was directed toward their RF. Meftah et al. (2002) showed a comparable effect of attention in the somatosensory cortex. Twenty percent of the S2 neurons (and virtually none in S1) showed a general increase in firing rate as soon as the monkey knew that he would receive a tactile instead of a visual stimulus. In a follow-up study in the same areas, the authors added a pre-cue delay of fixed duration, and observed an anticipatory activity very similar to the one we reported here (Meftah et al. 2009), with the same proportion of anticipatory neurons in S1 and S2. Interestingly, the attentional modulation seemed to be added on top of this anticipatory activity. Even though the anticipatory neurons had a tendency to show more attentional modulation than the non-anticipatory ones, the two phenomena did not seem to be related. For example, in S2 the activity of one third of the anticipatory «up» neurons was modulated by the information indicating the modality of the forthcoming cue, but no neuron in S1. This shows that in a task specifically designed to manipulate attention, anticipatory activity was observed but did not seem to be modulated by attention. Furthermore, as in our data, their anticipatory activity was not related to the behavioral performance of the monkey (error rates or RTs).

4.3.3.2. Time estimation

It was proposed that motor cortical areas might participate in a timing network, when a motor action is to be planned (see discussion in Roux et al. 2003). For instance, Lebedev et al. (2008) decoded the duration before or after the initiation of a self-triggered movement from the activity of PMd neurons, and Lucchetti & Bon (2001) showed that the discharge onset of motor cortical neurons depended on the delay before GO. These neurons exhibited a ramping activity (increasing or decreasing), similar to what we found during D1. Such a ramping activity has been shown to be a possible substrate of timing processes (e.g. Durstewitz 2004). fMRI studies in humans on implicit timing (i.e., the duration does not have to be overtly reported) show frequent activation of the premotor cortex (Coull & Nobre 1998; Coull et al. 2000), even when no motor act is involved (Schubotz & von Cramon 2001). Fujioka et al. (2012) showed that in absence of movement, the synchronization of beta oscillations of the MEG signal in motor cortex was timed to an isochronous signal. Taken together, these works support the idea that PMd is part of a temporal prediction network (Coull & Nobre 2008). Arnal (2012) proposed that sensory prediction in motor cortex relies on the simulation of movements via an internal

model, allowing the prediction of the timing of a stimulus and of its sensory consequences. Recently, Davranche et al. (2011) showed that the BOLD signal recorded in motor cortical areas (M1 and PM) and the left intraparietal sulcus (IPS) co-varied during a temporal orienting task, but only when the subject had to respond with a movement. This context-dependent recruitment may indicate the modulation of a motor anticipatory process in motor cortex by temporal information being processed in IPS. Applied to our task, this would mean that the anticipatory activity during D1 might reflect the involvement of the motor system in the estimation of the duration preceding the cue.

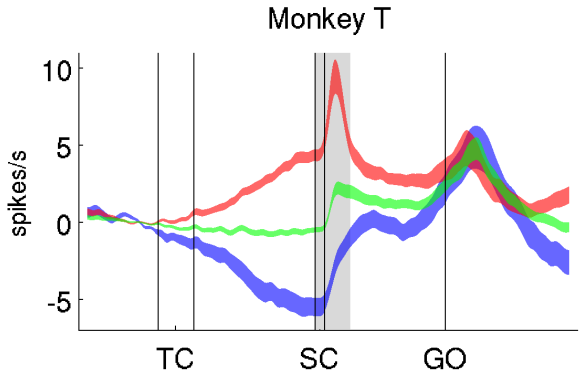
Some *in silico* models of time estimation rely on progressive changes in the conductivity of the membrane of neurons. For example, a modeling study by Reutimann et al. (2004) showed that interval timing could be attained by a pool of inhibitory neurons with a slowly decaying excitability. This would lead to the two patterns of activity that we observed during D1. However, one of the conditions for such a mechanism to be implemented is a smooth increase or decrease of activity by each neuron in all trials. Yet, the firing rate of the anticipatory neurons (especially the onset of the increase in firing rate) was extremely variable from trial to trial (see example in Fig. 4.1), as already mentioned by Vaadia et al. (1988). Alternatively, Okamoto et al. (2007) proposed a model of time estimation based on a network of neurons exhibiting long afterdepolarization potentials, leading to a bimodal mode of firing rate. However, both models predict that the mean activity of the time estimating network increases smoothly until the end of the timed period. For this reason, it seems unlikely that the anticipatory activity reflects the timing *per se*. The pattern of activity shown in monkey T is hard to reconcile with such a "brain hourglass" counting the time-in-passing (Figs. 4.4, 4.12 & 4.13). In this monkey, the firing rate shows an abrupt flattening of the slope in the middle of the long delay and stays stable until its end. This pattern of activity could only decode the elapsed time until the short duration is expired, and would get stuck into a static time afterward. In other words, even though the anticipatory activity tightly depends on timing, it likely reflects associated processes, as will be developed below.

4.3.3.3. "Priming and Breaking"

An alternative hypothesis is that before the cue appears, motor cortex enters into an optimal state to implement a "priming and breaking" mechanism (Prut & Fetz 1999; Cohen et al. 2010). This anticipatory state would actually be composed of two complementary processes: one (represented by D1up neurons) would tune up the gain of cue-processing networks, while the other (D1down neurons) would transiently inhibit the activity of the pyramidal movement-related neurons, to prevent any spurious movement triggering. An increasing anticipatory activity preceding the visual cues was observed in the caudate nucleus (Hikosaka et al. 1989). It was later described as being sensitive to different parameters (such as location or color) predictive of

the reward expected from reaching to the cue (Takikawa et al. 2002, Lauwereyns et al. 2002). The authors showed that in neurons with an anticipatory activity, the selectivity to the different directions of the movement did not improve in the rewarded vs. non-rewarded direction. In other words, this (reward-predictive) ramping-up anticipatory activity had an additive influence on the short-latency phasic response to a directional cue, similar to the one we report in sections 4.2.5 and 4.2.6. Unlike in the visual areas, where the expectation of a stimulus has the effect of improving the discriminability of the cued features (see Reynolds & Chelazzi 2004, for a review), corresponding to a multiplicative gain, it seems that in higher-order structures along the visuo-motor network, the anticipatory activity mainly increases the activity in response to a cue in an additive way. Lauwereyns et al. (2002) interpreted this activity as a general motivational bias toward a rewarding target, in order to "incorporate the reward value in the signal detection process". This motivational bias could then have an excitatory influence on a wide range of cortical areas. Among others, motor cortex could be directly influenced via the direct pathway (internal part of globus pallidus, substancia nigra, thalamus), or indirectly via the frontal cortex. Indeed, a similar feature-selective anticipatory activity has already been reported in a GO-noGO task in the dorso-lateral prefrontal cortex (Niki & Watanabe 1979; Sakagami & Niki 1994), which could in turn influence the motor cortex via its connections to PMd. The idea of a motivational bias is indirectly supported by an observation of Vaadia et al. (1988). When studying the same type of anticipatory activity as we did in PMd, they used a condition of their task in which the reward was given at random. They showed that after several unrewarded trials, the anticipatory activity tended to disappear. The influence of motivational factors on the activity of the motor cortical areas was already discussed in section 3.3.5: Roesch & Olson (2003, 2005a) shows that the expected reward modulates the activity of frontal areas associated with movement preparation. We can make the hypothesis that it also biases the representation of a cue leading to a rewarding motor act. This bias would not be necessarily restricted to visual cues or to motor cortex. As already mentioned, Meftah et al. (2009) showed a similar anticipatory activity in the somatosensory cortex, prior to a tactile or visual stimulus indicating a motor response.

The increasing anticipatory activity that we observed in motor cortex (D1up neurons) was accompanied by a "mirrored" modulation of activity by the D1down neurons. These two categories of neurons showed clear differences of modulations. For instance, their responses to the spatial cue were dramatically different. Whereas the D1up neurons often showed an early phasic response to the SC, such burst was less often in the D1down neurons (see Fig. 4.17, where the baseline is subtracted from the activity of each neuron).



4.17: PETH aligned on baseline activity before TC, short delay trials. D1up are in red, D1down in blue and D1flat in green. Note the early start of the anticipatory activity, as well as the similar time of onset for D1up and D1down neurons.

Another difference of activity between the D1up and D1down neurons is reported in section 4.2.7, where we show that the firing rate of the D1up neurons is usually higher following the cue, whereas that of the D1down neurons is higher during movement execution. Along this same line of evidence, the D1up and D1down neurons showed opposite modulations related to delay duration (section 4.2.11). Both neuronal categories modulate more their activity in short than in long delay trials, but in opposite direction. Finally and most importantly, the trial-by-trial fluctuations of firing rate during D1 are inversely correlated between neurons of the same category or of different categories (section 4.2.12). This "noise correlation" is thought to reflect the influence of a common noisy input (Cohen & Maunsell 2009). Here, this may be the reflection of the influence of one excitatory source projecting simultaneously on the D1up neurons and on inhibitory inter-neurons projecting in turn to the D1down neurons (for example, via the direct and indirect pathways of the basal-cortical loop. The hypothesis of a "proactive control" of movement - see below - through the indirect pathway was also mentioned in reviews by Aaron 2011 and Stuphorn & Emeric 2012). However, recent evidence obtained in mice using optogenetic and calcium imaging does not agree with this hypothesis (Cui et al. 2013). The authors showed that both the direct and indirect pathway were transiently active preceding movement initiation. Thus, a tonic regulatory activity of the motor cortical excitability would be hard to reconcile with such a phasic activation. Alternatively, the D1up neurons may receive an excitatory projection, and send inhibitory connections to the D1down. These two patterns of connectivity would enable a feed-forward inhibition between D1up and D1down neurons that may account for most of the modulations we observed, without affecting the directional selectivity of the two populations of neurons (section 4.3.8).

This inhibition would make sense if we make the hypothesis that the D1down neurons are mainly involved in the execution of the movement. During increased excitability of the motor cortex before the onset of a cue to improve its processing, the D1down neurons should be suppressed to avoid the triggering of a spurious movement or a disturbance of the processing of the cue. Indeed, motor cortex is activated at very short latencies following visual cues ("first sweep" Ledberg et al. 2007, and see also Fig. 3.19 of the preceding

chapter). This early activity can even trigger involuntary movements if not properly inhibited. Matsumura et al. (1991) showed that after injection of bicuculine methiodide (an agonist of the GABA-A inhibitory receptor) in M1, the neurons of this area started to show bursts of activity immediately following a non-informative warning signal, triggering EMGs in arm muscles and even movements. More directly, a behavioral study by Boulinguez et al. (2008) showed that the RT distribution of error trials in a delayed reaching task indicated that healthy, untreated human subjects responded involuntarily to the warning signal and not to the target. They postulated that a "proactive volitional inhibition" is set before the onset of the cue to prevent such unwanted movements (reviewed by Aron 2011). This concept is related to the "proactive control" of movement initiation described in the literature on monkey electrophysiology (see Stuphorn & Emeric 2012, for a review) and to the term "impulse control" described in studies on movement preparation using Transcranial Magnetic Stimulation (TMS, e.g., Duque & Ivry 2009). All of these concepts refer to the ability to modulate in advance the readiness to respond of the sensorimotor system, depending on the contingencies (history of past trials, likelihood of "catch" trials, presence of distracters...). This "proactive" inhibition of the sensorimotor system can be maintained over long durations and is part of the default state of the system (Criaud et al. 2012). This mechanism is assumed to operate at the spinal level in a selective way. Duque & Ivry (2009) showed that the EMG in arm muscles induced by a TMS pulse over the contralateral M1 was decreased only if the arm was cued for a future movement, whereas it did not change when the other arm was cued. Additionally, it has been shown on human participants that during motor preparation, the cortico-spinal excitability decreases (Hasbroucq et al. 1997; Davranche et al. 2007), especially when the participants are more ready to respond (e.g., in case of a short foreperiod), and the spinal motoneurons discharge less (i.e., are inhibited, Duclos et al. 2008). Furthermore, in the macaque monkey, the majority of the last-order spinal interneurons are inhibited during an instructed delay preceding a movement (Prut & Fetz 1999; reviewed in Fetz et al. 2002). Similar processes may take place in anticipation of a cue, as part of the "proactive inhibition" in order to counterbalance the strong response to the cue (e.g., in monkey M, the population response to the cue is larger than that during movement). In our case, the target of this inhibition would be the D1down neurons, supposedly more involved in movement execution. Additionally to the fact that the D1down are more active during movement than during the rest of the trial, one indirect evidence supports the motor role of the D1down neurons. Crammond & Kalaska (1996) report that most of the anticipatory neurons of PMd are ramping up before the onset of the cue (cue processing), whereas the ones recorded in the sulcus of M1 (zone with the highest density of direct corticomotoneuronal projections, see Rathelot & Strick 2009) are ramping down (motor output). This idea would be supported by results of Asher et al. (2009), showing that the MUAs recorded in the spinal cord show a transient inhibition in the 200ms following the onset of an informative cue

indicating the forthcoming movement, followed by a slow and steady increase of activity. This pattern is similar to the activity of the D1down neurons during D2.

This interpretation receives strong support by the modeling work of Moody & Wise (2000). Using a network of virtual neurons, they looked at the activity of the "hidden units" during two versions of a delayed matching task, with a predictable or unpredictable delay before the test stimulus. They showed an anticipatory activity before each test cue (they were presented in sequence), but only when the delay duration before the cues was predictable. Interestingly, they recorded neurons in the ventral prefrontal cortex of macaque monkeys performing the same tasks, which showed the same tendency. This confirms the idea that the predictability of the cue is necessary for an anticipatory activity to emerge (Vaadia et al. 1988). Some of the virtual units had a negative weight (each of their "spikes" decreased the firing probability of the post-synaptic unit). Removing them led to a systematic tendency to make "false-positive" errors. In other words, the network "responded" to the distractors. Other units had a positive weight, and were tuned to one or several test cues. Removing them led to "omission" errors. Then, they tested what was the minimal size for the network to be efficient. They showed that below a certain amount of units, the network made systematic "false-positive" errors, and responded to the first distractor: thus, below a critical size, most of the observed errors were false positive, i.e. lack of inhibition. In summary, Moody & Wise (2000) showed that when the timing of relevant cue was predictable, anticipatory activity arises naturally. Disturbing this anticipatory activity leads primarily to a lack of inhibition of the response to the cue, and secondarily a lack of a response to the cue. Even though it is a modeling work and their task is different from ours, their conclusion concurs with ours.

4.3.4. PMd as a good candidate to implement "priming and breaking"

More generally, PMd, the cortical area in which typically the anticipatory activity was recorded, was shown by several studies to be involved in both spatial cue processing and movement inhibition. Indeed, as presented in the Introduction, this area is thought to be involved in the ability to transform direct (Wise et al. 1983; Weinrich et al. 1984; Passingham 1985) or indirect cues (Kurata & Hoffman 1994; Yamagata et al. 2009) into a motor plan (short review in Hoshi & Tanji 2004). For example, Wallis & Miller (2003) showed that PMd was selective to abstract rules conditioning a motor response, and Yamagata et al. (2009) showed that PMd was involved in the mapping of abstract shapes to movement directions. Kurata & Hoffman (1994) showed that muscimol injection (GABA-A agonist) into PMd leads to directional errors in a conditional visual reaching task, whereas the dynamics and kinematics of the movement were not impaired. In human participants, Schluter et al. (1999) showed that a TMS pulse applied over PMd in human participants 144ms after a visual cue (i.e. during the signal-related activity) slowed down the execution of a movement, whereas it had no effect when

applied later. Additionally, the role of PMd in motor control may also imply the ability to send or withhold a motor command. Several studies showed an involvement of this area in movement inhibition. Kalaska & Crammond (1995) showed that the level of activity in PMd and the directional selectivity of PMd neurons were strongly modulated between GO and NoGO trials, whereas neurons from area 5 were not. Mirabella et al. (2011) showed that during a countermanding task, the discharge of PMd neurons might be compatible with the cancellation of a planned movement. Sawaguchi et al. (1996) showed that in a reaching task, the injection of bicuculine methiodide (GABA-A antagonist) provoked the random generation of short-lasting bursts of cortical activity (less than 200ms) during the anticipatory delay preceding a cue (exactly like our experiment), immediately followed by reaching movement toward a contralateral target. The authors interpreted this finding as the inability to withhold an over-trained movement when the GABA-A influence is impaired. In human participants, a study by Duque et al. (2012) showed that virtual lesions of PMd (by applying repetitive TMS) during the delay between a cue and a GO signal decreases the inhibition of M1, but only if a movement has to be made with the contralateral hand. This means that as a movement was planned but withheld with one arm, an inhibitory influence decreased the cortico-spinal excitability only for this arm. Hence, disturbing PMd would release this inhibition ("impulse control", Duque et al. 2009) and impair the inhibition of spinal motoneurons during a delayed movement preparation (Davranche et al. 2007; Duclos et al. 2008). Interestingly, whereas M1 projects mainly to spinal motoneurons, PMd neurons project to the intermediate layer of cervical spinal cord, where interneurons projecting to motoneurons of the upper-arm are localized (Dum & Strick 2002; Rathelot & Strick 2009). These premotor interneurons are supposed to be more involved in the modulation of the spinal circuitry rather than in the direct movement control (Fetz et al. 2002) and as such, would be compatible to act as a movement suppression mechanism.

4.3.5. Alternative mechanisms for movement inhibition

Alternatively, Cohen et al. (2010) proposed that PMd implement the "priming" part through cortico-cortical connections to M1, while at the same time enabling the "breaking" through cortico-reticulo-spinal projections, preventing the information transmission from the cortex to reach the spinal cord. Taken together, these findings support the hypothesis that PMd is at the same time involved in the two parallel processes of cue processing and movement inhibition. The anticipatory activity may be a pre-setting of the networks underlying these processes. Another possible mechanism would be a proactive regulation of the "readiness to respond" by the dorsomedial prefrontal cortex (Narayanan & Laubach 2006, 2009; see Stuphorn & Emeric 2012 for a review) and especially the SMA and the preSMA. For example, Chen et al. (2010) used a countermanding paradigm, during which the monkey was asked to reach to a target as quickly as possible, but was also cued to

cancel the movement before its execution in some trials ("stop trials"), while recording the neuronal activity in preSMA/SMA. They showed a higher power of the LFPs oscillations (10-50Hz) during movement execution in trials following a missed "stop trial" compared to the ones following a GO trial. They also found the same effect in the MUA and the activity of single neurons (SUA), as well as a strong lengthening of RTs. Their interpretation is that the recent history of missed cancellation modulated proactively the readiness of the cortex to respond, thus delaying movement execution. This proactive modulation may in turn influence PMd, since SMA is more connected to PMd and PMv than to M1 (Dum & Strick 2005b). In rats, Narayanan & Laubach (2006) showed that a reversible inactivation of the dorsomedial prefrontal cortex leads to an increased amount of premature responses, as well as decreasing the activity during the pre-movement delay in motor cortex. This was interpreted as a top-down influence to prevent an early release of the movement.

Another possible mechanism to prevent unwanted movements was described by Riehle et al. (2006). They showed that after the moment when a GO signal could have appeared but did not, a fraction of neurons recorded in M1 showed a transient change of preferred direction compared to that during movement execution. This transient preferred direction was opposite to the one determined during movement execution, leading to a reversal of the orientation of the population vector (Georgopoulos et al. 1983) between the moment when the GO signal could have appeared and the moment when it actually appeared. They interpreted this reversal as being the reflection of a "breaking" mechanism, by which the motor cortex may employ a directionally specific, inhibitory interaction in periods of uncertainty to prevent unwanted movements. It is conceivable that such a mechanism could also take place in our task, since the D1down neurons are also directionally tuned following the SC. This would lead to a prediction concerning the activity of the D1up and D1down neurons following the cue. The instantaneous population vectors of simultaneously recorded populations of D1up and D1down neurons would point in different directions from trial to trial (according to SC), but would systematically stay opposite to each other. The former would represent the planned movement, while the latter would cancel its immediate execution. To confirm this prediction, one would need to analyze data from simultaneous recordings of large populations of anticipatory neurons, which is not the case in our dataset.

4.3.6. Influence of delay duration on the anticipatory activity

We showed in part 4.2.12 that the anticipatory neurons in monkey M tend to have a larger firing rate modulation in short than in long delay trials. In other words, on average D1up neurons fire more and D1down neurons fire less in the last 200ms before SC in short compared with long delay trials. To interpret this result, several findings have to be returned to mind. 1) It seems that only Monkey M adopted the "exact timing"

strategy by using the temporal information provided by TC from the beginning of D1 to anticipate precisely the presentation of the cue. 2) The phasic response of the single neuron spiking activity to SC (see Fig. 3.6) and the VEP of LFPs (see Fig. 3.4) are generally larger in short than in long delay trials, especially in monkey M. This may reflect an early movement preparatory process or the awareness of an earlier available reward (see sections 3.3.4 and 3.3.5). 3) The phasic spiking response seems to "ride on top" of the anticipatory activity (see section 4.2.6). 4) Neurons with a ramping up activity exhibit usually a phasic response following the cue (Fig. 4.5).

We can thus make the hypothesis that in short delay trials, the steeper increase of firing rate of the D1up neurons “pushes up” the phasic response to the cue more than in long delay trials. The enhanced neuronal response to the cue may reflect either an early planning of the movement, or the expectation of a cue leading to an earlier reward (thus being subjectively more positive due to a reduced *temporal discounting*, Lowenstein & Elster 1992). In both interpretations, the processing of the cue has to be facilitated in short delay trials, as we already discussed in the previous chapter. In fact, it appears that this facilitation would begin even before the appearance of the cue, through a pre-cue increase in firing rate in short delay trials. According to the “priming and breaking” hypothesis that we formulated earlier, the degree of inhibition of the motor output depends on the facilitation of the preparatory processes. Short delay trials would thus also lead to a stronger suppression of the movement and a lower firing rate of the D1down neurons.

In monkey T, the influence of delay duration is weaker, maybe since the animal supposedly always expected the cue at the end of the short duration. However, by following this strategy, the amount of information that the monkey receives when the short duration has expired is higher than if he knew the delay duration beforehand. Indeed, in long delay trials, at the time when SC does not appear, monkey T has to reallocate (Correa et al. 2004) his attention to the next point in time when SC will appear. By contrast, this reallocation presumably does not occur in monkey M, since the correct timing is expected from TC onset. This difference of strategy is reflected in the amount of neurons modulating their activity in the middle of D1 in long delay trials. In section 4.3.3, we reported that about 25% of the anticipatory neurons in monkey T show a change in the modulation their firing rate at this precise moment (see Fig. 4.18), whereas less than 10% of the anticipatory neurons do so in Monkey M. Mauritz & Wise (1986, their Fig. 4) showed a neuron exhibiting a similar pattern than the up-down neurons. In their task, the monkeys were trained for months with a delay of 3 seconds preceding the cue. However, in some recording sessions, they used longer durations. Indeed, they showed a neuron recorded with a pre-cue duration of 6 seconds increasing its activity until the trained duration (3 seconds) and decreasing it afterwards. Their data thus confirm that the inflection of the pre-cue anticipatory activity happens when an expected cue did not appear. Since we did not observe obvious differences in the

activity during the early epoch of D2 between the D1up and the up-down neurons (see Fig. 4.18), we can only speculate about the why of such a pattern of activity. It seems that, when the information about the timing of the cue is used, the response to SC is pushed up in short more than in long delay trials (as in monkey M). The absence of cue at the end of the short duration would thus lead to a decrease of firing rate at cue onset in long delay trials.

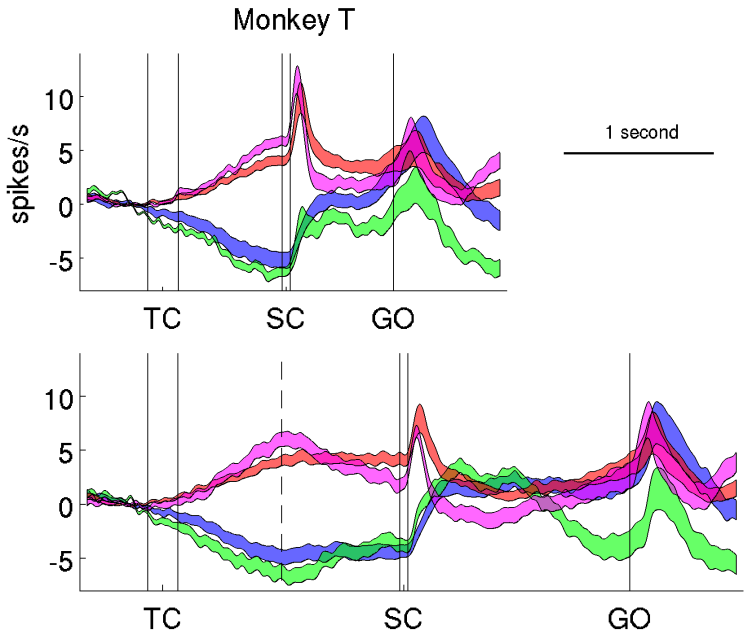


Figure 4.18: Mean firing rate of D1up, up-down, D1down and down-up neurons. The mean firing rates (±SEM) are displayed, with the D1up in red (N=79), the up-down in purple (N=31), the D1down in blue (N=70) and the down-up in green (N=21). The baseline activity before TC of each neuron is removed, to emphasize the difference of slope in the first part of D1. Note that the response to SC is highly similar in D1up and up-down, and in D1down and down-up.

4.3.7. Conclusion

In this study, we showed that many motor cortical neurons display an anticipatory activity preceding the onset of a relevant cue. Their activity during movement preparation is modulated according to their pre-cue activity, especially during the early epoch following the onset of the relevant cue and during movement execution. We propose that whenever there is temporal information available allowing cue anticipation, motor cortex, presumably along with other areas involved in time estimation processes during movement preparation, enters into an optimal preparatory state enabling a more efficient processing of the incoming spatial information and its transformation into a motor command ("pre-setting", Requin 1985), while preventing a premature movement in tasks which require to delay the motor response.

5. Summary and perspectives

In this last part, I will shortly summarize the results and conclusions that I described in the previous chapters, and then propose some experimental protocols which would allow to specifically test the hypotheses that I formulated.

5.1. Summary

Our actions are shaped by the spatial and temporal context in which they take place. With experience, we learn to extract the necessary spatial and temporal information, and use it to improve our sensorimotor behavior. Indeed, both perceptual (anticipation of external events) and motor performance (preparation for action) can be improved by such predictions. In my PhD thesis, I specifically studied the influence of the temporal context on the neuronal activity of the motor cortex (M1 and PMd) during movement preparation. To this end, we trained two monkeys to perform a task in which they could predict first the timing of a spatial cue and then the timing of an imperative (GO) signal requiring a reaching movement. These two signals were presented consecutively at predictable moments in time (see section 2.3-4). I showed that prior to the spatial cue, and in absence of any movement preparation, 40% of the motor cortical neurons exhibit a ramping anticipatory activity (see 4.2.2). Two-thirds of these anticipatory neurons increased their activity (D1up neurons), whereas one-third decreased it during the delay (D1down neurons). The slope of the anticipatory activity depended on the predicted timing of the cue, a short delay leading to a steeper slope of firing rate than a long delay. In other words, in short delay trials, just before cue onset the increasing neurons had a higher firing rate, whereas the decreasing ones had a lower firing rate (see 4.2.11). The pattern of anticipatory activity of these neurons was predictive of their activity during movement preparation. The D1up neurons typically responded with a short-latency phasic burst of spikes to that cue, whereas this burst was almost lacking in the D1down neurons (see 4.2.5). By contrast, the D1down neurons were generally more active during movement execution than the D1up neurons (see 4.2.7). This difference in activity during movement preparation between anticipatory increasing and decreasing neurons may suggest that these two populations of neurons are sequentially involved in movement preparation: the D1up neurons first (in the epoch following the SC), followed by the D1up neurons (around movement execution). We then hypothesized that a stronger involvement in movement preparation would be accompanied by a higher degree of directional selectivity. The supposed sequential involvement of the two populations of neurons would thus lead to a higher degree of directional selectivity at different epochs of the task. However, the two populations of anticipatory neurons were similarly directionally selective during the entire delay, supporting the idea that they are not sequentially involved in movement preparation (see 4.2.8).

In our task, the cue was so briefly presented that it was very difficult to perceive. In order to improve its perception, two different behavioral strategies may be possible, leading to different patterns of firing rate. Based on the firing rate preceding the cue, it appeared that one of the animals changed the strategy halfway during the recording sessions, allowing us to compare the possible influence of the strategy on the results presented above. However, no differences were observed in relation to the putative strategies. Therefore, the differences observed between the populations of anticipatory neurons do not seem to be influenced by the strategy that the monkey used (see 4.2.10).

We then looked at the trial-by-trial relationship between the firing rates of anticipatory neurons recorded simultaneously, separately for pairs of neurons having the same pattern of anticipatory activity and for pairs with different patterns. The firing rates of neurons with the same rate profile are significantly positively correlated, whereas neurons with different profiles are negatively correlated. In other words, the firing rates of two D1up neurons (or two D1down neurons) will co-vary in the same direction from trial to trial. If the firing rate of one neuron increases that of the other will also increase. By contrast, the firing rates of two neurons with opposite rate profiles (one D1up and one D1down neuron) will co-vary negatively, if the firing rate of one increases, the firing rate of the other systematically decreases (see 4.2.12). The anticipatory activity observed in motor cortex may therefore reflect two parallel mechanisms, complementary to each other and coupled trial-by-trial. One would facilitate the processing of a cue relevant to movement preparation, whereas the other would implement a proactive inhibition in order to prevent the triggering of a movement in response to the cue. PMd could play a central role in these two mechanisms, since it has been shown to be involved in both cue processing and movement inhibition. We can speculate that the facilitation of the cue processing may be partly caused by a motivational bias, since a reward-dependent anticipatory activity was recorded in a similar task than ours in the basal ganglia (Takikawa et al. 2002; Lauwereyns et al. 2002).

During movement preparation, the firing rates of the motor cortical neurons and the LFP evoked potentials were strongly modulated by the delay duration. The evoked potential of the LFP induced by the cue (VEP) was larger in short than in long delay trials, whereas the evoked potential induced by movement execution (MRP) was larger in long than in short delay trials (see 3.2.4). The firing rates of the neurons during the corresponding epochs were modulated similarly, but to a much weaker extent (see 3.2.5). The modulation due to delay duration does not seem to influence the directional selectivity of both signals (see 3.2.6-7). Instead, selectivity to duration and direction seem rather independent from each other, following different time courses. Indeed, as the trial progresses, the selectivity for movement direction increases whereas the selectivity for delay duration decreases (see 3.2.8). The selectivity to delay duration could be due to an early or late movement

planning depending on the available time before movement execution. Alternatively, the modulation due to expected and elapsed time could have different origins. The expected delay duration could cause a difference of arousal or motivation due to the reward available after a short or long delay, whereas the elapsed time would cause a difference in movement preparation.

The VEPs and MRPs were correlated trial-by-trial with RT. Whereas the VEP was weakly negatively correlated with RT, the MRP was strongly positively correlated with RT (see 3.3.9). This relationship between EP amplitude and RT is similar to the selectivity of the EPs to delay duration. Indeed, large VEPs tend to be associated with short delay durations and short RTs, whereas large MRPs are rather associated with long delay durations and long RTs. As did the EPs, the firing rate preceding movement initiation showed a similar relationship to delay duration and RT. In motor cortex, a subset of "preparatory" neurons shows a ramping up in firing rate preceding movement initiation. The onset of this activity was shifted according to the expected delay duration, that is, it had a constant latency in respect to movement onset. By contrast, the slope of the activity was basically unchanged (see 3.2.10). The same onset shift of the preparatory activity was observed between trials in which the monkey produced fast and slow RTs, regardless of the experimental condition (see 3.2.11). The similar modulation of the EPs and firing rate by RT and delay duration supports the idea that most of the variability in RT is due to the ability of the monkey to estimate precisely the timing of the GO. By contrast, other studies using a temporally unpredictable GO signal found that the RT was related to the slope of the firing rate (e.g. Hanes & Schall 1996). The predictability of the GO timing would thus be a central parameter in determining the relationship between neuronal activity and RT.

In summary, I showed that timing shapes several aspects of sensorimotor behavior. It modulates the motor cortical activity not only during movement preparation, but even during the anticipation of relevant information. The modulation of neuronal activity by delay duration is as important or even more as the one related to movement direction. However, the selectivities for these two task parameters seem rather independent from each other and follow different time courses. Finally, our data suggest that timing is impossible to dissociate from associated variables such as motivation and preparedness.

5.2. Perspectives: how can we test the hypotheses that I formulated?

5.2.1. "Pre-cue anticipatory activity is related to the visual cue, but only if it carries relevant information for movement preparation"

To test this hypothesis, Bjørg Kilavik and I developed a new experimental protocol and started to train two monkeys. This protocol is similar to the one I presented in this manuscript, but with the major difference that several spatial cues are presented in a row (Fig. 5.1). Each trial starts with a "selection cue" (SEL), indicating which of the three successively presented spatial cues (SC1, SC2, SC3) carries the relevant spatial information, and which cues are the distracters. For example, in the top row of Fig. 1.5, the cue on the top left is the target, which was indicated by SC1. SC2 and SC3 had thus to be ignored.

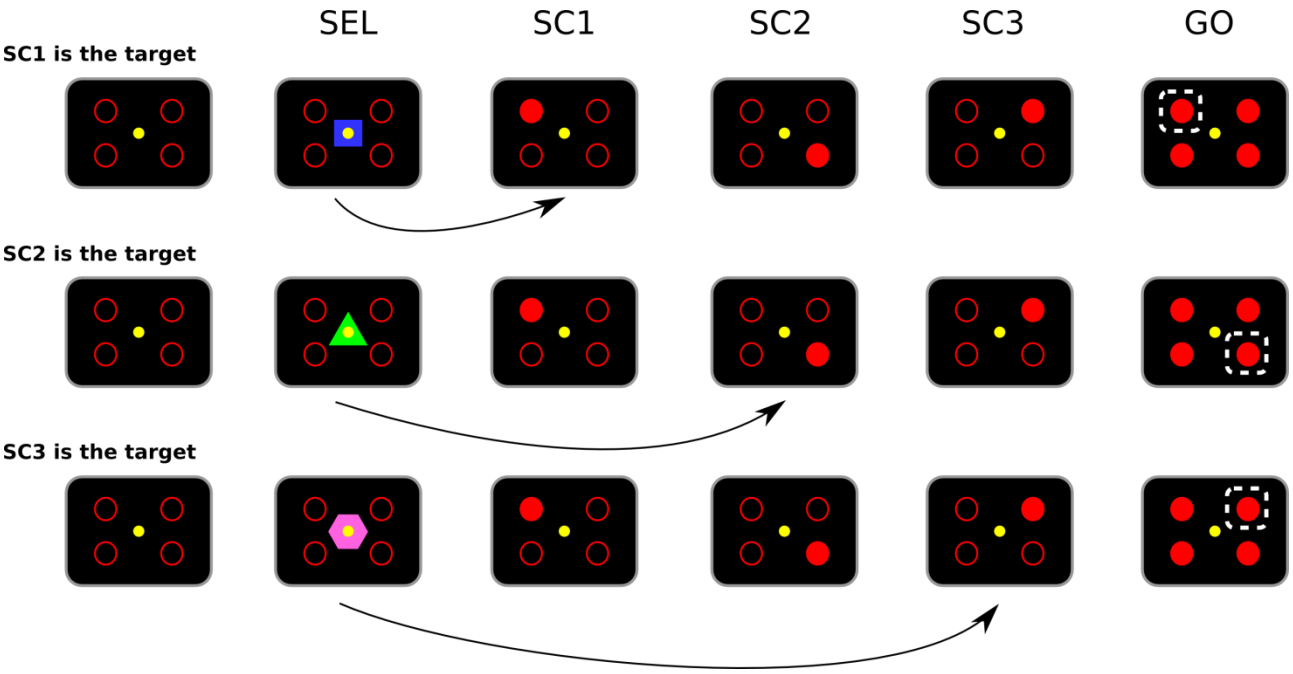


Figure 1.5: Experimental protocol of a task assessing the effect of cue relevance on anticipatory activity. Each column represents the on-screen display at a different step of the task. Each row is a different condition. The yellow dot is the fixation point, and indicates to the monkey where to move the cursor (not shown) to be held until the GO appears. The red circles indicate the possible locations of the SCs, they always stay on. The red discs indicate the SCs. SEL means selection cue, indicating which of the 3 SCs is the target. SC1, 2, 3 indicate the selection cues. In each trial, one SC is the target whereas the two others are distracters. GO requires the monkey to reach to the target. Each cue is presented for 200ms, and cues are separated by 1000ms. The arrows indicate the correspondence between the SEL and the correct SC. The white dashed square in the GO screen indicates the location of the target to be reached.

With such a protocol, by keeping the delay duration between the stimuli constant, the monkey knows in advance when the useful information will appear. It will allow us to compare the anticipatory activity preceding a relevant cue with that preceding an irrelevant cue. If the anticipatory pre-cue activity facilitates the processing of the cue used for movement preparation, it should be observed only before the relevant cue, not

before the distracters. Additionally, such a task may allow to directly compare the early responses of the spiking activity and the VEP to a relevant vs. an irrelevant cue. A similar question was addressed by di Pellegrino & Wise (1993a,b), but the additional influence of attention added another dimension to the task, and only the spiking activity was considered. Lastly, such a task involves a large amount of different cognitive processes, making it necessary to compare the activity of a wide range of cortical areas along the visuomotor chain when the monkey performs the same task.

5.2.2. "Anticipatory pre-cue activity induces an inhibition of motor output"

One of the main aspects of our task was to separate the activity related to the cue from the one related to movement preparation. The main drawback of this experimental feature is that we do not have a behavioral measure to which the activity preceding the onset of the cue could be correlated. The percentage of directional errors might indicate how well the animal paid attention to the cue, but it may also be influenced by the possible distraction of the animal during the second delay. We thus have to resort to another experimental method to assess if the D1 anticipatory activity induces an inhibition of immediate motor output. It has been shown that TMS (Transcranial Magnetic Stimulation) pulses over the arm area of M1 elicit EMG evoked potentials in the contralateral arm (e.g. Hasbroucq et al. 1997). The size of this evoked potential and the duration of the silent period that follows it (a short period of suppression of the EMG) was shown to be modulated by the degree of motor preparation (supporting the "priming and breaking" hypothesis of movement preparation, see Prut & Fetz 1999; Cohen et al. 2010), for example induced by different delay durations (Davranche et al. 2007). The amplitude of the EMG evoked potential and the duration of the silent period were assumed to reflect, respectively, the excitability of the cortico-spinal pathway and the recruitment of inhibitory cortical interneurons (see Hasbroucq et al. 1997). By using these physiological indices, we may thus have access to the "hidden" processes during the task, without behavioral output. If the anticipation of a cue induces an inhibition of the motor output, TMS pulses applied to M1 should trigger EMGs of different amplitudes at different distance in time from the cue onset, even in absence of movement preparation. The protocol that I propose could be used with human participants, they would perform the same task that I described in the experimental part of my PhD thesis, but only one delay duration would be used instead of two. A long training period would ensure that the participants (humans or monkeys) anticipate the timing of the cue. As they perform the task, TMS pulses would be applied on the participant's M1 area at different moments in time during the pre-cue delay. The EMG activity in the contralateral arm would be recorded. A modulation of the EMG amplitude with the timing of the TMS pulse related to that of the cue would validate our hypothesis.

5.2.3. "PMd plays a central role in proactive inhibition of the motor output"

If the effect predicted in the previous paragraph was indeed observed, the next step would be to test if PMd mediates it. In direct connection with the studies presented above, Duque et al. (2012) showed that suppressing the activity of PMd by repeated pulses of TMS (rTMS) cancels the increasing inhibition preceding movement onset. The authors interpreted this as a sign of "impulse control" (see Chapter 4), a mechanism by which a movement is prepared and timed at the desired moment. By adapting the exact same protocol to the delay preceding the cue, we could assess the involvement of PMd in the pre-cue inhibition of movement execution.

Furthermore, it might be interesting to replicate and refine the results of Kurata & Hoffman (1994), using other methods to suppress PMd than chemical injections, such as the uni- or bilateral cooling of this area. These authors showed an increase of directional reach errors after the inactivation of PMd. However, it would also be possible to use this protocol to test the involvement of PMd in a network of proactive inhibition of the motor output. As a monkey performs a delayed reaching task, with a delay separating an informative cue from a GO signal, the suppression of PMd should lead to an increase of unwanted motor responses following the presentation of the cue.

5.2.4. "The relationship between firing rate and RT depends on the temporal predictability of GO and on the availability of prior information"

Preceding movement execution, some neurons show a ramping activity. We observed that the onset of this ramping activity was correlated with RT, trial-by-trial, whereas its slope was less or not correlated with RT. This result is at odds with other works studying the same phenomenon. For instance, Hanes & Schall (1996) did show a correlation between RT and the slope of the ramping activity. I formulated the hypothesis that since in our task the timing of the GO was highly predictable, most of the RT variability was explained by the ability of the monkey to precisely estimate the delay duration. In a less predictable temporal context, RT variability may be mainly explained by other parameters, such as the ability of the monkey to quickly react to an unpredictable cue. In order to test this hypothesis, I propose a 2-directions reaching task with prior directional information including two conditions. The main difference between the two conditions would be the ability to predict the timing of the GO. One way to influence the predictability of the GO is to modulate its conditional probability of appearance. This variable, also called hazard rate, represents the probability that a cue appears, given that it has not appeared yet. Several reports have shown that RT was tightly linked to the hazard rate (e.g. Niemi & Nätäänen 1981; Requin et al. 1991; Riehle et al. 1997b; Ghose & Maunsell 2002; Janssen & Shadlen 2005; Praamstra et al. 2006; Van Ede et al. 2011). For example, in a simple RT task with a finite number of delay

durations preceding the GO, the RT will be determined by the relative probability of each delay duration to be drawn (see Fig. 5.2). If all delay durations are equally probable (Fig. 5.2 A), the conditional probability that the GO appears will increase as time elapses, leading to shorter RTs for longer durations. We thus propose two experimental conditions, having very different distributions of hazard rate over time.

In the first condition, the GO could only appear at one point in time, after a fixed delay. In this case, its conditional probability of appearance would stay at 0 during the whole delay and peak at only one moment. In the second condition, the conditional probability would stay flat during the whole delay, thus rendering the timing of the GO impossible to predict. Several methods can be used to produce a "non-aging interval", with a flat hazard rate function (see Niemi & Nätäänen 1981), like having a finite number of possible delay durations, with the short durations being more likely to be drawn than the long ones (Requin et al. 1991, see Fig. 5.2 B). These two conditions would have a very different temporal predictability of the timing of the GO signal, thus allowing us to assess the influence of this parameter on the relationship between firing rate and RT. Having only two movement directions would enable to record many trials in each condition, thus yielding enough statistical power to compute correlation analyses. Additionally, the data could be fit with several mathematical functions, to assess the precise relationship between RT and firing rate (e.g. linear, quadratic...).

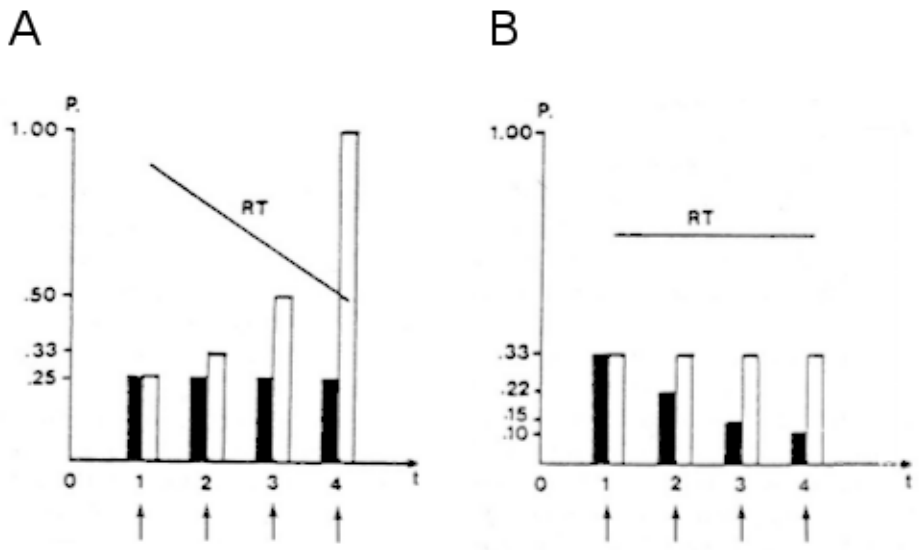


Figure 5.2: Schematic representation of the relationship between probability of delay duration, conditional probability and RT. The x-axis represents delay duration, the y-axis represents the probability that a given delay duration is drawn (solid bars), and the perceived conditional probability as time elapses during a delay (white bars). The thin lines above the bars represent the expected duration of the RT for each delay duration. A) The probability to draw each delay duration is equal, leading to an increasing conditional probability as time elapses during a trial along with a decreasing RT. B) The probability to draw a delay duration is inversely related to its duration, leading to a flat conditional probability and RT. Modified from Figure 4.6 in Requin et al. (1991).

The other factor that could influence the relationship between firing rate and RT is the availability of movement information. Riehle & Requin (1989) showed that without prior information about movement direction, it is difficult to plan a movement. If the directional information is available in advance, the monkey can plan its movement and needs only to time it properly in order to execute it at the correct time. By contrast, if the directional information is only provided by the GO signal, it has to be processed during RT. These two conditions may lead to different relationships between neuronal activity and RT. To test this hypothesis, the task of Riehle & Requin (1989), or of Crammond & Kalaska (1996), would be perfect. In a delayed reaching task, directional information is provided in advance in one condition, whereas in the other only the GO signal provides this information. According to our hypothesis, the correlation between firing rate and RT should be different in the two conditions. Riehle & Requin (1993) conducted a similar study, comparing the correlation between firing rate and RT with or without prior information about the movement. However, their work could be extended along two axes: first, they did not look for the correlation with a precise aspect of the firing rate (slope, onset, threshold), as we propose to do here. Second, they only analyzed the activity preceding the GO: during the trials in which the directional information was provided by the GO, the only correlation that could link RT and firing rate would be a general preparedness or arousal. However, the influence of the processing of the spatial information on the RT could not be evaluated.

5.2.5. "The modulation of motor cortical VEP and MRP (and of firing rate) are related (or not) to motivation"

Following Roesch & Olson's (2005a,b) footsteps, we could design a simple experiment that helps us to better understand the origin of the strong selectivity to delay duration observed in the LFP EPs (see Chapter 3). The task would be a delayed center-out reaching task, similar to our previous task. However, the experimental protocol (Fig. 5.3) would use two successive cues and a GO signal. The first cue would indicate both the delay duration between the second cue and the GO signal (short or long) and the size of the reward at the end of the trial (large or small). The second cue would indicate the direction of the movement required after the GO signal. We would thus have 4 conditions, with two sizes of reward and two delay durations.

Since the task would be directional, having too many directions would multiply the number of conditions (reward size * delay duration * movement direction), so the number of movement directions should be limited (here 3 directions are presented, leading to a total of 12 conditions, similar to the task I presented in this manuscript). The behavioral effects of both variables could be verified, the RTs should be longer in long delay trials (Niemi & Nätäänen 1981; this study), and the proportion of aborted trials should be higher for conditions with an expected low reward (e.g. Roesch & Olson 2003). By using this counter-balanced design, this

experiment may allow one to estimate the percentages of variance of the neuronal activity explained by both variables. Furthermore, it could assess the possible interactions between the effect of reward size and the putative effect of temporal discounting if an interaction delay duration * reward size is observed. Another aspect of this task would be to include a delay of fixed duration between the first cue and the spatial cue, thus allowing its anticipation. If the anticipatory activity is related to motivation (Lauwereyns et al. 2002), its slope or size should be influenced by reward size.

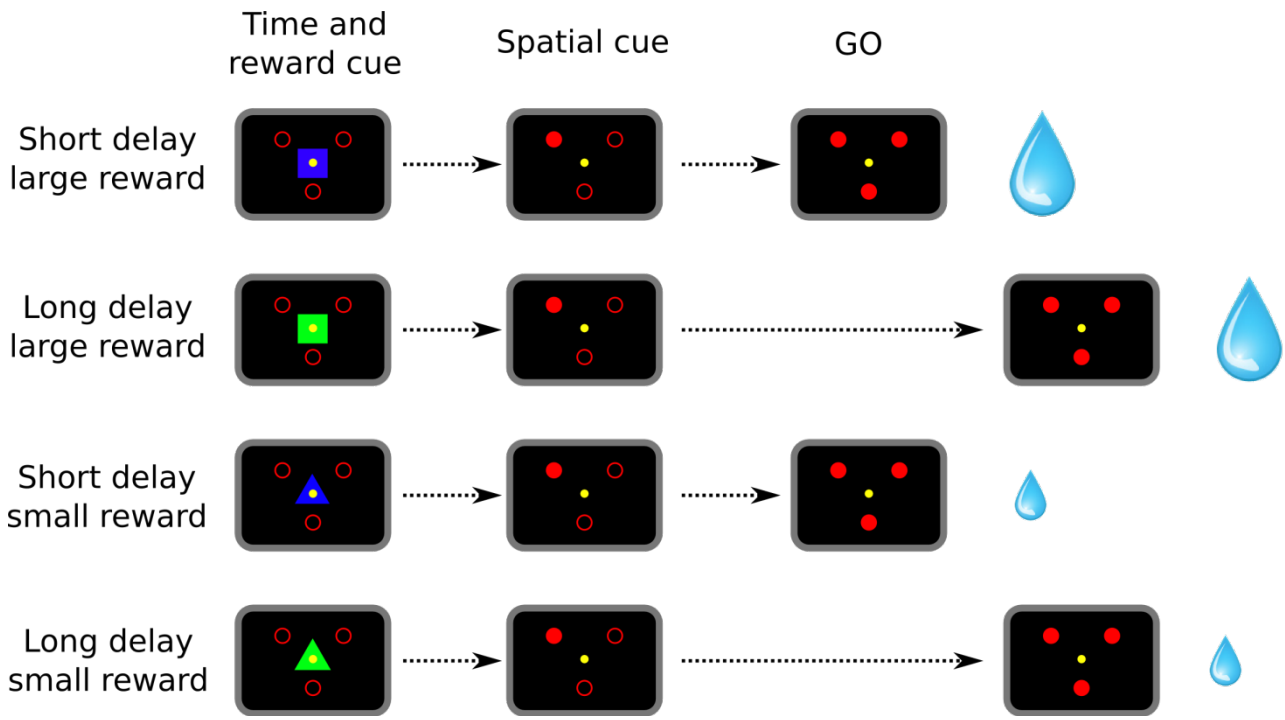


Figure 5.3: Experimental protocol to assess the motivational aspect of delay-duration effect during movement preparation. Each screen indicates a step of the task. The arrows correspond to the delays between the steps, and their length the duration of the delays (not scaled). The cues are similar as in Fig. 5.1. The drop of water symbolizes the reward, a big drop meaning a large reward. A blue (green) cue in the first screen indicates a short (long) delay between the spatial cue and the GO. A square (triangle) indicates that the trial, if successful, will be rewarded by a large (small) drop of water.

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7. Résumé étendu en Français

Introduction

La préparation d'un mouvement est un processus complexe, que de nombreux paramètres peuvent influencer, comme la disponibilité d'informations préalables concernant sa direction (Riehle & Requin 1989). En effet, afin d'optimiser nos actions, il semble que de nombreux aspects du mouvement sont traités bien avant son initiation, c'est à dire dès que les informations nécessaires sont disponibles (Requin et al. 1991 ; Riehle 2005). Cependant, la vie n'est pas une installation expérimentale, dans laquelle les informations sont fournies à un sujet passif. Dans la vie de tous les jours, ces informations sont activement extraites du contexte comportemental dans lequel nous évoluons. Toutes nos actions prennent en effet place dans un contexte spatial et temporel, et être capable d'extraire ces informations contextuelles permet d'avoir un comportement adapté. Prenons l'exemple de la chasse à l'arc au moyen-âge : si le chasseur aperçoit un oiseau disparaître derrière un bosquet d'arbre, il va utiliser les informations spatio-temporelles extraites de la trajectoire de l'animal afin de prédire où et quand il resurgira de l'autre côté des arbres. Cette prédiction de l'évolution probable de son environnement à permit au chasseur d'anticiper à la fois l'apparition d'un stimulus visuel afin de le traiter plus efficacement, et d'autre part de préparer son acte moteur (tirer une flèche) de manière à gagner du temps d'exécution. Plus généralement, nous utilisons donc constamment des informations spatiales et temporelles, afin d'optimiser nos comportements, autant perceptifs que moteurs. Ce manuscrit va donc tenter de mieux comprendre l'influence du contexte temporel (ou "timing") sur la préparation du mouvement et le traitement d'informations nécessaires à son exécution.

Pour étudier le substrat neuronal du comportement humain, le chercheur a différents outils à sa disposition. Il peut opter pour des méthodes non-invasives, permettant l'étude de la cognition à une grande échelle (cerveau entier), comme l'EEG (électroencéphalographie) ou l'IRMf (Imagerie par résonance magnétique fonctionnelle). Cependant, ces méthodes ont le défaut majeur de recueillir des signaux dérivés de l'activité cérébrale (l'activité électrique moyennée sur de larges surfaces pour l'EEG, et les fluctuations de débits sanguins locaux pour l'IRMf). D'autres méthodes, plus invasives, permettent d'avoir un accès direct à l'activité de populations de neurones, mais à une échelle spatiale bien plus réduite. Les enregistrements extra-cellulaires, qui sont la méthode utilisée dans ce manuscrit, limitée à des sujets non-humains, sont spatialement limité (la chambre d'enregistrement mesure 1.5cm de diamètre). Le chercheur doit donc décider quelle zone du cerveau il va étudier, et donc sur quelle fonction cognitive il va centrer son analyse. Notre étude porte sur la préparation du mouvement, nous avons donc décidé d'étudier l'activité du cortex moteur primaire (M1) et du cortex prémoteur dorsal (PMd) situés dans la partie postérieure latérale du lobe frontal, antérieurement à la scissure

de Rolando. Le cortex moteur primaire est caractérisé par ses denses projections vers la moelle épinière organisée somatotopiquement, et son rôle primordial dans le contrôle du mouvement. Par exemple, des lésions de M1 amènent à une paralysie dans certains membres controlésionnels. Le cortex prémoteur dorsal est plus impliqué (en tout cas chez le singe) dans le contrôle de mouvement de pointages dans l'espace. Plus précisément, il serait impliqué dans la transformation d'indice visuels en informations motrices (see Kurata 1994 ; Wise et al. 1996).

La tâche que nous avons développée étant plutôt complexe, des macaques rhésus (Macaca Mulatta) ont été choisis comme modèles expérimentaux, leurs capacité cognitives étant bien supérieure à celle des rats ou des chats. Même si leur structure cérébrale diffère de celle de l'humain, leur système moteur est assez proche pour permettre des comparaisons intéressantes avec le nôtre (Lemon & Griffiths 2005). Nous avons enregistrés l'activité intracorticale via 8 électrodes insérées à chaque session expérimentale à travers la dure-mère, nous permettant d'enregistrer les potentiels d'actions de neurones isolés, ainsi que l'activité moyenne de populations locales de neurones (les potentiels de champs locaux, ou LFP). Les potentiels d'actions sont émis par les neurones quand la somme des potentiels post-synaptiques atteint un certain seuil, et représentent donc par définition la "sortie" du neurone. Ils sont causés par l'entrée d'ions sodium à l'intérieur du corps cellulaire, amenant une dépolarisation du potentiel de membrane, et durent entre 1 et 3 ms. Les LFP sont obtenus en filtrant seulement les basses fréquences du signal neuronal enregistré sur les mêmes électrodes. Ils représentent majoritairement l'activité post-synaptique de plusieurs milliers de neurones (Logothetis et al. 2003), enregistrés sur plusieurs centaines de micromètres (Katzner et al. 2009). De manière similaire à l'EEG ou à l'activité électrique musculaire (EMG), les LFP montrent des potentiels évoqués par des événements importants de la tâche, que l'on peut faire ressortir en moyennant différents essais. Nous analyserons précisément deux types de potentiels évoqués : les VEP (potentiel évoqué visuel), suivant l'apparition de l'indice spatial, et les MRP (potentiel lié à l'exécution du mouvement), obtenus en alignant le signal LFP sur l'initiation du mouvement. Je dois préciser que ces deux types de potentiels évoqués furent enregistrés dans le cortex moteur.

Cortex moteur et influence de facteurs temporels

Certaines informations préalables sur le système moteur sont nécessaires à la compréhension des données que je vais exposer plus loin. Premièrement, une des particularités les plus connues de neurones des aires motrices (Georgopoulos et al. 1982) et des potentiels évoqués moteurs (Donchin et al. 2001) et visuels (O'Leary & Hastopoulos 2003) est qu'ils sont sélectifs à la direction du mouvement. En d'autre terme, leur activité (taux de décharge pour les neurones, amplitude pour les LFP) va varier selon la direction du mouvement effectué par

l'animal. Les neurones et les LFP ont donc une "direction préférée", leur activité décroissant de part et d'autre de cette direction en suivant une fonction de cosinus (Georgopoulos et al. 1982 ; Rickert et al. 2005 ; Amirikian & Georgopoulos 2000). Deuxièmement, l'activité neuronale du cortex moteur enregistrée avant l'apparition d'un signal instruisant l'exécution d'un mouvement est souvent corrélée essai-par-essai avec la performance de l'animal : un fort taux de décharge est associé à un temps de réaction (RT) court. Cette relation disparaît quand l'activité neuronale est alignée sur l'initiation du mouvement (Riehle 2005). Troisièmement, l'activité du cortex moteur pendant la préparation du mouvement est profondément influencée par les informations préalables sur ce mouvement (Requin & Riehle 1989 ; Crammond & Kalaska 1996, 2000 ; Riehle 2005 pour une revue de question). Lorsqu'un indice indique à l'animal la direction du mouvement (SC, pour "spatial cue") qui sera effectué après l'apparition d'un second indice (signal GO), de nombreux patterns d'activités sont observés chez les neurones du cortex moteur durant le délai séparant les deux indices. Premièrement, une activité phasique de courte latence (<200ms), et de courte durée (300ms maximum) suit l'apparition de l'indice. Cette activité, appelée "activité liée au signal" (Weinrich et al. 1984), semble apparaître suivant n'importe quel indice portant une information spatiale concernant le mouvement. La modalité de l'indice importe peu, vu que des indices visuels, auditifs et mêmes symboliques peuvent mener à ce type d'activité (Vaadia et al. 1986 ; Hoshi & tanji 2004). Cependant, l'indice doit porter des indices nécessaire à la production du mouvement, pas seulement du déplacement attentionnel accompagnant la production du mouvement (di Pellegrino & Wise 1993a,b). Cette activité phasique est principalement visible dans PMd et dans la partie rostrale de M1, et quasiment absente dans la partie sulcale de M1 (Weinrich et al. 1982, 1984 ; Crammond & Kalaska 1996, 2000 ; Riehle 2005). En plus de l'activité phasique suivant SC, certain neurones présentent une activité en forme de rampe durant une durée variable à l'intérieur du délai, menant jusqu'à l'exécution du mouvement. Cette "activité préparatoire" semble impliquée dans la préparation du mouvement, puisque son amplitude dépend de la précision de l'information directionnelle concernant le mouvement (Bastian et al. 2003). De plus, les indices menant le plus souvent à l'apparition de l'activité préparatoire portent généralement des informations menant aux plus fortes réductions de temps de réaction. Par exemple, une information préalable concernant la force frictionnelle d'un mouvement ou son amplitude amène à une réduction très faible du RT de l'animal (comparé à une condition sans information préalable), ainsi qu'à l'apparition d'une activité préparatoire chez un nombre réduit de neurones. Par contre, une information préalable sur la direction du mouvement amène une forte réduction du RT, l'apparition d'une activité préparatoire dans un nombre élevé de neurones et une forte corrélation essai-par-essai entre taux de décharge et RT (Riehle 2005).

Comme je l'ai expliqué plus haut, la préparation du mouvement se fait toujours dans un certain contexte, amenant à prédire l'apparition d'informations importantes, et à amorcer la préparation de mouvements. Le

paradigme "d'orientation temporelle" a été développé pour évaluer l'influence de l'information préalable sur la structure temporelle d'une tâche sur le traitement d'informations sensorielles et la préparation de mouvement (Nobre 2001; Nobre et al. 2007; Coull & Nobre 2008). La plupart de ces études ont été réalisées sur sujets humains, utilisant ainsi des méthodes non-invasives et donc limitées dans leur résolution temporelle ou spatiale. Elles ont cependant permis de rassembler un certain nombre d'éléments, autant comportementaux que physiologiques, amenant à penser que l'orientation temporelle agit sur différents niveaux cognitifs. En effet, les indices physiologiques liés aux traitements d'informations visuelles (Doherty et al. 2005; Rohenkohl & Nobre 2011) tout comme ceux liés à la préparation d'un mouvement au sens large (Miniussi et al. 1999; Praamstra et al. 2006; Correa et al. 2006 ; Correa & Nobre 2008; Rohenkohl & Nobre 2011) sont influencés par l'orientation temporelle. Cependant, les tâches généralement utilisées (soit une adaptation du paradigme de Posner (1980) dans le domaine temporel, soit une tâche d'occlusion de trajectoire) demandait un traitement de l'information sensoriel et la préparation simultanée d'un mouvement, rendant ainsi difficile la dissociation de ces différents processus. Chez le singe, le traitement du temps a été abordé dans d'autre contextes, comme le jugement temporel (Genovesio et al. 2009) ou la production de durée (Mita et al. 2009). Les données recueillies tendent à montrer que le timing est quasiment toujours associé à d'autres variables, comme la probabilité que la cible d'une saccade apparaisse (Leon & Shadlen 2005), ou la probabilité qu'un indice d'une certaine durée soit plus long qu'un autre indice préalablement présenté (Genovesio et al. 2009). Quant à l'influence du timing sur la préparation d'un mouvement, elle a principalement été étudiée dans le cadre des études sur la motivation (Roesch & Olson 2005).

Matériels et méthodes

Dans cette étude, nous proposons d'adapter le paradigme "classique" d'orientation temporelle, mais de dissocier temporellement l'activité liée au traitement des informations visuelles de celle liée à la préparation du mouvement. Nous avons donc eu recours au paradigme classique de pointage directionnel dans 6 directions (adapté de Georgopoulos et al. 1982) avec un pré-indiçage de la direction du mouvement. Cependant, le timing des deux indices (le SC donnant l'information spatiale) et le GO instruisant que le mouvement devait être effectué changeait d'essai à essai et était prédictible. Plus précisément, le singe manipulait une poignée qu'il pouvait déplacer dans le plan horizontal. Le mouvement de cette poignée contrôlait un curseur affiché sur un écran d'ordinateur disposé verticalement en face de l'animal. L'écran affichait une cible centrale, sur laquelle le singe devait maintenir le curseur jusqu'à l'apparition du GO, et l'emplacement des 6 cibles périphériques, disposées en cercle autour du point central. Chaque essai commençait par la présentation d'un indice sonore temporel ("temporel cue", TC), durant 200ms. La hauteur de ce son indiquait à l'animal quelle allait être la

durée des deux délais de la tâche. Cette durée pouvait être soit courte (700ms chez le singe T, 1000ms chez le singe M) soit longue (1500ms ou 2000ms respectivement chez les singes T et M). Nous parlerons donc "d'essais courts" et "d'essais longs" pour référer aux essais ayant des délais courts ou longs. Juste après TC, un premier délai (D1) commençait, à l'issue duquel le SC apparaissait pour 55ms et était immédiatement masqué. Le SC était une LED, brièvement allumée à l'un des 6 emplacements possibles, et ensuite masqué par l'allumage des 5 autres LED. Percevoir le SC était théoriquement extrêmement difficile, et même après 1 an d'entraînement et jusqu'à 1 an d'enregistrements, les singes faisaient toujours plus de 20 % d'erreurs directionnelles. Ainsi, afin de correctement percevoir le SC, les singes étaient sensé prendre en compte l'information temporelle portée par le TC, de manière à précisément estimer la durée de D1 et à anticiper l'apparition de SC. Les 6 LEDs restaient donc allumées pendant tout le deuxième délai (D2), de même durée que D1 (ainsi, si D1 était "court", D2 était "court" de même). À la fin de D2, toutes les LEDs étaient éteintes : c'était le signal de GO, indiquant à l'animal qui devait amener le curseur sur la cible préalablement indiquée par SC. En cas de succès, l'animal recevait une goutte d'eau. Ainsi, à chaque essai, le timing de SC et de GO changeait, mais l'animal pouvait le savoir en se servant de l'information temporelle portée par TC. D'un essai sur l'autre, la direction du mouvement et la durée des délais étaient choisies au hasard, mais avec la même probabilité pour une session donnée. À peu près 20 essais par condition expérimentale étaient enregistrés, ce qui amenait à environ 240 essais par session (2 durées de délai * 6 directions de mouvement * 20 essais). Les singes furent entraînés un an avant d'être opérés : une chambre d'enregistrement en acier de 1.5cm de diamètre ainsi qu'une barre de fixation furent alors fixés sur leur crâne par des vis et du ciment dentaire. Pendant les sessions expérimentales, le singe était assis dans une chaise pour primate, et réalisait la tâche avec le bras gauche, le bras droit étant attaché. Sa tête était maintenue fixe par une barre de fixation, et les mouvements des yeux n'étaient pas systématiquement contrôlés. Pendant ce temps, nous enregistrions l'activité corticale via 4 à 8 électrodes déplaçables individuellement et insérées transduralement dans le cortex moteur controlatéral au bras utilisé. Le signal de chaque électrode a été amplifié puis divisé en deux bandes de fréquences: les basses fréquences (1-250Hz) étant les LFPs, et les hautes fréquences (0.3 à 10kHz) étaient analysées en temps réel afin de détecter des potentiels d'actions, nous permettant d'enregistrer l'activité unitaire de 1 à 3 neurones sur la même électrode. Si nous avions des doutes concernant l'identification des potentiels d'actions, une seconde analyse off-line était effectuée. Toutes les analyses furent ensuite réalisées avec le logiciel Matlab.

Chez les deux singes, nous avons en tout enregistrés et analysés l'activité de 1210 neurones (470 chez singe T et 740 chez singe M) et 673 LFP (183 chez singe T et 490 chez singe M). Les deux singes avaient des temps de réaction plus rapides dans les essais courts que dans les essais longs, et faisaient plus d'erreurs directionnelles dans les essais longs que dans les essais courts. La différence de temps de réaction due à la durée du délai est

sans doute due à la capacité de l'animal d'en estimer la durée (Niemi & Näätänen 1981). En effet, plus la durée d'un délai est longue, plus l'estimation de sa durée est imprécise : cette propriété des mécanismes d'estimation temporelle est appelée la "scalarité de l'estimation du temps" (Gibbon 1977). Dans notre tâche, une estimation imprécise du timing du GO amène à une mauvaise préparation motrice, et donc à des RTs plus long.

Notre tâche étant divisée en deux délais, les tâches effectives effectuées pendant D1 et D2 sont complètement différentes : dans D1, l'animal estime la durée du délai afin de percevoir SC, et ne peut donc préparer aucun mouvement. Dans D2, l'animal sait quel mouvement devra être fait (vu que SC est apparu), ainsi que le timing du signal GO : il peut dès à présent préparer son mouvement, et attend que GO apparaisse afin de l'effectuer. Je vais d'abord présenter les résultats concernant l'influence d'informations préalables sur le timing de l'indice visuel, menant à son anticipation. Je vais ensuite aborder l'effet du contexte temporel sur la préparation motrice, c'est à dire pendant le deuxième délai.

Activité anticipatoire dans D1

Nous nous sommes d'abord intéressés à l'activité précédant l'apparition de SC. En effet, le cortex moteur étant principalement impliqué dans la préparation et l'exécution des mouvements, il serait surprenant si son activité était modulée bien avant même l'apparition d'un indice permettant de préparer le mouvement. Cependant nous avons observé que près de 40 % des neurones enregistrés modulaient significativement leur activité entre les 150 premières et dernières millisecondes de D1. En d'autres termes, 49 et 38 % des neurones des singes T et M, respectivement, changeait de taux de décharge avant l'apparition de SC. Parmi ces neurones, la majorité (~60%) montraient une augmentation d'activité, et les autres montraient une diminution d'activité. Nous avons nommé ces neurones "anticipatoires", et les avons divisé selon leur profil de décharge en deux "catégories" fonctionnelles : les D1up montrent une augmentation d'activité, et les D1down montrent une diminution. Les neurones ne montrant pas de modulation significative de leur activité avant SC furent classés dans la catégorie "D1flat". Il faut cependant garder en tête que ces différentes catégories ne sont que des subdivisions artificielles d'un continuum d'activité dans D1. En moyennant séparément l'activité de tous les neurones de chaque catégorie à travers tous les essais de chaque session, on voit que l'activité moyenne des trois catégories de neurones diverge complètement durant le second délai (c'est à dire quand le singe est en train de préparer le mouvement). Plus précisément, deux périodes de la tâches montraient les plus nettes différences d'activité entre neurones de différentes catégories : suivant l'apparition de SC et pendant l'exécution du mouvement. L'activité moyenne des D1up montrait une réponse phasique à courte latence suivant l'indice visuel, alors qu'elle retombait à un niveau d'activité plus bas par la suite. Au contraire, l'activité moyenne des D1down était

très basse et ne montrait quasiment pas de réponse phasique à la suite du SC, mais était plus élevée pendant l'exécution du mouvement.

La différence de réponse phasique fut analysée plus en détail, en regardant le pourcentage de neurones montrant une telle réponse dans les trois catégories. Une première analyse fut effectuée, confirmant un pourcentage de neurones avec une "réponse liée au signal" plus élevé dans la catégorie des D1up, puis des D1flat, et enfin des D1down. Cette analyse ayant été faite à travers les essais effectués dans toutes les directions de mouvement, elle était peut-être biaisée par une différence de sélectivité directionnelle entre catégories. La même analyse fut donc effectuée, en ne considérant que l'activité enregistrée dans la direction préférée du neurone : la différence de pourcentage était identique. Afin de caractériser plus précisément la différence d'activité phasique entre catégories de neurones anticipatoires, nous avons analysé la distribution des latences de leur réponse phasique. Il s'est avéré que même si cette analyse confirmait la précédente (les D1up ont beaucoup plus de chance d'avoir une réponse phasique à courte latence après SC), les raisons en étaient complètement différentes chez les deux singes. Chez le singe T, les D1up et D1down avaient la même proportion de neurones ayant une réponse phasique dans les 500ms suivant SC. Cependant, la latence médiane de cette réponse chez les D1up était de 200ms plus précoce que chez les D1down. Ce profil était inversé chez le singe M : la latence médiane des deux catégories était identique, mais la proportion de neurones avec une réponse phasique était bien plus forte dans les D1up que dans les D1down. En résumé, les neurones montrant une augmentation d'activité avant SC ont une forte probabilité d'avoir une réponse phasique suivant SC, alors que cette probabilité est faible chez les neurones avec une diminution d'activité précédent SC.

Nous avons ensuite évalué si l'activité anticipatoire avait une influence particulière sur la réponse phasique essai-par-essai, modulant son amplitude ou sa latence. Comme le pic d'activité devait être détecté essai par essai, seulement les neurones avec une réponse phasique ayant un fort rapport signal/bruit furent sélectionnés. Les analyses essai-par-essai étant très sensible au bruit, cette sélection drastique des neurones permettait d'avoir des résultats plus sûrs. Afin de maximiser le nombre de neurones analysés, les neurones furent groupés nonobstant leur appartenance à telle ou telle catégorie anticipatoire. Deux méthodes furent utilisées pour détecter le pic d'activité essai par essai, menant aux mêmes résultats. La première est basée sur les travaux de Nawrot et al. (1999), et consiste à convoluer les trains de potentiels d'actions de chaque essai avec un kernel triangulaire, de manière à reconstruire une estimation de taux de décharge sous-jacente. On obtient ainsi un signal continu au lieu d'un signal discret. À partir de ce signal, le pic est détecté en comparant chaque point contre ses deux voisins. L'autre méthode est basée sur Miller et al. (1992), et effectue un

comptage du nombre de potentiel d'action dans une fenêtre temporelle glissante, pour estimer à quelle latence est le maximum. Les deux méthodes de calcul utilisant des approches différentes mais menant aux mêmes résultats, les latences et amplitudes calculées paraissent sûres. Ces valeurs étaient ensuite corrélées avec le taux de décharge calculé dans une fenêtre temporelle précédent le SC (corrélation de rangs de Spearman). Le premier résultat est qu'il n'y a virtuellement pas de corrélation entre taux de décharge précédent le SC et latence du pic. Le deuxième résultat est qu'il y a une forte corrélation positive entre le taux de décharge précédent SC et l'amplitude du pic. Afin de mesurer si la relation entre l'activité précédent et suivant SC était de type additive ou multiplicative, nous avons effectués une régression linéaire entre ces deux valeurs. Si la pente de la régression était forte (>1.5), cela signifierait que la corrélation est multiplicative : le nombre de potentiels d'action précédent SC est multiplié pour atteindre l'amplitude de la réponse phasique. Par contre, si la pente est faible (<1), cela signifie que l'activité précédent SC influence peu celle suivant SC, et que la principale différence entre les deux valeurs est l'addition d'une valeur fixe. La distribution des valeurs de pente pour tous les neurones étaient inférieurs à 0.5 pour les deux animaux : chaque potentiel d'action précédent SC ajoute un demi-potentiel d'action après SC. Nous avons donc conclu que l'influence de l'activité précédant SC sur l'activité suivant SC est relativement faible, et que la réponse phasique "chevauche" l'activité anticipatoire comme si celle-ci était une modulation de la ligne de base.

Comme nous avons vu que l'activité moyenne des neurones divergeaient selon les catégories dans la période suivant le SC, mais aussi pendant l'exécution du mouvement, l'étape suivante était de comparer systématiquement l'activité dans ces deux périodes pour chaque neurones. Cette analyse confirmait l'idée tirée de l'activité moyenne : bien plus de D1up étaient plus actifs dans l'a période suivant SC que pendant le mouvement, alors que le profil inverse formait la majorité des D1down. Cette différence d'activité entre les catégories pendant les deux périodes importantes de la tâche amenait à penser que les neurones des deux catégories pourraient être impliqués l'un après l'autre dans la préparation du mouvement : les D1up pendant le traitement de l'information spatiale, et les D1down pendant l'exécution du mouvement. Pour tester cette possibilité, nous avons fait l'hypothèse que le degré d'implication dans la préparation d'un mouvement est reflété par le degré de sélectivité des neurones pour différents paramètres du mouvement, comme la direction (hypothèse aussi retenue par Cohen et al. 2010). Nous avons donc calculé la significativité de la sélectivité directionnelle de chaque neurone au cours du temps. En considérant que l'activité moyenne des neurones dans les 6 directions du mouvement pouvait être vue comme la somme vectorielle de 6 vecteurs d'amplitude différente (le taux de décharge dans chacune des directions), menant ainsi à un vecteur somme pointant dans la direction préférée du neurone (la norme du vecteur somme indiquant le degré de sélectivité). En mélangeant les essais à travers les directions, on peut obtenir un vecteur somme dû à la chance. Si on réitère

cette procédure 1000 fois, on obtient une distribution des normes de vecteurs sommes dues à la chance. Si le vrai vecteur somme est plus grand que 95 % de la distribution due à la chance, le neurone est significativement sélectif pour la direction du mouvement. En utilisant cette technique dans des fenêtres temporelles réduites, on obtient l'évolution du pourcentage de neurones directionnellement sélectifs au cours de l'essai. En appliquant cette méthode, pas de différences systématiques ne furent observées entre les neurones D1up et D1down. Certaines périodes montraient des différences significatives dans le pourcentage de neurones sélectifs, mais jamais de manière cohérente entre les deux animaux. Ainsi, basé sur la directionnalité des neurones il ne semble pas que les D1up et D1down ne soient séquentiellement impliqué dans la préparation du mouvement.

Une possibilité qu'il fallait éliminer afin de continuer l'analyse des données était que les animaux aient utilisée une stratégie mentionnée dans les travaux de Mauritz & Wise (1986). Dans leurs travaux, ils émettent l'hypothèse, basée sur les résultats comportementaux de l'animal, que l'activité enregistrée avant le SC est due à un choix précoce de l'animal d'une direction "par défaut" pour le mouvement à venir. Les neurones D1up seraient en fait les neurones dont la direction par défaut est la direction préférée, alors que les D1down seraient les neurones dont la direction préférée est opposée à la direction par défaut. De cette hypothèse découle le fait que les D1up et D1down devraient avoir des directions préférées opposées pendant le deuxième délai. La distribution des directions préférées des neurones des deux catégories fur donc analysée : les deux distributions de directions préférées étaient réparties de manière homogène. Ainsi, l'hypothèse de Mauritz & Wise (1986) ne peut pas expliquer nos données. Cependant, encore deux stratégies pouvaient avoir été utilisées par les animaux afin de résoudre la tâche, et nous nous sommes attachés à comprendre laquelle ils avaient utilisé. La première stratégie, appelée "exact timing", consiste à prendre en compte l'information temporelle contenue dans TC, et à attendre SC exactement au bon moment. L'autre stratégie, appelée "attentiste", consiste à ne pas prendre en compte l'information donnée par TC, et à toujours attendre le SC au bout du délai court. Si le SC n’apparaît pas à la fin de ce délai court, on en déduit que l'essai en question est un délai long. Le profil de taux de décharge associé à chaque stratégie est différent : si le taux de décharge des neurones (dans D1) dans les essais courts est similaire à celui des essais longs dans la première partie de D1, il est probable que l'animal utilise la stratégie "attentiste". Si par contre les taux de décharge des neurones diffèrent selon la durée du délai dès le début de l'essai, l'animal a forcément eu recours à la stratégie "exact timing". Le pourcentage de neurones montrant une différence significative d'activité due à la durée du délai a donc été calculé dans une fenêtre temporelle glissante, de TC jusqu'à SC dans les essais courts, et de TC jusqu'au moment correspondant dans les essais longs. Le singe T n'a montré aucune différence due à la durée du délai, et a donc vraisemblablement suivi la stratégie "attentiste". En effectuant la même analyse chez le

singe M, il s'est avéré que le profil de différence changeait au milieu des sessions expérimentales : dans les 6 premiers mois d'enregistrements, singe M montre le même profil de taux de décharge que singe T (stratégie "attentiste"). Par contre, dans les 6 derniers mois, le profil de décharge devint caractéristique de la stratégie "exact timing". Le singe M a manifestement changé de stratégie comportementale au milieu des sessions expérimentales. Nous avons donc divisées toutes les données acquises dans ces deux périodes et les avons analysées séparément : aucune différence qualitative ne fut observée. Ainsi, la stratégie de l'animal ne semble pas influer sur la relation entre activité liée à l'anticipation du SC et à la préparation motrice.

Notre prochaine hypothèse était que l'activité anticipatoire reflète un processus attentionnel, vu que l'activité de PMd peut être fortement influencée par des facteurs attentionnels (Boussaoud 2001; Lebedev & Wise 2001). Le taux d'erreurs directionnelles produites par le singe était assez élevé, montrant que cette tâche était particulièrement difficile. Nous avons émis l'idée que qu'au moins une part de ces erreurs étaient dues à des fluctuations attentionnelles, le singe étant distrait au moment du SC et ne le percevant ainsi pas. Nous avons donc analysé l'activité anticipatoire précédent SC avec l'idée que, si cette dernière reflète le niveau attentionnel de l'animal, les essais dans lequel une erreur a été commise montreraient une différence d'activité comparés aux essais corrects. Aucune différence ne fut trouvée. Ainsi, l'activité anticipatoire n'est pas liée à la probabilité de fournir une réponse juste à la fin de l'essai (un résultat déjà trouvé par Meftah et al. 2009 dans le cortex somatosensoriel primaire et secondaire).

Nous nous sommes ensuite intéressés à la modulation de l'activité anticipatoire par la durée du délai, vu que l'animal pouvait la prédire depuis le début de l'essai. L'activité moyenne de toute la population de neurones à la fin de D1 ne différait pas selon la durée de l'essai, chez aucun des deux singes. Cependant, une forte proportion de neurones individuels avait une activité différente entre ces mêmes périodes, mais il y avait autant de neurones montrant une activité plus forte dans les essais courts que dans les essais longs que de neurones ayant une activité plus forte dans les essais longs que dans les essais courts. La somme de ces différences s'annulait, menant à un taux de décharge moyen identique. Cependant, une différence nette était visible entre les deux singes, qui était liée à leurs stratégies comportementales. Chez le singe T, la préférence des neurones pour les délais courts ou longs semblait indépendante de leur profil anticipatoire. Par contre, chez le singe M, les D1up avaient tendance à être beaucoup plus actifs dans les essais courts que dans les essais longs, alors que les D1down montraient la tendance inverse. Comme décris plus haut il s'est avéré que seul le singe M prenait en considération les informations temporelles portées par TC, et attendait donc SC au bon moment. Les neurones du singe M montrent donc une modulation plus importante d'activité dans les essais courts que dans les essais longs. Ce résultat ajouté au fait que les neurones de ce singe montrent une

sélectivité pour la durée du délai dès le début de l'essai indique que dans les essais courts, les neurones anticipatoires adoptent une pente d'activité plus raide que dans les essais longs, menant à une activité plus fortement modulée précédent le SC. Cela mène au fait intéressant que les D1up et les D1down sont "inversement" modulés par la durée de l'essai.

Cette modulation inverse des D1up et D1down, ainsi que des données issues de la littérature, nous amena à émettre l'hypothèse que les D1up et les D1down neurones représentent deux facettes d'un même processus ayant pour but d'amener le cortex moteur dans un état optimal au moment de l'apparition de SC. Le premier aspect serait de faciliter le traitement de l'indice spatial en renforçant la réponse phasique suivant le SC, et le deuxième aspect, intimement lié, serait d'empêcher qu'un mouvement soit amorcé trop tôt. Cette hypothèse a déjà été avancée pour la préparation motrice, et est appelée "priming and breaking" ("préparer et freiner", Prut & Fetz 1999 ; Cohen et al. 2010). Elle stipule que la préparation d'un mouvement consiste à mettre le système dans un état optimal pour l'exécution du mouvement tout en retenant son exécution jusqu'à l'instant voulu. En effet, des études comportementales amènent à penser qu'une "inhibition proactive et volontaire" est régulée par le système moteur afin d'empêcher que des entrées sensorielles ne provoquent de mouvement involontaire (Boulinguez et al. 2008). Ces études ont en effet montré que la présentation d'un indice spatial amenait à l'émission de réponses motrices précoces et de potentiels musculaires si elles n'étaient pas inhibées correctement. Cette inhibition volontaire dépendrait du contexte comportemental (comme la présence d'essais dans lesquels la réponse motrice doit être retenue), et régulerait donc la réactivité du système moteur en fonction des contingences comportementales (Criaud et al. 2012). Des études utilisant la stimulation magnétique transcranienne (TMS) ont de plus montré que l'excitabilité cortico-spinale pendant la préparation du mouvement diminuait graduellement, mais seulement si le membre était impliqué dans la préparation du mouvement (Hasbroucq et al. 1997 ; Duque & Ivry 2009). Nous avons donc fait l'hypothèse que les neurones D1down seraient plus proches de la sortie motrice, et devraient donc être inhibés lors de l'apparition de SC afin d'empêcher que leur activation ne produise un mouvement parasite. Ces deux processus travaillant main dans la main, ils devraient être finement couplés essai-par-essai. De cette hypothèse naît la prédiction que les neurones de la même catégorie (deux D1up ou deux D1down) seraient positivement couplés essais par essais, alors que des neurones appartenant à des catégories différentes seraient négativement corrélés essais par essais. Nous avons donc sélectionné des paires de neurones anticipatoires enregistrés simultanément (nous avions entre 4 et 8 électrodes). Parmi ceux-ci, nous n'avons gardé que les paires de neurones enregistrés sur des électrodes différentes : en effet, si deux neurones sont enregistrés sur la même électrode, et qu'un faible pourcentage de potentiels d'actions est attribué au mauvais neurone par le logiciel de traitement des données, une corrélation positive artificielle sera induite entre l'activité des deux neurones. Nous avons ensuite calculé

le coefficient de corrélation entre les activités des paires de neurones de même ou de différentes catégories dans une fenêtre temporelle glissante, afin d'obtenir le décours temporel de cette corrélation. Le taux de décharge moyen des deux groupes ("même catégorie" et "différente catégorie") était similaire : les différences de corrélations ne peuvent être attribuées à des différences d'activité moyenne. L'analyse de la corrélation confirma notre hypothèse : les neurones de même catégorie étant en moyenne positivement corrélés, alors que les neurones de différentes catégories deviennent négativement corrélés à l'approche du SC. Ce résultat tend à soutenir l'idée que les D1up et D1down font partie d'un même réseau, leurs activités étant corrélés, mais que leurs modulations sont inversées. Nous soutenons donc l'idée que l'activité anticipatoire précédent SC reflète un mécanisme similaire au "priming and breaking" de la préparation motrice, dont le but est en partie de prévenir l'exécution précoce de mouvement en réaction à un indice visuel. PMd pourrait jouer un rôle central à la fois dans la facilitation du traitement des indices visuels et dans l'établissement de l'inhibition proactive. En effet, d'un côté Kurata & Hoffman (1994) ont montré que des lésions de PMd amenaient non à des problèmes moteurs per se, mais à des déficits dans des tâches de pointage vers des indices visuels. De nombreuses études ont de plus montré le rôle central de PMd dans le traitement d'indices spatiaux dans le cadre de la préparation motrice (par exemple : Kurata 1994 ; Wise et al. 1996 ; Yamagata et al. 2009). De l'autre côté, des lésions virtuelles de PMd par TMS répétée réduise l'inhibition cortico-spinale concomitante à la préparation du mouvement (Duque et al. 2012). De plus, Mirabella et al. (2011) ontmontré dans PMd l'existence de décharges neuronales précédant l'inhibition d'un mouvement. Des hypothèses alternatives seraient que l'activité anticipatoire a un rôle attentionnel, mais nos résultats comparant les essais corrects avec ceux comportant des erreurs directionnelles ne soutiennent pas cette idée. La dernière hypothèse serait que cette activité reflète l'estimation du temps per se. Cependant, l'activité du singe T pendant D1 n'est pas compatible avec une telle idée : au milieu du délai (quand la durée courte est écoulée), l'activité neuronale des neurones anticipatoire reste stationnaire. Une activité liée à l'estimation du temps devrait permettre le décodage d'une durée du début à la fin du délai, or celle-ci ne le permet pas.

Influence du timing sur la préparation motrice et le traitement de l'indice spatial

Dans cette seconde partie, je vais présenter l'analyse de l'activité neuronale (potentiels évoqués des LFP et taux de décharge des neurones individuels), principalement en relation avec le traitement de l'indice spatial (VEP des LFP et "activité liée au signal" du taux de décharge) et en relation avec la préparation (augmentation lente du taux de décharge des neurones) et l'exécution (MRP des LFP et augmentation du taux de décharge liée au mouvement).

En moyennant le signal LFP suivant l'apparition de l'indice spatial, un signal caractéristique est visible : le VEP, composé de 3 composantes, la N1 (composante négative de latence 50 à 120ms), la P1 (composante positive de latence 120 à 200ms), puis la N2 (de 200 à 300ms). Seulement les deux premières composantes furent systématiquement analysées, la N2 étant moins fréquentes que les deux autres. De même, en alignant les LFP sur l'initiation du mouvement, un MRP caractéristique est visible : d'abord une composante positive P1 précède l'initiation du mouvement et dure autour de 100ms, puis une négative N1 est centrée sur son initiation, et dure aussi 100ms. Enfin, P2 est plus tardive et dure 200ms. Les MRP sont vraisemblablement liés à l'exécution du mouvement, y compris la composante précoce P1. En effet, son timing pourrait laisser penser qu'elle est une réaction au signal de GO. Au contraire, sa latence est relativement fixe par rapport à l'exécution du mouvement, alors qu'elle est très variable quand alignée sur le GO : son timing est donc étroitement lié à l'initiation du mouvement, et il semble très improbable qu'elle soit la trace de la perception du signal de GO. Nous avons d'abord détecté la présence de tels EP, en comparant l'activité dans les deux périodes d'intérêts (suivant SC et à l'initiation du mouvement) à une période contrôle précédent SC, séparément dans les essais courts et longs. Il faut de plus préciser que seulement les basses fréquences (1-15Hz) des EP ne furent conservées, afin d'éliminer d'autres signaux comme les oscillations. Le pourcentage de EP détectés dépendait de la période d'intérêt et de la durée du délai : beaucoup plus de VEP étaient détectés dans les essais courts que dans les essais longs, alors que beaucoup plus de MRP étaient détectés dans les essais longs que dans les essais courts. Cette différence de proportion semblait indiquer que les EP pourraient avoir une différente amplitude selon la durée séparant le SC du GO, rendant les EP moins grands plus difficile à détecter. La taille des Es a donc été systématiquement comparée entre délais courts et longs : l'amplitude de la grande majorité des VEP était en effet plus grande dans les essais courts que dans les essais longs, alors que le profil inverse était observé chez les MRP. En résumé, les VEP et MRP montrent une modulation inverse liée au délai attendu et écoulé.

Nous avons ensuite comparé ce résultat à la même analyse effectuée sur le taux de décharge des neurones enregistrés dans la même période. En effet, l'activité liée au signal a un timing similaire au VEP, son pic étant observable exactement entre N1 et P1. Nous avons donc exclusivement sélectionné les neurones répondant phasiquement à SC, et dont le pic de la réponse avait une latence de 250ms ou moins. De même, nous avons sélectionné tous les neurones actifs pendant l'exécution du mouvement, et avons comparé leur activité moyennée dans la même fenêtre temporelle que celle utilisée pour calculer le MRP entre essais courts et longs. Le résultat obtenu était similaire que ceux obtenus pour les EP, mais la proportion de neurones concernés était bien plus faible : 28 % des neurones montraient une différence d'activité phasique liée au délai, et 16 % des neurones montraient une différence d'activité motrice. Par comparaison, 76 % des VEP et 52 % des

MRP montraient une différence d'amplitude liée au délai. De plus, les différences observées chez les neurones, bien que significativement biaisée dans le même sens que les EP, n'étaient pas aussi claires.

Comme je l'ai expliqué plus haut, l'amplitude des EP est modulée par la direction du mouvement (Donchin et al. 2001), même dans la période suivant l'apparition de l'indice (O'Leary & Hatsopoulos 2003). Cette sélectivité pour la direction du mouvement est-elle influencée par la sélectivité pour la durée du délai ? Plus généralement, comment ces deux sélectivités interagissent-elles ? Utilisant la même méthode que celle décrite pour évaluer la sélectivité directionnelle des neurones dans la section précédente, nous avons déterminé qu'autour de 40 et 20 % des VEP étaient directionnellement sélectifs chez les singes T et M, ainsi que 60 et 40 % de leurs MRP. Ainsi, l'importance de la sélectivité des EP pour la direction du mouvement et pour la durée du délai est du même ordre de grandeur. Effectuant le même test pour les neurones, autour de 35 % des neurones étaient significativement sélectifs à la direction du mouvement dans la période suivant le SC, alors que ~70 % montraient cette sélectivité pendant l'exécution du mouvement. En regardant la distribution circulaire des directions préférées des EP et des neurones, une forte différence apparaît : alors que les distributions de directions préférées des neurones sont plus ou moins réparties de manières homogènes autour des 360 degrés, les directions préférées des EP étaient clairement regroupées en 1 à 3 "groupes" de directions préférées. Cette différence dans la répartition des directions préférées des neurones et des LFP est un résultat intriguant, mais qui a déjà été rapporté ailleurs (O'Leary & Hatsopoulos 2003). Sélectionnant ensuite les EP et les neurones dont l'activité était sélective à la direction dans les essais courts et longs, nous avons analysé la distribution des différences angulaires de directions préférées entre essais courts et essais longs. L'idée derrière cette analyse était que si la sélectivité à la durée du délai influence la sélectivité à la direction du mouvement, la distribution des différences angulaires des signaux sélectifs à la durée du délai devrait différer de celle des signaux non sélectifs à la durée du délai. Les résultats pour les EP et pour le taux de décharge des neurones étaient équivalents : toutes les distributions de différences angulaires étaient centrées sur 0, signifiant qu'en moyenne, la direction préférée des signaux était similaire entre essais courts et essais longs, même quand le signal électrophysiologique en question était sélectif à la durée du délai. Il semble donc que les sélectivités pour les deux paramètres de la tâche sont plutôt indépendantes.

Cependant, il est possible que l'influence de la durée du délai et l'influence de la direction du mouvement aient des décours temporels différents. Afin d'évaluer ce point, nous avons calculé la sélectivité à la durée du délai et pour la direction du mouvement de chaque composante des EP séparément, pour voir si la proportion entre le nombre de composantes modulées par l'un ou l'autre paramètre changeait au cours de l'essai. En effet, les pourcentages de neurones sélectifs à la durée du délai ou à la direction du mouvement n'étaient pas

équivalents entre la période suivant SC et celle centrée sur l'exécution du mouvement (voir plus haut) : il semblerait que la durée du délai ait beaucoup plus d'influence sur le taux de décharge des neurones au début de l'essai, alors que la direction du mouvement a beaucoup plus d'importance pendant l'exécution du mouvement. Plusieurs observations furent faites : 1) même la composante la plus précoce des VEP montrait une forte sélectivité à la direction et à la durée du délai. 2) la seconde composante des VEP étaient beaucoup plus sélective à la direction du mouvement que la première, alors que la différence de sélectivité à la durée du délai était moins grande (voire inexistante chez un singe). 3) les MRP étaient en général plus sélectifs à la direction du mouvement que les VEP, et moins sélectifs à la durée du délai. 4) la sélectivité à la direction du mouvement augmente graduellement à travers les trois composantes du MRP : la dernière composante étant plus sélective à la direction que les deux autres. Plus généralement, en représentant le nombre de composantes sélectives à l'un ou l'autre paramètre sous forme de ratio, une tendance générale émerge : au début de l'essai, au cours de l'essai, les composantes des EP sont de moins en moins sélectives à la durée du délai, et de plus en plus sélectives à la direction du mouvement : la composante avec le ratio EP sélectifs à la durée / EP sélectifs à la direction le plus positif étant la première composante des EP, alors que celle avec le ratio le plus négatif est la dernière composante du MRP. En résumé, il semble que pour l'activité des neurones et l'amplitude des EP, l'influence des deux paramètres de la tâche a un différent décours temporel, la durée du délai étant plus importante à la suite de la présentation du SC, tandis que la direction est plus importante alors que le mouvement approche.

Nous avons retenu deux interprétations pour tenter d'expliquer ces résultats : une sélectivité à la durée du délai inverse pour les VEPs et les MRPs (ainsi que de l'activité des neurones dans les mêmes périodes), et la différence des décours temporels de la sélectivité aux deux paramètres de la tâche. La première interprétation postule que la relation inverse entre VEP et MRP pourrait être due à une stratégie motrice. Dans les essais courts, la planification du mouvement pourrait avoir lieu dès la présentation du SC. Par contre, dans les essais longs, la contrainte de devoir garder la préparation du mouvement "active" pendant un long délai, ajouté à la difficulté d'estimer la durée d'un long délai afin d'anticiper la préparation du mouvement, rendrait cette stratégie moins efficace. En conséquence, dans les essais longs, la préparation du mouvement serait moins importante suivant le SC et plus importante pendant l'exécution du mouvement elle-même. Cette hypothèse expliquerait le rapport inverse des EP à la durée du délai, et le fait que les VEP soient fortement sélectifs à la direction. Cependant, une étude du taux de décharge dans différentes aires du lobe frontal, y compris PMd, a déjà montré que les neurones de cette aire avaient une forte sensibilité à la durée du délai attendu (Roesch & Olson 2005a). Ces auteurs montrèrent par la suite que les neurones sensibles à la durée du délai étaient aussi sensibles à la taille de la récompense attendue. Ils interprétèrent ces résultats en invoquant le concept de

"perte de la valeur de la récompense liée au délai" ("temporal discounting", Löwenstein & Elster 1992). Selon cette interprétation, la différence de taux de décharge liée à la durée du délai serait en fait liée à la valeur subjective de la récompense, qui dépendrait du délai précédent sa disponibilité. Nos données pourraient donc être interprétées de cette manière : au début du délai, des facteurs motivationnels auraient une importance très forte, agissant sur l' "excitation" générale ("arousal") de l'animal, ou son niveau de préparation. Cela serait reflété par un VEP plus ample dans les essais courts que dans les essais longs, et bien plus sélectif à la durée du délai qu'à la direction du mouvement. Alors que le délai s'écoule, la préparation motrice serait plus importante, amenant une plus forte représentation de la direction du mouvement par rapport à la durée du délai. À l'apparition du GO, la durée écoulée du délai influence la préparation du mouvement : il est bien plus difficile de préparer en avance un mouvement dans un délai long que dans un délai court, car le timing du GO est plus difficile à estimer. Utilisant la TMS, Davranche et al. (2007) ont par exemple montré que la diminution d'excitabilité cortico-spinale observée pendant la préparation du mouvement était plus faible dans un délai long que dans un délai court. Ainsi, l'impossibilité de préparer un mouvement en avance donnerait un plus gros poids aux entrées sensorielles pendant la préparation du mouvement, amenant à un MRP plus large dans les essais longs. Une nouvelle expérience manipulant à la fois durée du délai et valeur de la récompense devrait être menée afin de valider l'une ou l'autre de ces hypothèses.

Nous nous sommes ensuite intéressés à la relation entre les EP et le comportement. En d'autres termes, est-ce que l'amplitude des EP est corrélée avec le RT ? Pour ce faire, différentes analyses furent effectuées. Dans la première, nous avons corrélé essai-par-essai le RT avec l'amplitude des VEP et MRP. Une corrélation positive très forte fut trouvée dans de nombreux MRP, alors qu'une corrélation négative avec le RT fut trouvée dans quelques VEP. Cependant, cette analyse était conduite à travers toutes les directions du mouvement, pouvant ainsi mener à un biais du résultat lié à la directionnalité des EP. Nous effectuâmes donc une analyse supplémentaire, en groupant les 50 % des essais ayant les RT les plus lents et les plus rapides dans chaque condition. En comparant l'amplitude des EP dans ces deux groupes d'essais, on contrebalançait l'effet de la direction du mouvement. Des résultats similaires furent trouvés : de nombreuses relations significativement positives furent trouvées entre MRP et RT, alors que peu de relations significativement négatives furent trouvées entre VEP et RT. Enfin, nous avons établi la différence d'amplitude moyenne entre RT rapides et lents pour chaque EP, afin d'obtenir une distribution de ces différences pour toute la population de EP. Cette dernière analyse montra que même si le nombre de relations significatives entre VEP et RT était très faible, la distribution des différences d'amplitudes pour tous les EP était significativement négative. En résumé, au niveau des EP individuels et de la population, les MRP sont positivement corrélés avec le RT, alors que les VEP sont faiblement négativement corrélés.

La même analyse fut effectuée pour le taux de décharge des neurones, mais en n'utilisant que la méthode groupant les RT en deux groupes selon leur vitesse. Le premier résultat est que l'activité suivant l'apparition du SC ne montre aucune relation avec le RT. Pour l'analyse de l'activité dans la période entourant l'exécution du mouvement, les résultats sont plus intéressants. Le fait que le taux de décharge précédent le signal de GO est négativement corrélé avec le RT est bien connu (Riehle 2005). Cependant, cette relation entre taux de décharge et RT peut être expliquée par différents modèles de la préparation motrice. En effet, le RT peut avoir de différentes relations avec le taux de décharge des "neurones préparatoires" dont j'ai parlé dans l'introduction, et nous avons examiné trois d'entre elles. Le premier modèle de préparation du mouvement indique que la latence du taux de décharge précédent l'apparition du GO est relativement fixe précédent l'initiation du mouvement. De plus, un seuil de taux de décharge fixe, lui aussi, doit être atteint pour qu'un mouvement soit initié. Cependant, la pente de cette activité peut varier, et une pente plus raide amène à franchir le seuil plus vite, et donc à une initiation du mouvement plus précoce et un RT plus court. Nous avons appelé ce modèle "pente variable". Le second modèle que nous avons considéré postule que la pente de l'activité précédent l'initiation du mouvement reste fixe, tout comme le seuil d'activité à atteindre, mais la latence de l'augmentation d'activité varie d'un essai à l'autre. Ainsi, une latence courte amènerait à une initiation du mouvement précoce et donc à un RT court. Ce modèle fut donc nommé "latence variable". Le dernier postule que la latence et la pente de l'activité restent fixent, mais que le seuil de taux de décharge change d'un essai à l'autre. Ainsi, quand le seuil est plus bas il est atteint plus vite, menant à des RTs plus courts. Ce dernier modèle était nommé "seuil variable". Ces trois modèles de préparation du mouvement amènent à différentes prédictions sur la relation entre taux de décharge et RT. En calculant la relation du taux de décharge avec le RT dans trois fenêtres temporelles différentes, on peut être capable de voir quel modèle de préparation du mouvement est le plus adapté pour expliquer nos données. Selon notre analyse, le modèle "latence variable" est de loin le modèle expliquant le mieux la relation entre taux de décharge et RT.

Nous nous sommes intéressés à l'influence de la durée du délai sur l'activité préparatoire précédant l'initiation d'un mouvement. En effet, les animaux connaissant la durée du délai par cœur, ils peuvent commencer à préparer leur mouvement à différents délais en fonction de la leur durée. Nous avons sélectionné des neurones préparatoires, basés sur leur haut taux de décharge et sur leur activité augmentant pendant les 600 dernières millisecondes précédant l'exécution du mouvement. Par inspection visuelles, trois personnes différentes ont jugés si l'activité préparatoire subissait un changement de latence, un changement de pente, ou les deux en fonction de la durée du délai. En effet, l'activité précédant l'initiation du mouvement pourrait toujours commencer à la même latence suivant SC, mais augmenter avec différentes pente jusqu'à l'exécution du mouvement. Alternativement, la pente de l'activité serait la même dans les essais courts et longs, mais

l'activité commencerait à augmenter à différentes latence après SC selon la durée du délai. Notez que différente latence par rapport à SC peut signifier une latence identique comparée au GO. La très grande majorité des neurones que nous avons inspectés montraient une différence de latence, et quelques-uns une différence de latence et de pente. Seulement 3 neurones montraient une différence de pente sans différence de latence.

Ces résultats, combinés à ceux portant sur le modèle de la préparation du mouvement, permettent de formuler une hypothèse. Dans notre tâche, l'individu savait quel mouvement produire dès le début du délai, et le timing du GO est hautement prédictible. Ainsi, dans notre cas, la variable permettant le plus d'estimer la performance de l'animal ne serait pas sa capacité à intégrer l'information rapidement (elle est disponible depuis SC) ou sa réactivité quand le GO arrive (il peut prédire son apparition), mais sa capacité à estimer la durée du délai. Cela expliquerait pourquoi le même changement de latence de l'activité préparatoire est observé quand l'animal anticipe un délai de différente durée, et quand il estime la même durée mais montre une variabilité de performance. Le même mécanisme sous-jacent expliquerait cette similarité. Notre hypothèse est donc que le contexte comportemental (comme la prédictibilité du signal GO) influence fortement la relation entre taux de décharge et RT. Ainsi, plusieurs différences entre nos résultats et ceux d'autres études pourraient être expliqué. Asher et al. (2010) ont en effet trouvé une corrélation négative très forte entre VEP et RT, alors que la relation que nous avons trouvée est très faible. Dans leur tâche, le délai précédent GO était imprédictible : la variable qui aurait le plus expliqué le RT serait un niveau de préparation général, reflété dans le VEP. Cette relation est absente dans nos résultats, car l'animal ne réagit pas au GO mais anticipe son apparition. De plus, la plupart des études étudiant la relation entre RT et taux de décharge montre une différence de pente (Hanes & Schall 1996 ; Lebedev et al. 2008). Dans ces études, le timing du GO n'est pas prédictible, ou le mouvement est initié librement.

Dans ce manuscrit, j'ai donc montré les différentes facettes de l'influence du timing sur la préparation motrice, autant pendant l'anticipation d'un indice visuel que pendant le traitement de l'indice ou l'exécution du mouvement. Connaître le timing d'un indice spatial va permettre son anticipation, et ainsi la mise en place de processus permettant un traitement optimal de l'information qu'il porte tout en empêchant un mouvement d'être déclenché. Pendant l'exécution du mouvement, l'activité corticale motrice va être inversement modulée par le délai attendu et écoulé. Cette sensibilité au délai est vraisemblablement indépendante de la sélectivité directionnelle observée dans le cortex moteur, et possède un décours temporel différent. Elle pourrait refléter la mise en place d'une stratégie motrice ou l'influence de facteurs motivationnels.