The Binding of Reaching and Grasping Movements
in the Parietal Cortex: An Electrophysiological Study

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Abstract

Prehension is a complex, multi-joint arm movement that involves coordinated reaching and grasping. Prehension movements are known to be planned and controlled by multiple brain areas, but little is known about the mechanisms involved in binding together the activity in these areas to produce a smooth and coordinated movement profile. In the present study I trained macaques to perform a delayed prehension task that required them to reach, grasp and hold different objects presented in different directions, and examined simultaneously recorded neural activity in multiple areas within the posterior parietal cortex (PPC). The behavioral task and recording techniques of the present study were specially designed for testing synchrony-based models of neural coding, particularly in the context of across-area coordination.

The first objective of this study was to examine whether reaching and grasping are indeed anatomically and functionally segregated in PPC, as assumed previously. This question is addressed in the first chapter of the Results section. My findings indicate that segregated models of PPC consisting of “reaching areas” vs. “grasping areas” and “direction neurons” vs. “object neurons” are clearly oversimplified. On the one hand, the sensorimotor cortical maps based on passive and active mapping of multi-unit activity indicated only partial segregation of distal and proximal joint representations. In particular, the superior parietal lobule (SPL), expected to be involved in reaching, was characterized by a majority of proximal sites, whereas in the inferior parietal lobule (IPL), expected to be dedicated to grasping, distal sites were more common than in SPL. On the other hand, segregation of direction and object sensitivity, tested in well-isolated single units, was either much weaker or non-existent. A clear functional segregation was also not observed, since a considerable fraction of the single units in both SPL and IPL were tuned to both parameters, and units with complex properties were typically more common than (or at least as common as) units with pure object preference.

The second objective of this study was to examine the possibility that the coordination of prehension movements is achieved by precise synchrony of single units and/or by phase locking of local field potential (LFP) oscillations. Analyses related to this issue are presented in the second and the third chapters of the Results section. Analyses of cross-correlations of single unit pairs in PPC during prehension did not support models of binding by sharp synchronization. Cross-correlation histograms of single units in PPC were generally “flat” and
not characterized by sharp and significant peaks. Some sharp peaks in cross-correlations were observed, but their overall incidence in the population was typically not higher than 1% (the significance level of the test used to identify them). Moreover, fractions of significant correlations, as well as correlation strengths, did not change when monkeys were informed about target direction and object. In-depth investigation of the few pairs with significant correlations did not indicate a clear relation between the properties of the pair members and the behavioral condition in which the significant correlation was observed. Taken together, the characteristics of correlations observed in our data could not serve as evidence for the functional significance of neural correlations. This negative finding is of course insufficient for refuting time code models altogether, yet it can serve as an example of the limitations of applying conservative cross-correlation analysis in similar types of experiments.

Field potential oscillations are an additional focus of interest in the ongoing search for a high level neural code. Gamma-band (40-70 Hz) oscillations in LFP were reported to appear in visual cortex following specific complex stimuli, and it was suggested that coherence between oscillations at spatially separate sites plays a role in the perceptual binding of stimulus attributes. Gamma oscillations in frequency ranges similar to those reported in visual cortex were not a typical phenomenon in our data. A wide-band (30-100 Hz) increase in Gamma-band power was observed throughout the task, and especially during movement. However, I showed that this phenomenon is more likely to merely be a reflection of an increase in multi-unit firing rates, rather than a reflection of an oscillatory generator.

In contrast, I found that bouts of Beta-band (13-30 Hz) oscillations were frequent in LFP of PPC sites, especially during pre-movement delay and/or during post-movement grip. Beta oscillations were most prominent in area MIP. These oscillations were often tuned to target direction and/or object, inconsistent with a general “preparatory” attention state. In addition, the observation of Beta oscillations in PPC is not congruent with their hypothesized role in optimizing motor output, since parietal areas do not directly project to the spinal cord. Interestingly, across-area coherence in the Beta range tended to decrease during the task, despite increases in Beta power. This finding is inconsistent with the hypothesis that across-area coordination is achieved through coherence in the Beta band.

Whereas studies of LFP in visual cortex hint that signs of across-area coordination should be searched for in the Gamma range, I found that coherence of across-area (SPL-IPL) LFP signal
pairs was maximal in lower frequencies (<13 Hz), and that increases in coherence were observed not only during target presentation or movement, but also during pre-movement delay. This was in contrast to the tendency of Beta-range coherence to decrease during delay. Moreover, tuning of across-area coherence during the task was in above-chance fractions only in the lower frequency bands, but not in the Gamma band. These findings suggest that future empirical and theoretical studies of cortical oscillations should not be limited to Gamma-range activity but focus on low-frequency oscillations as well.

The third objective of this study was to compare single unit activity and LFPs with respect to their sensitivity to the independent variables of the task; namely, target direction and object. I found that LFP is very often tuned to target direction or object. This was true both for tuning curves of evoked potentials (EPs) and for tuning curves of LFP power spectrum in different frequency bands. LFP power analysis showed that fractions of significantly tuned LFP sites are similar to fractions of tuned single units, but LFP was inferior to single unit activity in two respects: the signal-to-noise ratio and non-independence of observations from different channels. This was reflected in the tendency of samples of preferred directions computed from LFP tuning curves to deviate from a uniform distribution. In contrast, preferred directions of single unit samples did not show deviation from uniform distributions, consistent with previous literature. Thus these findings indicate two clear disadvantages of the LFP signal with respect to the development of clinical applications that receive a limited number of noisy input signals.

Like LFP power, EPs were frequently tuned to target direction or object, and showed a significant deviation from a uniform distribution of preferred directions. Direction or object tuning of movement-evoked potentials typically emerged only around or after movement onset, whereas activity in multi-unit spikes frequently showed significant tuning before movement onset. Thus, pre-movement processing, which was reflected in the tuning of neurons, was not reflected in the evoked potentials. This finding was especially surprising because many movement-evoked potentials started around 100 ms before movement onset, typically with a positive peak. Thus, early activity observed in the EP may not reflect cortical pre-movement processing, but rather some external input that is less sensitive to target properties. This difference between spike and EP signals is also critical in the context of future clinical devices, since decoding has to focus on predicting movement intention from pre-movement signals. In contrast, pre-movement tuning of LFP was frequently found when
tuning curves were constructed from LFP power. Thus, my results suggest that if LFP is to be used for a brain-machine interface, spectral methods seem more promising than EP-based methods. Analyses of evoked field potentials included LFP data from premotor cortex, recorded by Eran Stark. These additional data gave me the opportunity to test the accepted feed-forward view of information flow in the cortex: from the parietal cortex to the motor cortex. Distributions of latencies of significant visual EPs reported in the current study did not support a model of serial activation of the parietal and premotor cortex, but rather their parallel activation, whereas latencies of motor EPs were in accordance with a premotor-to-parietal flow.
# Table of Contents

**Introduction** .................................................................6-13

**Methods** .................................................................14-23

**Results** .................................................................24-86

1. Mixed representation of reaching and grasping by single units in Posterior Parietal Cortex .................................................................24-42

2. Correlations between single unit pairs in PPC during prehension do not support models of binding by sharp synchronization .........................43-60

3. Direction and object sensitivity revealed in the spectral content of PPC local field potentials during prehension ..................................................60-73

4. Evoked local field potentials in PPC and premotor cortex during prehension do not support feed-forward parietal-premotor activation ..........74-86

**Discussion** .................................................................87-99

**Abbreviation list** ...........................................................100

**References** ...............................................................101-113
Introduction

Prehension: coordinated reaching and grasping

When we reach our hand to take an object, we perceive this as a simple unified act. However, psychophysical, physiological and neurological research suggests that prehension movements are composed of two distinct elements: Reaching - moving the arm towards the object, and Grasping - applying the appropriate forces to grip and manipulate the object (Jeannerod et al., 1995). Reaching movements are mediated mainly by the movements of the more proximal arm joints (shoulder and elbow), whereas grasping involves the more distal joints (wrist and finger joints). For a successful reach, the brain needs information about the position of the object in space (usually visual information) and about the position of the hand (proprioceptive as well as visual). Thus, reaching involves a sensorimotor transformation aimed at matching the coordinates of the hand and those of the target object. Grasping, in contrast, does not depend on the object’s position, but rather on its shape, size and orientation. In humans, grasping of a tool is also affected by the high-level motor programs involved in using it (e.g., monkeys will probably grasp a fork and a pencil in similar ways, whereas humans will use different grasping techniques).

Whereas reaching and grasping are distinct components of prehension, they are not carried out in simple sequential order. Grasping starts with preshaping of the hand (separating the relevant fingers and adjusting the orientation of the hand, using the wrist joint), which is visible when the arm is still reaching towards the target.

Jeannerod (1984) was one of the first to investigate human prehension by studying natural, unconstrained movements. His main finding was that grasping landmarks (such as maximal finger aperture) were highly time locked to landmarks in the reaching profile. Jeannerod concluded that prehension is composed of two parallel but interdependent components. Interdependence of reaching and grasping is a necessary condition for prehension execution, since calculation of the reaching path must be related to the time course of hand preshaping, for example when we make a curved reaching path to avoid obstacles. Indeed, psychophysical studies have shown that sudden perturbations of target parameters (e.g. location, size, orientation) affect the time course of both movement components (Haggard and Wing, 1995). Developmental studies with babies show gradual maturation of motor skills starting with
reaching alone, through grasping that is uncoordinated with reaching, to coordinated prehension (Wimmers et al., 1998).

In contrast to this expected and observed interdependence, studies of monkey physiology and anatomy, supported by imaging studies and neurological syndromes, have placed greater stress on the modular, independent processing of the two components, and have not examined how these components are coordinated.

**Electrophysiology of reaching and grasping**

Neural activity related to reaching has been observed in the primary motor cortex, the dorsal premotor area, area F4 in the ventral premotor area, the supplementary motor area, and a variety of posterior parietal cortex (PPC) areas, mainly in the superior parietal lobule (SPL, Caminiti et al., 1998; Kalaska et al., 1997; Rizzolatti et al., 1997). Georgopoulos et al. (1982) and many others used the center-out reaching paradigm and found cells in these areas that were sensitive to the direction of the movement. The majority of the cells in these "reaching-related" areas were reported to have a non-uniform, typically broad tuning curve, with a preferred direction (PD). In addition, Georgopoulos et al. (1982) reported that a population vector, reconstructed from the activity of such cells, could serve to signal the movement’s direction and velocity with great precision. These findings indicated the involvement of primary motor cortex in computing kinematic parameters (i.e. motion in external space coordinates), rather then dynamic ones (i.e. motion in coordinates of muscle-contraction and joint angles), as implied from Evarts’ (1968) classical studies. However, subsequent studies which dissociated kinematic and dynamic parameters (Alexander and Crutcher, 1990; Kakei, Hoffman, and Strick, 1999; Scott and Kalaska, 1995, 1997) found in primary motor cortex (MI) as well as in the supplementary motor area (SMA) a mixture of “extrinsic” and “muscle-like” cells, and also “extrinsic” cells which modulated their activity and their tuning curves in response to variations in arm posture (an “intrinsic” parameter). Other studies, in which the sensory stimulus was dissociated from the hand trajectory (Shen and Alexander, 1997ab), revealed the presence of “visual” neurons in MI, dorsal premotor cortex (PMd), and SMA: these neurons responded to the visual target’s position and not to the arm trajectory. Attempts to compare cortical areas with respect to activation latencies relative to movement onset (Crutcher & Alexander, 1990; Ashe & Georgopoulos, 1994; Kalaska et al., 1983) revealed between-area differences but also extensive overlap of latency distributions.
Taken together, these studies indicate that each motor area was involved in several levels of the visuomotor coordinate transformation (i.e., sensory, kinematic, and dynamic), and that the notion of serial-hierarchical order of information flow (from PPC to SMA and PMd, and then to MI), suggested by earlier studies (e.g., Wise, 1985), was inaccurate. Several studies reported an interesting gradient in the spatial distributions of cell categories: in the frontal motor areas, “higher-order” or sensory neurons were more prominent in rostral regions whereas “lower order” neurons were more prominent in caudal regions (Alexander and Crutcher, 1990; Johnson et al., 1996; Shen and Alexander, 1997ab). Johnson et al. (1996) found a similar gradient in the medial intraparietal area (MIP), but in the opposite direction. Moreover, they found that parietal and frontal subregions with similar functional properties were strongly interconnected through association fibers. Overall, the extensive study of the reaching movement served as a model for the complexity of the sensorimotor cortex in general. However, apart from challenging earlier models, in which large cortical areas with simple functions (“target location”, “choice selection”, etc.) were serially connected, the current findings still do not seem to converge into a unified theory for the computation of reaching.

In contrast to the elaborate electrophysiological study of reaching, until recently grasping movements have only been studied by a few groups. Neural activity related to grasping was observed in the primary motor cortex (Muir and Lemon, 1983), area F5 in the ventral premotor area (PMv, Murata et al., 1997; Rizzolatti et al., 1988), anterior intraparietal area (AIP) and area 7b in the parietal lobe (Gallese et al., 1994; Murata et al., 1996; Sakata et al., 1995; Taira et al., 1990, Gardner et al., 1999), and the SMA (Kazennikov et al., 1999). Single cells in areas AIP and F5 show similar patterns of activity during preshaping and grasping, and many are tuned to specific grasping motions, such as precision grip (thumb and index) and power grip. Thus, whereas in "reaching-related" areas, many cells tend to have a preferred direction, cells in "grasping-related" areas are reported to have a preferred object (PO).

Murata et al. (1996) studied the response of AIP neurons during grasping of 3D objects in the light/dark, and during fixation. Approximately half of the task-related cells were bimodal (visual and motor). These cells showed an increase in firing rates even when the monkey was supposed to fixate on the object. Both bimodal and motor cells showed significant object shape selectivity. The same group reported similar results from neurons in F5 (Murata et al., 1997). Rizzolatti et al. (1988) found that many F5 cells could be classified into subsets, each
of which increased its firing during a specific phase/s of the grasping. Gallese et al. (1994) and Fogassi et al. (2001) found that reversible inactivation of either F5 or AIP (using muscimol) led to strikingly similar effects: disruption of preshaping and object grip, but no observed deficits in reaching.

Rizzolatti and colleagues (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti and Fadiga, 1998) reported a unique group of neurons, called “mirror neurons”, in area F5. These neurons discharge not only when the monkey performs specific grasping movements, but also when it sees another monkey, or the experimenter, performing the same movement. This discovery led to speculations about the evolution of imitation abilities in primates, and of language in humans (Rizzolatti and Arbib, 1998; Rizzolatti and Fadiga, 1998). Recently, "mirror neurons" were also reported in parietal area AIP (Fogassi et al., 2005).

In contrast to the extensive research on the involvement of motor cortex in reaching, PPC activity related to arm and hand movement has only been the subject of a few studies, conducted by a small number of research groups. These groups have used tasks that involve either reaching alone, or reaching and grasping of several objects, but in the same direction. The labeling of a cortical area as "reaching-related" or "grasping-related" has not involved a formal examination of the sensitivity of neurons in this area to manipulations of both target object and direction. Our setup and task design were constructed to examine the sensitivity of each neuron to both variables: direction and object.

**Anatomical segregation of reaching and grasping**

Recent tracing studies have stressed the existence of multiple segregated parieto-frontal circuits (Luppino & Rizzolatti, 2000). For example, reports of dense connections between F5 and the inferior parietal lobule (IPL, including AIP and Area 7b), but not between these two and their neighboring within-lobe areas, taken together with the physiological findings mentioned above, hint at the possibility of a parieto-frontal "grasping circuit". Similarly, several segregated circuits have been observed ("reaching", "saccades", "head vicinity", etc.). However, in contrast to classical feed-forward views of information flow and transformation from visual through parietal to motor cortex, tracing studies have found an abundance of recurrent connections. The segregation of reaching and grasping is even more conspicuous at the corticospinal level (Dum and Strick, 1991). Further evidence supporting anatomical
segregation comes from recent imaging studies which report dissociation of cortical activation in reaching vs. grasping tasks. For example, Binkofski et al (1998) reported grasping-specific activation in the human homologue of macaque AIP. Finally, there are several neurological reports of dissociations of reaching and grasping lesions (Battaglia-Mayer, 2002; Binkofski et al., 1998; James et al., 2003)

To conclude, while behavioral findings stress the mutual dependence and the coordination of reaching and grasping movements, evidence from physiology, anatomy, imaging and neurology have tended to stress their anatomical and functional segregation. Our main goal in this study was to ask how, if not by strong hard-wired connections, this coordination is accomplished. In fact, the need to coordinate the reaching and grasping components can be viewed as a special case of a general theoretical problem: how are anatomically and functionally distinct cortical modules bound together to produce unified perception and coordinated movement?

**The binding problem and prehension as a model for motor binding**

The distributed nature of brain function and its specializing pathways constitute a theoretical challenge; namely, how to account for unified perception and coordinated movement in the absence of a single "command room". For example, how does the brain associate the shape, color, and location of the same object, which are analyzed by separate visual pathways? Christoph von der Malsburg (1981, 1995, 1999) termed this question “the binding problem”, and highlighted the difficulty of using classical models of neural networks to deal with it successfully. Several solutions (which are not mutually exclusive) have been proposed, among them the “binding by time” hypothesis (von der Malsburg, 1981, 1999), attentional mechanisms such as the “search light hypothesis” (Crick, 1984), and combination coding cells (Barlow, 1972, Shadlen and Movshon, 1999).

Von der Malsburg claimed that a combinatorial problem arises if higher-level cortical cells are to represent - by their firing rate - all possible combinations of simple features represented by the lower-level cells. Instead, the “binding by time” hypothesis suggests that groups of cells (for example, cells representing different features of the same object) are bound to each other by signal synchrony on a time scale of milliseconds, and that the activity in the brain is a series of “microstates”, each defined by the simultaneous activity of a certain set of neurons.
This suggestion is supported by reports of fine temporal structures found within the highly irregular firing patterns of cortical cells (e.g., Abeles et al., 1993; Riehle et al., 1997). Von der Malsburg acknowledged the existence of combination coding cells, at least for basic features, and suggested that temporal binding is used in novel situations and during learning. Singer and Gray (1995) suggested that neural synchrony is achieved through phase-locking of field potential oscillations in the $\gamma$ frequency range. Abeles (1991) suggested that temporal precision of neural ensemble firing is conserved through feed-forward Synfire chains. Thus, this model predicts that precise temporal correlations between single units will also be found in non-zero lags.

Critics of the “binding by time” models (e.g., Ghose and Maunsell, 1999; Shadlen and Movshon, 1999) raised several caveats regarding the plausibility and utility of binding by temporal synchrony, and criticized the empirical evidence supporting it. A central argument was related to the ongoing debate regarding the role of the cortical neuron (Abeles, 1982; König et al., 1996; Shadlen and Newsome, 1994; Softky, 1995). Shadlen and Movshon claimed that synchrony is not a useful neural code, because neurons are unable to distinguish between synchronous and asynchronous inputs. In their view, changes in firing rates and combination coding cells are sufficient for transmitting all perceptual information. Finally, they suggested that binding was accomplished in the posterior parietal cortex. This proposal was based on neurological evidence (Balint’s syndrome) and on findings from single unit recordings (mainly in the lateral intraparietal area, Shadlen and Newsome, 1996).

In recent years the binding debate has grown fashionable, mainly among researchers of the visual system, yet very few attempts have been made to test the suggested hypotheses directly (e.g., Lamme and Spekreijse, 1998, who found no support for binding by synchrony). Apart from the lack of direct empirical evidence for either side, the issue of binding suffers from fuzziness and inconsistency of definitions. In an attempt to solve the latter problem, Treisman (1996) distinguished seven types of binding. The binding of grasping and reaching movements is the motor parallel to what Treisman called “location binding” (i.e., binding a perceived object with its current location, or simply binding “what” with “where”). It is not clear, however, whether the nature of the problem and its solution at the sensory level (i.e., having a unified percept of target direction and object) is the same as the problem and solution at the motor level (i.e., executing a smooth, coordinated movement).
The behavioral task and recording apparatus of the present study were designed for testing synchrony-based models of neural coding. Specifically, I was interested in examining the possibility that correlations and/or oscillatory activity are used for coordination or "binding" cortical populations.

**Local field potential as an additional neural signal**

In recent years there has been growing interest in population measures in the electrophysiological literature. This focus stems from greater attention in research to both the basic study of cell assemblies and the development of clinical applications. Specifically, many groups have started to analyze local field potential (LFP), the slow waves recorded by extracellular microelectrodes used for recording spiking activity (Bullock, 1997). LFP is thought to reflect the summation of synaptic currents from a radius of up to a few mm (Mitzdorf, 1985). Specifically, the LFP signal has been studied by several groups that tested synchrony-based models for binding (Frien & Eckhorn, 2000; Roelfsema et al. 1997). Other groups (e.g., Mehring et al., 2003; Pesaran et al., 2002) compared simultaneously recorded LFP and single unit activity as potential sources for decoding observed behavior. This approach is useful both for the basic understanding of the much less studied LFP signal, and also for future development of brain-machine interfaces.

**Research objectives**

1. **Does the segregation of reaching and grasping indeed hold?**
   Each single unit (or LFP channel) can be tuned to direction, object, both, or none. A classical view of PPC predicts anatomical segregation: neurons recorded at "reaching areas" of the SPL, such as MIP and Area 5, are predicted to be tuned to directions and not (or at least, much less) to objects, whereas neurons at "grasping areas" in rostral IPL, such as AIP and Area 7b, will tend to be tuned to objects and not to directions. In addition, this view predicts functional, within-neuron segregation: a direction-sensitive neuron is unlikely to be object-sensitive, and hence the direction-sensitive and object-sensitive neuron sets are predicted to have little overlap. Alternatively, the "combination coding" view predicts that many neurons in PPC, which is considered a “high-level” area, will be tuned to both variables, and will prefer specific combinations of object and direction. The extreme case of combination coding is
sharp tuning to both variables (e.g., a neuron fires only when a certain object is presented in a certain direction).

2. Are correlations and/or oscillations possible candidates for coordination? Single units are typically tagged as “task-related” or as “coding information” if they change their firing rate in a consistent manner with respect to a stimulus or behavior. Models that assign any functional significance to synchrony of neuron pairs (measured by cross correlation), or to oscillatory activity, predict that these synchrony measures will be task related. Such predictions include, for example: (a) dynamics in the strength of synchrony during the course of the trial; (b) consistent relations between the strength of synchrony and behavioral efficiency; and (c) sensitivity, or tuning, of correlations to target direction and object.

Moreover, models of binding by synchrony predict not only that correlations be tuned, but that their preferred target can be predicted from the properties of the pair of units or channels participating in the correlation (e.g., one unit "prefers" rightward movement, and the other "prefers" power grip, the correlation between them is maximal in the condition of right movement and power-grip). The degree of support for such models depends on the distance between recorded units in a pair. Whereas local correlations can be explained by common input or synaptic connections, correlations over distances, and especially between areas that are known to be sparsely interconnected, are more surprising. Such correlations may indicate the use of synchrony for binding distributed cell assemblies.

3. What are the differences between single unit measures and population measures? On the one hand, population signals may be characterized by higher signal to noise ratios, compared to single unit signals, due to averaging. On the other hand, since population signals do not reflect the activity of segregated functional assemblies, they may be less informative, or less precise, than single unit signals. In the current study I adapted methods of analysis commonly used for single unit data to local field potentials, both in single channel analyses, and in analyses of signal pairs. This approach permitted me to observe the similarities and differences between single unit measures and population measures, and assess whether the latter can be reliably used for decoding of stimuli and movement.
Methods

The following section includes details of the experimental procedures which were used in this study, and the preprocessing steps in data analysis, which are relevant to more than one chapter in the Results section. Each chapter in the Results includes an additional methods subsection that describes data analysis techniques used specifically in that chapter.

Subjects

Two monkeys (Macaca fascicularis, females, D and J, 2.5/3.2 kg, respectively) were used in this study. Both monkeys used the right hand for task performance, and recordings were made from the contralateral hemisphere. All surgical and animal handling procedures were according to the NIH Guide for the Care and Use of Laboratory Animals (1996), complied with Israeli law, and were approved by the Ethics Committee of the Hebrew University.

Behavioral setup

A light and sound isolated chamber was used for training and recordings. Monkeys were brought from the animal house to the recording chamber in a plastic primate chair. The primate chair was positioned in the chamber within a stabilized metal frame so that the workspace center was situated approximately 10 cm in front of the monkey. During training and recording sessions, the left arm was restrained and a barrier prevented the monkey from inserting its legs into the workspace. The head was fixed during recording sessions only.

Monkeys were trained to reach, grasp and hold 3 different objects presented at 6 equally spaced directions in the horizontal plane. All objects required a similar orientation of the shoulder, elbow, and wrist joints when presented in a given direction, but different finger configurations for correct grip. The objects were designed by Eran Stark and built by Moshe Nakar in the fine mechanics workshop of the Physiology Department. Each object was fitted with two micro-switches that only made contact when the object was grasped in the intended manner. Objects were presented by two robotic arms, each of which presented objects at 3 targets (left/right). Two identical copies of each object type (one for each robotic arm) were used. A touch pad was located directly in front of the monkey’s right mid-clavicular line at
chest level, in the center of the workspace, and at the same height as the horizontal target plane. This resting position permitted movements of equal amplitude with minimal elevation changes, in all six directions (6.5/7.5 cm, monkey D/J; different amplitudes were required in order to adapt the setup to different lengths of the monkeys’ arms). The touch pad consisted of 3 buttons with LEDs installed and was 5.5 x 2 cm in size. A horizontal half-mirror was placed between the workspace (touch pad and objects) and the monkey’s head, and prevented eye contact with the workspace during most of the trial sequence. Light sources in the recording chamber were as follows: (1) two 6V DC light bulbs located above the half mirror were constantly turned on during the experiment; (2) during object presentation, a light bulb (24V DC) located below the half mirror was turned on, and made the object visible to the monkey; (3) a similar (24V DC) light bulb was located above the half mirror and was turned on during inter-block intervals. This light served as a cue for a relatively long break, and also as a precaution against dark adaptation. Juice reward was administered through a metal tube.

The behavioral control hardware included two interconnected personal computers. One of the PCs controlled a custom-designed behavioral and data logging hardware and software system (JDAS, Jerusalem Data Acquisition System, developed by Moshe Abeles). This PC was used as a state machine that controlled the trial, block and session sequence, and was responsible for randomization of trials and blocks. In addition, this system controlled reward administration. The other PC directly controlled the robotic arms, the touch pad LEDs, and light conditions, and monitored the behavior of the monkey (state of touch pad buttons and object micro-switches). Software for this system was developed by Eran Stark.

**Behavioral protocol**

A trial started when the monkey pressed a central button on the touch-pad in response to a visual cue (LED of the central button illuminated in green), to indicate that it was ready for a trial. After a variable control period (500-1000 ms), an object was made visible to the monkey for a brief period (200-400 ms) and then obscured, by changing lighting conditions in the recording booth. After a variable delay (1000-1500 ms), a visual Go signal was given (all LEDs illuminated in orange), and the monkey reacted by releasing the touch pad, reaching to grasp the object and holding it gripped in place. Reaction time (from Go signal to movement onset) was limited to 500/1000 ms (monkey D/J), movement time (from movement onset to full grasp) was limited to 1000/2000 ms, and holding of the object was required for a variable
period of 580-620/700-1000 ms. The trial ended when the monkey, upon visual cue, released the object and returned its hand to the touch pad. A drop of juice rewarded correct accomplishment of a trial. Trials were separated by an inter-trial interval of 2-3 seconds, and were arranged in blocks of 15-18 trials per block. In some of the trials, the target object was visible throughout the delay and movement.

During each session, 3 objects were used. For monkey D, one object required a power-grip hand configuration, another required a precision grip, and another was a simple button-press object, requiring reaching only. In some of the sessions a fourth object, grasped by finger opposition, was used instead of the power grip object. Preliminary data analyses revealed that the button-press object evoked considerable activity in cells and muscles (recorded in parallel with premotor cortex recordings) related to distal movements, and hence we could not regard trials with this object as “reaching only” trials. Therefore, for monkey J, the finger opposition object was used in all sessions instead of the reaching object. In order to allow pooling of data from the two monkeys, I focused my analyses on 2 objects per session.

Within block, objects were fixed on the two arms (e.g., power grip on left arm, precision grip on right) and could be of the same or different object-type. Target directions were pseudo-random within a block. During inter-block intervals, objects were switched in one arm, in both arms, or in none of them. Object switching between blocks was pseudo-random so that objects were presented an equal number of times in different directions. Ideally, objects should have been switched during inter-trial intervals. This design was impractical due to the duration of each switching sequence. Thus, within a given block, except for the first trials, the monkey could have memorized the object used in each direction set (right targets vs. left targets), and knew in advance the identity of the target based on its direction (i.e., direction and object were not uncorrelated within a block).

**Monkey training process**

The objective of monkey training was to teach the monkeys to perform the task with high success rates, for long sessions of many trials and with minimal trial-by-trial variability. Monkey training essentially uses basic principles of operant conditioning. The primary drive for learning and execution of the task is thirst, caused by liquid deprivation during the time the monkey spends at its cage. Task accomplishment is rewarded by fruit juice, and good conduct
is rewarded by small pieces of fruit and nuts. Care must be taken to ensure that the monkey does not dehydrate. This was done by daily inspection of the monkey's weight, urine, general appearance and mood, in addition to regular kidney function tests made by the supervising veterinarian.

The first stages of monkey training included acquaintance with the trainer and with the setup. Next, the monkey learned the method of reward application, and associated the reward with a simultaneous beep sound, indicating correct performance (this learning involves classical conditioning). The monkey was taught that its arm movements could produce reward, and learned the visual command signals used during the trial (press/release touch pad). The monkey gradually learned to reach, grasp, and hold various objects (reaching pad, power grip, finger opposition and precision grip), and the concept of delay was introduced. Finally, monkeys were trained to perform prehension in the dark, after a brief presentation of the object, and in sessions involving multiple directions and multiple objects. Apart from the serial complications of the trial sequence, task parameters (e.g., maximal reaction time, delay between presentation and Go signal) were gradually adjusted to allow maximal delay times and minimal reaction and movement times. Overall, training of monkeys took about 15 / 12 months (monkey D/J, respectively, 5-6 training days per week).

Each training period involves times of gradual improvement and times of crisis. Typically, a crisis is prone to appear when a major or "conceptual" change in the task is introduced (e.g., from reaching only to reaching and grasping), or when the rate of parameter adjustment is too fast, and the success rate of the monkey drops. While such crises are part of almost all complex learning processes, the involved frustration (of the monkey, but also of the trainer) may delay and jeopardize the learning process. These situations were more frequent during the training of the first monkey, which progressed in parallel with the development of the setup, and affected task adjustments. On the one hand, building a new setup and a new task required us to use the first monkey as a "test pilot", in order to pinpoint unpredicted design faults. On the other hand, this situation was sometimes a cause for monkey stress and even helplessness. Originally, our task included simultaneous presentation of two candidate targets in each trial, followed by a visual cue indicating a single dimension of the target identity (direction or object). I failed to teach the monkey to perform this task, and was forced to use a simpler, single target, trial sequence.
Surgical procedures

Chamber implant surgeries, which followed monkey training, were performed in aseptic conditions and under general anesthesia (halothane and N₂O, induced by ketamine (3mg/kg) and medetomidine hydrochloride (Domitor, 0.1 mg/kg)). During surgery, the monkey’s head was fixed to a stereotaxic frame and a square piece of bone was excised using an electrical drill from the region overlying the motor and parietal cortex of the left hemisphere. Titanium screws were implanted in adjacent skull locations and a 44mm x 22mm plastic chamber was placed over the drilled hole. The chamber was then fixed to the skull and the titanium screws using dental acrylic. Analgesics (pentazocine (Talwin), carprofen (Rymadil)) and antibiotics (ceftriaxone) were used peri- and postoperatively.

A week before the implant surgery, the monkeys were anesthetized with ketamine-Domitor anesthesia, and water filled glass beads were implanted on the skull surface. Using consecutive coronal MRI images (Biospec Bruker 4.7T animal system, fast spin echo sequence; effective echo time, 80 ms; repetition time, 2.5 sec; 0.5 x 0.5 x 2 mm resolution) we planned the position of the recording chamber relative to the beads. Additional MRI scans were conducted after chamber implantation (both monkeys), and post-mortem (monkey D only).

Approximately every 2-3 weeks, when the dura mater thickened (due to tissue growth), and consequently electrodes broke as they were advanced through it, the external tissue on the dura was scraped. During scraping, the monkey was seated in a primate chair with its head fixed, anesthetized (ketamine-Domitor), and the overlying tissue was scraped using sterile metal instruments. In monkey J, 5-Flurouracil solution (25 mg/ml) was used in order to minimize dural growth and reduce the frequency of scraping surgeries (Spinks et al., 2003). However, this treatment apparently caused parietal and frontal lesions in the monkey. This procedure was therefore abandoned and recordings ceased as soon as behavioral effects were observed.

After completion of experiments, the monkeys were deeply anesthetized (ketamine, pentobarbital (Nembutal, 30mg/kg)), prepared for perfusion, euthanized by an overdose of pentobarbital, and perfused transcardially with buffered saline followed by 4% formaldehyde.
in buffered saline. Prior to perfusion, pins were inserted in defined chamber locations to allow reconstruction of penetration sites relative to cortical landmarks.

**Neural and behavioral recordings**

During each session, up to 16 glass-coated tungsten micro-electrodes (impedance 0.2-2 MΩ at 1 KHz) were individually inserted into two cortical areas in the left hemisphere (EPS 1.31, Alpha-Omega Engineering, Nazareth, Israel). Electrodes were arranged in two circular guide tubes (Double MT, Alpha-Omega Engineering, 1.5mm inner diameter), such that inter-electrode spacing within a circle was ~300µm. The Double MT apparatus allowed independent positioning of the two guide tubes (4 degrees of freedom per guide tube: lateral-medial and anterior-posterior position on cortex surface, lateral-medial and anterior-posterior angles).

Before inserting the electrodes on each daily session, the recording chamber was filled with 4% agarose solution in saline to constrain brain movements due to blood pressure fluctuations. Next, electrodes were individually lowered at steps of a 100-200µm until the brain surface was encountered. Then, a pause of about 15 minutes was taken to allow the brain tissue to reach a stable state with respect to the electrodes. Subsequently, electrodes were lowered in smaller steps (~10-50 µm) until extra-cellular neuronal activity was discerned. Neuronal activity was monitored both by listening to the audio signal from each electrode through headphones, and by viewing it on oscilloscopes. After stable spiking activity was found in all (or most) electrodes, single neuron activity was isolated using the MSD hardware (Alpha Omega Instruments, Nazareth Israel) that detects spikes with a template matching algorithm. Once units have been isolated in each of the electrodes, the behavioral program was activated and the session started. During the session, we continuously monitored the spike signals as detected by the MSD and graded the units according to how distinct their spike shapes were from other neurons recorded by the same electrode. Online-sorted spike times were used only for exploratory purposes. All subsequent spike data analyses were based on spikes that were extracted and sorted offline (see below).

The continuous signal from each electrode was amplified (10k), band-pass filtered (1-10000 Hz), and sampled at 25 kHz (Alpha-Map 5.4, Alpha-Omega Engineering). This signal was used for offline spike and LFP extraction. Behavioral events (e.g., visual cues and touch pad
button configuration) were sampled at 6 kHz. Eye movements were recorded using an infrared beam system (Oculometer, Dr. Bouis, Karlsruhe, Germany) tracking movements of one eye. The 2D signal from this system, as well as the position of the robotic arms, was sampled at 400 Hz. The workspace and monkey’s movements were monitored using 3 infrared CCD video cameras synchronized to the task and recorded on VHS tapes.

**Recording area identification**

Allocation of the recording area of a given site was based on MRI scans, conducted prior to the recording period, and post-mortem (monkey D only). On the basis of MRI scans, extensive mapping sessions were conducted prior to the recording period and after each session. MU activity, evaluated by listening to each of the 16 channel inputs through audio amplifiers, was tested for responses to passive movement of limb joints, light stroking of the skin, and palpation of muscles. Responses to tactile stimulation of the face were tested with eyes covered. Visual receptive fields were examined using a hand-held LED. In addition, MU activity was tested while the monkey reached for and grasped various lab objects, or collected food from a variant of the Kulver board, requiring manipulation of different objects. Mapping results, together with summary information regarding online recorded units, as well as information about the session (penetration coordinates, monkey behavior, etc), were fed to a Microsoft Access database.

Based on sensorimotor mappings, each electrode was tagged by two properties: response modality (motor, tactile, and/or visual) and response organ (shoulder, elbow, wrist, digit, face, eye, and so on). Sulci coordinates derived from MRI scans, together with the results of the mapping sessions were used to determine approximate extent of the relevant recording areas. Rostral IPL recordings focused on locations anterior to the lateral intraparietal area (where saccade-related activity was observed), and posterior to finger areas of primary somatosensory area (Murata et al., 1996). SPL recordings focused on locations posterior to arm representations of primary somatosensory area, and lateral to the post-central dimple (Figure 1.1). The depth of each recording site relative to the surface of the cortex was used to determine whether the electrode tip was recording from a gyrus area (Area 5 and Area 7b) or from a peri-sulcal area (MIP and AIP). Relative depths >2.5 mm were considered as recordings from a sulcus bank area, provided their location matched a sulcus vicinity based on
the MRI scans. This criterion was based on typical macaque cortex thickness and on depth data collected during mapping and recording sessions in gyri areas.

**Neural data pre-processing**

The recorded continuous signal was cleaned of 50 Hz noise caused by the AC power line, using pulse-triggered averaging. Line cycles were recorded using one bit, and the line-triggered average over 1000 cycles was subtracted from the original signal. The line-triggered average was adapted to possible changes by gradual modification such that

\[
\bar{X}_{i,j} = 0.999 \cdot \bar{X}_{i-1,j} + 0.001 \cdot X_{i,j}
\]

where \( X_{i,j} \) is the j-th sample of the raw signal in the i-th cycle. Subsequent spike and LFP extraction used the line-cleaned signal.

**Spike data preprocessing**

Spikes were detected by a modified second derivative algorithm (7 samples backwards and 11 forward), accentuating spiky features; segments that crossed an adaptive threshold were identified. Within each segment, a potential spike peak was defined as the time of the maximal derivative. If a sharper spike was not encountered within 1.2ms, 64 samples (10 before peak and 53 after) were registered. Principal component analysis-based software (Alpha-Sort 4.0, Alpha-Omega Engineering) was used to sort offline extracted spikes to single units (Abeles and Goldstein 1977). Separation quality of sorted units was graded by ISI histograms, individual spike shapes, and cluster overlap. Spike trains were down-sampled to 1 ms resolution and cut to include only correctly performed trials (4 sec before to 2 sec after Go signal in monkey D, 5 sec before to 3.5 sec after Go signal in monkey J), and sorted by behavioral condition. This step used the "Cut Engine" software programmed by Itay Gat. Next, long-term trial-to-trial stationarity of the responses of each unit was determined by an algorithm based on a time-varying Poisson counting process, devised by Eran Stark, and validated by visual inspection of raster plots. Only well isolated units that had at least 5 stationary trials per behavioral condition (12 behaviors) were included in single unit analyses.

Spike trains of stable trials were realigned and cut by timing of behavioral events into seven 400 ms long epochs:
1. The *Control* epoch (CE) was defined as 100 ms to 500 ms after trial start. During this epoch, the monkey’s hand pressed the central button of the touch pad, and the monkey had no information on the behavioral condition of the trial.

2. The *Signal* epoch was defined as 50 to 450 ms after object presentation. During this epoch, the monkey’s hand pressed the central button of the touch pad, and the monkey had visual information on the behavioral condition of the trial. Note that objects were presented for a variable period, always shorter than 450 ms. Hence eye contact with the object was available only for a certain fraction of the epoch (uniformly distributed between 37.5% and 87.5%).

3. The *Set1* epoch was defined as 450 to 850 ms after object presentation. During this epoch, the monkey’s hand continued to press the central button of the touch pad. During most trials, the target was not visible to the monkey, but could be stored in working memory. During 20% of the trials, the object was also visible during delay.

4. The *Set2* epoch was defined as 850 to 1250 ms after object presentation. All behavior and stimulus conditions were the same as for *Set1*.

5. The *PreGo* epoch was defined as 400 to 0 ms before the Go signal. All behavior and stimulus conditions were the same as for *Set1*. On average, there was a 100 ms overlap between this epoch and epoch *Set2*. Hereafter, the latter three epochs (*Set1*, *Set2*, and *PreGo*) are often referred to as “Delay epochs”.

6. The *Reaction and Movement time (RTMT)* epoch was defined as 150 ms before to 250 ms after movement onset (defined as touch pad release). Due to quick responses of monkey D, it was not possible to analyze activity during reaction time and during movement separately.

7. The *Hold* epoch was defined as 100 to 500 ms after object grasp. During this epoch, the monkey’s hand constantly gripped the object, with minimal movement of proximal joints.

Comparison of analysis results across different epochs required a common duration for all epochs. The 400 ms duration of the analysis window was chosen as a compromise between time resolution and stationarity on the one hand, and signal to noise, on the other. While the epochs represent different behaviorally defined states of the task, they do not imply within-trial firing rate stationarity. For example, many units tended to gradually increase their firing rates during the delay epochs. The RTMT epoch produced even more extreme and rapid firing rate increases or decreases.
Multi-unit (MU) spike data for each electrode were obtained by summing over the activity of all good quality spikes extracted offline. Quality was judged by an error measure between the original spike shape and its PC reconstruction (I used a threshold of 1). These MU spike trains are a more localized measure of site activation than common measurements of MU activity obtained by high-pass filtering of raw analog data. Long-term trial-to-trial stationarity of MU data was determined using the same procedure as for single units, and analysis was restricted to stationary sections, realigned and cut to behavioral epochs.

**LFP data preprocessing**

The line-cleaned signal was band-pass filtered (1-100 Hz, 2-pole Butterworth) and down-sampled to 500Hz. LFP traces were cut to include only correctly performed trials and sorted by behavioral condition (“Cut Engine”). Next, trials were realigned and cut by behavioral epochs, as described above for single units. Outlying trials (within epoch) were detected and removed using a moving average of the RMS (window length = 10 ms; ±2.58 standard deviations threshold). Exploratory analysis of LFP data revealed that outlier detection was enough to obtain trial-to-trial stationarity in the LFP data, in contrast to single unit stationarity. Outlying trials constituted less than 3% of all recorded trials, and for a given behavior, the number of rejected trials was typically 0 (59% of 345 channels times 12 behaviors). Only channels with at least 5 stationary LFP trials per behavioral condition (12 behaviors), and in which spiking activity was recorded (multi unit activity with minimal average rate of 1Hz over the whole trial), were included in analyses.
Results

1. Mixed representation of reaching and grasping by single units in Posterior Parietal Cortex

My first research objective in this study was to examine directly whether the cortical representation of reaching and grasping in PPC is indeed anatomically and functionally segregated, as implied by the current literature. I addressed this question at two resolution levels: the cortical map level, using results of sensorimotor mapping, and the single neuron level. Segregation at the cortical map level predicts that the "reaching areas" will be activated mainly during active and passive movements of proximal joints, whereas the "grasping areas" will be activated during active and passive movements of distal joints. At the single neuron level, anatomical-functional segregation predicts that neurons recorded at "reaching areas" will tend to be tuned to directions and not (or at least, more than) to objects, whereas neurons at "grasping areas" will tend to be tuned to objects and not to directions. However, involvement in proximal movements does not necessarily imply directional tuning, and involvement in distal movements does not necessarily imply object specificity, as other movement parameters are likely to be represented by the neurons involved. Therefore, these two predictions are a-priori independent, and rejection of one does not necessarily lead to the rejection of the other.

My results show that sensorimotor cortical maps clearly indicated anatomical segregation of distal and proximal joint representations. This effect was stronger in SPL than in IPL. Differences between areas with respect to direction and object sensitivity of single units were much weaker, but still, in most cases, significant in the expected direction. A considerable fraction of the single units in SPL and IPL showed complex properties, either with or without interaction effects. Moreover, units with complex properties were typically more common than (or at least as common as) units with pure object preference. These findings run counter a simplistic anatomical-functional dichotomy of reaching areas vs. grasping areas.

Methods
Sensorimotor mapping sessions were conducted at the end of each recording session (see general Methods chapter). Each recording penetration was tagged by two properties: response modality (e.g., motor, tactile, visual, or combinations of these) and response organ (shoulder, elbow, wrist, digit, face, eye, and so on). Standard $\chi^2$ tests were used to test differences between distributions of modalities and organs in the areas of interest.

For the purpose of spike count analyses, spike trains of stable trials were realigned and cut into seven 400 ms-long behavioral epochs (see general Methods chapter). In order to examine whether PPC units change their firing rate during the prehension task, spike counts during 6 task epochs were compared with the counts during the Control epoch. For each unit, both two-way analyses (with condition and epoch as independent variables) and one-way analyses (with data from all conditions pooled together), followed by pair-wise planned comparisons, were applied. Next, each unit was examined using a two-way test for direction, object and interaction effects, separately for each behavioral epoch. Non-parametric (Kruskal-Wallis and Mann-Whitney) tests were used instead of the more conventional ANOVA and t-test, because assumptions of normality and equality of variances were often violated in the spike count data. Population analysis (e.g., comparison between cortical areas) used $\chi^2$ tests.

Apart from performing a statistical test for direction and object effects, it is important to estimate the sizes of each effect. This is because units can be sensitive to more than one parameter, but to variable degrees. Furthermore, it might be presumed that “reaching areas” and “grasping areas” have similar fractions of directionally tuned (or object sensitive) units, but that the signal-to-noise ratios are much higher in units of “reaching areas” than in “grasping areas”. I used the $\eta^2$ effect size measure (Fisher, 1925), defined as

$$\eta^2 = \frac{SSe}{SSt}$$

where SSe is the sum of squares of a given effect (direction, object, or interaction), obtained from the ANOVA table, and SSt is the total sum of squares, which includes the 3 effect sums of squares, and the error sum of squares (trial-by-trial variability unrelated to any effect). $\eta^2$ can be considered as a signal-to-noise measure. It is additive for different effects, and bounded between 0 and 1. The use of effect size measure serves to quantify the degree to which each
unit is more “direction-related” or more “object-related” on a single continuum. This is achieved by a direction-object index (DOI) defined as the ratio

\[
\frac{\eta^2_d - \eta^2_o}{\eta^2_d + \eta^2_o}
\]

This index is bounded between -1 and 1. For random normal noise, when both direction and object have the same number of degrees of freedom (i.e., same number of directions and objects, same number of trials per combination), this index displays a symmetrical U-shaped distribution centered at around 0. However, in the current experiment the direction effect has 5 degrees of freedom, and the object effect has only 1 degree of freedom. Under such conditions the DOI index is heavily biased towards positive values (for simulated normally distributed noise, mean 0.67±0.02, for 300 trials). Since this bias cannot be accurately compensated for, I computed the DOI twice: using all data, and using data from only 2 reaching directions (right and left). Results of single unit and correlation analyses were not sensitive to the choice of DOI.

Results

1.1 Neuronal database

Out of 1086 PPC single units that passed the inclusion criteria, 810 were identified by anatomical coordinates as belonging to the four areas of interest, and were considered for further analysis. These units were recorded in 346 penetrations (39 recording sessions). Table 1.1 shows the distribution of these units and penetrations by monkeys and cortical areas. In the following subsections, population analyses compare pooled data of SPL (MIP and Area 5, so-called “reaching-related” areas) vs. IPL (AIP and Area 7b, so-called “grasping-related” areas). Some differences between gyri areas (Areas 5 and 7b) and sulci areas (MIP and AIP) were observed, but these did not change the major findings presented below. These differences are presented and discussed separately in the final subsection.

1.2 Sensorimotor cortical maps indicate substantial but incomplete segregation of distal and proximal representations

Data collected during the sensorimotor mapping procedure conducted at the end of
each recording session were used to test the segregation at the cortical map level. Figure 1.1 presents cortical maps of penetrations in the two monkeys. Only sites that were used for single unit analysis were included. As expected, SPL was characterized by a majority (66%) of proximal sites. In IPL distal sites were more common than in SPL, but did not constitute a majority (40% in AIP, 25.7% in Area 7b, compared to 5.3% in SPL). Differences between SPL and IPL were significant ($\chi^2$ test). Interestingly, proximal and distal sites within the IPL were not uniformly distributed. Rather, proximal sites tended to be located caudally, adjacent to area LIP, where saccade-related activity was found, whereas distal sites tended to be located in rostral parts of AIP and Area 7b. This observation is in line with a previous report (Ferrari et al., 2003, Soc. Neurosc. Abstracts). Thus, results of sensorimotor mapping generally support classical views of PPC, which assume that representations of proximal and distal joints are anatomically segregated.

Table 1.1: Numbers of single units (penetrations) in the database, by monkey and recording area

<table>
<thead>
<tr>
<th>Monkey / Area</th>
<th>SPL MIP</th>
<th>SPL Area 5</th>
<th>IPL AIP</th>
<th>IPL Area 7b</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey D</td>
<td>183 (82)</td>
<td>195 (80)</td>
<td>171 (76)</td>
<td>49 (29)</td>
<td>598 (266)</td>
</tr>
<tr>
<td>Monkey J</td>
<td>39 (15)</td>
<td>80 (29)</td>
<td>78 (29)</td>
<td>15 (7)</td>
<td>212 (80)</td>
</tr>
<tr>
<td>Total</td>
<td>222 (97)</td>
<td>275 (109)</td>
<td>249 (105)</td>
<td>64 (35)</td>
<td>810 (346)</td>
</tr>
</tbody>
</table>

1.3 Set-related activity is slightly more common in SPL, whereas peri-motor activity is slightly more common in IPL

Analysis of neural data concentrated on multiple behavioral epochs. This approach allowed some degree of temporal resolution in data analysis and reduction of firing rate variability that was due to variability in stimuli or behavior. However, as an exploratory measure, it was important to examine whether neurons indeed changed their firing rates during the task, and whether all behavioral epochs were equally relevant for data analysis. In the extreme theoretical case, it is clearly unjustified to compare cortical areas with respect to their direction and object selectivity properties during an epoch in which one of the areas is typically inactive (or, more precisely, active at baseline levels).
For this purpose, the effect of behavioral epoch on the spike counts of single units was tested (Kruskal-Wallis test). A main effect of epoch was observed in 94.8/95.6% out of 810 units (one- or two-way test, respectively). Planned comparisons were used to test changes of firing rate in each epoch relative to baseline (2-tailed Mann-Whitney test, data from all behaviors pooled together). Fractions of units that changed their rate relative to baseline were maximal during movement (RTMT epoch), yet changes in firing rate were often observed in pre-movement epochs (Figure 1.2). Visual-related activity was about equally common in SPL and in IPL. Set-related activity was more common in SPL, and movement-related activity was more common in IPL (binomial test). Most of the units which showed a significant change in
firing rate tended to *increase* their firing. This effect was most conspicuous during the RTMT epoch and least conspicuous during the Hold epoch, where rate decreases relative to baseline were most common (but still the minority), especially in SPL.

According to the planned comparisons, each unit could be tagged as ‘visual-related’ ‘set-related’ and/or ‘motor-related’ (Figure 1.3). Although most of the units did not change their firing rate during all task epochs, the probability of a unit to display visual-related activity was not independent of the probability to display movement-related activity ($\chi^2$ test for independence). Rather, the probability of observing both responses was higher than expected from the product of the probabilities to observe each. Similarly, set-related activity was not independent from either visual- or movement-related activity. These effects were consistent across areas.

In sum, PPC single units displayed complex rate patterns, characteristic of visuomotor areas, and large fractions of the units changed their firing rate during multiple epochs. However, observed differences between SPL units and IPL units may affect a-priori probabilities to observe direction or object sensitivity, due to “floor effects”. Specifically, SPL units are more prone to be sensitive to *either* direction or object during delay epochs, whereas IPL units are more prone to be sensitive to *either* direction or object during RTMT and Hold epochs.

### 1.4 Directionally tuned units and object sensitive units are found in reaching-related as well as in grasping-related areas of PPC

According to existing electrophysiological literature, neurons in SPL are expected to show selectivity mainly to target direction, whereas neurons in rostral IPL are expected to show selectivity mainly to target object. Figure 1.4 shows examples of two such neurons. The unit in Fig. 1.4 A, recorded in area MIP, shows clear sensitivity to target direction, but not to target object. Maximal activity was evoked by leftward movements towards both objects, after target presentation and immediately prior to movement onset. The unit in Figure 1.4 B, recorded in area AIP, shows practically no sensitivity to target direction, but shows elevated firing during movement towards and grasping of the power grip object (right), and inhibited firing during precision grip trials (left). However, many PPC units had preferences that were different or even opposite to those expected by their anatomical location. For example, units in AIP were
sensitive to direction but not to object (Fig. 1.5 A), and units in MIP were sensitive to object but not to direction (Fig. 1.5 B).

![Graph showing fraction of units with significant rate change with respect to baseline, as a function of behavioral epoch.](image1.png)

**Figure 1.2:** Fractions of units with significant rate change with respect to baseline, as a function of behavioral epoch. Error bars are binomial confidence intervals (α=0.01). White: rate increase relative to baseline; gray: rate decrease relative to baseline. Sig: Signal epoch, PG: Pre-Go epoch.

![Venn diagram depicting numbers of 'Visual', 'Set' and/or 'Motor' units](image2.png)

**Figure 1.3:** PPC units tend to change their rate with respect to baseline during multiple epochs. Venn diagram depicting numbers of ‘Visual’, ‘Set’ and/or ‘Motor’ units, for all cortical areas (total N=810). Units that changed their rate during the Signal epoch were labeled ‘Visual’; Units that changed their rate during the Set 2 epoch were labeled ‘Set-related’; Units that changed their rate during the RTMT epoch were labeled ‘Motor’. Numbers indicate numbers of units. Numbers in parentheses indicate numbers expected if the probabilities of each unit to belong to each class were independent.
A. Directional tuning

B. Object preference

Figure 1.4: Examples of single units with simple effects predicted by anatomy. Directional tuning without object preference in an MIP unit (A) and object preference without directional tuning in an MIP unit (B). Each set of 6 raster plots and peri-event-time histograms shows the response of one unit in trials of 6 directions and one object (left: power grip, right: precision grip). Vertical line marks movement onset.

Directional tuning was observed in 30-60% of the units during the task epochs (Fig. 1.6). Object sensitive units were generally less frequent than directionally tuned units, regardless of anatomical location. Units with directional tuning were more frequent in SPL than in IPL (binomial test, all epochs except CE). In contrast, units with object preference were more frequent in IPL areas during the RTMT and Hold epochs, but less frequent during the Set1 and Set2 epochs. Fractions of direction or object sensitive units in both SPL and IPL during CE were not higher than chance levels (binomial test). Comparison of effect sizes showed a similar pattern: direction effect sizes of directionally tuned units in SPL were higher than in IPL (Mann-Whitney test, all epochs except for Hold epoch), whereas object effect sizes in object sensitive units were similar in SPL and IPL, except for the Hold epoch, where IPL units had stronger effect sizes (Fig. 1.6 C-D).
Since sensorimotor mapping results do not show perfect anatomical segregation, it is also important to test for differences between samples of ‘proximal’ and ‘distal’ units, according to the labeling of the recording site by sensorimotor mapping. Specifically, it could be claimed that apparent similarities between populations of SPL and IPL units stem from the inclusion of many ‘proximal’ units in the IPL sample (Fig. 1.1), and that “true grasping areas” in PPC constitute only a limited portion of rostral IPL (Ferrari et al., 2003, Soc. Neurosc. Abstracts). I therefore repeated the test, this time contrasting populations of units classified by the properties of their recording site (N = 411/150 ‘proximal’ vs. ‘distal’ units, respectively, 80% of the units in each class were recorded in the ‘expected’ location). Fractions of direction sensitive and object sensitive units in the two classes were almost identical to those obtained by the anatomical grouping.

Figure 1.5: Examples of single units with simple effects not predicted by anatomy
Direction tuning without object preference in an AIP unit (A) and object preference without directional tuning observed in an MIP unit (B). Conventions are as in Fig. 1.4.
In sum, analysis of direction and object effects in single units either did not support anatomical segregation predictions (Set1 and Set2 epochs, object sensitivity during Signal and PreGo epochs), or weakly supported them (RTMT and Hold epochs, directional tuning during Signal and PreGo epochs). This is in contrast to the clearer anatomical segregation of proximal and distal sites observed in sensorimotor mapping. A possible explanation for this difference between the single-unit properties and the population activity observed during sensorimotor mapping is the functional-spatial organization of single units (Ben-Shaul et al., 2003). Stark et al. (submitted) found in premotor cortex a striking similarity between proximal and distal recording sites (typically labeled by ICMS) with respect to directional tuning and object sensitivity. Stark et al. suggested that if single units in proximal sites are spatially organized with respect to their directional preferences (i.e., nearby units have similar PDs) whereas

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**Figure 1.6:** Fractions of units with significant directional tuning (A) and object preference (B) in SPL units (solid line, N=497) and IPL units (dashed line, N=313), as a function of behavioral epoch. Error bars are binomial confidence intervals. (C) Average direction effect sizes ($\eta^2_d$) of directionally tuned units. (D) Average object effect sizes ($\eta^2_o$) of object sensitive units. Error bars are standard error confidence intervals ($\alpha=0.01$). Epoch abbreviations are as in Figure 1.2.
single units in distal sites are spatially organized with respect to their object preferences, then threshold-stimulation in proximal sites will yield movement of proximal joints, and threshold-stimulation of distal sites will yield movement of distal joints.

Following this line of reasoning I examined SUs in PPC for evidence supporting spatial organization of PDs. Three predictions arise from this possible explanation for differences between single unit properties and mapping results. First, within-electrode unit pairs should have smaller PD differences than across-electrode unit pairs. Second, this effect should only be observed in proximal sites of SPL (a “reaching-area”) rather than in distal sites of IPL (a “grasping area”). Thus, I also expected within-electrode PD differences to be smaller in SPL than in IPL. Figure 1.7 shows pair-wise PD differences of within-electrode and across-electrode SUs in SPL (A) and in IPL (B). Within-electrode unit pairs tended to have slightly smaller PD differences than across-electrode pairs in SPL (all epochs, Mann-Whitney test). This result replicates the findings reported by Ben-Shaul et al. (2003) based on recordings in motor cortex, mostly in proximal sites. In contrast, this tendency was observed only during the RTMT epoch in IPL. Within-electrode PD differences were smaller in SPL than in IPL during the Signal and PreGo epochs. Average across-electrode PD differences in SPL were only slightly smaller than 90 degrees (the expected average assuming a uniform distribution of PD differences) in all epochs but Hold, and not smaller than 90 degrees in IPL in any of the task epochs.

**Figure 1.7:** Mean differences in PDs of single unit pairs, as a function of task epoch. (A) SPL proximal sites (B) IPL distal sites. Solid lines are average PD differences of SU pairs from the same electrode. Dashed lines are average PD differences of across-electrodes SU pairs. For each epoch, only pairs in which both units had significant directional tuning were considered. Error bars are SEM-based confidence intervals ($\alpha=0.01$). Note that under the null hypothesis of independence of PDs, differences are expected to distribute uniformly between 0 and 180 degrees with a mean of 90 degrees (horizontal dashed lines).
1.5 Why are single units in PPC more prone to show directional tuning than object sensitivity? Several possible explanations

Generally, the probability of a unit to be tagged as directionally tuned was much higher than its probability to be object sensitive, regardless of anatomical location (Fig. 1.6 A-B, 47% vs. 12.7% out of all 810 units, fractions are averages over task epochs). Specifically, even in the so-called “grasping-related” areas, the probability of a single unit to be directionally tuned was higher than its probability to be object-sensitive (all task epochs, except Hold). Moreover, direction effect sizes in directionally tuned units were generally higher than object effect sizes in object sensitive units (Fig. 1.6 C-D). An interesting interpretation of this result is that directional information is better represented in the activity of PPC units than object information. This interpretation fits the well-documented observation that representations of proximal joints occupy larger areas of the parietal and frontal cortical surface than representations of distal joints (although distal limb parts are still over-represented in cortical maps compared to their relative sizes). It could further be speculated that larger cortical areas are related to reaching because it is more important for the primate to plan reach parameters than grasp parameters, since the latter are easier to modify on-line, when tactile information is available.

However, there are two alternative explanations to this bias towards directional tuning. First, the activity of single units may have been modified by confounding variables that were correlated with reach direction but not with object type. For example, we found that eye position during the task depended, as expected, on target direction, but was not sensitive to target object. If many units in PPC were sensitive not only to target direction, but also (or only) to eye position (Buneo et al., 2002), this could explain why more units were tuned to target direction than to the identity of the object. Moreover, if units in “reaching areas” are more prone to be sensitive to eye position than units in “grasping areas”, across-area differences in directional tuning probably stem from confounding with eye position. I used linear regression to identify units tuned to eye position. About 10% of the units showed significant relation to eye position during each epoch, but differences between cortical areas were not significant (13.48% vs. 13.74% during CE, 8.75% vs. 9.0% during the task epochs). Fractions of direction or object sensitive units, when eye position was used as an additional explaining variable in a multiple regression model, were not different from those found using the 2-way analysis. Similar results were observed when reaction or movement times were
considered as potential confounding variables.

Second, the bias in favor of directional tuning could stem from task design. In the present experiment, 6 directions and only 2 (or 3) objects were used in each session, whereas reaching experiments typically involve a uniform sampling of 6 or 8 directions, and grasping experiments (e.g., Murata et al., 2000) typically also sample a similar number of different objects. Therefore, our design created a statistical bias favoring directional tuning effects. A partial solution to this bias is by post-hoc “dilution” of the data by inspection of trials from 4 conditions only (2 directions (right and left) x 2 objects), and repeating the two-way analysis. Interestingly, fractions of directionally tuned units, as well as those of object sensitive units decreased by a factor of 2 (24% / 6.3%, respectively, averaged over all task epochs), but the differences between SPL and IPL were preserved. Moreover, even in this case directional sensitivity was almost four times more common than object sensitivity, and direction effect sizes were higher than object effect sizes.

However, this analysis still cannot compensate for bias caused by the non-trivial dimensionality of grasp types and by monkey training. First, we cannot assume uniform sampling of the object space by any 2 objects used in a session. A uniform sampling of the parameter space is optimal in any given sampling resolution. Uniform sampling is well-defined for reaching directions, but is not a trivial issue for grasp types (Santello et al., 1998). Thus, rightward and leftward movements are opposite reaching directions, but precision grip cannot be considered the opposite of power grip (or of finger opposition, used in some sessions). Second, task training involved 6 reaching directions and only 3 objects, and it could be claimed that training caused over-representation of direction, which does not reflect the situation in the cortex of a naïve monkey. Therefore, we cannot conclude whether directional information is indeed better represented in the studied cortical areas than object information.

1.6 Complex unit properties and interaction effects are quite common

In the previous sections, cortical areas were compared with respect to fractions of units with direction effect and fractions of units with object effect. This may create a false impression of an existing functional segregation: i.e., there are “direction units” and “object units” in the cortex, and it only remains to see whether, or to what degree, there is also anatomical segregation between the two sets. Functional segregation was tested directly using the two-
way nonparametric analysis of variance. This method tests three independent effects in each unit: direction, object, and interaction between the two factors. Thus, units can be categorized into eight exclusive classes: units with no significant effect, units with a single significant effect (direction effect, object effect, or interaction), or with combinations of 2 or 3 effects. If functional segregation indeed holds, we expect to find in our database mostly ‘simple’ units that are sensitive to one parameter (either direction or object), but very few ‘complex’ units that are sensitive to both parameters, and/or show interaction effects.

All theoretically possible units with complex properties were indeed observed in our data (Fig. 1.8). Various combinations of main effect and interaction effects essentially express several types of differences between directional tuning curves of the same unit with 2 target objects. Tuning curves can be different in their shape (e.g., cosine, von-Mises, bimodal), their PD (direction of peak), the mean response over directions, and their modulation depth. For example, two non-flat tuning curves, which differ only in the mean activity over directions, produce direction and object effects without interaction (Fig. 1.8 A). Two non-flat tuning curves, which differ only in their PDs, may produce direction and interaction effects, or interaction alone, depending on the PD difference (Fig. 1.8 B and C). All three effects may be significant in units which showed no response to one object, regardless of direction, but directionally tuned response to the other object (Fig. 1.8 E and F). In some cases, response was limited to only one combination of direction and object (Fig. 1.8 F). Tuning curves of the unit in Figure 1.8 G differ in both mean response and modulation depth, whereas tuning curves of the unit in Figure 1.8H differ in mean response, modulation depth and PD.

Figure 1.9 summarizes the fractions of units with various combinations of direction, object, and interaction effects in the different epochs. For purposes of statistical comparison, all classes with at least an interaction effect (with or without direction or object effects) were pooled. Area differences were significant in all epochs except for the Signal epoch ($\chi^2$ test), and maximal during the Hold and Set1 epochs. Single units with pure object effect were relatively rare, except for the Hold epoch in IPL. Instead, most units which showed an object effect also showed a direction and/or interaction effect, and interaction effects were as common as object effects. This pattern is very different from that predicted by a functional segregation model, and implies that it is improper to simply label single units as “object units” or “direction units”. The next section deals with quantification of the degree to which units are related more to direction or to object.
1.7 SPL and IPL have similar distributions of direction vs. object sensitivity

The findings presented above indicate that single unit data do not support simplistic notions of “reaching areas” vs. “grasping areas” (implying functional *and* anatomical segregation), and furthermore that they do not support the notions of “direction units” vs. “object units” (implying functional segregation). However, if effect sizes of direction and object sensitivity can be quantified, one can estimate the degree to which each unit is more direction-sensitive or more object sensitive. For this purpose we used the DOI (see Methods). By construction, units with positive DOI values are more direction sensitive than object sensitive, and units
with negative DOI values are more object sensitive than direction sensitive. This labeling is useful for comparing samples of units recorded in different cortical areas. For example, one may hypothesize that in “reaching areas” the DOI distribution will have more positive values than the DOI distribution in “grasping areas”. Such labeling is also useful for pair-wise analyses (Results, chapter 2). For example, one may hypothesize that the degree of correlation between any 2 units is related to the similarity of their DOIs.

Figure 1.9: Distributions of direction, object, and interaction effects in units recorded from SPL (left, N=497) vs. IPL (right, N=313). Each pie chart relates to one behavioral epoch. Note the relative dominance of pure direction effects and the paucity of pure object effects, except for the Hold epoch in IPL.
Figure 1.10 presents distributions of DOIs for SPL and IPL units for the 6 task epochs. For this figure, DOIs were computed from trials including only rightward and leftward movements, and 2 objects. This figure shows the tendency of single units in PPC to have stronger direction effects than object effects, even when the number of directions and the number of objects used is equal (see section 1.5). Differences between areas were generally non significant (Mann-Whitney test, except for the Hold epoch). When all trials were included (not shown), DOIs had modes in values around +1, and almost no negative values. Similar distributions SPL and IPL, except for the Hold period, were observed also when all data from all trials were used. Therefore, labeling used for correlation analyses (Results, Chapter 2) was based on DOIs computed from rightward and leftward movements alone, together with a condition on existence of significant direction and/or object effects. To conclude, distributions of direction vs. object sensitivity, quantified by the DOI, were generally similar for SPL and IPL (except for the Hold epoch), and tended to be biased towards direction effects.

Figure 1.10: Direction-Object index (DOI) distributions in IPL and SPL largely overlap. Each subplot displays distributions of DOIs for IPL (red) and SPL (blue) units, obtained from analysis of data from 2 directions (right and left) and 2 objects, in 6 task epochs. Vertical solid lines indicate medians for each set.

1.8 Sulci areas are more “visuo-motor” than gyri areas

Up to this point, so-called “reaching related” areas of the SPL were compared with “grasping related” areas of the IPL. In this section, I report differences observed within these lobules, namely between MIP and area 5, and between AIP and area 7b. These differences are not directly related to the main objectives of this study, but contribute to characterization of PPC areas. The major finding, which was consistent in SPL and IPL, was that both sensorimotor
mapping and SU activity indicated greater involvement of peri-IPS areas MIP and AIP in visuomotor processing, relative to the neighboring Area 5 and Area 7b. During sensorimotor mapping, each recording site was labeled as Motor, Visual, Somatosensory, and or Oculomotor (Fig. 1.11), in addition to the organ labeling (proximal, distal, etc., Fig. 1.1). In all 4 cortical areas, over 50% of the sites were tagged as “Motor” or “Motor-Visual” according to somatosensory mapping. Approximately 20% of the sites in AIP and Area 5 were labeled “Motor-Somatosensory”, and 11% of the sites in AIP and Area 7b were tri-modal. In contrast, pure somatosensory or visual responses were relatively rare (0-5%). Between-area differences were significant ($\chi^2$ test). Specifically, visuomotor responses were more common in sulci areas (MIP and AIP) relative to gyri areas (Areas 5 and 7b), where pure motor responses were more common. This finding is in line with previous reports (Colby & Duhamel, 1996; Taira et al., 1990; Buneo et al., 2002).

Visual-related activity was also observed in responses of single units (Fig. 1.2). In contrast to mapping results, fractions of units with significant rate change in the Signal epoch compared to baseline rates were similar in sulci and in gyri areas (binomial test). However, by analyzing units’ responses in trials where prehension was accomplished with visual feedback, area differences emerged. During 35 out of 39 of sessions, the prehension task included standard trials and additional trials in which eye contact with the target object was available throughout the delay and prehension epochs. This type of trials was used to enable another formal test of visual related activity, in addition to contrasting activity during the Control epoch with activity...
during the Signal epoch (Murata et al., 1996). Light sensitivity was examined separately for each behavioral epoch, when trials from all directions and objects were pooled (2-tailed Mann-Whitney test). Statistics of light sensitive units highlighted the difference between the two “reaching related areas”, Area 5 and MIP (Figure 1.12).

![Graphs showing light sensitivity](image)

**Figure 1.12: Fractions of units which displayed sensitivity to light conditions during the trial, as a function of behavioral epoch.** White: rate increase in prehension in the light trials relative to dark trials; gray: rate decrease in light trials. Dashed lines indicate chance levels. Other conventions are as in Fig. 1.2. Note that during both Control and Signal epochs, lighting conditions are the same for the two trial types hence a fraction of chance level is expected.

Whereas in Area 5 fractions of light sensitive units did not differ from chance levels at any epoch, light sensitivity was observed in up to a quarter of the units in the other areas. Most light-sensitive units increased their firing rates when the object was visible during delay and movement. In Area 7b 100% of the light sensitive units increased their firing during prehension in the light trials. Interestingly, this finding is not consistent with the single report of enhanced responses of Area 7b neurons during reaching in the dark (MacKay et al., 1990), compared to reaching with visual feedback.
2. Correlations between single unit pairs in PPC during prehension do not support models of binding by sharp synchronization

The role of precise timing in the neural code is one of the most debated issues in theoretical and experimental neuroscience. Specifically, time codes have been suggested as a means for transmitting high-level aspects of external stimuli and behavioral commands and for binding anatomically distributed representations. Such models require precise (on the order of a few ms) temporal relations in the firing of cortical neurons. In the case of neuron pairs, the standard tool for examining temporal relations is the cross-correlation histogram (CCH, Perkel et al., 1967; Abeles, 1982). A "flat" CCH indicates that there is no orderly temporal relation between the firing of the two neurons. Sharp peaks in the CCH may indicate either a common input or a synaptic connection between the two neurons. Synchronization in visual cortex was typically observed in near-zero time lags (reviewed by Singer, 1999), whereas Synfire models (Abeles, 1991) predict that sharp correlations will also be observed in non-zero time lags. In this chapter I report the analysis of CCHs computed for simultaneously-recorded single units, in search of evidence supporting or refuting synchrony-based models of brain function. Specifically, I was interested in correlations between single units related to different aspects of prehension (e.g. reaching direction vs. object type) and/or correlations between single units located in separate cortical regions.

I found that CCHs of single units in PPC were not characterized by sharp (1 ms scale) and significant peaks. Some sharp peaks in CCHs were observed, but their overall frequency in the population was typically not higher than the fraction expected by the significance level of the tests used to identify them. This general finding was robust to different methods and different parameters used. Specifically, scarcity of sharp correlations in across-electrode units did not seem related to a lack of potentially more “reliable” data, collected during many trials from units with very high firing rates. Moreover, fractions of significant CCHs, as well as correlation strengths, were similar during Control and during Delay. In-depth investigation of the few pairs with significant correlation did not indicate a clear relation between the properties of pairs and their single unit members. Taken together, the characteristics of correlations observed in our data could not serve as evidence for the functional significance of neural correlations. Specifically, I found no evidence to support models of binding distant neurons by precise synchronization. However, these findings are by no means enough to
conclude that precise timing has no functional significance for the role of PPC in prehension, let alone for neural coding in general (see Discussion).

Methods

Requirements for sufficient amounts of data

The sparse nature of neural firing in the cortex requires large amounts of stationary data for a reliable estimation of pair-wise correlations by the CCH. Specifically, data durations that are sufficient for spike-count analyses in single units are not always sufficient for cross-correlation analyses. The expected mean count per bin (CPB) in a given CCH is

$$f_1 \cdot f_2 \cdot T \cdot \Delta T$$  \hspace{1cm} (Perkel et al., 1967),

where $f_1, f_2$ are mean firing rates of the units, $T$ is the total recording time (epoch duration multiplied by number of trials) and $\Delta T$ is the bin size. I was specifically interested in sharp features of CCHs, and therefore used a bin size of 1 ms without smoothing. The following criteria were used to include CCHs in the analysis:

1. Number of trials threshold for determining valid pairs: Only well isolated single units, recorded in anatomical areas of interest were used in the analysis. Trials taken for analysis were the intersection of stationary trials for the two units. CCHs were computed only for pairs with at least 10 valid trials per behavioral condition, for all 12 conditions.

2. Mean count per bin threshold for determining valid CCHs: Only CCHs with an observed mean CPB $\geq 0.1$ were used. For 25 trials of 1 second, this threshold is equivalent to a threshold of 4Hz$^2$ on the product of firing rates. Hence, for a given unit pair and epoch, 0 to 12 CCHs could be valid CCHs. The use of more conservative CPB thresholds (e.g., as used by Prut and Perlmutter, 2003) is optimal in theory, but led to rejection of $>95\%$ of the CCHs.

Results were not sensitive to the above criteria or to the small bin size used. In the last section of the results I specifically examine whether the reported results are due to lack of sufficient data (low rates, too few trials, short epochs, etc.).

Analysis epochs

The analysis epochs used for cross correlation analyses were longer than those used in spike counts analyses (Results, chapter 1), in order to increase the amount of available data at the
expense of temporal resolution. I used two analysis epochs, each 1 sec long: (1) a Control epoch (-0.5 to 0.5 sec from trial start, monkey D / 0 to 1 sec from trial start, monkey J), and (2) Delay epoch (1 sec preceding the Go signal). During both epochs the monkeys had their hand pressing on the central key of the Touch Pad and no sight of the target object. Thus, the only behavioral difference between the 2 epochs was that during the Delay epoch the monkey had acquired knowledge about target location and required grasp type in each trial. In addition, it is important to note that firing rates of almost half of the single units increased during delay, relative to baseline rates (Results, chapter 1). Cross correlations were also computed for whole trials (-4 to 2 sec from the Go signal, monkey D / -5 to 3.5 sec from the Go signal, monkey J). Whole trial data contain more spikes, but are contaminated by larger sensory or motor trial-by-trial variability, which may produce false positive (or false negative) detections of CCH peaks (Ben Shaul et al., 2001).

Ideally, one would like to compute cross correlations in a method that allows maximum temporal resolution both in the lag dimension and in the trial course dimension (e.g., time resolved cross correlation, Baker et al, 2001, or the joint-PSTH, Aertsen et al., 1989). Time-resolved cross-correlations were computed for several pairs, but these seemed unreliable, due to lack of sufficient data. I therefore did not apply this method on all pairs.

**Computation of the CCH predictor and the surprise vector**

Raw CCHs (coincidence counts) were computed for each epoch and behavioral condition, up to a maximal lag of ±100 ms, using a 1 ms bin. To compute a predictor for the CCH, I used the Abeles & Gat (2001) method, and convolved the CCH with a Gaussian (SD= 25 ms) with a value of zero at its central sample. This predictor is assumed to be less vulnerable than previously suggested predictors to false detections related to trial-by-trial non-stationarity (Ben Shaul et al., 2001), and is specifically designed for locating sharp features in the CCH (rather than wide peaks, which could be related to effects of rate non-stationarity during the trial). The predictor was used to compute, for each bin in the raw CCH, the probability of obtaining the observed count or a higher count given the expected value (the predictor) under the assumption that counts follow a Poisson distribution (Abeles and Gat 2001). The bin-wise probability vector can be conveniently visualized by the $-\log_{10}(P)$ transform yielding a “surprise” vector.

**Identifying subsets of significant and precise CCHs out of the valid CCHs**
I used two independent methods to test the significance of CCHs.

1. “Bin-wise significance”. This is a parametric method, assuming a Poissonian distribution of counts in each bin. A given CCH was considered significant if its surprise vector crossed a threshold in at least one bin. To correct for multiple comparisons, this threshold was set to $-\log_{10}(0.01 / 201) = 4.3$ (Bonferroni correction for 201 delay bins). Hence by chance 1% of the CCHs are expected to be labeled significant. Note that this method quantifies correlation strength, but uses the strength of the surprise given the predictor, rather than the strength of the raw peak (by standardization of the raw CCH).

2. “Jitter sensitivity”. This is a non-parametric method, to assess the temporal precision of the CCH. This method is explained in detail in Abeles & Gat 2001. Briefly, we would like to consider cases of a strong single peak in a CCH as well as cases of numerous weaker peaks as significant, since both are very unlikely under the null hypothesis of no correlation. For a set of critical p-values (0.01, 0.005, 0.001, ... 0.00001), we count how many bins are surprising by these values. We compare these counts to the counts expected by Poisson, and call the smallest p-value the “least likely event”. We estimate the significance of this statistic by jittering the spike train 1000 times (+/-4 ms rectangular window) and computing the statistic for the jittered trains.

Testing the existence of excess significant correlations

Observed fractions of significant CCHs were compared to those expected by chance using a binomial test. For fractions of significant pairs, I used a Bonferroni correction. Since each pair may have 1 to 12 opportunities to have significant correlations (the number of valid conditions), we need to divide the critical p-value for the CCHs of each pair by the number of valid conditions for this pair, and then tag a pair as significant if one or more conditions are tagged as significant using the corrected critical value. The fraction of significant pairs is computed from the number of pairs with one or more valid conditions. In addition, I estimated the empirical significance level of the two methods used for detecting significant correlations by shifting the spike trains of one unit in the pair by one/two trials, and repeating the analysis. To avoid non-stationarity problems created by cyclic trial shifting, the first 2 and last 2 trials of one unit in the pair were trimmed, and remaining trials were paired with shifted trials from the 2nd unit.

In depth examination of the subset of significant CCHs
To test whether significant CCHs are more frequent among single unit pairs with similar properties or among pairs of neighboring units, I compared the statistics of the significant CCHs (and pairs with significant CCHs) with the whole sample of valid CCHs (pairs), using \( \chi^2 \) tests. For this purpose, PDs, POs and their differences were re-calculated for each unit pair, using only the trials included in the cross-correlation analysis. PD differences were classified into 4 categories: similar (<60 degrees), intermediate (60-120), different (>120), and “other” (none or only one of the units was directionally tuned). PO differences were classified into 3 categories: same PO, different PO, and “other” (none or only one of the units was object-sensitive).

Similarly, I used the DOI (Results, Chapter 1) to categorize units as “direction units”, “object units” or “other”, and thus pairs were categorized as “direction-direction”, “object-object”, “direction-object” and “other”. In addition, I tested whether the condition in which a CCH is significant is expected by the properties of the single units, using binomial tests.

**Results**

**2.1 Pair and CCH database**

After applying a threshold of 10 trials per behavior on all 12 behaviors, CCHs were calculated for 9273 pairs from 34 recording days. The CCHs of these pairs were screened by mean count per bin (CPB), as a standard tool for rejecting CCHs that were unreliable due to lack of sufficient data (low firing rates and/or number of trials). Table 2.1 summarizes the number of valid CCHs (with mean CPB \( \geq 0.1 \)) and pairs remaining for each epoch. Differences between epochs in the sizes of the valid data stem from the typical increase in firing rate during pre-movement delay (Results, Chapter 1). Any valid pair, as defined by the CPB and the number of trials criteria, could contribute 1-12 valid CCHs to the database. However, as the table shows, most pairs tended to contribute multiple valid CCHs (median: 9 and 7; mode: 12 and 12 for the Control and Delay epochs, respectively).

**2.2 Significant correlations among pairs of neurons are relatively weak and rare, regardless of task epoch**

The vast majority of the CCHs were not significant (“flat”), yet some CCHs with sharp peaks were detected within the data set. Figure 2.1 shows six examples of sharp correlations found
during the delay epoch. These examples were taken from within-electrode, within-area and across-area unit pairs. Using the bin-wise method, fractions of significant CCHs were not significantly higher than chance. Moreover, fractions were similar across epochs (binomial test, 0.84% and 0.98% out of N=17284 and 21530 valid CCHs during the Control epoch and Delay epoch, respectively). Thus, at least in the time resolution examined no tendency of either increase or decrease in cross correlation was observed when monkeys got sensory information and prepared for movement. Using the jitter-sensitivity method, fractions of significant correlations were slightly but significantly higher than chance, but were similar during Control and Delay (binomial test, 1.41% and 1.34%). The methods only partially overlap: 0.64% and 0.78% of CCHs were identified by intersection of the two methods.

Table 2.1: Database sizes: numbers of valid CCHs (and pairs contributing to these CCHs, in parentheses), by analysis epoch and pair location.

<table>
<thead>
<tr>
<th>Epoch, monkey / Pair location</th>
<th>SPL same electrode</th>
<th>IPL same electrode</th>
<th>SPL across electrodes</th>
<th>IPL across electrodes</th>
<th>SPL-IPL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control epoch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>958 (124)</td>
<td>309 (43)</td>
<td>6931 (858)</td>
<td>1264 (192)</td>
<td>4942 (702)</td>
<td>14404</td>
</tr>
<tr>
<td>J</td>
<td>269 (29)</td>
<td>48 (7)</td>
<td>1228 (138)</td>
<td>74 (21)</td>
<td>1261 (154)</td>
<td>2880</td>
</tr>
<tr>
<td>Total</td>
<td>1227 (153)</td>
<td>357 (50)</td>
<td>8159 (996)</td>
<td>1338 (213)</td>
<td>6203 (856)</td>
<td>17284</td>
</tr>
<tr>
<td>Delay epoch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1161 (160)</td>
<td>320 (45)</td>
<td>8784 (1184)</td>
<td>1475 (220)</td>
<td>6912 (1088)</td>
<td>18652</td>
</tr>
<tr>
<td>J</td>
<td>215 (29)</td>
<td>77 (9)</td>
<td>1089 (129)</td>
<td>146 (29)</td>
<td>1351 (179)</td>
<td>2878</td>
</tr>
<tr>
<td>Total</td>
<td>1376 (189)</td>
<td>397 (54)</td>
<td>9873 (1313)</td>
<td>1621 (249)</td>
<td>8263 (1267)</td>
<td>21530</td>
</tr>
<tr>
<td>Full trial&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1242 (145)</td>
<td>550 (64)</td>
<td>9456 (1083)</td>
<td>2780 (335)</td>
<td>9105 (1142)</td>
<td>23133</td>
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<tr>
<td>J</td>
<td>281 (31)</td>
<td>136 (15)</td>
<td>1293 (133)</td>
<td>389 (48)</td>
<td>1860 (197)</td>
<td>3959</td>
</tr>
<tr>
<td>Total</td>
<td>1523 (176)</td>
<td>686 (79)</td>
<td>10749 (1216)</td>
<td>3169 (383)</td>
<td>10965 (1339)</td>
<td>27092</td>
</tr>
</tbody>
</table>

<sup>1</sup> CPB thresholds for full trials were adjusted to be equivalent in terms of firing rates to those of the 1 second-long epochs (0.6 and 0.85 for monkey D, J, respectively).

Visual inspection suggested that the jitter method could be over-permissive, because many of the CCHs identified as jitter-sensitive were characterized by two or more weak sharp peaks of similar strength that often did not seem “to the naked eye” to be true deviations from a flat
correlation, or as cases of weak oscillatory correlation. In order to estimate empirically the significance level of the two methods, I examined the fraction of significant CCHs computed from trial-shifted spike trains (see Methods above) during Delay. Average (±SD) fractions of significant CCHs over 4 shifts (1 or 2 trials, backward and forward) were 1.26 % (±0.11%) for the jitter method and 1.15 % (±0.04%) for the bin-wise method. Therefore, it is possible that the jitter method was indeed slightly over-permissive with respect to its significance level, and the above-chance fractions found using this method in non-shifted data should be regarded with some caution.

Most significant correlations were relatively weak, as quantified by the bin-wise surprise values at their peaks or by standardizing the raw or predictor-corrected CCHs (Figure 2.2). Thus, sharp correlations were not common, but in addition even those identified as significant did not have surprisingly strong peaks. For example, during Delay, 211 of 21530 valid CCHs (0.98%) were significant with a p-value smaller than $10^{-2}$, of which only 23 (0.107%) had a p-value smaller than $10^{-3}$, and only one with a p-value between $10^{-4}$ and $10^{-3}$. Peak strengths during Delay were not higher than during Control (Mann-Whitney test on surprise peaks, p=0.7; for standardized peaks, Control was significantly stronger, but this can be attributed to firing rate differences). Over 90% of the pairs with any significant CCH gave rise to only one significant CCH. To estimate the fraction of pairs with significant CCHs I used a specific Bonferroni correction for each pair, depending on the number of its valid CCHs. After this correction, fractions of pairs with significant correlations were 1.06% and 0.85% for the Control and Delay epochs, using the bin-wise method. Using the jitter method, fractions were 1.41% and 1.11%, respectively, but the number of pairs with significant peaks was not significantly higher than expected by chance.

As stated above, in the few pairs that showed a significant correlation, it was typically restricted to one behavioral condition. It may be tempting to view the latter finding as indicating “sharp tuning” of correlations that differ from the typically wide tuning curves reconstructed from firing rates of single units. However, given the low probability of observing a significant correlation in the database, this is an expected finding, and a flat tuning of correlations (i.e., similar correlation strength for all behavioral conditions) is the truly unexpected event. If we assume that CCHs from different behavioral conditions in the same pair are independent observations (an assumption which cannot be rejected in our data), and all pairs are sampled from a homogeneous population, then the number of significant CCHs in
each pair can be predicted from the binomial distribution with N (the number of valid CCHs for this pair) and $\hat{P}$ (the observed fraction of significant CCHs from the total number of valid CCHs). If significant CCHs are found to be rare, then the incidence of 2 or more significant CCHs in a pair will be very rare. Thus, having only one condition (out of 12) with significant CCH is likely to be a chance event, and cannot be taken as evidence for tuning of CCH.

In sum, the tendency of single unit pairs in PPC to be synchronized during the Delay epoch was (a) not different or only slightly higher than chance levels, and (b) similar to levels observed during the Control epoch. Even significant peaks in CCHs tended to be weak. Taken
together, population statistics of cross correlations of single units in PPC appear inconsistent with models that ascribe functional significance to precise neural synchronization. However, these findings may be misleading. It could be the case that overall neural correlations are weak and rare, but that they are more common among pairs with specific anatomical or physiological properties. The next two sections examine this possibility.

**Figure 2.2:** Strong, precise correlations are relatively rare in PPC during prehension. Left: Control epoch; Right: Delay epoch. Numbers are counts of valid CCHs in each epoch. Top: Distribution of standardized peak strengths. For each valid CCH, raw count at peak was corrected by subtracting the predicted count and transformed to a z-score. Middle: Distributions of peak surprise values. For each CCH and its predictor, a surprise vector was computed based on Poissonian assumption. A CCH was considered significant if its surprise vector crossed a threshold equivalent to a Bonferroni corrected significance level of 0.01. Numbers are counts of observations above the significance threshold (red line). The blue distribution is the distribution of peak surprise values for a uniform random sample of p-values. Bottom: Distribution of jitter p-values. Numbers are counts of observations above the significance threshold (red line). The blue distribution is the distribution of surprise values for a uniform random sample of p-values.
2.3 Significant correlations among pairs of neurons are relatively rare, regardless of inter-unit distance

I tested whether the probability of observing a significant CCH depended on cortical location or on inter-unit distance. The recording technique used in our experiment yielded a highly non-uniform distribution of inter-unit distances. I therefore grouped CCHs into 5 categories, according to recording area (SPL vs. IPL) and distances between units (Table 2.1). About 8% of the CCHs were computed from within-electrode pairs (distances up to tens of microns), about 53% from across-electrode within-area pairs (median distance, 1.1 mm), and the rest from across-area pairs (median distance, 9.7 mm). Figure 2.3 shows fractions of significant CCHs during the two epochs, as a function of the anatomical category. Fractions of significant correlations tended to be similar across areas and distance categories, and were typically not higher than chance levels (exceptions: SPL across-electrode in monkey D during Control, and SPL-IPL in monkey D during Delay, jitter method only). These results were qualitatively similar in the two monkeys, and did not depend on the method used for detecting a significant CCH.

An interesting difference between within-electrode unit pairs and across-electrode unit pairs was in the distribution of peak delays (Figure 2.4). Overall, there was a slight tendency for maximal correlation to be near zero, but this tendency was unique to within-electrode pairs, and was also observed in non-significant correlations. This finding could be attributed to common input and monosynaptic connections that are expected to be more common among neighboring cells. Whereas this tendency of within-electrode pairs seems to support zero-lag models of synchrony (Singer & Gray, 1995), there was no evidence supporting the possibility of “binding” distant cortical areas by zero lag correlation.

To test directly whether there is a tendency for near-zero lag synchrony in PPC single unit pairs, as predicted by zero-lag synchrony models (Singer, 1999), I recomputed the fraction of significant CCHs as though the correlation was computed only for delays of ±3 ms. In this case, the Bonferroni corrected threshold for bin-wise significance is the significance level 0.01 divided by 7 instead of 201. Fractions of CCHs with peaks at lags of ±3 ms which crossed this threshold were not significantly higher than expected by chance (0.86% and 0.98% during Control and Delay, respectively, using only across-electrode pairs).
A. Strong CCH peaks

B. Precise CCH peaks

Figure 2.3: The near-chance fraction of significant correlations is not distance dependent or epoch dependent. Fractions of significant correlations identified by amplitude of peak in the surprise vector (“bin-wise method”, A) or by the precision of peaks by a jitter test (B), are displayed separately for the two monkeys (Monkey D, left; Monkey J, right), and for categories of anatomical locations and distances of pair members. SPLs: units recorded from the same electrode in SPL; IPLs: same electrode in IPL; SPLd: different electrodes in SPL; IPLd: different electrodes in IPL; SPL-IPL: one unit in SPL and the second in IPL. Error bars are binomial confidence intervals ($\alpha=0.01$, Ns are as in Table 2.1).
2.4 Precise pair-wise correlations in PPC are not related to preferences of pair members

I checked whether pairs in which significant correlations were detected were a special subset of the data set in terms of single unit properties, i.e. preferred directions and objects. This analysis was applied to the Delay epoch only, because single units tended not to show tuning
during the Control epoch (around 1% tuned, see Results, Chapter 1). The analysis was repeated twice, using the two (only partially overlapping) subsets of significant CCHs, identified by the two methods. Results were consistent across methods. For simplicity, I report below the results for significant CCHs identified by the jitter method. Taking only within-electrode, only within-area, or only across-area pairs did not change the results on any of the tests reported below.

I tested whether pairs with similar preferred directions (or objects) were more prone to be correlated than other pairs (Figure 2.5). This prediction relates sharp cross correlations to the notion of “signal correlation”, or correlation of spike-count tuning curves (e.g., Kohn and Smith, 2005). Fractions of pairs with similar PDs or same POs were similar in the sample of correlated pairs and the sample of non-correlated pairs ($\chi^2$ test, $p=0.93, 0.08)$. Next, I tested whether “directional units” were more prone to be correlated with “direction units” (or “object units” with “object units”) than other pairs. For this purpose, I used the direction-object index, calculated based on effect sizes (Results, Chapter 1). Fractions of “object-object”, “direction-direction”, and “direction-object” were similar in the sample of correlated pairs and the sample of non-correlated pairs ($\chi^2$ test, $p=0.20$).

Focusing solely on the subset of pairs with significant CCHs, I tested whether the condition in which a significant correlation was observed could be predicted from the properties of single units. One possible prediction is the direction closest to the mean preferred direction (chance level is 1/6). Out of 270 pairs with significant CCHs, predictions based on mean PD existed for 138 pairs. Out of these, the mean PD predicted the direction in which the correlation was significant in 27 cases (19.6%, not different from chance level). An alternative possibility to examine the relation between signal correlation and cross correlations is predicting that the condition with a sharp correlation will be the condition with the maximal product of firing rates (chance level is 1/12). This method does not set any demands on statistical significance for single unit tuning, and thus predictions existed for all 270 pairs. This prediction was met in 28 out of 270 pairs (10.4%, not different from chance level).

Finally, I tested the intriguing possibility that correlations are used to bind, or “build”, complex representations from simpler representations achieved by single units. In this case, if one unit has a preferred direction, and the other unit has a preferred object (or vice versa), the
significant CCH is predicted to be in the combination of this direction and object (chance level is 1/12). This prediction was met in 9 out of 82 pairs (11%, not different from chance level).

<table>
<thead>
<tr>
<th>A. PD differences</th>
<th>B. Object Preferences</th>
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<tbody>
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<td><img src="image" alt="B. Object Preferences" /></td>
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<table>
<thead>
<tr>
<th>C. Direction/Object Units, 6X2</th>
<th>D. Direction/Object Units, 2X2</th>
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<tbody>
<tr>
<td><img src="image" alt="C. Direction/Object Units, 6X2" /></td>
<td><img src="image" alt="D. Direction/Object Units, 2X2" /></td>
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**Figure 2.5:** No relation between similarity of single units’ preferences and the probability of a pair to be tuned. Significant (gray) vs. nonsignificant (white) pairs are compared according to tuning properties of single units. A. PD differences grouped in 4 categories: <60 degrees, between 60 and 120, above 120, and other (only one of the units, or none of them, had directional tuning). B. PO differences grouped into 3 categories: same preferred object, different preferred object, and other (only one of the units, or none of them, had object preference). C-D: Pairs classified by effect sizes of single units in pairs (see Methods) as pairs of 2 object units (OO), 2 direction units (DD), one direction and one object unit (DO) and other. Classification is either based on all available data (C) or on data from rightward and leftward movements to two objects (D). Fractions are taken from the size of each sub-sample: pairs with one or more significant CCHs (N=270), pairs with no significant CCH (N=2802).
2.5 The low fractions of significant correlations are probably not due to insufficient amounts of data

A major limitation of the results presented above is that they are based on data which may be simply insufficient for detecting weak, sharp correlations. For example, taking only valid CCHs during Delay, the median number of trials was 22, and median single unit rate was 4.5Hz, leading to about 100 trigger spikes per CCH. It could be claimed that fractions of significant correlations should be much higher than those reported above if sufficient amounts of data are available (e.g., recording hundreds of trials per condition, using longer analysis epochs, or using only data from units with highest firing rates). In this section I use the available data to examine whether our data support this claim.

The most intuitive test for the “not enough data” hypothesis is by post-hoc manipulation of the inclusion criteria (see Methods) used to label a CCH as valid for analysis. If this hypothesis is correct, the fraction of significant CCHs should rise monotonically as stricter inclusion criteria are used. Figure 2.6 shows the dependence of the fraction of significant CCHs on the number of trials and mean CPB thresholds. Using stricter CPB thresholds did not seem to increase the probability of finding a significant CCH in our data, regardless of the method used (compare left column with middle column in Fig. 2.6). This finding indicates that correlations are found in similar fractions in pairs with low and high firing rates. Thus, it seems that cross correlation analysis should not be limited solely to pairs with high firing rates, as implied from using strict CPB thresholds. In fact, the best examples of significant correlations were observed in CCHs where the mean CPB was less than 1 (Figure 2.1, right column).

The effect of the N trials threshold was unclear. On the one hand, fractions of significant CCHs are higher than expected by chance only when low trial number thresholds are used (Fig. 2.6, top left, jitter method). On the other hand, the highest fractions were observed when strict thresholds on number of trials and the observed CPB were used (right column of Fig. 2.6). However, these fractions were not significantly higher than chance due to sample size (one out of ten CCHs with >=5 counts per bin and >=40 trials was significant, but 10% is not significantly higher than 1% when N=10). Although the maximal number of trials per CCH in the data was around 60, these findings indicate that designing an experiment with fewer experimental conditions (thereby increasing the number of trials per condition) would not have changed our major finding. On the contrary, it is possible that above-chance fractions of
significant CCHs found by the jitter method are partly due to over-permissiveness of this method (see above, section 2.2) that is specific to small numbers of trials.

In addition to controlling for the effect of minimal data thresholds, I examined the effect of three possible methods to increase the number of spikes used for generating CCHs, given the available data: (1) using MU spikes, by pooling spikes from different units recorded in the same electrode, (2) Pooling data from all 12 behaviors, and (3) using data from the whole trial (see Table 2.1). All three methods are of course sub-optimal in theory: the use of whole-trial data and/or data from different behaviors increases both within-trial and across-trial non-stationarity. Non-stationarity of spike trains has been shown to increase fractions of “false alarms” in the detection of significant correlations (Brody, 1999; Ben Shaul et al, 2001).

Figure 2.6: Fractions of significant correlations are not a result of using inappropriate thresholds for minimal data size. Each subplot displays the fraction of significant CCHs, by either the jitter method (top), or the bin-wise method (bottom) as a function of the trial number threshold, using a specific CPB threshold. Left: “permissive” threshold (0.1, used throughout this chapter); middle: “intermediate” threshold; right: “strict threshold”. Dotted lines are binomial confidence intervals ($\alpha=0.01$). Dashed line is the expected fraction under the null hypothesis. Fractions of significant CCHs are higher than expected by chance only when permissive CPB and trial number thresholds are used (top left, jitter method only). However, the highest fractions were observed when strict thresholds were used (top right, note different Y scale), but excess relative to chance levels was not significant due to small sample size.
In contrast, computing correlations between MU spike trains (e.g., Singer and Gray, 1995) has been shown theoretically to result in an underestimation of the true correlation between single units (Gerstein, 2000), and therefore may lead to an underestimation of the fraction of correlated unit pairs. Note as well that the 1st and 2nd methods dramatically reduce the numbers of valid CCHs, since pooling data from different behaviors means 0 or 1 valid CCHs per unit pair, and using MU spikes means that the number of available pairs per session depends on the number of channels.

A. Strong CCH peaks

![Graph showing fraction of significant correlations for Monkey D and Monkey J, with categories of anatomical locations and distances of pair members.]

B. Precise CCH peaks

![Graph showing fraction of significant correlations for Monkey D and Monkey J, with categories of anatomical locations and distances of pair members.]

**Figure 2.7:** The near-chance fraction of significant correlations in between-electrode pairs is not related to data size limitations. Fractions of significant correlations identified by amplitude of peak in the surprise vector (“bin-wise method”, A) or by the precision of peaks (“jitter method”, B), are displayed separately for the two monkeys (Monkey D, left; Monkey J, right), and for categories of anatomical locations and distances of pair members. Fractions are taken from the number of valid CCHs, computed for the 1 sec Delay epoch (red), for the whole trial (blue), for the delay epoch using all behavioral conditions (magenta), for the whole trial using all behavioral conditions (black). Other conventions and abbreviations are as in Figure 2.3. Using whole trial data, and/or pooling data from different behaviors causes the fraction of significant CCHs to increase up to around 30%, but this effect is limited to within-electrode pairs (IPLs, SPLs), and is not observed even in neighboring units (IPLd, SPLd).
Fractions of significant CCHs between MU pairs were similar to those observed between single units (not shown), and did not exceed chance level in either the Delay or the Control epochs. Our data were therefore not consistent with the theoretical claim made by Gerstein (2000), but this is probably due to a floor effect. In contrast, using the whole trial for CCH computation, pooling data from all behaviors, or both, increased the fractions of significant CCHs (Figure 2.7), up to 29% and 25% (bin-wise and jitter methods, respectively, data from both monkeys). However, this effect was almost exclusively restricted to within-electrode CCHs, and fractions of significant across-electrode correlations were not different from chance levels. We conclude that the lack of sufficient amounts of data could be a decisive factor in the observed low fractions of significant CCHs, but only for within-electrode unit pairs.

3. Direction and object sensitivity revealed in the spectral content of PPC local field potentials during prehension

The third research objective of this study is to compare single unit measures and LFP measures. Specifically, in this chapter I treat the spectral content of LFP as the neural signal. The LFP spectrum is analyzed with respect to the monkeys’ behavior, and compared with analyses of the most commonly used neural signal: firing rates of single units (Results, Chapter 1). This approach has been used previously in tasks involving eye movements (Pesaran et al., 2002) or reaching movements (Scherberger et al., 2005; Rickert et al., 2005) in the context of examining the utility of LFP as an input signal for Brain-Machine Interfaces.

I found that the LFP spectrum shows complex dynamics across the trial duration, but that there were several between-area differences in these dynamics. For example, increases in Beta-band oscillations during delay (Scherberger et al., 2005) or during Hold (Baker et al., 1997), were very frequent in MIP, but rare in the adjacent Area 5, and in area AIP. No clear evidence for Gamma oscillations was observed in PPC during prehension. A wide-band increase in Gamma power (Rickert et al., 2005) during the task was observed, but this effect is shown to be a residual of spiking activity. LFP spectrum is very often tuned to target direction or object, and fractions of tuned LFP channels were similar to fractions of tuned single units. However, LFP is typically inferior in 2 aspects: its signal-to-noise ratio and non-independence of channels, observed not only within recording sessions, but when data from all recording
sessions were pooled. Finally, I found that coherence of between-area (SPL-IPL) LFP signal pairs was maximal in lower frequencies (<13 Hz), and that a coherence increase in this range was observed not only during target presentation or movement, but also during delay. Tuning of between-area coherence during the task was in above-chance fractions only in the Delta to Beta band, but not in the Gamma band. These findings suggest that coordination across cortical areas may be achieved through low-frequency activity, rather than through the more widely studied Gamma oscillations.

Methods

Only channels with at least 5 stationary LFP trials per behavioral condition (12 behaviors), and in which spiking activity was recorded (MU rate >1Hz over the whole trial), were included in analyses. LFP traces were realigned and cut into seven 400 ms long behavioral epochs, the same as those used for single unit analyses (see general Methods section). Multi-taper spectral analysis (NFFT = 256 samples, frequency resolution 1.95 Hz, 5 Slepian data tapers) was applied to each epoch in each trial. The multi-taper technique (Mitra and Pesaran, 1999) was chosen since it provides a formal method to obtain estimates of the spectrum with optimal bias and variance properties. Next, the power in frequency bands was summed for each epoch and trial. Frequency bands were defined as follows: Delta (1-4 Hz), Theta (4-8 Hz), Alpha (8-13 Hz), Beta (13-30 Hz), lower Gamma (30-60 Hz), and higher Gamma (60-100Hz). In each site, changes in the power of the 6 different frequency bands, with respect to baseline, were tested in each of the 6 task epochs (Kruskal-Wallis test, followed by 2-tailed Mann-Whitney planned comparisons). Next, each site was examined in a two-way non-parametric test for direction, object and interaction effects, separately for each behavioral epoch. Since results for Delta, Theta, and Alpha frequency bands were very similar, some of the figures below present data for the whole 1-13 Hz frequency range, but differences between bands are noted in the text.

Pair-wise coherence between LFP sites (Jarvis and Mitra, 2001) was computed by multi-taper methods (NFFT = 256 samples, 5 Slepian data tapers). Significance of coherence was tested by a parametric threshold. Tuning of coherence was tested by a 2-way non-parametric test (Kruskal-Wallis) on the single-trial coherence estimates, averaged for each frequency band (i.e., 6 tuning curves for each channel pair).
Results

3.1 LFP database

Out of 481 PPC sites that passed the inclusion criteria, 345 were identified by anatomical coordinates as belonging to the four areas of interest, and were considered for further analysis. Data were collected in 43 recording sessions (39 SPL and IPL, 4 SPL and PMd). Table 3.1 shows the distribution of these sites by monkeys and cortical areas.

<table>
<thead>
<tr>
<th>Monkey / Area</th>
<th>SPL</th>
<th>IPL</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>MIP</td>
<td>Area 5</td>
<td>AIP</td>
</tr>
<tr>
<td>Monkey D</td>
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<td>79</td>
<td>72</td>
</tr>
<tr>
<td>Monkey J</td>
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<td>26</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>114</td>
<td>105</td>
<td>99</td>
</tr>
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</table>

3.2 LFP spectrum shows complex dynamics over the course of the trial

Parietal LFPs are dominated by frequencies below 30Hz, regardless of behavioral epoch and anatomical location (Fig. 3.1A), but the relative weight of Beta (13-30Hz) and lower frequencies depended on the behavioral epoch: shifts towards the lower frequencies were observed during target presentation and during movement, while shifts towards the Beta frequencies were observed during the delay and Hold epochs. In order to better visualize epoch-related changes in the spectral composition, the spectral power estimate for each recording site was divided (bin by bin) by the spectral power during the Control epoch, and then averaged across sites (Fig. 3.1 B). This normalization highlights a strong, wide-band increase in the Gamma frequency bands (30-100 Hz). This increase was observed during all task epochs, but was strongest around movement, and, in area MIP, after target presentation.

In order to examine whether the population averages presented in Figure 3.1 are indeed representative of the task-related changes in the spectrum of recorded sites, task epochs were compared with the Control epoch for each frequency band separately. Figure 3.2 summarizes statistics of changes in power with respect to the baseline (2-tailed Mann-Whitney test).
A. Raw power

Figure 3.1: Average LFP power spectra as a function of cortical area and behavioral epoch. Each spectrogram displays the average power spectrum of recording sites from a cortical area. Color scale depicts in logarithmic scale the absolute power spectrum (A, units are µV^2) or change in each frequency bin relative to the Control epoch (B, units are % of power). Thin horizontal lines separate the frequency bands used in the analysis (see Methods). The frequency scale was adjusted such that each of the 6 frequency bands received equal weight (original frequency resolution: 1.95 Hz).
Generally, significant changes in LFP power with respect to the baseline were more common than changes in firing rate of single units (compare Fig. 3.2 with Fig. 1.2). Dynamics of lower frequency ranges were complex, and depended on both behavioral epoch and cortical area. Delta, Theta and Alpha activity tended to decrease during the task epochs, but increases were also frequently observed, especially during the Signal, RTMT, and Hold epochs. Increases of low frequency elements of the LFP were locked to Cue and/or movement onset, and were thus additionally examined by time-domain analysis (Results, Chapter 4). Prominent Beta oscillations (Scherberger et al., 2005) were observed during delay and Hold epochs in area MIP, but in the adjacent area 5, all epochs were characterized by a decrease in Beta activity. Results in areas AIP and 7b were mixed. Power in the Gamma range increased during the task in 64.8% of the recording sites (Fig. 3.2, bottom row; results for the 30-60 and 60-100 bands were qualitatively similar).
3.3 Dynamics of LFP in the Gamma range are associated with dynamics in firing rate, due to residual contribution of the spike shape

Rickert et al. (2005) reported a wide-band increase in high-Gamma (63-200 Hz) activity in primary motor cortex around movement onset, similar to that reported above. This effect was accompanied by an increase in the directional information available from the high-Gamma frequency band. They claimed that this effect was not due to contamination of LFP by residual spiking activity. Data from the current experiment show an association between single-/multi-unit activity and high-Gamma activity, which indicates that this claim may be unjustified.

First, differences between cortical areas with respect to Gamma range power (Figs 3.1 and 3.2) were similar to differences between cortical areas in mean firing rates (Results, Chapter 1, Fig. 1.2). For example, in area MIP, cue-related rate increases (i.e., rate in Signal epoch higher than Control) were as common as movement-related rate increases. This property of MIP units was also observed in high frequencies of the LFP in this area. In contrast, cue-related rate increases in Area 5 were less common than movement-related rate increases. Similarly, the effect of cue presentation on high frequencies of the LFP was weaker in area 5 than the movement-related effect. The degree of similarity between rate dynamics and LFP power dynamics was quantified by the Spearman rank correlation between the average MU spike counts during 7 epochs, and the average power in each frequency band (Figure 3.3, N= 324 recording sites). Distributions of correlation coefficients were highly skewed towards values close to 1 when the correlation was computed between spike counts and LFP in either 30-60 or 60-100 Hz (median r=0.57 / 0.75, respectively), indicating that firing rate increases were typically associated with an increase in the LFP power in Gamma frequencies. This tendency was common to all examined cortical areas, and could be observed as well when MU spike counts and LFP power during 7 epochs of single trials were rank-correlated (N=84013 trials from 324 sites).

Second, a moderate signal correlation (defined as the correlation between tuning curves, Lee et al., 1998) was observed between tuning curves constructed from MU firing rates and tuning curves constructed from high-Gamma power (Figure 3.4A). Once again, the average signal correlation was near zero for the lower (<30 Hz) frequency bands. The average noise correlation (i.e., correlation between trial-to-trial fluctuations of spike counts and LFP power) between high-Gamma power and MU rates was low, but significantly higher than zero (Figure
3.4B). The observed magnitude of the noise correlation was similar to that reported by Rickert et al. (2005).

![Graph showing distribution of Spearman rank correlations between mean MU spike counts and mean power in each frequency band.](image)

**Figure 3.3: Average power dynamics in Gamma range is positively correlated with MU rate dynamics.** Each histogram shows the distribution of Spearman rank correlations between mean MU spike counts in 7 epochs and mean power in each frequency band (N=324 sites). Vertical solid lines mark median values for each distribution.

Third, using spike-triggered averages (STA) of the LFP, I found no constant phase relation of “Gamma oscillations” with SU/MU spike timing, as might be suspected from the co-occurrence of rises in firing rate and in Gamma power. Instead, the STA of the LFP recorded from the same channel as that of the trigger unit often showed a sharp feature around the time of the spike, not present in STA of the same unit with LFPs of nearby electrodes (observed also by Arieli, 1986 in the anesthetized cat, and by Goldberg, 2004, in monkey motor cortex).

Finally, the spectral content of single unit spike shapes, when analyzed without prior high-pass filtering, showed a prominent peak around 500 Hz and its harmonics, but also non-zero power...
across the entire spectrum from the lowest frequencies up to the Nyquist frequency (Fig. 3.5). The lesson from this figure is that even if a very strict and high-order low pass is used for LFP extraction, some residue of the spike shape still remains in the filtered LFP. In summary, the evidence presented above suggests that the peri-movement “Gamma oscillations” reported by Rickert et al. (2005), and evident in our data as well are merely residuals of spike shapes. In contrast, Beta-band oscillations (Baker et al., 1997; Scherberger et al., 2005), which were observed in our data mainly in area MIP, are not residuals of spiking activity. These observations should be kept in mind when we move to examine the sensitivity of different LFP frequency bands to target direction and object in the next section.

![Figure 3.4](image-url)

**Figure 3.4: Positive signal and noise correlation between high Gamma activity and MU firing rate.** Each subplot shows average signal (A) and noise (B) correlation between MU rates and LFP power as a function of time epoch in 3 frequency bands (N=324 sites). LFP-MUA signal correlation measures similarity of LFP and MUA tuning curves, whereas noise correlation measures association of trial-to-trial variability in the two signals. Error bars are SEM-based confidence intervals ($\alpha = 0.01$). Epoch 1: Control epoch, 2: Signal epoch, 3: Set1 epoch, 4: Set2 epoch, 5: Pre-Go epoch, 6: Rtmt epoch, 7: Hold epoch.
3.4 Direction and object sensitivity LFP power spectrum is at least as common as direction and object sensitivity in firing rate of single units

In the first chapter of the Results section, I compared the tuning of single units in “reaching-related” and “grasping related” areas to target direction and object type. In this section, the tuning of single units is compared to the tuning of simultaneously recorded LFPs. Figure 3.6 displays fractions of direction and object sensitive LFPs separately for 3 representative frequency bands and 7 behavioral epochs, with fractions of sensitive single units displayed as reference (N = 345 LFP sites, 810 single units, data from all areas pooled). The general impression from this figure is that (1) fractions of direction or object sensitive LFP sites are similar to, or even higher than fractions of direction or object sensitive single units, (2) LFPs and single units alike show maximal directional tuning during movement and during the visual stimulus, but maximal object sensitivity during Hold, especially in the low frequency ranges. (3) The observation that SUs recorded in the current experiment were typically more prone to be directionally tuned than object sensitive was replicated in the analysis of LFP sites (with the exception of the Hold epoch, but see also Fig. 1.6). (4) As expected, fractions of tuned LFP sites during the Control epoch did not exceed chance level. Inspection of the same data, parsed by recording area (not shown), revealed that fractions of directional tuning and object sensitivity in the high Gamma range were maximal in Area 5, whereas fractions of directional tuning in the Beta range during Delay epochs were maximal in MIP.

**Figure 3.5: The spike waveform itself also contains non-zero energy in slow frequencies.** The average power spectrum of 1212 single unit spike shapes (solid line), length 2.56 ms, and sampling rate of 25 KHz (NFFT = \(2^{12}\)) as a function of frequency (logarithmic scale). The dotted line shows average spectrum of 1212 random sections of the same duration taken from the same recording channel and same data file. Both curves are normalized by the maximum of the spike curve. The vertical solid line indicates low-pass used for LFP in our study. Dashed black line is 300 Hz, a commonly used high-pass for spike extraction.
3.5 However, single units are superior to LFPs with respect to their signal-to-noise ratios and the uniformity of preferred directions and objects

Comparing fractions of stimulus-sensitive neural signals, such as SU activity vs. field potential power, or SU activity in different cortical areas, is clearly not sufficient to make the claim that these signals are equally informative. One important additional feature is the strength of the main effect/s ("signal") relative to the trial-by-trial variability ("noise") in each signal. To quantify the signal to noise ratio of LFPs, I used the $\eta^2$ effect size (Fisher, 1925, see Results, Chapter 1). Figure 3.7A shows average direction and object effect sizes of significantly tuned single units and LFP sites. Single units tended to have higher effect sizes than LFP sites, regardless of the frequency band (direction effect - all 6 epochs, object effect – 4 out of 6 epochs). This advantage of single units was consistent across areas. Thus, a single LFP signal is as likely to be informative about target direction or object as a single spike-count signal, but is likely to be noisier than the spike-count signal.

**Figure 3.6:** Directional tuning (A) and object selectivity (B) of single units and spectrum of LFP sites, as a function of behavioral epoch. Fractions of significantly tuned single units (SU) or LFP sites (N = 810 single units, 345 LFP sites). Single units are compared in each epoch to three frequency bands: Low (Delta to Theta, 1-13 Hz), Beta (13-30 Hz), and high Gamma (60-100 Hz). Error bars are binomial confidence intervals ($\alpha = 0.01$).
In addition, the quality of a neural signal should be assessed when multiple channels of the same signal type are available. For example, in the case of directional information, it is clearly preferable to decode target direction from a set of N inputs with a uniform PD distribution than from a set of the same size, but with similar PDs that do not span the entire phase plane. While the PD distribution of tuned single units did not deviate from uniformity (Rao’s test, \( p > 0.27 \)), LFPs often had non-uniform PD distributions (Fig. 3.7B, top). PD distributions of LFPs were biased towards far targets during the Signal epoch (three lower frequency bands), towards near targets during Delay epochs, and towards contra-lateral (right) directions during Hold (Beta and Gamma bands). The degree of similarity between significant PDs was quantified by the circular variance, which ranges from 0 to 1 (1 indicating maximal dispersion). The average circular variance of simultaneously recorded single units was about twice the circular variance of LFP channels (Fig. 3.7B, left). Moreover, even when PD samples included pooled data from all recording sessions, the circular variance computed from PDs of SUs was slightly higher than the circular variance computed from PDs of LFPs (Fig. 3.7B, right, all epochs but the Signal and Hold epochs).

### 3.6 Slow waves and oscillations, rather than Gamma band oscillations, are possible candidates for coordinating activity across distant areas

Dynamics in the spectral content of single LFP signals during the task show between-area differences (Fig. 3.1 and 3.2). Therefore, for the analysis of between-area measures, which may indicate cooperation or “binding”, I used pair-wise coherence, which is computed as a function of frequency, and bounded between 0 and 1. Coherence dynamics of within-area (SPL-SPL, IPL-IPL) pairs were very similar to the dynamics of single-channel spectra (not shown). Figure 3.8 displays population results for SPL-IPL coherence. Coherence in the Delta and Theta ranges tended to increase during the Set1, RTMT and Hold epochs (Kruskal-Wallis test, followed by multiple comparisons to Control). Coherence in the Alpha range tended to increase during the Signal, Set1, and RTMT epochs. In contrast, a peak in coherence in the Beta range, which was observed during the Control epoch, tended to decrease during the task (significant only for Set1 epoch) and rebound during Hold. Coherence values in frequencies above 30 Hz (Gamma range) were close to 0 across the entire trial duration, but were slightly higher during target presentation (Fig. 3.8B, Signal epoch higher than Delay and RTMT epochs, but not significantly higher than Control).
A. Average effect sizes

![Effect Size Graph]

B. Dispersion of preferred directions

![Circular Variance Graph]

**Figure 3.7: Advantages of single unit (SU) signals over the LFP signals.** (A) Average direction and object effect sizes ($\eta^2$) of significantly tuned units/sites, as a function of task epoch. Error bars are SEM-based confidence intervals ($\alpha = 0.01$). (B) Top: Distributions of significant preferred directions during the PreGo epoch. Color code indicates data type (single units or LFP frequency band). Black lines show the magnitude and angle of the mean preferred direction. Bottom: Circular variance of significant PDs of significantly tuned units/sites, as a function of task epoch. The left panel shows the circular variance within each recording session, averaged over sessions. The right panel shows the circular variance of significant PDs pooled from all sessions.
However, observation of increases in between-area coherence is not sufficient to indicate functional significance. Thus, I tested the sensitivity of coherence in each frequency band to target direction and object (Fig. 3.9, directional tuning only). Generally, fractions of tuned LFP channel pairs were lower than fractions of tuned single channels (or single units) by at least an order of magnitude (compare Fig. 3.9 with Fig. 3.6). Fractions of tuned pairs during Control were not significantly higher than expected by chance, regardless of the frequency
band. In contrast, fractions of tuned pairs during the task were often higher than expected by chance, especially in the lower frequency bands (<30 Hz).

No association was observed between the single-trial coherence (in any band) and efficient or more motivated behavior, indicated by shorter reaction or movement times.

![Graph showing directional tuning in SPL-IPL coherence.](image)

**Figure 3.9: Directional tuning in SPL-IPL coherence.** Each subplot shows the fraction of directionally tuned pairs (N=656) in a given frequency band, as a function of behavioral epoch. Error bars are binomial confidence intervals ($\alpha = 0.01$). The dashed horizontal line is the fraction expected by chance. Epoch 1: Control epoch, 2: Signal epoch, 3: Set1 epoch, 4: Set2 epoch, 5: Pre-Go epoch, 6: Rtmt epoch, 7: Hold epoch.
4. Evoked local field potentials in PPC and premotor cortex during prehension do not support feed-forward parietal-premotor activation

In the previous chapter I characterized the LFP recorded in PPC, using frequency-domain analysis. This analysis is better suited for describing oscillatory phenomena, found mostly during the Delay and Hold epochs. In this chapter, I use time-domain analyses, in order to examine evoked potentials (EPs) that were prominent around the onset of arm movement and/or visual stimuli. EPs are computed by averaging over trials in order to reduce noise from superimposed ongoing activity that is assumed to be unbiased. EPs reflect activity that is locked to a sensory or a motor event, whereas other task related phenomena (e.g., oscillations) are smoothed out. Donchin et al. (2001) reported the presence of motor evoked potentials (MEPs) in LFP recorded from macaque primary and supplementary motor cortex during reaching. I examined whether EP phenomena prevail to similar extents in various cortical areas, whether they reflect effects observed in neural firing rates, and whether they can be reliably used for decoding movement parameters. Data used in the present chapter included, in addition to field potentials recorded in PPC, LFP data from premotor cortex (same monkeys and task, but different recording sessions), courtesy of Eran Stark. Thus, I had the opportunity to compare posterior parietal cortex with premotor cortex, in terms of incidence of EPs, their tuning properties, and their latency.

I found that significant visual and motor EPs are frequently observed in premotor and posterior parietal cortices. The distribution of EP peak latencies does not support a parietal-to-premotor recruitment pattern. Instead, visual EPs indicate parallel activation of premotor and parietal areas, whereas motor EPs support a premotor-to-parietal flow. Approximately 50% of the EPs show significant tuning to target direction or object, independent of recording area. However, distributions of preferred directions and objects of EPs are not uniform, in contrast to the relatively uniform distributions observed in multi-unit spike data. In contrast to spike counts, motor evoked potentials become tuned to target direction or object only around or after movement onset.
Methods

LFP and EP database
Only channels that exhibited at least 5 stationary LFP trials and at least 5 stationary MU spike trials per behavioral condition (12 behaviors), and in which spiking activity was recorded (MU rate >1Hz over the whole trial) were included in analyses. The EP wave (average over traces) and standard error of the mean of the stationary traces were computed. For each EP, a parametric confidence interval was computed, using the standard error of the mean.

EP peak significance, amplitude and latency detection
I was interested in comparing cortical areas with respect to their peak latencies, and specifically in testing (a) whether premotor and parietal areas are activated sequentially or in parallel, and (b) whether activation in “reaching areas” PMd and SPL is synchronous with activation in “grasping areas” PMv and IPL. However, inspection of the whole EP database (see below, Fig. 4.2) showed that it is clearly unjustified to express the rich variance in multiple-peak EP waveforms by a single latency value for each EP vector (and a corresponding amplitude value). I therefore followed the approach of Donchin et al. (2001) and searched for global extrema in multiple time windows that matched typical peak latency and polarity observed in the data.

Visual evoked potentials (VEPs) tended to have a negative deflection around 60ms after Cue onset, a wide positive deflection around 400ms from Cue onset, and one or two peaks between the early and late peaks (Fig. 4.2A). Therefore, 4 non-overlapping time windows were defined: 40-80 ms, 80-140 ms, 140-200 ms, and 300-500 ms after Cue onset. MEPs tended to have a weak pre-movement positive deflection and a late positive peak (400 ms from movement onset), around grasp time, and 1-3 peaks in between (Fig. 4.2B). Three MEP time windows were defined: between -100 and 0 ms (positive peak), -30-200 ms (negative or positive peak), and 250-450 ms (positive peak) from movement onset. The partial overlap between the first and second windows was required due to overlap between latencies of the first (positive) peak in SPL and those of the second (negative) peak in PMd (Fig. 4.2B, left). In cases where the global maximum between -100 and 0 ms was also the maximum in absolute value between -30 and 200 ms, this point was defined as the first peak and the second peak was defined as the global maximum in absolute value occurring between the first peak and 200 ms from movement onset. The significance of each peak was tested by a parametric
confidence interval computed from single trials. Significance testing was based on the assumption that the amplitude of the LFP signal over the trial is normally distributed, and this is indeed the case for our data (null hypothesis of a normal distribution not rejected in 79.1% / 76.5% of the trials. Jarque-Bera / Lilliefors test, respectively, during delay). The significance level required for each peak was computed by a Dunn-Sidak correction for the multiple comparisons used (i.e., the number of time samples per search window).

**Directional tuning and object selectivity**

For each channel and event (VEP, MEP, control), 6 EPs corresponding to 6 directions were computed, by pooling trials from 2 objects in each direction. The root mean square (RMS) amplitude of each EP was computed, to yield a tuning curve. A vector sum of the 6 elements of the tuning curve was computed, with its angle indicating the preferred direction (PD) of the channel, and its length R indicating the tuning modulation depth. I used resampling methods that were previously employed on spike counts (Crammond & Kalaska, 1996; Stark & Abeles, 2005) to test for significance of the tuning. This involved 1000 repetitions of a shuffling procedure in which single trials were randomly reassigned to different movement directions, 6 EPs were computed and the resultant vector of the shuffled data was determined. A channel was considered directionally tuned if the length of the actual mean vector exceeded at least 990 out of the 1000 shuffled mean vectors (p<0.01).

Object selectivity, or the existence of a preferred object (PO), was assessed in the same way by pooling across reaching directions, and conducting a two-tailed resampling test on the difference between average responses. Thus, a channel could be identified as preferring the first object, the second object, or as nonselective. For each cortical area I tested the null hypothesis that significant preferred directions were uniformly distributed, using Rao’s test for equal spacing. Similarly, the overall object preference of a cortical area was tested against the null hypothesis of equal probability to prefer each of the two objects (binomial test). Between-area comparisons used Kruskal-Wallis non-parametric analysis of variance and multiple comparisons with Bonferroni corrections.
Results

4.1 EP database

Out of 925 sites with sufficient data, 784 were identified by anatomical coordinates as belonging to the six areas of interest, and were considered for further analysis. Data were collected in 87 recording sessions (44 premotor, 39 parietal, and 4 PMd-SPL), with an average of 9 (±3.6) channels per session. Each EP was computed by averaging over 30 ± 11 trials of the same direction and object. Table 4.1 shows the distribution of these sites by monkeys and cortical areas. From each recording site, I obtained EPs for all behavioral events, computed separately for the 12 experimental conditions. Visual inspection of averaged traces (and in some cases, also of single trials) frequently showed a conspicuous MEP, and, in many cases, a VEP as well (Figure 4.1). The optimal alignment for MEPs was movement onset, rather than the Go signal or the grasp events. Other behavioral events tested, i.e. the start of the trial, or turning the visual cue off, typically did not reveal a significant EP. Subsequent analyses included EPs computed by aligning LFP traces on 3 behavioral events: trial start (used as control), object presentation (VEPs), and movement onset (MEPs).

Table 4.1: Numbers of sites in the ERP database, by monkey and recording area

<table>
<thead>
<tr>
<th>Monkey / Area</th>
<th>Premotor Cortex</th>
<th>SPL</th>
<th>IPL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PMd</td>
<td>PMv</td>
<td>MIP</td>
<td>Area 5</td>
</tr>
<tr>
<td>Monkey D</td>
<td>177</td>
<td>170</td>
<td>94</td>
<td>79</td>
</tr>
<tr>
<td>Monkey J</td>
<td>60</td>
<td>51</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>237</td>
<td>221</td>
<td>108</td>
<td>105</td>
</tr>
</tbody>
</table>

4.2 EP peak latencies do not support feed forward parietal-to-premotor models

MEPs recorded in macaque primary motor cortex during reaching (Donchin et al., 2001; Rickert et al., 2005) were reported to have a typical 4-peak shape (P1-N1-P2-N2), with approximate peak latencies -50, +50, +200, and +500 ms. The most conspicuous peak of the four was N1. The peaks were reported to have little variation in their latency and relative amplitudes, and could thus theoretically serve for between-area comparison.
I examined whether MEPs recorded in the current experiment indeed fit the typical 4 peak shape described by Donchin et al. (VEPs were not reported by this group, due to their task design, which did not separate the visual cue from the Go signal). Figure 4.2 shows all EPs in the VEP and MEP database, normalized and sorted by the latency of the strongest absolute peak. EPs were computed for the interval between half a second before to half a second after the relevant behavioral event. The four-peak typical MEP shape described by Donchin et al. and their respective peak latencies fitted only less than half of the MEPs in area PMd, which lies closest to proximal arm representation in primary motor cortex (Fig. 4.2B, top left). A similar waveform was observed in MEPs of SPL (Fig. 4.2B, bottom left), but peaks were delayed relative to PMd and MI. In PMv and IPL, the strongest peak was also delayed relative to PMd, and was often inverted (positive). Thus, differences between our data and those of

**Figure 4.1: Field potential traces and movement-evoked potential (MEP) recorded from anterior intra-parietal cortex.** Averaging is based on 38 repetitions of the same condition (power-grip object, direction 60 degrees), of which only the first 7 are shown. Traces are aligned on movement onset. Note different scales for raw traces and average (each scale bar is 50 µV; MEP is magnified 5 times relative to single trials). Confidence interval around the MEP (orange shading) is ±1.96 standard errors (computed for each sample point). Vertical marks indicate behavioral events. Note that the MEP and the visual evoked potential are also evident in the single trials but the latter is smeared in the average trace because of misalignment.
Donchin et al. and Rickert et al. apparently do not stem only from the different task used, or from a different filtering process, but rather from a rich variation in EP shapes, at least partly related to anatomy.

A second look at the whole population of EPs also revealed invariance in peak latency within and between cortical areas. VEPs tended to have a negative deflection around 60ms after Cue onset, a wide positive deflection around 400 ms from Cue onset, and one or two peaks between the early and late peaks. The 400 ms peak was probably not related to Cue offset, because of the large across-trial variance of Cue duration (distributed uniformly between 200 and 400 ms), and because it was not observed when traces were aligned on Cue offset. MEPs tended to have a weak pre-movement positive deflection similar to Donchin et al’s “P1” during reaction time (average reaction time across sessions and conditions was around 250 ms) and a late positive peak (400 ms from movement onset), around grasp time, and 1-3 peaks

**Figure 4.2:** variance and invariance in EP peak latency. All recorded VEPs (A) and MEPs (B) from each cortical area are presented, sorted by the latency of the strongest peak. Each EP is plotted as a horizontal row in the colored image (Red, positive voltage, blue, negative voltage, EPs were normalized to have zero mean and a norm of 1). The black vertical line notes Cue onset (A) or movement onset (B). The sorting visualizes cumulative distribution functions of the maximal peak, but also facilitates inspection of invariant features in the EP database, observed as vertical stripes, such as a pre-movement positive peak in MEP, most conspicuous in SPL, but still detectable in other areas.
in between. For both VEPs and MEPs, variance in peak latency was minimal in the earliest peak and maximal in the latest peak. Interestingly, polarity inversions were uncommon in the earliest and latest peaks, but very common in the middle peaks, especially in parietal cortex.

As Fig. 4.2 shows, using the Donchin et al. (2001) 4 peak definitions for our data is clearly inappropriate, yet it is also unjustified to reduce the dimensionality of the data to a single latency and amplitude value for each EP vector. In order to compare EPs from different areas with respect to their amplitude and latency, I searched for extrema in multiple time windows that matched typical peak latency and polarity observed in the data. Figure 4.3 displays average latencies of significant VEP and MEP peaks. VEPs showed a clear pattern of parallel activation. Differences between areas with respect to latencies of the 1st peak were non-significant (Kruskal-Wallis test, followed by planned comparisons). Other between-area differences were occasionally significant, but could be either congruent or non-congruent with a simple feed-forward model (e.g., SPL 9 ms before PMd in 2nd peak, but 7 ms after PMd in 3rd and 4th peaks). In contrast, premotor MEP latencies were slightly earlier than parietal peak latencies in all 3 peaks, inconsistent with a parietal-to-premotor flow model. Interestingly, the latency of the first peak also showed a small but consistent “reach-grasp” effect, since PMd had a 7 ms advantage over PMv, and SPL had a 6 ms advantage over IPL. This effect was observed in the 2nd peak, but in premotor cortex only, and reversed in the 3rd peak in parietal cortex.

4.3 Non-uniform direction and object selectivity observed in VEPs and MEPs

Visual inspection of EPs computed for different directions and objects often revealed sensitivity of the EP amplitude to task parameters (Fig. 4.4). In order to quantify the directional tuning of each channel, the RMS amplitude of EPs in the 6 reaching directions was computed, and a tuning curve was obtained. Significance of tuning was assessed by a resampling test. Tuning was rare or inexistent (1.02%, data from all cortical areas pooled) for control EPs, when the monkey was not moving and had no prior knowledge about target direction. However, both VEPs and MEPs were often directionally tuned (Fig. 4.5A). Preferred directions were not uniformly distributed (Rao’s test, all samples but MEPs in IPL), and right-left directions were under-represented. Analysis of POs only used data recorded in sessions where the power grip and the precision grip objects were used. MEPs in all areas except PMd showed a preference of the power grip object over the precision grip object.
**Figure 4.4: Example of directional tuning of visual and motor evoked potentials.**

Each subplot shows visual (A) or motor (B) evoked potentials from 6 reaching directions (power grip object). Same channel as in Figure 4.1. The same traces were aligned either on object presentation (A) or movement onset (B). Confidence intervals around EP traces indicate $\pm 1.96$ standard error of the mean. Horizontal bars indicate time windows used for effect amplitude. Insets: polar tuning curves of EP amplitudes. The preferred direction is the angle of the vector sum of 6 responses.

**Figure 4.3: Distribution of VEP (A) and MEP (B) peak latencies in the 4 cortical areas.** Only significant peaks were included. Error bars are parametric confidence intervals ($\alpha = 0.01$). Horizontal lines indicate time of visual cue (A) or movement onset (B). See text for peak definitions.
Figure 4.5: Directional tuning (A) and object sensitivity (B) in the EP data set.

(A) Top: Fractions of channels with directional tuning within each cortical area, for control EPs, VEPs and MEPs. Error bars indicate binomial confidence intervals ($\alpha=0.01$). Bottom: distributions of significant preferred directions only, from different areas and epochs. An asterisk below a sample indicates rejection of the null hypothesis that PDs were drawn from a circular uniform distribution. (B) Each bar presents the relative fraction of channels with significant preferred objects from different epochs and cortical areas. An asterisk above a bar indicates rejection of the null hypothesis of equal probability for both objects. Data were taken only from sessions where the precision grip and power grip objects were used.
Directional tuning and/or object selectivity were often observed in the MU spike data. Hence, I examined the relation between tuning properties of MU activity and tuning of EPs. An example of MU spike data from one channel is presented in Figure 4.6. The spike-count tuning curve computed for the visual epoch (Fig. 4.6A, solid line polar plot) displays sharp tuning to leftward movements, whereas the tuning curve for the movement epoch reveals a preference towards the far-right quadrant (Fig. 4.6B). A comparison of these curves with the tuning curves of VEPs and MEPs recorded by the same channel (dashed lines) indicates that in both epochs the directional information in the LFP clearly deviated from that of the MU spikes. This is true both for the shape of the tuning curve and for the PD.

![Figure 4.6: Directional tuning of multi unit activity.](image)

Raster plots, peri-event time histograms and polar plots of multi unit spikes recorded simultaneously with field potential in the same channel and same behavioral conditions as in Figure 4.4. Raster plots and histograms are aligned either on object presentation (A) or movement onset (B). Bolded vertical ticks on the spike raster plots indicate behavioral events: object presentation and movement onset. Horizontal bars indicate time windows used for effect amplitude. Insets: polar tuning curves of spike count amplitudes (solid lines) are different from those of the EPs recorded in the same channel (dashed lines, taken from Figure 4.4).

Inspection of directional tuning curves typically showed that EPs tended to have wider tuning than MU spike counts. The width of the tuning curve was quantified by the length of the vector sum of responses, which ranges from 0 to 1, where 0 indicates insensitivity to direction
(all responses are equal). Median R values for VEPs / MEPs (only channels with significant PDs) were 0.187 / 0.105 respectively, whereas median R values for MU spikes during the same time windows were 0.195 / 0.162 (Mann-Whitney test, p=0.056, p<0.01).

The non-uniform preferences of EPs were not a reflection of preferences in the MU data. Figure 4.7 shows distributions of preferred directions (A) and objects (B) for the MU spike count data recorded by the same electrodes used for LFP recordings. For all 4 areas, and for both PDs and POs, there was no significant deviation from a uniform distribution. For channels in which a significant PD was observed in both MU activity and the EP in the same epoch, the angular PD difference was computed. The distributions of PD differences did not deviate from a uniform distribution (Kolmogorov-Smirnov test), indicating that there were no consistent relations between preferences observed in the two signals.

4.4 Direction and object selectivity in MEPs, but not in spike counts, emerges after movement onset

I tested whether the time window used for determining tuning had similar effects on both the EP and MU data sets. The analysis described above was repeated for all possible time windows of different durations between -500 to +500 ms relative to movement onset, in increments of 50 ms. Figure 4.8 shows the probability of obtaining significant directional tuning for possible 200 ms time windows. Similar results were obtained for object preference (not shown). This analysis highlighted another clear difference between the LFP signal and MU spikes. Spike counts were in many cases informative of object identity and direction before movement onset, as revealed from fractions of statistically significant preferred objects and/or directions. In contrast, MEPs computed from strictly pre-movement time windows were almost always uninformative. The result of uniform distribution of preferred directions for the spike data, but non-uniform distribution for the MEP data was consistent across time windows.
A comparison between cortical areas reveals that time courses of probability of tuning were similar across areas for MU spike counts, supporting parallel processing in premotor areas and parietal areas on the one hand, and among subdivisions of premotor and parietal cortex, on the other. However, tuning of EPs in premotor areas emerged approximately 50 before tuning of EPs in parietal cortex (right shift of solid curves in bottom row vs. top row). This finding is
related to the differences between parietal and premotor areas in the latency of the first MEP peak (Fig. 4.3B), and consistent with Rickert et al.’s report that this peak is typically not tuned to reach direction.

![Graph showing time course of directional tuning](image)

**Figure 4.8: Time course of directional tuning.**
Tuning of MEPs (solid lines) and multi-unit activity (MUA, dashed lines) was tested in multiple time windows with a length of 200 ms. The fraction of tuned channels is plotted against time of window end, thus time zero indicates that the 200 ms window ends at movement onset. Each subplot concerns one cortical area. Numbers of channels per area are the same as in Fig. 4.4.
Discussion

In the present study I investigated neural activity in posterior parietal cortex of the macaque during prehension. Prehension movements include reaching elements and grasping elements. Thus they were chosen as an example of a complex, multi-joint movement, which is planned and controlled by multiple cortical areas. The design and execution of such movements necessarily involves coordination, or “binding” of joint movements, in order to produce a smooth and efficient motion, but the coordination mechanism is unclear. Moreover, it is not clear whether it can be anatomically localized to a certain level in the nervous system. The first objective of this study was to examine whether reaching and grasping are indeed anatomically and functionally segregated in PPC, as assumed by previous models (Luppino and Rizzolatti, 2000). The second objective was to examine the possibility that the coordination of prehension movements is achieved by precise synchrony of single units (Triesch and Von der Malsburg, 1996) and/or by phase locking of LFP oscillations (Gray and Singer, 1989). The LFP signal has received special attention recently not only in the context of the ongoing search for a high-level neural code, but also in the context of its clinical utility for brain-machine interfaces (Andersen et al., 2004). Therefore, my third objective in this study was to compare single unit activity and LFP with respect to their sensitivity to the independent variables of the task; namely, target direction and object.

In the following paragraphs, I first review and discuss the results related to the anatomical-functional segregation question. The next two sections deal with results related to coordination and binding. Next, I discuss the results of the comparison between LFP signals and the rate signal. Finally, I discuss EP latency results with relation to the question of parallel vs. serial cortical processing. I conclude the discussion with a personal view on a preferred direction (pun intended) for students of the neural code.

1. Segregated models of PPC, consisting of “reaching areas” vs. “grasping areas” and of “direction neurons” vs. “object neurons” are clearly oversimplified

Based on current literature, I expected to find an anatomical segregation in PPC, separating “reaching areas”, where neurons are sensitive to reaching parameters, from “grasping areas”, where neurons are sensitive to grasping parameters. In addition, I expected to find functional, within-neuron segregation, which implies that direction-sensitive neurons are unlikely to be
object-sensitive, and vice versa. I found that sensorimotor cortical maps, based on passive and active mapping of multi-unit activity, indicated partial segregation of distal and proximal joint representations (Fig. 1.1). As expected, SPL was characterized by a majority of proximal sites, whereas in IPL distal sites were more common than in SPL (but did not constitute a majority). In addition, many sites were sensitive to movement or somatosensory stimulation of multiple arm joints. In contrast, segregation of direction and object sensitivity, tested in well-isolated single units, was either much weaker or non-significant (Fig. 1.6). A clear functional segregation was also not observed, since a considerable fraction of the single units in SPL and IPL were tuned to both parameters, either with or without an interaction effect. Moreover, units with such complex properties were typically more common than (or at least as common as) units with pure object preference (Fig. 1.9).

My study was part of an experimental project that included recordings in premotor areas of the same monkeys, during performance of the same task (Stark et al., submitted). The relative similarity of single units’ tuning properties in PMd (a so-called “reaching area”) and in PMv (a “grasping area”) was even more pronounced in premotor cortex, where between-area differences in fractions of tuned units as well as in effect sizes were typically not significant. It could be speculated that multi-joint coordination, manifested as the incidence of “unexpected” single units (e.g. “reaching neurons” in “grasping areas” and vice versa) is expressed more in motor areas than in associative areas, such as the PPC. However, recordings from primary motor cortex during the same task are necessary to examine whether this speculation is warranted.

Taken together, these observations are inconsistent with a simplistic anatomical-functional dichotomy of “reaching areas” vs. “grasping areas” found in current Neuroscience textbooks (for instance, Kandell et al., 2000). However, this simplistic dichotomy is based mainly on anatomical studies (see review by Luppino & Rizzolatti, 2000), and on partial physiological evidence. Most electrophysiological studies of forelimb areas in SPL involve reaching tasks, whereas studies of IPL hand representation involve grasping of different objects, without controlled variation of target direction. A single exception is the study by Fattori et al. (2004), who investigated the activity of neurons in area V6A during reach-to-grasp and reach-to-point tasks. Area V6A, which lies posterior to area MIP, had been considered a visuo-motor area related to reaching, but Fattori et al. found that a considerable fraction of recorded units in this area were sensitive to the task (pointing vs. grasping). A limitation of our study, common to
all experiments with trained animals, is the possibility that single unit tuning properties are qualitatively modulated by the process of training. Hence, it is possible that functional-anatomical segregation of single units would have been observed in the untrained animal. This limitation is relevant as well for the putative function of neural synchrony (see below). The Fattori et al. study complements our observations: although it did not involve tests for direction and object tuning, it included analyses of neural activity during spontaneous, untrained, prehension movements. Many units in area V6A increased their firing only with relation to finger flexion, indicating their involvement in grasping. Thus, this study supports our conclusion that the simplistic dichotomy of “reaching areas” vs. “grasping areas” is indeed unjustified even for early stages of visuomotor processing.

A multitude of studies on the motor cortex of macaques (e.g., Ben-Shaul et al., 2004; Donchin et al., 1998; Hoshi and Tanji, 2002; Kakei et al., 2001) have already shown that many neurons in primary motor cortex do not respond like “low-level” units that are expected to be involved in single-joint movements and not affected by task context. Moreover, differences between properties of single unit populations in MI and premotor cortex were much weaker than expected under simplistic models ascribing different functions to different areas. For example, sensitivity to sequential context, previously thought to be coded in the supplementary motor area, was also found in premotor and even in primary motor cortex (Ben Shaul et al., 2004). Our results are in line with these observations, which all converge to wave a warning signal at attempts to model the sensorimotor cortex as a series of box-modules with exclusive functions, each with anatomically defined boundaries. Apart from the Fattori et al. (2004) study, this approach is not represented in studies of parietal cortex. Instead, key electrophysiological studies (e.g., Murata et al., 2000; Snyder et al., 1997) are even inclined to define cortical areas by task-dependence of neurons, as is commonly done in fMRI studies. This approach leads to biased sampling of neural populations, which in turn conserves the simplified view of anatomical-functional segregation.

Before moving on to discuss analyses of pair-wise correlations and field potentials, an interesting issue related to properties of single units needs to be discussed: What could account for the difference between tuning properties of single units and sensorimotor maps? As noted above, the sensorimotor mapping results were more compatible with classical models of the PPC. One possible explanation for this observation is biased sampling. During recording sessions I did not attempt to obtain uniform sampling of PPC surface, but rather
focus on task-related sites, which approximately matched reports in previous literature. Thus, during planning of each recording session, my objective was to find “distal” sites in IPL and “proximal” sites in SPL, based on all available data from previous mapping and recording sessions. In contrast, there was no bias in choice of single units according to their task-dependence, or their fit to a theoretical tuning model. An alternative explanation is spatial organization of single units (Ben Shaul et al., 2003). Sensorimotor mapping procedures were applied to the channel level, rather than at the single unit level. If single units in SPL and IPL are equally likely to be directionally tuned, but PDs of nearby units in SPL are more similar than those of units in IPL, listening to multi-unit hash might give the impression that IPL sites are less related to proximal movement than SPL sites. PD differences of within-electrode and across-electrode unit pairs provide some support for this explanation (Fig. 1.7). Finally, it is important to note that during sensorimotor mapping, it is much easier to observe peri-movement activity, which in PPC was slightly more segregated than activity during delay.

2. Correlations between single unit pairs in PPC during prehension do not lend support to models of binding by sharp synchronization

Von der Malsburg (1981) suggested that sharp synchronization of single unit firing is used by the brain to bind high-level, distributed representations in the visual system. Since the theoretical grounds for the “binding problem” in sensory systems also apply to binding of movement elements (sometimes referred to as “primitives”), I tested the existence of sharp synchrony between single-unit pairs in PPC, using epoch-resolved cross correlation histograms. The use of multi-electrode recording techniques allowed me to test correlations between single units related to different aspects of prehension (e.g. reaching direction vs. object type) and located in separate cortical regions. However, synchrony-based models do not make quantitative, refutable predictions regarding the expected frequency and strength of correlations. Therefore, I searched for several types of evidence supporting the functional significance of pair-wise synchrony, such as dynamics in the strength of synchrony during the course of the trial, and association between tuning of correlations and properties of single units.

I found that CCHs of single units in PPC were generally “flat” and not characterized by sharp and significant peaks. Some sharp peaks in CCHs were observed, but their overall incidence in the population was typically not higher than the fraction expected by the significance level of
the tests used to identify them. Moreover, fractions of significant CCHs, as well as correlation strengths, were similar during Control and during Delay (Figs. 2.2 and 2.3). In-depth investigation of the few pairs with significant correlations did not indicate a clear relation between the properties of pairs and their single unit members (Fig. 2.5). Taken together, the characteristics of correlations observed in our data could not serve as evidence for the functional significance of neural correlations. Specifically, I found no evidence to support models of binding distant (across-area) neurons by precise synchronization.

However, lack of positive evidence in a single experiment, and using a specific analysis method, cannot be regarded as refuting time code models altogether. First, it could be claimed that coordination by precise neural synchrony is accomplished not in PPC but elsewhere in the brain. If one accepts evidence for grouping of visual stimuli by neural synchrony in visual cortex (Singer and Gray, 1995), then perhaps precise timing related to movement planning and execution should be looked for in motor cortex (e.g., Shmiel et al., 2005), or even in the spinal cord. Second, it could be claimed that the 1 second pre-movement delay, in which correlation was tested and not found, is not the right epoch. Third, it could be argued that synchronization between single units is too weak to be observed when data is too sparse (tens of trials per behavior, 1 sec. epoch, and low firing rates). My data did not support this possibility (Figs. 2.6 and 2.7), but did not make it possible to eliminate it altogether. Specifically, it could be claimed that correlation dynamics in time are very rapid, and can only be observed by time-resolved methods (Baker et al, 2001; Aertsen et al., 1989), for which I clearly did not have enough data. Finally, it could be claimed that neural synchrony is used to bind distributed representations solely during learning (Triesch and Von der Malsburg, 1996), and therefore it is not surprising that they are not frequently found in the over-trained animal.

Is it possible, then, to plan an “ultimate experiment” for testing binding by precise timing? The answer to this question is not straightforward. First, and as said above, ample theoretical work is needed to supply elaborate predictions regarding the degree of expected synchrony under different conditions. For example, it is possible that weak synchrony between specific neurons facilitates computation, but strong synchrony, as observed in pathological states (Goldberg et al., 2002) is destructive for cortical function. Alternatively, it could be claimed that different distributions of correlation peak lags represent different computation modes. Second, by trying to solve the over-training limitation by recording during animal learning, one risks amplifying problems of insufficient data size and trial-by-trial variability (Ben-Shaul et al., 2001), which
could lead to under- or overestimation of the true degree of synchrony in the system under investigation.

3. Slow waves and oscillations as possible candidates for coordinating activity across distant areas

Studies in the visual cortex (Singer & Gray, 1995) showed that Gamma oscillations in LFP appeared following a specific pattern of stimuli, and that coherence between oscillations at spatially separate sites could be interpreted as having a role in the perceptual binding of stimulus attributes. Subsequently, 20-40 Hz oscillations were also reported in motor cortex (Murthy and Fetz, 1996; Baker et al., 1997; Donoghue et al., 1998), and in parietal cortex (Mackay and Mendonca, 1995; Scherberger et al., 2005). However, these reports differed with respect to the association of oscillatory activity with behavioral state, and hence, with respect to its hypothesized function. For example, Baker et al. (1997) found maximal oscillatory activity in MI during application of steady-state precision grip force. These oscillations were abolished during movement, and coherent with measured EMG oscillations. Baker et al. therefore suggested that oscillatory states promote efficient cortico-motor output, whereas asynchronous states are better for highly-demanding computation associated with movement execution. In contrast, Donoghue et al. (1998) found oscillations in MI and premotor cortex that occurred either during pre-movement delay (in a trained delayed-reaching task) or during movement itself (in untrained reaching). This group concluded that oscillations reflected general mechanisms of planning and preparatory functions.

I found that bouts of oscillatory activity were frequent in LFP of PPC sites. Oscillations were typically in the Beta range (13-30 Hz), and were observed during pre-movement delay (consistent with Donoghue et al., 1998; Scherberger et al., 2005) and/or during post-movement grip (Hold epoch, consistent with Baker et al., 1997). Oscillations were most prominent in area MIP (Fig. 3.1 and 3.2). Beta oscillations were often tuned to target direction and/or object, inconsistent with a general “preparatory” attention state. The observation of Beta oscillations in PPC is not congruent with their hypothesized role in optimizing motor output (Baker et al., 1997), since parietal areas do not directly project to cortico-motor pathways. However, since our experimental project did not include recordings from MI, I could not test the possibility that two (or more) different sources of Beta oscillations exist in the macaque cortex. Some support for this speculation comes from epidural recordings in a
single monkey by Mackay and Mendonca (1995), who found maximal activity in 20 Hz over MI, and maximal activity in 21-29 Hz over medial PPC. Interestingly, across-area coherence in the Beta range tended to decrease during the task, despite increases in Beta power (Fig. 3.8). This finding is inconsistent with the hypothesis that across-area coordination is achieved through coherence in the Beta band.

Gamma oscillations, in frequency ranges similar to those reported in visual cortex, were not a typical phenomenon in our data. However, a wide-band (30-100 Hz) increase in Gamma-band power was observed throughout the task, and especially during movement (Fig. 3.1 and 3.2). A similar phenomenon was found by Rickert et al. (2005) in MI during reaching, and was impressively used for decoding movement direction. However, I showed that this phenomenon is more likely to merely be a reflection of an increase in multi-unit firing rates, rather than a reflection of an oscillatory generator (Fig. 3.3 and 3.4). Thus, decoding stimulus or movement information from Gamma power is to a large extent a decoding based on MU spikes. Similarly, claiming that Gamma LFP is superior to spikes for decoding is equivalent to claiming that MU activity has a better signal-to-noise ratio than SU activity.

Whereas several studies of LFP in visual cortex hint that signs of across-area coordination should be searched for in the Gamma range, I found that coherence of across-area (SPL-IPL) LFP signal pairs was maximal in lower frequencies (<13 Hz), and that increases were observed not only during target presentation or movement, but also during delay. This was in contrast to the tendency of Beta-range coherence to decrease during delay. Moreover, tuning of across-area coherence during the task was in above-chance fractions only in the Delta to Beta band, but not in the Gamma band (Fig. 3.9). These results are in line with task-related changes in LFP-LFP and spike-LFP coherence recently reported by Pesaran et al. (Soc. Neurosci. Abstracts, 2005). In this study single-units and LFPs were recorded in MIP and PMd simultaneously during reaching tasks. Whereas within-area Beta coherence (15-40 Hz) in areas PMd and MIP tended to show an increase during pre-movement delay, across-area (PMd-MIP) coherence showed an increase only in frequencies between 5 to 20 Hz. Our results suggest that coordination across cortical areas may be achieved through low-frequency oscillations that are not necessarily generated in the cortex. Theoretical works (e.g., Von der Malsburg, 1999) stressed the utility of high-frequency oscillations for perceptual binding requiring high temporal acuity. Low-frequency oscillations, which by definition have long cycle durations, might seem inadequate for computational processes requiring fast and
accurate responses, such as execution of smooth, complex movements. Therefore, future empirical and theoretical studies of cortical oscillations should not be limited to Gamma-range activity but focus on low-frequency oscillations as well (Kahana et al., 2001).

4. Single units in PPC are superior to simultaneously recorded LFPs as sources for stimulus or movement decoding

Recently, the LFP signal was proposed as a potential input to neural prosthetic machines (Andersen et al., 2004), in addition to previously studied EEG and single unit spike signals. The LFP lies between these two signal types. Like extra-cranial EEG, LFP represents the activity of neuron populations, but like SU signals, LFP requires invasive recording techniques. However, LFP is easier to record than SU activity, and is more stationary over long time periods (orders of weeks and months). In contrast to EEG, LFP was recently shown to provide highly specific information regarding stimuli and movement parameters (Pesaran et al., 2002; Scherberger et al., 2005; Rickert et al., 2005).

In this study I compared the directional tuning and object sensitivity of spiking activity and LFP, using both time-domain (evoked potentials) and frequency-domain analysis. Brain-machine interface developers use engineering-based approaches, and typically compare different algorithms (or different neural signals), by their single-trial decoding abilities. I preferred the approach of the classical physiologist, and did not try to develop decoding algorithms, but rather tested tuning curves of evoked potential amplitude, LFP power, and LFP coherence for their statistical significance and trial-by-trial variability. In other words, I treated LFPs in the same manner as most neurophysiologists usually treat spike counts (or rates).

I found that LFP is very often tuned to target direction or object. This was true both for EP tuning curves (time domain analysis) and for tuning curves of LFP power spectrum in different frequency bands (Figs. 4.5 and 3.6, respectively). LFP power analysis showed that fractions of significantly tuned LFP sites are similar to fractions of tuned single units, but LFP was inferior to SU activity in two respects: the signal-to-noise ratio (or effect size, if social science terms are used) and non-independence of observations from different channels (Fig. 3.7). Non-independence related not only to simultaneously recorded LFPs from nearby electrodes, but for pooled data from the entire recording period of the two monkeys. This was
reflected in the tendency of samples of significant PDs computed from LFP tuning curves to deviate from a uniform distribution. In contrast, SU (as well as MU) activity did not show deviation from uniform distributions, consistent with previous literature (Figs. 3.7 and 4.7). These findings indicate two clear disadvantages of the LFP signal with respect to the development of clinical applications that receive a limited number of noisy input signals. Contrasting these disadvantages with the advantage of long-range stability of LFP recordings (Andersen et al., 2004) was outside the scope of the current study.

Like LFP power, LFP evoked responses were frequently tuned to target direction or object, and showed a significant deviation from a uniform distribution of PDs and POs (Fig. 4.5). Using time-domain analysis facilitated the comparison of spiking activity and LFP as a function of time. I found that direction or object tuning of MEPs typically emerged only around or after movement onset, whereas activity in MU spikes frequently showed significant tuning before movement onset (Fig. 4.8). A similar result was observed by Mehring et al. (2003, supplementary information) and Rickert et al. (2005) in primary motor cortex. Thus, pre-movement processing, which was reflected in the tuning of neurons, was not reflected in the MEPs. This finding was especially surprising because many MEPs started around 100 ms before movement onset (Figs. 4.2, 4.3), typically with a positive peak (‘P1’ in Donchin et al., 2001). Mitzdorf (1985) related early EP peaks to subcortical inputs, and late peaks to local cortical feedback. Thus, early activity observed in the EP may not reflect cortical pre-movement processing, but rather some external input that is less sensitive to target properties. This difference between spike and EP signals is also critical in the context of future clinical devices, since decoding has to focus on predicting movement intention from pre-movement signals. In contrast, pre-movement tuning of LFP was frequently found when tuning curves were constructed from LFP power, consistent with the reports by Rickert et al. (2005), and Scherberger et al. (2005). Thus, our results suggest that if LFP is to be used for a brain-machine interface, spectral methods seem more promising than EP-based methods.

5. Why do different neural signals convey different preferences? Two speculative explanations

Apart from the application-centered view, the above findings leave us with an unsolved puzzle: How is it that the LFP carries ample information on target parameters, but does not relay the same message conveyed by simultaneously recorded spikes? The LFP signal
measures the activity of a neuron population, and this population is typically characterized by uniform distributions of preferred directions and objects. Therefore, the most intuitive predictions are that the LFP will either show little sensitivity to task parameters, due to cancellation, or that LFP will have similar preferences to neurons recorded in the same site. Interestingly, our findings, which do not fit either of these predictions, are in line with previous reports that compared LFP and spike tuning in other cortical areas. Donchin et al. (2001) reported that MEPs in primary motor cortex had a preference for the contralateral arm over the ipsilateral one, and that this preference was not observed in the spike data. Similarly, Eggermont & Mossop (1998) found stronger contralateral preference in LFP than in spike data recorded from cat auditory cortex.

Understanding the difference between tuning properties of single unit populations and LFPs requires a better understanding of the sources of the LFP signal, which could not be fully achieved with our recording methods. For this purpose, multiple electrodes should be placed at equal distances on the cortex face, and at different depths, such that different cortical layers are sampled systematically. As shown by Mitzdorf (1985), LFP does not result from summation of multi-unit spiking activity in the vicinity of the electrode. Similarly, our (and others’) findings show that the LFP signal does not reflect the number of activated neurons, or their firing rates. If tuning of LFP simply means that more units are recruited for certain movements (e.g., power-grip vs. precision-grip), then a preference for these movements would have been observed in the MU data as well (e.g., more units with power-grip PO).

One possible explanation for the discrepancy between LFP-based and spike-based tuning may relate to the spatial organization of neurons (see above, in the first section of the discussion). If neurons that are sensitive to a task parameter (e.g., target direction) are distributed in a non-random manner within a cortical area (for example, in patches or columns of similar PDs), significant parameter tuning could be obtained from population measures such as the EP. Some evidence for such non-randomness in motor cortex has been reported (Amirikian & Georgopoulos, 2003; Ben Shaul et al., 2003). If, furthermore, this non-uniform spatial organization is biased towards specific parameter values, the population measure tuning may not represent spike tuning. Consider an extreme example, where patches of neurons with contralateral preference dwell within a messy population of neurons with random preferred directions, such that for the whole population PDs are uniformly distributed. In such a case,
many EPs recorded from this cortical area will show significant directional tuning, and a non-uniform PD distribution.

An alternative explanation is that the LFP (or EP) amplitude is associated with the degree of local population synchrony, just like electrocardiographic phenomena. In this case, EP peaks reflect fast changes in local synchrony, which are too weak to be observed by cross-correlation analyses of single unit pairs. Similarly, LFP oscillatory bouts could be related to synchronized activity of specific neural populations (e.g., inhibitory interneurons), which constitute a minority among the SUs that are typically recorded simultaneously with the LFP signal.

6. Evoked local field potentials do not support feed forward parietal-to-premotor models

Response latency detection is important for inferring directionality of information flow within the cortex, and its relation to external events. Most models of visuomotor transformation (e.g., Fagg and Arbib, 1998) tend to adopt a feed-forward view of information flow from the visual, through the parietal, to the motor cortex. In contrast, recent anatomical studies of parieto-frontal circuits (Luppino & Rizzolatti, 2000) report strong bi-directional connections between the parietal and frontal areas. Distributions of latencies of significant VEPs reported in the current study did not support a model of serial activation of the parietal and premotor cortex, but rather their parallel activation, whereas latencies of MEPs were in accordance with a premotor-to-parietal flow. Previous attempts at comparing activation latencies between cortical areas based on spike data (Crutcher & Alexander, 1990; Ashe & Georgopoulos, 1994; Kalaska et al., 1983) revealed between-area differences but also extensive overlap of latency distributions. Consistent with our findings, these studies indicate that models assuming strict serial feed-forward flow of information from parietal to premotor and then to primary motor cortex are over-simplified, and that processing is much more parallel than serial.

An exception to the evidence supporting parallel activation of the examined cortical areas is the finding that latencies of the first MEP peak in the dorsal “reaching related” areas slightly preceded latencies of the ventral “grasping related” areas (Fig. 4.3B). This “reach-before-grasp” lag (effect size of only 6-7 ms) disappeared in the later (and also stronger) EP peaks. This finding does not fit predictions based on psychophysical studies revealing co-occurrence of reaching and grasping components, or the parallel activation of proximal and distal joints in humans (Jeannerod, 1984) and monkeys (Roy et al., 2000, 2002; Mason et al., 2004). One
possible explanation for this apparent difference is the temporal resolution of behavioral measurements (e.g., sampling rate of 60 Hz in the studies of Roy et al.), which is insufficient for observing lags on the order of few ms in the activation of proximal and distal joints. Alternatively, it is possible that reaching and grasping components are initiated separately, but are gradually synchronized during the course of movement, with the help of sensory feedback mechanisms.

7. A concluding note on the neural code

Classical systems neurophysiology regards neurons’ firing rates as the dependent variable of the experiment. Regardless of the spike extraction, unit sorting, and data-logging techniques, spike occurrences are usually summed over defined time windows, and spike counts (or rates) are used to test the effect of an extrinsic independent variable (i.e., experimental manipulation, such as stimulus direction) or of an intrinsic independent variable (observed behavioral response, such as reaction time). Rate analysis assumes that the exact temporal profile of neuron responses, either at the single-trial level, or at the average response (PSTH) level, does not have a critical functional significance. Rate analysis also assumes that since neurons are the building blocks of the computation network, the activity of single units is the purest and therefore most appropriate neural signal for study. Finally, rate analysis typically assumes that a given sample of recorded (or simulated) neurons is a random sample of independent observations. This assumption is used in the development of theoretical models as well as in statistical testing of empirical data. Undoubtedly, those three assumptions have simplified experimental methods and thus facilitated knowledge accumulation for decades.

In recent years, however, important theoretical questions have been raised with respect to the capacity of the rate code to serve for higher brain function. The binding problem (Von der Malsburg, 1981) is but one example of this type of question. The introduction of multi-electrode recording techniques has made it possible to examine the assumption of independent firing, and increased the interest in analyzing responses of neuron pairs, triplets or larger assemblies (Averbeck and Lee, 2004). Interest in assembly cooperation has contributed as well to the interest in population signals such as the LFP (Bullock, 1997). In contrast to the simple, perhaps over-simplistic assumptions and techniques used by proponents of the rate-code approach, there is little agreement regarding the methodology of choice for studying alternative or complementary coding: Should one study data of multiple single units, or of
population signals? Should one concentrate on precise correlation effects, or use wide smoothing windows (‘noise correlations’)? Is frequency domain analysis preferable to time-domain analysis? In other words, the a-priori definition of the dependent variable, a fundamental element of any research design, is highly variable across research groups.

Moreover, while complex analysis methods are continuously introduced, little effort has been made for the purpose of unifying and standardizing the rapidly growing field of “neurostatistics” (an exception is the open-source software developed by P.P. Mitra’s laboratory, which I used for the spectral analysis of LFP). Instead, different research groups, which focus on different neural signals, devise their own analysis tools and statistical tests. The lack of standardization is a serious obstacle to the advancement of any scientific discipline, since weak positive results are often discarded by opponents of a theory as being artifacts of a suspicious method, and negative (typically unpublished) results may be the outcome of using an over-conservative method with low statistical power. Under these conditions, there is little likelihood of obtaining sufficient empirical evidence to prompt a “scientific revolution” (Kuhn, 1962) in Neuroscience, acknowledging the importance of time codes and/or assembly-based coding, which may coexist with the simpler rate code.

In my view, the greatest challenge for future neural code-crackers is finding the dependent variable of choice, and agreeing on its operative definition and statistical testing methods. However, it is still not clear whether it is possible to dissociate the “location” or “substance” of the code from the “key” to deciphering it, as was the case in Genetics research. This challenge can only be met by contrasting different analysis approaches by testing them on data from various types of experiments.
Abbreviations list

AIP anterior intra-parietal area
CCH cross correlation histogram
CE control epoch
CPB mean count per bin
DOI direction-object index
EEG electroencephalogram
EP evoked potential
ICMS intra-cortical micro-stimulation
IPL inferior parietal lobule; in this study the term refers to the anterior part of IPL (AIP and Area 7b).
LFP local field potential
MEP motor evoked potential
MI primary motor area
MIP medial intra-parietal area
MRI magnetic resonance imaging
MU multi unit
PD preferred direction
PMd dorsal premotor area
PMv ventral premotor area
PO preferred object
PPC posterior parietal cortex
PSTH peri-stimulus time histogram
RMS root mean square
RTMT reaction time and movement time epoch
SMA supplementary motor area
SPL superior parietal lobule; in this study the term refers to MIP and Area 5.
STA spike-triggered averaging
VEP visual evoked potential
References


Stark, E., Asher, I., Drori, R., & Abeles, M. Dissociation between local population and single-unit properties in premotor cortex during preparation for prehension. (submitted to *Neuron*)


