DYNAMICS OF NEURONAL ACTIVATION
AND INTERACTIONS IN CORTICAL FRONTAL
FIELDS DURING ADAPTIVE SENSORIMOTOR
ASSOCIATIONS

Thesis submitted for the degree of
“Doctor of Philosophy”

by

Dorrit Inbar

Submitted to the Senate of the Hebrew University of Jerusalem

December, 2010
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This work was carried out under the supervision of:

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לאבי, שלמה ארצי ז"ל
בגעגועים גדולים
בנטנופים שונים
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Abstract

Processing information in the brain is carried out by densely interconnected neurons. Research has shown that neurons encode information not only as single encoders, but also as participants in ensembles. Therefore, in order to explore the function of the brain it is necessary not only to address the response properties of the single neurons, but also their interactions and co-activation in a network. Although the correlated activity between cortical neurons is statistically weak, several previous studies suggested that the information content can be high even in such low correlated networks.

Here we study the co-activity of pairs of cells, and its relation to the response properties of their constituent single cells. To do so, we recorded activity of single cells in the motor cortices of behaving monkeys, by using micro-electrode arrays. The monkeys performed a sensorimotor task of center→out reaching movements of the arm, guided by visual cues. We approached the study by first classifying the cells according to the signal they carry in their firing rates around the period of movement onset. Next, we examined the co-activation of pairs of cells using noise correlation and analyzed its temporal dynamics along the trials by Joint-peri-event-time-histogram (JPETH).

Our main findings point out a clear relation between similarity in the single cells’ preferred directions (PD) and their pair-wise correlation: Pairs with close-PDs have higher noise correlation than pairs with opposite PDs. Moreover, similar relation was found following learning of arbitrary mapping of stimulus color to movement direction; during the learning process new properties emerged in single cells response. We found
that noise correlation of pairs with both constituent cells having learning-induced-color-sensitivity was higher than the noise correlation of the other pairs.

However, an intriguing variety in the temporal dynamics and average strength of correlation arise in thorough examination of the results. In some pairs the temporal dynamics show increases or decreases of the level of correlation; these modulations are found to be related to (A) behavioral events and (B) the difference between the preferred-directions of the constituent cells. Other pairs show on-going correlation along the trial with minor, or no temporal modulations. In addition, concerning the variability in the mean strengths of correlations, we find that some pairs featured by opposite PDs are positively correlated, and vice versa – some of the close PDs pairs have negative correlations. Thus, the correlation strength ranges from positive to negative even when pairs are grouped to subpopulations according to their PD difference. Interestingly, among neighboring cells we find also negative correlations; however only the subpopulation of opposite-PDs, and not the close-PDs, has a significant group of cells with negative correlation.

Taken together, the outcomes of this study imply an organization of partially overlapping neural networks in the motor cortices. We found that different sensorimotor associations give rise to network activity in different patterns. These patterns of dynamics of neuronal activations and interactions imply an emergence of two major types of network organizations: First, a network with dynamic connectivity, reflected in the correlation's temporal dynamics related to behavioral events. Second, a network with consistent interaction, reflected in on-going correlation strength unrelated to events.
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1. Introduction

Neurons in the central nervous system process information that eventually (or cyclically) leads to desired interaction with the environment. The cerebral cortex is known to be densely populated with highly inter-connected neurons. For example, in the human motor cortex the neuronal density is estimated by ~10,000/mm³, where each neuron receives inputs from tens of thousands synapses (Abeles M, 1991). To understand information processing in the brain, it is necessary to understand the interactions between the neurons, and to unveil the relations between neuronal network activity and the behavior of the animal. An outside observer of the brain would notice that pair-wise correlations are statistically weak and infrequent (the order of magnitude of correlation-coefficient is 0.01 for neighboring cells in our study), and the synaptic strength is much lower than its naïve portrayal. However, this is by no mean surprising, taking into account the huge number of inputs a cortical neuron receives; in case the neurons’ activities were much more correlated, the probable consequences would have been (1) burst of synchronous firing across the brain, and (2) high redundancy of encoding the same information by many neurons.

Single cells in various cortical regions are known for having task related activity, for example by changing their firing rate as a function of stimulus properties or behavioral parameters (Hubel & Wiesel, 1959; Georgopoulos et al., 1982). It has also been shown in previous studies, in our group among others, that cells in motor cortices modify their
firing properties in relation to learning of novel stimulus-response couplings (Paz et al., 2003; Paz et al., 2005; Zach et al., 2008; Mandelblat-Cerf et al., 2009; Arce et al., 2010). Addressing the role of single cell’s activity in the brain, Barlow (1972) attributed the representation of a complex object to a single neuron (the concept of “cardinal-cell”). One of the challenges this concept poses is the need of almost an infinite number of neurons to represent the nearly infinite number of objects in the world. In contrast, a more feasible concept of representation in the brain is by neuronal population, where a particular combination of several activated neurons holds the representation of a complex object. Representation of complex objects by a neuronal ensemble allows almost an infinite number of possible representations. For example, von-der-Malsburg (1981) dealt with “the binding problem”, claiming that the correlated firing of neurons would be a way to establish links which associate several simultaneous features. Extending the theoretical arguments made by von-der-Malsburg to motor cortex, statistical interactions among motor cortical neurons could combine elementary motor representations to generate a coordinated motor action.

The issue of neuronal networks has been addressed by many studies from different perspectives- theoretically and experimentally. The smallest network is a pair of single cells, and a common measure for the co-activity of the two single cells recorded in-vivo extracellularly is the cross-correlation between their spike trains (cross-correlation histogram (CCH), Perkel et al. (1967)). Previous studies (for example: (Zohary et al., 1994; Vaadia et al., 1995; Lee et al., 1998; Hatsopoulos et al., 1998; Smith & Kohn, 2008)) have shown that although pair wise correlations in the cerebral cortex is featured by low values, they still support representation of information on larger-scales networks
(Salinas & Sejnowski, 2000; Salinas & Sejnowski, 2001; Averbeck et al., 2006; Schneidman et al., 2006).

These pair-wise correlations were frequently attributed to a shared anatomical input (Fetz et al., 1991), or reflect a dynamic process of emergence into a functional neuronal network (Abeles et al., 1993; Grun, 1996; Riehle et al., 1997; Prut et al., 1998). The term "noise-correlation" is commonly used (Zohary et al., 1994; Lee et al., 1998; Yanai et al., 2007) to describe the degree of trial-by-trial correlated variability beyond the expected level. The notion of “expected level” relates to the mean response to a certain parameter across trials. In order to further explore the temporal dynamics of pair wise correlation in different time lags (in relation to a certain behavioral event), Aertsen et al. (1989) developed the Joint-peri-stimulus-time (JPSTH) analysis. Henceforth “noise correlation” is referred simply by “correlation”; and JPSTH is termed Joint-Peri-Event-Histogram (JPETH) to denote relation to behavioral event rather than merely stimulus related co-activity.

Though some studies suggested that the contribution of correlations to information procession is negligible (Shadlen & Newsome, 1998; Nirenberg et al., 2001; Kayser et al., 2010), others demonstrated that correlations vary with sensory inputs (Gray et al., 1989; Ahissar et al., 1992; Laurent & Davidowitz, 1994; deCharms & Merzenich, 1996), motor output (Maynard et al., 1999; Hatsopoulos et al., 2003; Kilavik et al., 2009), attention (Riehle et al., 1997; Steinmetz et al., 2000), past experience (Hoffman & McNaughton, 2002), and combination of movement parameters (Stark et al., 2008).
Relations between noise correlation and similarity in tuning curves of the single cells has been previously shown in a few cortical areas: for example, in sensory processing Zohary et al. (1994) and Bair et al. (2001) demonstrated higher noise correlation between cell pairs in medial temporal (MT) cortex, when the tuning curves for visual stimulus of moving dots were similar. In primary auditory cortex, Rothschild et al. (2010) showed that the noise and the signal correlations are significantly positively correlated; however the variability was high and there were still pairs with higher signal correlation that had no noise correlation, and vice versa. The relations between directional tuning curves and noise correlation in motor cortices were addressed in previous studies. Lee et al. (1998) showed dependency between noise correlation and signal correlation of pairs of adjacent cells in M1 and parietal cortex; Stark (2007) found in pre-motor cortex that noise correlation tended to be higher when signal correlation was higher, or when units were anatomically closer. This relation was described to be stronger during pre-movement epoch, than during the movement-onset.

*In our study we seek evidence for possible relations between the parameters that single neurons encode, and the level of pair-wise correlations between them.* We address the question of dependencies between correlated cells’ activity in pairs, and various task parameters. The main question in this study concerns the dynamics of the correlated activity of cell pairs, asking whether the correlations persist along the recording session regardless of behavioral events; or rather depend on such events.

Encoding of task properties in motor cortices is not limited to kinematic and dynamic parameters, but also to other arbitrary parameters which the animal has learned to map and associate to movements (reviewed by Wise & Murray (2000)). For example, Mitz et
al. (1991) showed in a conditional learning task encoding of visual stimuli, which served as non-directional cues to the direction of movement. A previous study in our lab (Zach et al. (2008) with shared data of this thesis) showed learning-related changes of firing rates of cells in motor and pre-motor cortices in response to *color*. Meaning, when learning a rule of mapping colors to movement directions, cells changed their firing rates when presented with these colors; in contrast, the cells did not change their activity in response to other colors (“naïve” colors) that were not mapped to movements.

*In our study we thus ask whether the pair-wise correlations are related to the constituent cells’ responses to arbitrary visual cues when related to movement direction in comparison to “naïve” cues that have no such relation.*

Further, previous studies have demonstrated learning related changes in motor cortices: from long-term-potentiation (LTP)-like changes (Buitrago et al., 2004; Wankerl et al., 2010), to single cell firing rate changes during and after learning a visuomotor task (Wise et al., 1998; Gandolfo et al., 2000; Li et al., 2001; Cohen & Nicolelis, 2004; Paz et al., 2005; Zach et al., 2008), and fMRI activation of M1 during learning of a finger motor sequence (Karni et al., 1995; Karni, 1996; Shadmehr & Holcomb, 1997; Karni et al., 1998).

*Thus, our study addresses the question of learning induced changes in the correlated activity of cells in the motor cortices: would learning modify the strength and pattern of correlation between cells?*
To address these issues we recorded the neural activity from monkeys’ motor cortical fields, while the monkeys were performing a visuomotor task of center → out arm movements. The results of our study demonstrate that noise correlation varies between neuronal pairs, and shows dependency on the response properties of the constituent cells. Pairs with close preferred directions (PDs) have higher noise correlation than pairs with opposite PDs. Further, pairs with both constituent cells having learning-induced-color-sensitivity, have higher noise correlation than other pairs which have cells that do not share this property. However, an intriguing heterogeneity exist, both in the average level of correlation, and in the temporal patterns of the correlations (which varies from event related dynamic changes to ongoing persistent correlation strength). These outcomes presented here imply an organization of cells into partially overlapping functional networks, which is generated to some extent by the properties of the input signals. Further, the changes of noise correlations after learning lend support to the notion of reorganization of neuronal networks in the motor cortices during learning.
2. Methods

This study was based on electrophysiological experiments on two behaving monkeys K and R. Neural activity was recorded by microelectrodes situated in motor cortices of the monkeys, which performed a visuomotor reaching task. Training sessions of the monkeys and surgical procedures preceded the mentioned experiments.

Animal care and experimentation were in accord with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals and with the Hebrew University guidelines for the use and care of laboratory animals in research, approved and supervised by the Institutional Animal Care and Use Committee. Before, during, and after experimentation, monkeys were assessed for general wellbeing. When the health of the monkeys so required, the experiment was terminated or appropriate treatment was applied as recommended by the head veterinarian.

When the experiments came to conclusion, the recording chamber (monkey K), microelectrodes array (monkey R), and head holder were removed, and the skin was sutured. Following a recovery period the monkeys were sent to a primate sanctuary (http://monkeypark.co.il).

The acquired data was further analyzed on custom written software in MATLAB (Mathworks).
2.1 Experimental procedures

Behavioral task

During each training and recording session the monkeys (*Macaca fascicularis*) were sitting in a primate chair, and holding a handle with their dominant hand, while the other hand was restrained. In front of them was a screen presenting visual cues and a cursor that tracked the instantaneous position of the handle. The task consisted of center→out reaching movements to eight directions from a central position to a peripheral position, in the two dimensional workspace. The x-y coordinates of the handle’s position were sampled and stored, available for further analysis.

The arm and the handle were concealed from the monkey’s sight during task performance.

Trial flow

Each trial began with the screen display of an “origin” (cue in the center of the workspace). The monkeys positioned the cursor at the origin and held it there for a randomized variable period until a target appeared in one of eight possible locations in the periphery of the workspace. After a second delay (varied randomly) from target onset (TO), the origin disappeared (“Go” signal (GS)) and the monkeys were supposed to reach the target within 1s from go signal. Upon reaching the target, the monkeys held the position to get a reward (fruit puree), cued by change of target color and an auditory note.

An inter-trial interval followed trial end, during which the workspace was blanked.

The trial flow and the specifications for both monkeys are detailed in Figure 1.
Figure 1 Trial flow. Black figures illustrate the visual presentation on the monitor from beginning of trial (“Origin Onset”) and leftwards to end of trial (“Reward” and inter-trial interval). The durations of epochs within the trials are detailed below for both monkeys (monkey R – green caption; monkey K – amethyst caption). Captions denote behavioral events and epoch names within the trial.

**Monkey R**

The monkey (male, ~4kg) held the handle of a lightweight robotic arm (Phantom Premium 1.5 High Force, SensAble Devices, Cambridge, MA) with its right hand. A 3D monitor projected a stereo image onto a mirror. A horizontal workspace plane was created via force boundaries applied by the robotic arm along the vertical axis.

The origin and the target were 10mm radius, and the distance between their centers was 4.24cm. (These specifications were presented earlier in a study from the same data (Arce et al., 2010)).

**Monkey K**

The monkey (female, ~3 kg) held a two-joint manipulandum with its left (dominant) hand at the elbow level. The manipulandum was moving in a two-dimensional plane. The origin and targets were 7mm radius. The target was colored with one of four possibilities,
in a semi-random order between the trials. These colors were meaningless during the trials of the “standard” block, but served as a reference to the arbitrary learning block.

In the arbitrary association learning task, presented to monkey K, the color of the target, rather its spatial location, signaled the direction of the movement. During this block, two colors were in use, whereas the other two colors were not presented. Note that all four colors were presented during the standard (non-learning) blocks.

The mapping between the colors and the movement directions varied between training and recording sessions.

Detection of movement onset

The behavioral event of “movement onset” (MO) is often being regarded in this thesis. The detection of initiation of movement after the Go signal was determined off-line by an algorithm developed by Amos Arieli. The main outlines are as follows:

*Reference velocity* – defined as the average across all trials, of the median velocity around the Go signal (±50msec for monkey R; ±10msec for monkey K).

*Peak velocity* – defined as maximum of the velocity profile.

If the conditions for a certain step are fulfilled, that step replaces the movement onset definition of the previous one in the hierarchy. The four steps are:

1. Movement onsets when the tangent at 2/3 of *peak velocity* on the velocity profile, crosses the *reference velocity*. 


II. Movement onsets when the line intersecting the points of 1/3 and 2/3 of peak velocity, crosses the instantaneous velocity curve (preceding the point of 1/3 peak velocity).

III. Movement onsets at the latest time point with negative slope of the instantaneous velocity curve (preceding the point of 1/3 peak velocity).

IV. Movement onsets at the time point of 1/5 of the mean slope of the instantaneous velocity curve (preceding the point of 1/3 peak velocity).

Surgical procedures and identification of cortical locations

Following training sessions, the monkeys went through a surgical procedure to allow extra-cellular recordings by microelectrodes in the motor cortices.

*Monkey R* was chronically implanted with a 100 microelectrode array (Cyberkinetics Neurotechnology Systems) on the arm region of the left M1. 96 out of the 100 microelectrodes (ME) are used for recording neural activity. The cortical location was identified using MRI and passive limb movement. See Figure 2 I for location of the array on the cortex.

*Monkey K* was implanted with a 27x27mm recording chamber above the frontal area of the right hemisphere. The identification of cortical location based on bone surface landmarks, MRI analysis with the generous help of Dr. Tanya Orlov, intra cortical micro-stimulation (ICMS) and passive limb movements. Preliminary ICMS mapping sessions were conducted before initiation of the recording sessions. Penetration sites where ICMS
evoked single joint shoulder or elbow movements at \( \leq 20\mu A \) were regarded as M1 arm area. Areas more lateral were regarded as premotor area, both dorsal and ventral, relative to the principle sulcus. See Figure 2 IIA for penetration map of the electrodes on the cortex.

When a critical number of electrodes failed to penetrate the Dura Mater (usually once in a couple of weeks), a scrapping procedure of the tissue was performed.

**Recordings and data acquisition**

*Monkey R*

In each recording session a fine cable was attached to the on-surface connector of the implanted chronic array. The signals from 96 microelectrodes were amplified, online sorted, sampled at 30 kHz, and stored by Cyberkinetics Neurotechnology Systems.

Experiments on monkey R were conducted by Yael Mandelblat, Itai Novick and Fritzie Arce.

*Monkey K*

In each recording session an MT (Microdriving terminal, Alpha-Omega) with up to 32 moveable microelectrodes was positioned on the chamber and adjusted in the horizontal plane. The 32 microelectrodes were inserted via a penetration grid in the MT, allowing for diverse spatial arrangement of the electrodes. The minimal distance between grid
holes was 1mm; however, due to technical constrains the electrodes were not usually positioned in adjacent holes, resulting with at least 2mm distance. Due to this arrangement of electrodes in the grid, “nearby” pairs are those with inter-electrodes distance up to three grid holes in a diagonal, corresponding to $\sqrt{8}$ mm $\approx$ 3mm. See Figure 2 IIB for demonstration of arrangement of 32 electrodes in the grid.

Glass-coated tungsten microelectrodes with impedance of 0.2-1MΩ at 1kHs (prepared by Varda Sharkansky) were used for recordings. We inserted each electrode with a PC controlled system (Electrode-positioning-system, EPS, Alpha-Omega). Signals from the electrodes were amplified and filtered by the MCP-plus (Alpha-Omega). Then, sampling and manual online sorting were carried out by the Multi-spike-detector (MSD, Alpha-Omega, 16 channels, 25 kHz) and the Alpha-spike-detector (ASD, Alpha-Omega, 16 channels, 30 kHz); the Alpha-Map (Alpha-Omega) was used for acquisition of the signals.

Experiments on monkey K were conducted by Neta Zach, Yael Grinvald and me.

Database of recording forms

During the recording sessions we filled up paper forms; these forms contained information regarding the session (monkey weight, task specification), the electrodes (coordinates of their location, ICMS and passive movements), and the units (isolation grades and remarks). This information was further fed into an MS-Access database, custom made with the kind help of Uri Inbar. The database was accessible from Matlab for further analysis.
Figure 2 I Cortical map of monkey R: location of the 4X4mm 100-microelectrodes (ME) array in relation to the central sulcus, in the left hemisphere. Figure constructed from a digital photograph taken at the surgery of implantation.

II (A) Cortical map of monkey K: location of cells in the right hemisphere included in the analysis. Figure constructed from bone landmarks and MRI. (B) An example of spatial arrangement of 32 microelectrodes in the MT’s grid, during acute recordings of monkey K.
2.2 Data analysis

Single unit activity

Stability of neuronal activity

Each unit was graded during an on-line sorting and recording session. For further analysis we chose units that were graded as reasonably isolated single-unit.

We evaluated by careful eye-balling each unit for stability for firing rates during whole recorded period, by examining the pre-target-onset hold epoch (MATLAB software developed by Yoram Ben-Shaul). We excluded from further analysis units that did not demonstrate stable firing rate along the session, units with low firing rates (<1Hz), and specific trials with bursting activity.

Behavioral parameters of movement

For each trial in the data set of monkey R we calculated five different parameters of performance of movements:

1. *Path length*- calculated from the hand position in movement onset time (MO) till target reach (TR).

2. *Reaction time*- the duration time from “Go” signal (GS) till MO.

3. *Movement time*- the duration from MO till TR.
4. *Time to peak velocity*- duration time from MO till time of maximal velocity along the movement.

5. *Distance from straight line*- calculated from the Cartesian distance from the point of peak velocity on the trajectory curve, to the straight line connecting the beginning and the end of the trajectory curve (i.e. at point of MO and at point of TR).

For each recording day we calculated separately the mean and the standard-deviation of each movement parameter. A trial was denoted as having low behavioral variability, if in all the listed above parameters it did not exceed the mean ±1*STD of the distribution for that recording day (i.e. session).

**Directional tuning**

The mean firing rate of each single cell was calculated from -100msec to +300 around movement onset. This was calculated for all trials in the session that passed the previous mentioned criteria, with a prerequisite of successful trials (i.e. the monkey performed it successfully) to at least six (out of possible eight) different directions. (If a session also included a learning block of trials, in addition to standard trials, they were excluded from calculation of directional tuning). Then, we performed one-way-ANOVA on the firing rates grouped according to the direction of movement. A cell was defined as directionally tuned if the p value was smaller than 0.05. Meaning, the cell responded with a different firing rate to at least one direction of movement.
The preferred direction (PD) of each directionally tuned cell was defined as the direction (one out of possible eight) which elicited the maximum firing rate around movement-onset.

Furthermore, we performed for each cell the linear fit to the standard uni-modal cosine tuning as described in (Georgopoulos et al., 1982). The goodness of fit was assessed by computing the coefficient of determination, R2 (Georgopoulos et al., 1982; Amirikian & Georgopoulos, 2000) A cell was defined as fitting a cosine function if R2 exceeded the value of 0.55.

*Similarity of preferred directions (PDs) between pairs of cells:*

The term “Close-PDs pairs” describes cell pairs with PD difference of up to ±45°. The term “Opposite-PDs pairs” describes cell pairs with PD difference of 180±45°.

Trials with movements towards the PD include also the ±45° trials from that direction; Movements towards the null direction include trials to the directions of 180° from the PD ±45°.

*Learning-induced-color-sensitivity*

Monkey K was introduced with a block of arbitrary learning trials, in several recording sessions. Two blocks of well-known standard trials preceded and followed the learning block.

The mean firing rate of each cell was calculated from -100msec to +400 around movement onset, of the learning trials, and from the pre-learning (1st standard (STD1)) of
the exact matching type (i.e. same movement direction and same color as in the learning trial). The cell was defined as having "learning induced color sensitivity" if two-sample t-test between the learning and the pre-learning firing rates had p-value lesser than 0.05.

Cell pairs were further marked as “both” if the two constituent cells had learning-induced-color-sensitivity; “one” if one cell passed the criterion for sensitivity, while the other cell did not pass; “none” if both did not pass the criterion. Please note that pairs were not further subdivided according to a response to particular color, but only according to the response to movement-related-colors vs. response to colors without any relevance to movement. This is based on previous finding form our group (Zach et al., 2008) demonstrating that cell’s firing response did not differ according to a particular color, but merely on the basis of behavioral relevance of the color to movement.

**Correlation analysis**

The main results of our study focus on the co-activation of cell pairs beyond the expected from their firing rates, meaning the noise correlation of the pair.

**Cross-correlation-histogram / Cross-correlogram (CCH)**

The cross-correlation-histogram, also termed cross-correlogram (CCH, (Gerstein & Perkel, 1969; Aertsen et al., 1989)), measures the correlation between spike trains in different time lags between the trains. It is calculated as follows:

The *raw* histogram: Trial-by-trial cross correlation.
The predictor histogram: Cross correlation between the Peri-event-time-histograms (PETH). Used for correcting for common firing rates modulations.

The standard-deviation (STD) histogram: Depicts the STD for each time lag, and normalizes the noise-correlation into correlation-coefficient (cc) units.

The $CCH = (\text{raw-histogram} – \text{predictor}) / \text{standard-deviation}$

Joint peri event time histogram (JPETH)

The Joint-peri-event-time-histogram (JPETH) analysis gives a measure for pair-wise noise-correlation in different time bins along the trial. We calculate it in a similar approach to the CCH above, including the raw matrix, PETH-predictor matrix, standard-deviation matrix and normalized-JPETH. The following conventions are used: $n$ the number of coincidences in a time bin; $i,j$ neuron; $u,v$ time bin; $k$ trial; $K$ total number of trials (from Aertsen et al. (1989)).

The “raw” matrix:

$$\langle n_{ij}(u,v)\rangle = \frac{1}{K} \sum_{k=1}^{K} n_{ij}^{(k)}(u)n_{ij}^{(k)}(v) = \frac{1}{K} \sum_{k=1}^{K} n_{ij}^{(k)}(u,v)$$

The Peri-event-time-histogram (PETH):

$$\langle n_{i}(u)\rangle = \frac{1}{K} \sum_{k=1}^{K} n_{i}^{(k)}(u)$$

The PETH-predictor:

$$\tilde{n}_{ij}(u,v) = \langle n_{i}(u)\rangle \langle n_{j}(v)\rangle$$

The corrected (un-normalized) JPETH:

$$D_{ij}(u,v) = \langle n_{ij}(u,v)\rangle - \tilde{n}_{ij}(u,v) = \langle n_{ij}(u,v)\rangle - \langle n_{i}(u)\rangle \langle n_{j}(v)\rangle$$

The standard-deviation matrix:
Thus, the JPETH after correction and normalization:

\[
\tilde{s}_{ij}(u,v) = s_i(u) \tilde{s}_j(v) = \left\{ \frac{1}{K} \sum_{k=1}^{K} \left( n_i^{(k)}(u) - \langle n_i(u) \rangle \right)^2 \cdot \frac{1}{K} \sum_{k=1}^{K} \left( n_j^{(k)}(v) - \langle n_j(v) \rangle \right)^2 \right\}^{1/2} = \left\{ D_{ij}(u,u) D_{ij}(v,v) \right\}^{1/2}
\]

\[
C_{ij}(u,v) = \frac{D_{ij}(u,v)}{\tilde{s}_{ij}(u,v)} = \frac{D_{ij}(u,v)}{\left\{ D_{ij}(u,u) D_{ij}(v,v) \right\}^{1/2}}
\]

Henceforth, the term “JPETH” relates to the normalized corrected JPETH.

Note that the main diagonal of the JPETH matrix corresponds to zero lag between the spike trains. Further, averaging along a particular diagonal yields the corresponding CCH value of that time lag. Therefore, in our study we calculated the CCHs out of the JPETHs.

The bin size of the JPETH matrices is of 50msec.

Firing rate induced modulations of correlation: The most prominent results with temporal modulations of the JPETH’s main diagonal were demonstrated in correlation analysis of monkey R. Therefore in addition to using the PETH-predictor correction, we added a further criterion for the correlation analysis: JPETH matrices were eye examined for modulations in horizontal and vertical bands (rather than the diagonal band), which might stem from un-corrected modulations of firing rates of the cells. Number of pairs which passed this criterion: 195 out of 312 pairs with close-PDs; and 127 out of 285 with opposite-PDs.

Group of trials: The correlations were corrected and normalized separately for different trials, grouped according to the different stimulus or action: trials with movements towards the preferred direction of the cells, vs. trials towards the null direction; trials
with cues of *learned* colors, vs. trials with cues of “*naïve*” colors; trials *before* the learning epoch, vs. trials *after* the learning epoch.

This trial grouping is another step towards exclusion of undesired possible effects on the correlation analysis, by response latency or excitability (Brody, 1999) or stimulus and action dependent trial-by-trial variability of firing rate (Ben-Shaul et al., 2001).

*Population-JPETH:* We extended the JPETH analysis of a single neuronal pair to population of pairs, by averaging the JPETH matrices. Each matrix was corrected and normalized prior to averaging across the population (Joshua et al., 2009). The population-JPETH matrices were smoothed using two-dimensional Gaussian window (STD of 1bin).

From the population-JPETH we construct the population-CCH, in a similar manner of constructing a CCH from a single JPETH (each CCH bin is the average of a JPETH’s diagonal, corresponding to a certain time lag).

The significance evaluation of difference between population-JPETHs is performed by analysis of variance (ANOVA) comparing the main diagonals of the population-JPETHs. Further, we perform multiple comparison test using the Tukey–Kramer method (Sokal & Rohlf, 1995).

*Quartile analysis*

We ranked pairs in the population according to their correlation strength, and then divided them into quartiles. The correlation strength was calculated by summing the central diagonal of the JPETH and its two adjacent diagonals from either side, corresponding to the central peak of the CCH ± 150ms (unless specified otherwise).
Negativity of lower quartile: To evaluate the significance of the negativity of the lower quartile, we calculate the fraction of pairs with significant negative peak out of the total number of pairs in the population. The peak was considered negative if it exceeded 2*STD from the mean of the tail of the CCH (corresponding to baseline correlation): monkey R time lag range [+1 +2]sec of the right tail, monkey K time lag [+1.5 +4]sec of the right tail. When the fraction was larger than 0.05, the negativity was considered significant in this population.

Pairs from the same electrodes (neighboring cells)

We calculated for monkey K the correlation for pairs from the same electrode.

An additional criterion for further analysis, excluded pairs of neighboring cells with more than 5% of inter-spike-intervals (ISI) smaller than 3msec.

For further precaution, we excluded from any further statistical tests of significance, and ranking of correlation strength (e.g. in quartile analysis), the central bin (of 50msec width) to exclude any potential influence of mixing spikes from the constituent cells in the pair. The central bin has also been omitted from presentation in the CCHs of neighboring cells.
3. Results

Neural activity was recorded from motor cortical fields of two monkeys: K and R. During each recording session the monkeys performed a visuo-motor center-out task to eight directions. Recording sessions of monkey K also included blocks of learning trials, in addition to the previously extensively trained center-out trials (see methods).

From monkey R neural activity was recorded using a chronic array of 96 micro-electrodes. 206 neurons were isolated in three recording days; out of them only 77 cells passed our criteria to construct 322 pairs for the correlation analysis. These pairs were constructed only of cells that were recorded from different electrodes, to avoid possible artifacts due to imperfect spike sorting in recordings from the array.

To study the correlation between neighboring cells (recorded by the same electrodes) we used data from monkey K with careful spike sorting of cells. In this monkey we used acute simultaneous recordings by up to 32 micro-electrodes. From 40 recording days we used a pool of 688 cells (out of 2129 isolated stable cells), which had a matching cell on the same electrode; however only 105 intra-electrode pairs (of 191 cells) passed the criteria for further analysis. From the same pool we constructed 257 pairs (of 244 cells) from nearby electrodes (distance <3mm), and 2063 pairs (of 403 cells) from farther apart electrodes (distance >3mm).

[Note: the above enumerations relate to the correlation analysis, after the pairs were chosen according to the difference of their preferred-directions (“close PDs” and “opposite PDs”, to be presented below), among other criteria for selection of cells (stability, minimal firing rate, inter-spike-interval, etc.) – see methods].
3.1 Directional tuning of cells and pair-wise correlations

For each cell we determined whether it is significantly tuned to the direction of arm movement, using ANOVA analysis (see methods). Figure 3 shows the spike activity of simultaneously recorded tuned cells, in a trial where the arm was reaching rightwards (target at location of 0°). Directional tuning was calculated from the firing rate during the epoch around movement-onset (see epoch between two vertical lines in Figure 3).

![Figure 3 Dot-display of 30 tuned cells recorded simultaneously during a single trial, aligned at movement-onset (Time = 0). Each dot denotes a single spike, and each line represents a spike train from a single cell. Arrows denote the time of behavioral events; epoch between two vertical lines marks the time around movement onset, during which the tuning of cells was calculated. Recorded from monkey R.](image-url)
Fitting the cells’ movement related firing rates to a cosine function (see Methods) assisted us in evaluated the uni-modality of the cells’ directional tuning function. Out of the cells that were defined directionally tuned (using the ANOVA analysis as described above in this section): 92% in monkey R and 55% in monkey K also fit a cosine function, and therefore inherently having a uni-modal directional tuning function.

Figure 4 A shows the number of pairs recorded from the same electrode (monkey K) that had two, one or none of the cells tuned (n = 156, 105, 33 respectively). The histogram in Figure 4 B shows that when the two cells of the pair were directionally tuned, most had close preferred-directions (PDcellII – PDcellIII ≤ 45°). However, notice also that about 25% of the neighboring cells had opposite PDs (PD-difference larger than 135°). This is consistent with previous studies demonstrating some spatial organization of cell’s PDs in the motor cortices (Ben-Shaul et al., 2003; Amirikian & Georgopoulos, 2003; Stark et al., 2009); however the organization is not strict, allowing a large number of cells (25%) with opposite PDs to reside in the same site.
Figure 4 Directional tuning properties of cell pairs recorded from the same electrode. (A) Number of cell pairs with both cells significantly tuned (ANOVA, p < 0.05), only one cell tuned (ANOVA, p < 0.05), or none of the cells in the pair is tuned. (B) Distribution of preferred-direction (PD) difference (degrees) within the cell pairs. Both cells in the pairs are significantly tuned (leftmost bar in (A)).

Relation between cells’ pair-wise correlations and similarity of preferred-direction

To address the main goal of our research we examined the relations between directional tuning of cells and their functional connectivity.

Figure 5 shows the noise correlation as a function of the preferred-direction (PD) distance between the cells. The linear fitting model is:

\[ y = -0.0031611 \times x + 0.012204 \]

Where x is the PD distance and y the noise correlation of the cell-pair, and the norm of residuals = 0.0011425. The high variability of the distribution of noise correlation values
in each PD distance is clearly noticeable and the noise distributions fail to be significant different in the different PD distances (one-way ANOVA, \( p = 0.065 \)). Taken together with significant negative slope of the linear fit, we continued our analysis by grouping the cell pairs according to the PD difference.

![Box plot of noise correlation as a function of preferred-direction distance](image)

Figure 5 Noise correlation as a function for the preferred-direction (PD) of the constituent cells in the pair. Monkey R: 804 pairs of cells from different electrodes. Inset- display for clarification: The mean noise correlation as a function of PD distance (blue line), excluding the box-plot. Red line- linear fit model \( y = -0.0031611 \times x + 0.012204 \), norm of residuals = 0.0011425.

We classified two groups of pairs based on the directional tuning of their constituent cells: (1) Pairs with cells that had “close-PDs” (PD difference up to ±45°); and (2) the pairs with cells that had “opposite-PDs” (180°±45°). We then computed
crosscorrelograms (CCH) and Joint-peri-event-time histograms (JPETH) for each pair. In order to test the relation between the correlations in each group and the movement direction we averaged the CCH and the JPETH (see *methods*) in four different combinations. Table 1 depicts these combinations, between two groups and two movement directions.

<table>
<thead>
<tr>
<th>Behavioral condition</th>
<th>Movements to PD of first cell in pair</th>
<th>Movements to null-direction of first cell in pair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Close preferred-directions</td>
<td>Trials towards the (common) PD</td>
<td>Trials towards the (common) null direction</td>
</tr>
<tr>
<td>Opposite preferred-directions</td>
<td>Trials towards PD of first cell (PD1)</td>
<td>Trials towards PD of second cell (PD2)</td>
</tr>
</tbody>
</table>

Table 1 Four groups of combinations of cell-pairs and behavioral conditions. (Colors of captions will be further used in relation to these combinations).
**Averaged cross-correlation analysis**

The cross-correlogram is a measure of the correlation-coefficient (y-axis) between spike trains of two cells, and it depicts the correlation at different time lags (x-axis). The cross-correlograms in this study are corrected to show the correlation beyond the expected level for average firing rates of the two independent spike trains. Thus, they serve as a measure for the noise correlations (see *methods*).

For each cell-pair we constructed a CCH; then, we averaged CCHs from several pairs of a certain group. Figure 6 shows the four averaged CCHs for the four combinations specified in Table 1. The figure shows a clear difference in strength of correlations for both monkeys. Pairs of cells with close-PDs had higher correlation peaks (blue and green lines) as compared to pairs with opposite-PD (red and magenta lines). These results confirm previous studies that showed dependency between the signal correlation and the noise correlation of cell-pairs (Lee et al., 1998). Moreover, the correlation in the pairs with close-PDs shows a trend to be higher when the arm moves towards the common PD of the cells, compared to moving towards the null direction. While the same trend is found in both monkeys, it was found to be statistically significant only in monkey R (see Figure 7 I Fand IIF).
Figure 6 Crosscorrelograms (CCHs) in the four combinations. Cell-pairs recorded from different electrodes. (I) monkey R - Close PDs pairs n = 195; Opposite PDs pairs n = 127; (II) monkey K - Close PDs pairs n = 133; Opposite PDs pairs n = 124.
Joint-peri-event-time-histogram (JPETH) between cells from different electrodes

The CCH analysis shown above illustrates the average correlation-coefficient across the entire set of spike trains, and on each time lag. To study the temporal dynamics of the pair-wise cross-correlation along the trial, we use the Joint-peri-event-time-histogram with modification for population analysis (population-JPETH, see methods).

As for the CCHs, the JPETHs were constructed for each of the four combinations between the cell-pair populations and behavioral conditions (see Table 1).

Figure 7 shows these four JPETHs for each of the monkeys (I for monkey R and II for monkey K) and illustrates significant differences between them (one-way ANOVA, p<<0.001 for both monkeys). The main interest is in the main diagonal. First, as expected from the CCH analysis, the JPETH for the group of pairs with close PDs (Figure 7 A, B in I and II) has significantly higher correlation than the two JPETHs of the opposite-PDs pairs (Figure 7 C, D). Second, the strongest correlation can be found in close-PDs pairs with movements to the common PDs.

In monkey R we can see a significant difference in the correlations of close-PDs pairs: when the hand is at rest, before movement onset, the cells activity ceases from being correlated, and when the movement starts- the correlation increases if the movement is toward their common preferred-direction. If the movement is in the null-direction, the increase of correlation is hardly detected. In monkey K however, we cannot see these dynamics of correlated activity; the JPETH exhibits on-going correlation with hardly noticeable modulation along the trial, regardless of movement directions (null versus PD).
While the numbers of pairs are quite similar in the two monkeys (in monkey R: Close PDs n = 195, Opposite PDs n = 127; in monkey K: Close PDs n = 133, Opposite PDs n = 124), in monkey K the average correlations strengths are weaker (see CCHs in Figure 6), and the JPETHs are much noisier.

The differences between the monkeys are discussed in detail below (see *discussion*). Briefly, the results suggest that the main dynamical difference lies in the prominent relation to arm movement in monkey R than in monkey K, due to the exact location of the recording sites in relation to the primary motor cortex.
Figure 7 JPETH in the four combinations, for pairs from different electrodes. (I) monkey R; (II) monkey K (inter-electrodes distance < 3mm). The JPETH corresponds to the CCH in Figure 6 above. Number of pairs same as for the CCHs in the figure above.

(A-D) Averaged Joint-peri-event-time-histogram's (JPETHs) across populations of cell-pairs. Bin width 50ms, pixel size 50X50ms, spike trains ((I) monkey R [-2 1]s; (II) mokey K [-2.5 2.5]s) around the behavioral event of movement-onset (MO, at t = 0s, see methods), color bar: correlation coefficient (CC). The JPETHs were smoothed with a two dimensional Gaussian window (standard-deviation = 1 bin).

Marginal histograms: Averaged firing rates (spikes/sec = Hz) Peri-event-time-histogram (PETH) of the population of the first cells in each pair (left edge of the JPETH matrix) and the population of the second cells in each pair (bottom edge of the JPETH matrix).

(A) JPETH of population of cell-pairs with close-PDs, during trials with movement towards PD.

(B) JPETH of population of cell-pairs with close-PDs, during trials with movements towards null-direction.

(C) JPETH of population of cell-pairs with opposite-PDs, during trials with movements towards PD of the first cell in the pair.

(D) JPETH of population of cell-pairs with opposite-PD, during trials with movements towards PD of the second cell in the pair.

(E) Main diagonals of the four JPETHs in A-D with corresponding colors blue, green, red and magenta- respectively. Monkey R: main diagonal ±1 adjacent diagonal; monkey K: main diagonal ±3 adjacent diagonals.

(F) Multiple comparison analysis between the main diagonals of the four JPETHs in A-D. Monkey R: main diagonal ±1 adjacent diagonal; monkey K: main diagonal ±3 adjacent diagonals.

Plotting the JPETH for the two monkeys (Figure 8) shows that alignment on behavioral event of target onset instead of movement onset (shown in Figure 7) produces similar results of significant difference between the four combinations (for both monkeys: one-way ANOVA, p<<0.001). For monkey R we can see the difference between the combinations even more bluntly in this alignment than the previous, with a clear negative peak of the CCH for pairs with opposite PDs (Figure 8 I, (C-D)).
Figure 8 JPETHs for monkey R (I) and monkey K (II). Trials aligned around the event of target-onset (TO, at t = 0s). (A-E) Legends same as in Figure 7 (A-D, F, respectively). (F) CCHs of the four combinations: Blue and green – pairs with close-PDs, during movements towards PD (blue) and null direction (green); Red and magenta – pairs with opposite-PDs, during movements towards PD of the first cell (PD1, red) and the PD of the second cell (PD2, magenta).
Arm movement parameters and JPETH of populations with close or opposite PDs

Different parameters of movement performance vary across repetitions of the same movement. These variations in movements can be reflected in the neuronal activity of cells in M1. As previously mentioned above in the Methods, such variations in terms of firing rates might cause apparent noise correlation (Georgopoulos et al., 1982; Ben-Shaul et al., 2001). Figure 9 and Table 2 illustrate the distribution of movement parameters for each day in five histograms (path length, reaction time, movement time, time to peak velocity, and distance from straight line) and hand trajectories (in Figure 9) of trials with low behavioral variability in these five parameters. In addition we pooled together these movement parameters across the days, for illustration of overall behavioral performance. The selected trials, restricted to low variability in all five parameters, served as a data set for correlation analysis shown in Figure 10.
Figure 9 Histograms of movement parameters, and hand trajectories plots of monkey R.
Figure 9 Histograms of movement parameters, and hand trajectories plots of monkey R. Each row represents a different movement parameter, and each column a different recording day (last column, background color cyan, pools together the data from all three recording days). Y axis of histograms – counts of trials. First row- Path length (mm) calculated from movement onset time (MO) till target reach (TR); Second row- Reaction time (ms) from “Go” signal (GS) till MO; Third row- Movement time (ms) duration of time elapsed from MO till TR; Forth row- Time to peak velocity (ms) duration of time elapsed from MO till time of maximal velocity along the movement; Fifth row- Distance from straight line (mm) is the distance from the trajectory curve at point of peak velocity, to the straight line connecting the beginning and the end of the trajectory curve (i.e. at point of MO and at point of TR). Red marks on the histograms denotes the mean ±1*STD of distribution. Sixth row plots the trajectory curves from MO to TR of trials with low variability of the movement parameters (i.e. all the trials did not exceed 1 standard deviation from the mean (calculated for each recording day separately) in all five parameters of movement).

<table>
<thead>
<tr>
<th></th>
<th>Day I</th>
<th>Day II</th>
<th>Day III</th>
<th>All days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Path length (mm)</td>
<td>52.5 (11.3)</td>
<td>48.7 (8.1)</td>
<td>48.7 (9)</td>
<td>49.5 (9.2)</td>
</tr>
<tr>
<td>Reaction time (ms)</td>
<td>108 (97.1)</td>
<td>121.5 (92.1)</td>
<td>146.5 (90.4)</td>
<td>123.2 (93.7)</td>
</tr>
<tr>
<td>Movement time (ms)</td>
<td>258.3 (67.4)</td>
<td>268.9 (68.8)</td>
<td>265.5 (57.4)</td>
<td>265.9 (66.6)</td>
</tr>
<tr>
<td>Time to peak velocity (ms)</td>
<td>180.4 (36.7)</td>
<td>190.1 (43.6)</td>
<td>190.9 (60)</td>
<td>188.1 (45.8)</td>
</tr>
<tr>
<td>Distance to straight line (mm)</td>
<td>1.3 (1.2)</td>
<td>1.2 (1.2)</td>
<td>1.3 (1.8)</td>
<td>1.2 (1.3)</td>
</tr>
</tbody>
</table>

Table 2 Behavioral parameters of movements in three recording days, and across all days (last column). Mean (standard deviation). The Table corresponds to histograms in Figure 9.
The correlation analysis presented in Figure 10 illustrates again the relation between noise correlation and the PD difference: the population of cell pairs with close PDs share higher correlation than pairs with opposite PDs. This is noticeable despite the lower signal to noise ratio.
Figure 10 JPETH in the four combinations, for pairs from different electrodes, monkey R.
Figure 10 JPETH in the four combinations, for pairs from different electrodes, monkey R.

(A-D) Averaged Joint-peri-event-time-histogram's (JPETHs) across populations of cell-pairs. Same as in figure Figure 7 I.

(E) Crosscorrelograms (CCHs) corresponding fo the four JPETHs in A-D with corresponding colors blue, green, red and magenta- respectively.

(F) Main diagonals of the four JPETHs in A-D with corresponding colors blue, green, red and magenta- respectively. Main diagonal ±1 adjacent diagonal.

(G) Multiple comparison analysis between the main diagonals of the four JPETHs in A-D. Main diagonal ±1 adjacent diagonal.
Quartile analysis of pair-wise correlations of populations with close or opposite PDs

The results shown so far for the pair-wise correlation demonstrate that the population of pairs with close-PDs has a stronger correlation than the population with opposite-PDs. Each of these populations is comprised of many pairs with a wide range of correlation strengths, as illustrated in Figure 11. The distribution of CCH peaks of the four sub-populations is significantly different for both monkeys, and it is significantly higher than zero for the close-PDs pairs (one-way ANOVA, p < 0.05 for monkey R; p < 0.001 for monkey K). Furthermore, Figure 11 demonstrates the wide range of correlation strengths within each sub-population, and overlapping ranges between sub-populations. Therefore we examine the populations of pairs on the basis of grouping them according to their strength.

In this section we explore the populations by examining first the lower and upper quartiles of the correlation peaks (see methods). Figure 12 A shows for the pairs with strongest correlations (upper quartile) and Figure 12 B the pairs with weakest correlations (lower quartile) out of the population of pairs with close-PDs; similarly, Figure 13 (A-B) show the upper and lower quartiles for the pairs with opposite-PDs (in both Figure 12 and Figure 13 : I- monkey R, II- monkey K).

By definition, the upper quartiles in both populations show higher and clearer correlations both in the CCH analysis and in the main diagonal of the JPETH analysis (Figure 12 and Figure 13).
Figure 11 Distribution of peaks of Crosscorrelograms (CCHs). (I) Monkey R; (II) Monkey K.
(A) Histogram of correlation strength (cc) of pairs in the four combinations (colors are indicated in the figure legend).
(B) Multiple comparison analysis of correlation strength distribution.
(I) Monkey R. n = 47 pairs with trials to the PD (A, C, E); n = 47 pairs with trials to the null-direction (B, D, F).
(II) Monkey K. $n = 24$ pairs with trials to the PD (A, C, E); $n = 22$ pairs with trials to the null-direction (B, D, F).
Figure 12 Upper and lower quartiles of the population of close-PDs pairs. (I) Monkey R; (II) Monkey K.
(A–D) JPETH and marginal PETH same as in Figure 7 (however note that all subfigures here relate only to the population of CLOSE-PDs). Left inset- CCH of the corresponding JPETH: noise correlation (CC) as a function of time lag (sec).
(A) Upper quartile of population of pairs with close-PDs, in trials towards the PD.
(B) Upper quartile of population of pairs with close-PDs, in trials towards the null direction.
(C) Lower quartile of population of pairs with close-PDs, in trials towards the PD.
(D) Lower quartile of population of pairs with close-PDs, in trials towards the null direction.
(E–F) Histogram of peaks of CCHs in the lower quartile of the population. Units are in standard-deviation from the tail of each corresponding CCH. Peaks with more than 2*STD from the tail, are considered significantly negative. Vertical axis- number of pairs; Horizontal axis- STD from the mean of the population.
(E) Histogram of lower quartile of the population of close-PDs in trials towards the PD. (I) Monkey R: 0.122 of the pairs in the population are negatively correlated (0.48 of the pairs in the lower quartile). (II) Monkey K: 0.01 of the pairs in the population are negatively correlated (0.04 of the pairs in the lower quartile).
(F) Histogram of lower quartile of the population of close-PDs in trials towards the null direction. (I) Monkey R: 0.124 of the pairs in the population are negatively correlated (0.49 of the pairs in the lower quartile). (II) Monkey K: 0.011 of the pairs in the population are negatively correlated (0.045 of the pairs in the lower quartile).
(I) Monkey R. n = 31 pairs with trials to the PD of the first cell (PD1) (A, C, E); n = 30 pairs with trials to the PD of the second cell (PD2) (B, D, F).
(II) Monkey K. n = 22 pairs with trials to the PD of the first cell (PD1) (A, C, E); n = 20 pairs with trials to the PD of the second cell (PD2) (B, D, F).
Figure 13 Upper and lower quartiles of the population of opposite-PDs pairs. (I) Monkey R; (II) Monkey K.
(A–F) Legends of JPETH, marginal PETH, inset CCH and histograms (E–F) are same as in Figure 12.
(A) Upper quartile of population of pairs with opposite PDs, in trials towards the PD of the first cell (PD1).
(B) Upper quartile of population of pairs with opposite PDs, in trials towards the PD of the second cell (PD2).
(C) Lower quartile of population of pairs with opposite PDs, in trials towards PD1.
(D) Lower quartile of population of pairs with opposite PDs, in trials towards PD2.
(E) Histogram of lower quartile of the population of opposite-PDs in trials towards PD1.
(I) Monkey R: 0.187 of the pairs in the population are negatively correlated (0.742 of the pairs in the lower quartile). (II) Monkey K: 0.034 of the pairs in the population are negatively correlated (0.136 of the pairs in the lower quartile).
(F) Histogram of lower quartile of the population of opposite-PDs in trials towards PD2.
(I) Monkey R: 0.168 of the pairs in the population are negatively correlated (0.667 of the pairs in the lower quartile). (II) Monkey K: 0.012 of the pairs in the population are negatively correlated (0.05 of the pairs in the lower quartile).

Figure 12 and Figure 13 show results of quartile analysis. The results of monkey R (I in both figures) and monkey K (II in both figures) show quite similar trends. However, the correlations are clearly noisier in monkey K.

The analysis, which classifies pairs of cells by the correlation strength, exposes the following results for the four groups (specified in Table 1):

1. Figure 12(A-B) demonstrates that the sub-population of pairs having close-PDs exhibit stronger correlations (upper quartile) for both monkeys. These pairs exhibit on-going correlated activity as shown by the correlations before the target is presented and while the hand is at rest, having the cursor inside the origin position.

The upper-quartile of the pairs shows similar modulations of correlation after movement onset for monkey R (Figure 12 I(A-B)) with increased correlation at movement onset towards the common PD. In monkey K, however, the upper-quartile of pairs shows
hardly distinguishable modulations (Figure 12 II(A-B) for monkey K), similarly to what was found in the whole population (Figure 7).

2. Figure 12 (C-D) shows the lower-quartile sub-population of pairs with close-PDs for both monkeys (I monkey R, II monkey K), and demonstrates the opposite relations compared with the upper quartile—these pairs show on average negative correlation (as seen also in the CCH). This ongoing negative correlated activity is also modulated by movements – it is decreased (becomes less negative) around movement onset. For monkey K the correlation is negative, though not statistically significant. See Figure 12 I(E-F) and Figure 12 II(E-F) for histograms of negative peaks in the lower quartile.

3. Figure 13 (A-B) shows the upper quartile of pairs with opposite PDs. These JPETHs for monkey R (Figure 13 I(A-B)) show opposite pattern of modulation as compared with the pairs with close-PDs; they show higher and stronger correlated activity which decreases at movement onset (by definition, the movements are to the PD of one cell which is the null of the other cell). For monkey K, however, we see again in Figure 13 II(A-B) that movement-related modulations of correlations are hardly evident.

4. Figure 13 (C-D) shows the lower-quartile of pairs with opposite-PDs. For both monkeys, the JPETHs exhibit a similar trend as the lower quartile of the close-PDs population: The JPETHs have an on-going negative diagonal which decreased around movement onset. The negative correlation trend is evident and strong in monkey R, but fails to reach significance in monkey K.

What can we learn from the middle quartiles of the populations? To answer the question after examining the two extreme quartiles, and to complete our view on the population
using the quartile analysis, Figure 14 (for the close-PDs pairs) and Figure 15 (for the opposite-PDs pairs) present the middle quartiles of monkey R (we did not include the middle quartile analysis of monkey K on this section, since the extreme quartiles were already very noisy; therefore, the middle quartiles of monkey K are expected to be even less informative). In these quartiles ([0.25 0.5] termed “lower-mid”, and [0.5 0.75] “upper-mid”) we clearly find intermediate values for correlation strengths, with the same trends: The upper-mid quartile of the close-PDs pairs shows higher correlation than the upper-mid of the opposite-PDs pairs (the latest is closer to 0 correlation values). We can also see in the close-PDs pairs a trace of movement related dynamics of the correlation. The four quartiles altogether comprise a population that shows on average the movement-related patterns of correlations, as described above (see Figure 7 I and related section in the results).
Figure 14 Middle quartiles of the population of close-PDs pairs, of Monkey R. n = 48 pairs with trials to the PD (A, C, E); n = 46 pairs with trials to the null-direction (B, D, F).

(A-B) Upper-middle quartile includes the range of [0.25 0.5]; (C-F) Lower-middle quartile includes the range of [0.5 0.75]. (E) None of the pairs are significantly negative. (F) A single pair (0.005 of the population) is significantly negative (0.022 of the pairs in the quartile). (A–F) Legends of JPETH, marginal PETH, inset CCH and histograms are same as in Figure 12 I.
Figure 15 Middle quartiles of the population of opposite-PDs pairs, of Monkey R. n = 31 pairs with trials to the PD of the first cell (PD1) (A, C, E); n = 30 pairs with trials to the PD of the second cell (PD2) (B, D, F).

(A-B) Upper-middle quartile includes the range of [0.25 0.5]; (C-F) Lower-middle quartile includes the range of [0.5 0.75]. (E-F) None of the pairs are significantly negative. (A–F) Legends of JPETH, marginal PETH, inset CCH and histograms are same as in Figure 12 I.

To conclude the quartile analysis presented above, we point out that in both monkeys the pairs comprising each population exhibit a variety of correlation strengths and patterns.
Nevertheless, as mentioned in previous sections of the results and to be addressed below in the discussion – pairs of monkey R in different parts of the population show dynamics of correlations which are much more related to movement, compared to monkey K. The results of monkey K exhibit more a trend of on-going correlation which is less related to behavioral events such as movement onset.

**Correlations between cell pairs from different electrodes farther apart**

The analysis presented thus far showed the correlations between pairs that were recorded by different electrodes. Previous studies (Lee et al., 1998; Stark, 2007) noted that the correlation between cells recorded from different sites, not only became weaker as the distance between the sites grows larger, but also became less informative.

Figure 16 (I for monkey R and II for monkey K) show the correlation for pairs that were recorded by electrodes at distances larger than 3mm, in the four groups discussed so far (and described in Table 1). In monkey R, although the correlations become much weaker, we can still notice the signature of difference in the correlation strength between the subpopulations (one-way ANOVA, p = 0.005). It is especially evident that cells with close PDs are correlated during movements to the common PD (see multiple-comparison analysis in Figure 16 I E). In contrast, the correlations in monkey K are even weaker and noisier and we can see only a reminder of the trace of higher correlation between pairs with close-PDs in comparison to opposite-PDs pairs, and we can no longer find significant difference among the groups of pairs (one-way ANOVA, p = 0.035). Therefore, the results discussed in this study for monkey R include all the pairs regardless of the distance between the cells; whereas the results for monkey K concerning pairs
from different electrodes exclude the pairs with inter-electrode distance of 3mm and above. For distribution of pairs in different inter-electrode distances see Figure 17.

I Monkey R. \( n = 41 \) pairs with close-PDs (A-B); \( n = 33 \) pairs with opposite-PDs (C-D). Notice difference in correlation strength between the groups.
(II) Monkey K. n = 1033 pairs with close-PDs (A-B); n = 1030 pairs with opposite-PDs (C-D). Notice hardly any difference in correlation strength between the groups.

Figure 16 JPETHs of pairs with inter-electrode distance $>\sqrt{8}$ mm. (I) Monkey R; (II) Monkey K. Trials aligned around the event of Movement Onset (MO at time 0sec). (A-F) Legends are same as in Figure 8.
Figure 17 Histograms of distances between electrodes in monkey R (I) and monkey K (II). The histograms include all pairs of cells from different electrodes, which were included in the results presented thus far. Monkey R: n = 322 pairs; Monkey K: n = 2320 pairs.

Correlations between neighboring cells (recorded by the same electrode)

We have described so far the results of pair-wise correlation analysis between cells from different electrodes. The recording setup of monkey K enables us to study co-activation of neighboring cells (i.e. recorded by the same electrodes), while taking into consideration the required precautions to minimize possible artifacts due to unsatisfactory spike sorting quality (see the methods section for detailed description of these measures).
First, we show in Figure 18 a comparison of the correlations between neighboring cells (from the same electrode) to more distant cells (recorded by different electrodes). The figure shows averaged correlations across pairs from monkey K classified according to the distance between the cells. As expected (Lee et al., 1998; Stark, 2007), the average correlations between neighboring cells are generally much stronger (note that bin 0 in these correlations was ignored). The correlation strength decreases as the distance between the cells grows: peak of CCH for pairs recorded by the same electrode \(CC=0.028\) (mean value of adjacent bins from either sides), pairs recorded by different electrodes at distances \(<3\text{mm}\) \(CC=0.005\), and pairs from electrodes farther apart (distances \(>3\text{mm}\)) \(CC=0.002\) (see Figure 18).

![Figure 18 CCHs of pairs from monkey K grouped by the distance between the electrodes: Black- cells from the same electrode (neighboring cells, \(n = 309\) pairs); Blue-cells from different electrodes with distance \(\leq \sqrt{8}\text{mm}\) (~3mm, \(n = 752\) pairs); Magenta-cells from different electrodes with distance \(> \sqrt{8}\text{mm}\) (~3mm, \(n = 5472\) pairs). The central bin (corresponding to 0 time lag) was removed in CCH of cells from the same electrode.](image)
Next, we analyzed in more detail the nature of correlations between neighboring cells, similarly to what was shown above for the distant cells. Figure 19 shows the average of the CCHs and the JPETHs for the four combinations specified in Table 1. As shown for pairs from different electrodes (Figure 6 II for the CCHs and Figure 7 II for the JPETHs), Figure 19 shows significantly different strengths of correlations (one-way ANOVA, p<<0.001): pairs of cells with close-PDs show significantly higher correlation peaks in comparison to pairs with opposite-PDs, with no statistical significant difference between behavioral conditions (see multiple-comparison analysis in Figure 19 E).

Examination of the main diagonal of the four JPETHs in Figure 19 A-D reveals a clear continuous co-activation of the pairs through the entire trial even before the movement onset (at time 0sec) and even before any previous knowledge of the impinging movement (up to around time -1s in the figure).

Aligning the JPETHs on target-onset (TO, see methods) instead of on the movement onset (MO) event, produced the same results, as shown in Figure 20. Except for alignment of trials, the data in Figure 20 is identical to the one in Figure 19 (with the same two populations of pairs and the same sets of trials).
Figure 19 JPETHs of neighboring cells (pairs recorded by the same electrodes), from monkey K. n = 70 pairs with close-PDs (A-B); n = 35 pairs opposite-PDs (C, D). Trials aligned around the event of Movement Onset (MO at time 0sec). (A-F) Legends are same as in Figure 8. (F) CCHs constructed from the un-smoothed corresponding JPETHs; the central bin (0 time lag) is omitted.
Figure 20 JPETHs of neighboring cells (pairs recorded by the same electrodes), from monkey K. n = 70 pairs with close-PDs (A-B); n = 35 pairs opposite-PDs (C, D). Trials aligned around the event of Target Onset (TO at time 0sec, cut range: [-1 4]s). (A-F) Legends are same as in Figure 8. (F) CCHs constructed from the un-smoothed corresponding JPETHs; the central bin (0 time lag) is omitted.
Upper and lower quartiles of correlations of neighboring cells

The results produced for pair-wise correlation of neighboring cells, similarly to what was found in pairs from different electrodes, demonstrate again clearly higher correlation values for pairs with close PDs than for pairs with opposite PDs. Assuming that the variability of correlations is high across pairs of cells, even if they are anatomically adjacent, we performed the same quartile analysis (see methods) for the population of neighboring cells. Interestingly, we find difference in the properties of the lower quartiles; the lower quartile of the close-PDs population averages to around zero, whereas the opposite-PDs population averages to a significant negative value of the correlation coefficient. This is demonstrated both in the CCH analysis and the main diagonal of the JPETH (Figure 21 and Figure 22, the close and opposite PDs populations, respectively). The histograms in Figure 21 E-F and Figure 22 E-F show the significance test for the negativity of the lower quartile of pairs. The close-PDs do not have a significant fraction of the population with negative values, whereas among the opposite-PDs cell-pairs a significant fraction of the correlation values is negative (CC<-2*STD).

The upper quartiles demonstrate as expected, higher correlation for the close-PDs population as compared to the upper quartile of the opposite-PD, both in the CCH and the JPETHs (Figure 21 A-B and Figure 22A-B).
Figure 21 Upper and lower quartiles of the population of close-PDs pairs, of neighboring cells (pairs recorded by the same electrodes) from monkey K. (A-F) legends as in Figure 12.

(E) Histogram of lower quartile of the population of close-PDs in trials towards the PD. 0.046 of the pairs in the population are negatively correlated (0.182 of the pairs in the lower quartile).

(F) Histogram of lower quartile of the population of close-PDs in trials towards the null direction. 0.049 of the pairs in the population are negatively correlated (0.2 of the pairs in the lower quartile).
Figure 22 Upper and lower quartiles of the population of opposite-PDs pairs, of neighboring cells (pairs recorded by the same electrodes) from monkey K. (A-F) legends as in Figure 13.

(E) Histogram of lower quartile of the population of opposite-PDs in trials towards the PD of the first cell (PD1). 0.16 of the pairs in the population are negatively correlated (0.667 of the pairs in the lower quartile).

(F) Histogram of lower quartile of the population of opposite-PDs in trials towards the PD of the second cell (PD2). 0.174 of the pairs in the population are negatively correlated (0.667 of the pairs in the lower quartile).
To summarize, the results for intra-electrode correlations suggest that (1) neighboring cells show an intriguing non-homogeneity: First, about 30% of the pairs are comprised of one tuned cell and another which is un-tuned (see Figure 4 A); Second, out of the pairs with both cells tuned- about a quarter have opposite preferred directions (see Figure 4 B). Third, in regard to the correlation analysis, we see that the population on average has ongoing co-activation. However the quartile analysis exposes the wide variety of correlation patterns. Each population has pairs with strong or weak correlations; moreover, the population of opposite-PDs pairs has pairs with negative correlation, whereas an insignificant minority of close-PDs pairs is negatively correlated.

2. Observing the dynamics of the correlations using the JPETHs, we find that the correlation between neighboring cells is quite stable regardless of the behavioral events that emerge throughout the time course of the trial, and regardless of alignment of the JPETH on different behavioral events. Thus, it seems that if there are any modulations of correlations between neighboring cells, they are very small in comparison to the high baseline correlation. This may reflect the functional anatomy of groups of cells that have common function and are likely to receive similar common inputs.

Comparing the intra- and inter-electrode correlations, we see that on-going correlation is more prominent in intra-electrode (neighboring) pairs while temporal dynamics of correlations is more evident in inter-electrodes (distant) pairs. However, ongoing correlation is also observed in distant pairs that share stronger correlations (upper-quartile of close-PDs pairs). Patterns of negative correlations can be found in populations both from the intra- and inter-electrodes. Note that, distant cells with close-PDs may show
negative correlation values (lower quartile) while neighboring cells with close-PDs rarely do.
3.2 Learning induced color sensitivity and pair-wise correlations

The analysis so far has been related to aspects of spatial mapping from the direction of a visual instruction to the direction of arm movement (see results). Cells in the motor areas can also show relation to other task parameters and non-spatial cues – like the color of a cue, when instructing the direction of the movements (as we described in Zach et al, 2008 for the data of this thesis). Monkey K performed an arbitrary learning task of color coding of movements directions, thus allowing the study of neural correlates of learning an arbitrary-association task. The previous publication included analysis of the single cells responses. Here, we describe the pair-wise correlations, showing that cells which share similar learning induced color-sensitivity showed higher correlations as compared to other pairs, which did not share this sensitivity. Moreover, we show that the correlation increases after learning, and it is significantly higher during the post-learning block than the correlation during pre-learning block.

To test which factors affect the correlation strength in relation to learning-induced-color-sensitivity, we performed multi-way ANOVA. For this purpose of significance examination of the main diagonal of the JPETHs, we used 5 adjacent diagonal from either sides (corresponding to the range of [-250 250]msec around the central bin of the CCH); we concatenated the length of the diagonal (diagonal bin range [1 65] corresponding to [-2500 750]msec around movement onset), in order to exclude possible artifacts due to broad and higher correlation values at the last segment of the diagonal (bins > 65). The multi-way ANOVA, together with the CCH and JPETH analysis, yielded the following results.
Relation between cells’ pair-wise correlation and learning-induced-color-sensitivity

We examined the correlation strength separately for different populations of the following types: A. None of the cells in the pair is sensitive to color (termed: “None”, n = 277 pairs); B. One of the cells in the pair is sensitive to color, and the other is not sensitive (termed: “One”, n = 261 pairs); C. Both of the cells in the pair are sensitive to color (termed: “Both”, n = 79 pairs). For more details see also methods.

Figure 23 shows the averaged CCH and the JPETH for pairs grouped according to their color sensitivity (“none”, “one” and “both”). Figure 23 (B, D, F) presents the correlations after learning, during performance of standard trials (STD2). From multi-way-ANOVA (see Table 3) and multiple-comparison-analysis (see Figure 24 A) we see that the factor of the sensitivity (“none/one/both”) was found to be significantly affecting the correlation strength. The correlation of cell pairs when both were color sensitive (“Both”) is significantly higher than the correlation between pairs in the “None” and “One” groups (p = 0.0001). Moreover, the JPETH analysis reveals that the difference in correlation strength is evident even before any visual cue is presented, while neither the color nor the movement direction can be predicted by the subject ([-2.5 -1.5]sec in Figure 23). This ongoing persistent pattern of correlation in the “both” group, is quite similar to the pattern of persistent correlation for the cells with “close-PDs” in the same monkey (monkey K) (see Figure 7 II and Figure 19).
Figure 23 Correlations between pairs of cells grouped according to the cell’s learning-induced-color-sensitivity. All pairs are from different electrodes, recorded from monkey K. Trials aligned around Movement Onset (MO, ranges [-2.5 2.5]). (A-F) In each subplot the central image is the JPETH; Marginal histograms are the PETHs of the cells (spikes/second (=Hz) as a function of time in the trial); Inset is the CCH constructed from the diagonals of the JPETH (correlation (CC) as a function of time lag (sec)).
(A) Correlation between pairs of cells with no learning-induced-color-sensitivity, during pre-learning trials (STD1).
(B) Correlation between pairs of cells with no learning-induced-color-sensitivity, during post-learning trials (STD2).
(C) Correlation between pairs of cell with one cell having learning-induced-color-sensitivity and the other cell not sensitive, during pre-learning trials (STD1).
(D) Correlation between pairs of cell with one cell having learning-induced-color-sensitivity and the other cell not sensitive, during post-learning trials (STD2).
(E) Correlation between pairs of cells which both have learning-induced-color-sensitivity, during pre-learning trials (STD1).
(F) Correlation between pairs of cells which both have learning-induced-color-sensitivity, during post-learning trials (STD2).

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Table 3 Multi-way ANOVA, between three factors: Block- pre-learning (STD1) or post-learning (STD2) block of trials; Sensitivity- learning-induced-color-sensitivity of the cells in the pair (None sensitive, One sensitive, Both sensitive); Colors- spike trains from trials with certain target colors (learned colors, or naïve colors with no association to movement).
Figure 24 Multiple-comparison analysis of ANOVA (see Table 3) for each single factor separately: (A) Factor of Block of trials- pre (STD1) or post (STD2) learning standard trials. (B) Factor of Sensitivity of the pairs- None of the cells in the pair with learning-induced-color-sensitivity; One of the cells is sensitive and the other is not; Both cells in the pair are sensitive. (C) Factor of the Color of the visual cue in the trials- targets with Learned colors; targets with naïve colors which do not appear in the learning block.
Correlation strength before learning block differs from after learning

We compared the correlations in standard trials before the learning (STD1) to trials after the learning block (STD2). The learning block by itself was not included in this correlation analysis due to insufficient number of trials. Comparison of the JPETHs demonstrates that the correlation is higher after learning (Figure 23 B, D, F), compared with the correlation in trials before the learning block (Figure 23 A, C, E). This is also shown in the multi-way-ANOVA (Table 3) and multiple comparison analysis (Figure 24 B) where the factor of Block of trials has a significant effect on the correlation strength (p<<0.001).

Moreover, multi-way ANOVA reveals interaction between the factors of Block and Sensitivity: the correlation between the pairs both sensitive to learned color, is significantly higher especially during the post-learning block, than any other combination of pairs and trials (see Supplementary Figure 5 A).

Finally, the analysis demonstrates that the differences in correlation strengths are kept in trials with learned colors as well as non-learned colors. (Correlations in subfigures (A,C) vs. (B,D) in: Supplementary Figure 2, Supplementary Figure 3, Supplementary Figure 4; Multi-way-ANOVA in Table 3; multiple-comparison-analysis in Supplementary Figure 5 (B-C), and Supplementary Figure 6). Notice, that this trend exists even though pairs were classified to subpopulations (“none/one/both”) according to their responses to learned-color (the Sensitivity factor). This suggests that the correlation is induced by learning and requires sensitivity to the learned colors; but it also becomes a feature of the cells’ activity at all times and not just during responses to specific stimuli or actions. This trend of differences in correlation strength irrespective of the group of trials (even though the
trials are grouped in relation to the single cells’ responses), reminds a similar trend found in the same monkey (monkey K) in relation of PD differences: As presented above, the correlation in pairs with close-PDs is higher, both in trials towards their common PD or in trials to their null-direction, as compared to the correlation in pairs with opposite-PD.
4. Discussion

Our study demonstrates that co-activation of cell-pairs in the monkey's motor cortices shows dependency on the single cells tuning properties: Adjacent cells that share close preferred-directions are on average co-activated more than cells that have opposing preferred-directions. Between anatomically distant cells the correlations are weaker, but show the same dependency on the cells properties. Moreover, learning-induced color sensitivity of single cells implies their pair-wise correlation strength; pairs with both cells being sensitive to learned colors, have on average higher correlation. We further found that pair-wise correlation after learning a new association task may become stronger as compared to pre-learning trials.

However, an intriguing heterogeneity of correlation strength and dynamics exist in every subpopulation (grouped according to the signal correlation). Therefore, our findings imply a much more elaborated arrangement of functional connection between cells, than has been described thus far.

It is generally agreed that coarse temporal correlations (tens and hundreds of milliseconds (Gawne & Richmond, 1993; Zohary et al., 1994; Lee et al., 1998; Reich et al., 2001)) and precise temporal synchrony (millisecond resolution (Toyama et al., 1981; Abeles, 1982; Kwan et al., 1987; Prut et al., 1998; Kohn & Smith, 2005; Sakurai & Takahashi, 2006)) exist between cortical neurons. In our study we analyze coarse temporal correlations between neuronal pairs; by this, we address correlations of firing rates processes, which
might be attributed to a broader network organization, rather than direct modulation of single synaptic connection.

The findings concerning the co-activation of cell pairs were studied using noise-correlation analysis that aims to extract the level of correlated activity beyond the expected from correlation of two independent neurons, given their firing rate. For example, noise correlations exclude the common activation of the two cells by a common controlled behavioral parameter, thus implying network connectivity between the cells in the pair (Perkel et al., 1967; Aertsen et al., 1989).

Noise correlation literature points out several mechanisms, besides network connectivity. Main caveats in noise correlation analysis might stem from the variability of behavior and stimuli, causing variations in the cell’s firing rate. Thus, they might introduce uncorrected signal correlation into the noise (Ben-Shaul et al., 2001). This is particularly relevant in motor behavioral studies, where the behavioral parameters of the movements vary inevitably. In our study, however, we took several precaution measures in behavioral task design and selection of trials and the cell pairs (see Methods). In addition, we examined the effects of variability of behavioral parameters; by calculating noise correlation for trials with only low variability of movement parameters. Comparison of the noise correlation built from low noise trials, to correlation from all trials, show analogous relation between the noise correlation to the signal the cells’ carry (to be discussed later in details), although with some disparities due to smaller sample size of trials. Other factors that could, in principle, cause apparent noise correlation are: In experiments conducted under anesthesia, correlations may arise from spontaneous oscillations that are absent in behaving animal – however, in our study the animals were
awake and behaving; Recordings from electrodes that are not chronically implanted often suffer from instabilities in the electrodes’ positions - however, in our study we analyzed data sets (separately) from acute micro-electrode array, and chronically implanted micro-electro array (see Methods), yielding significant close results; suboptimal single-unit isolation is a concern when multiple cells are recorded from the same electrode - but in our study we separated the data sets of cell pairs from within- and between-electrodes, and based the majority of the results on pairs from different electrodes. Despite the difficulties in measuring and interpreting correlations, our results agree with a number of studies (Pillow et al., 2008; Kohn et al., 2009; Truccolo et al., 2010; Cohen & Kohn, 2011) that promote the characterization of the correlation properties and how these depend on network state and behavioral experience.

The temporal dynamics of pair-wise correlation, and their relation to behavioral events have been demonstrated previously (Vaadia et al., 1995; Hatsopoulos et al., 1998; Baker et al., 2001). To examine the dynamics of interactions between cells in our study, we used the Joint-peri-event-time-histogram (JPETH) analysis of a single cell-pair (Aertsen et al., 1989). However, our research questions required task design which did not allow for sufficient spike count from trials that pass our criteria (see methods), to yield statistically significant results from the JPETH analysis for single cell pairs. Instead we studied correlations in subpopulations of cell-pairs by extending the use of JPETH analysis to groups of cell-pairs (see Joshua et al. (2009)). This was done by averaging the corrected correlation-coefficient values for each bin, across all the pairs in a selected group (see Table 1 for definitions of such groups).
Although the role of correlations between cell pairs has been studied before (as mentioned above in the *introduction*), the relations between the response properties of the cells and noise correlation remain a fundamental question. Such relations between noise correlation and similarity in tuning curves of the single cells have been previously demonstrated in several cortical areas, among them also in the motor areas. For example, noise correlation tended to be higher when signal correlation (the correlation between the cells’ directional tuning curves) were higher, and/or the units were closer anatomically – in M1 and parietal cortex (Lee et al., 1998) and in premotor cortex (Stark, 2007). Our results are consistent with the notion that noise and signal correlations are mutually dependent, and extend investigation of this notion by examination of the dynamics of correlations along the trials and around different behavioral events- before and after onset of cues and movements (see *results*). Further, we examined the dynamics of noise correlations in subpopulations grouped both by the signal and by the strength of noise correlation. This examination suggests that the baseline correlation between selected cell-pairs that share similar properties can remain unvarying (especially between neighboring cells, see *results* and Figure 19) while other pairs can be temporally modulated in relation to behavior, exhibiting specific, event related patterns of correlations (see *results* and especially Figure 7 I, Figure 12 I, and Figure 13 I).

*Movement related modulations of the correlations*

Previous studies have addressed the question of correlation strength during a small number of trial epochs. For example, Rothschild et al. (2010) show in the auditory cortex that the noise correlation before a tone stimulation was lower (and more variable) than
during the stimulus onset. In premotor cortex Stark (2007) shows higher noise correlation during the hold period of a motor task, as in some of the pairs in our study – the pairs with highest correlation (upper quartile) among the opposite-PDs population in monkey R (see results and Figure 13 I(A-B)). However, other pairs show higher correlation during movement than on hold (see upper quartile of close-PDs in monkey R in the results and Figure 12 I). Notice the difference between the studies in recording area (merely premotor cortex) and the task design which required memorization; while in our study the recording area was the primary motor cortex and the cue was constantly presented and memorization was not required during hold. The study by Lee et al. (1998) shows averaged correlation during hold and during movement epochs in motor and pre-motor cortices, but no particular period (hold or movement) was described with consistently higher correlation values (across inter-electrode distances and recorded areas (M1 and PM)).

Our study extended the previous ones by using a different analysis approach. First we grouped our pairs according to the cells’ activity during movement related period. Then, we examined the temporal dynamics of correlations not only during the period by which the cells were classified (the movement period), but throughout the whole trial from the beginning to its end. Our findings indicate that movement related modulations of the main diagonal in the JPETH analysis are evident in several sub-populations. For pairs with close-PDs in trials toward the common PD (see Figure 7 IA), the correlation value rises from near zero during the hold period, to higher level during arm movement. In contrast, some of the pairs with opposite PDs (Figure 13 I(A-B)) show higher level of correlation during the hold period, but when the movement starts the correlation
decreases; while in other pairs with opposite-PDs (Figure 13 I(C-D)) the correlation during hold is negative, and becomes weaker during movement. Note that these results of movement related modulations of the correlations could be obtained only in monkey R (see discussion below).

**PD-difference and correlations between neighboring cells**

Pair-wise correlations of *neighboring cells* (meaning recorded by the same electrode) clearly show that pairs with close-PDs tend to have stronger correlated activity than neighboring cells with opposite-PDs (Figure 19). Further, our results (Figure 4) show a non-homogeneous distribution of PD differences between neighboring cell-pairs, with higher probability to share close PDs (at least 25% of the tuned neighboring cells have PD difference of less than 45°). This is consistent with previous studies demonstrating a non-random spatial organization of cell’s PDs in the motor cortices ([Ben-Shaul et al., 2003; Amirikian & Georgopoulos, 2003; Stark et al., 2009]; local field potential (LFP) in M1 is tuned to direction of movement ([Donchin et al., 2001; Mehring et al., 2003]; directional organization evident in fMRI ([Eisenberg et al., 2010]).

Previous studies ([Haalman & Vaadia, 1997]) showed that neighboring pairs are positively correlated. The correlations of the neighboring close-PDs pairs in our study agree with this description (see results, lower quartile of close-PDs neighboring pairs, monkey K, showing insignificant fraction of the subpopulation with negative correlation, Figure 21 (E-F)), however the neighboring opposite-PDs pairs in our study do show for some pairs negative correlation values along the trial (Figure 22 (E-F)). In agreement with our study, Okun and Lampl (2008) demonstrated negative correlations between two nearby neurons in spontaneous and evoked activities, in intra-cellular recordings of the rat somatosensory
cortex. However, here we do not attribute the correlated activity fully to a direct single synaptic connection, but rather to a broader network arrangement.

The described correlations between neighboring cells reflect an organization of inputs to these cells causing similar response properties to emerge, thus suggesting that these cells share similar functionality. In a network with seemingly strong random component, we find principles of organization; partially overlapping functional networks of neighboring cells are organized by the similarity of the cells’ PDs, while negatively-correlated neighboring cells have opposing functionality.

The following section shows that the correlation patterns of cells sharing common response properties is even richer, when examined in specific subgroups and specific dynamics.

**Heterogeneous patterns of correlations**

The discussion thus far focused on the common properties of cells with similar signal correlation. However, our analysis show more detailed features; even for cells with close-PDs we observed different patterns of correlations. The quartile analysis in both monkeys reveals clearly that although each population was grouped according to the directional tuning of the cells, it is comprised of pairs with different strengths and temporal patterns of correlations: on-going positive, on-going negative, and movement related increases or decreases of the correlation. Heterogeneity in strength of average noise correlation given signal correlation, were described also for primary auditory cortex by Rothschild et al. (2010). Moreover, Renart et al. (2010) theoretically demonstrated that recurrent neural
networks can generate an asynchronous state, resulting in low mean correlation. Thus, a shared input does not inevitably imply correlated activity.

The heterogeneity in strength and temporal dynamics of correlations, adds to the fact that on average the population of pairs sharing close PDs has clearly higher correlation than the population of opposite PDs pairs. Meaning, we see in our results that, although a clear trend of difference of correlation strength exists between populations (each population characterized according to its single cell firing rate properties), they are comprised of pairs belonging to partially overlapping sub-networks. Thus, we infer that not only common inputs create a common preferred-direction of single cells, but there is a balance between several trends. These trends range from cooperative (expressed by positive noise correlation), to competitive (expressed by negative correlation or de-correlations in relation to previous higher correlation).

*Dependency of correlation on learning-induced-color-sensitivity*

The next goal of the study was to test the notion, that learning new sensorimotor associations between stimulus properties and required movement, may be associated with tuning of correlation strength between the task relevant cells.

This notion was supported by observing the relations between the pair-wise correlations and learning dependent changes of single cells’ responses to a newly learnt arbitrary association of color to movement direction. First, we see that pairs with both cells sensitive to learned colors have higher correlations than pairs which none or just one of their constituent cells are sensitive to the learned colors (Figure 23 (A-D vs. E-F), Table 3, and Figure 24(A)). Second, we find that following a learning block, the correlations
became stronger when both cells were sensitive to the learned instruction color (Figure 23 (E vs. F), Table 3, Supplementary Figure 4(A-B vs. C-D), Supplementary Figure 5(A)). This trend was detectable and statistically significant although the cells are not adjacent and the JPETHs are rather noisy.

Two monkeys – two different patterns of correlations?

Throughout the results section of this study, the findings from two monkeys are presented in detail, rather than simply combined by averaging. This was done in order to allow careful comparisons between the individual monkeys. At a first glance we see one monkey with clear dynamics of main diagonal (monkey R), while the other monkey (K) presents on-going correlated activity with little or no modulation of the main diagonal (for example in Figure 7 I and II, respectively). However, taking into account the different recording methodologies, it is likely that the results from the two monkeys taken together may yield a more comprehensive understanding of correlated activity in the motor cortical fields.

The recordings from monkey R were performed by a chronic array of microelectrodes, with inter-electrode distances ranging between 0.4 – 4mm (see methods and Figure 17), while the pairs from monkey K were from the same electrode or from different electrodes with distances in the range of 1-16mm. The different distributions of pairs shown in Figure 17, demonstrate that smaller distances were much more abundant in monkey R than in monkey K, which might yield the noisier results of monkey K. Moreover, the microelectrodes in monkey K recorded neural activity from broader area, including premotor cortex, while in monkey R the chronic array was implanted in the arm region in
M1. Indeed, the neuronal activity in monkey R was more clearly related to onset time and direction of arm movement, and a considerable higher fraction of the directionally tuned cells in monkey R exhibited uni-modal tuning function than in monkey K. This could also be the reason that movement related modulations on the main diagonal in the JPETH analysis of monkey R are more easily detected as compared to monkey K.

Interestingly, we can see from the quartile analysis that the upper quartile (meaning the pairs with strongest correlations) of the pairs with close-PDs of monkey R had on-going correlation, though modulated with movement (Figure 12 I). This pattern of correlation resembles the pattern of on-going correlation in monkey K, in particular in the pairs of neighboring cells (i.e. from the same electrode, Figure 19). We can infer from this that despite the different dynamics of correlations, in both monkeys we see pairs with on-going correlations. This on-going correlation was not emphasized in the literature thus far, which has focused on the movement-related pattern of correlation. However it may be of special interest, since it supports the notion that some basic organization exists in motor cortex comprising of cells which are more closely connected (especially neighboring cells) and are constantly correlated. On top of this, we can observe dynamic formation of functional groups with timing related to specific events and movements, hence taking part in dynamic grouping that assemble and disassemble according to specific parameters of performance of movements.
Reference List


Appendix

Pair-wise correlations between pairs during movements toward orthogonal directions to the PD:

Supplementary Figure 1 demonstrate that these pairs have intermediate values of positive correlations among the other sub-populations presented thus far (i.e. pairs with close-PDs and pairs with opposite-PDs). This is both in terms of average correlation (presented in the CCH analysis) and of temporal dynamics of correlations (presented in the diagonal analysis of JPSTHs). Please note that in the current experimental settings, the behavioral paradigm consisted of center→ out task to eight directions, allowing for only two types of trials to be considered as “toward orthogonal direction”, whereas three types of trials considered as “towards PD” or “towards null direction” (see Methods). This imbalance in number of behavioral trials could affect the results of correlation analysis, which are very sensitive to variance in firing rates. Therefore, we do not include further comparisons to correlations during movements to orthogonal directions in this study.
Supplementary Figure 1 Comparison of correlation during orthogonal movement directions, to preferred and null directions. (I) Monkey R; (II) Monkey K.
(A) Crosscorrelogram (CCH) of pairs with close PDs (blue- towards the PD; green- towards the null direction; cyan- towards the direction orthogonal to PD), and with opposite PDs (red- towards PD of the 1st cell; magenta- towards PD of the 2nd cell; yellow- towards the direction orthogonal to the PDs). The color code is same as in figure legend.
(B) Diagonal of the JPSTH showing the temporal dynamics of the correlations between the pairs of cells during different behavioral conditions. Color code- same as in A.
Supplementary Figure 2 Correlations between pairs of cells with no learning-induced-color-sensitivity (*None*). All pairs are from different electrodes, recorded from monkey K. Trials aligned around Movement Onset (MO, ranges [-2.5 2.5]). (A-D) In each subplot the central image is the JPETH (color-bar codes for the noise-correlation (correlation coefficient (cc))); Marginal histograms are the PETHs of the cells (spikes/second (=Hz) as a function of time in the trial); Inset is the CCH constructed from the diagonals of the JPETH (correlation (cc) as a function of time lag (sec)).

(A) Trials during pre-learning (*STD1*) block, having only with colors that appear in the learning epoch (*learned-col*).

(B) Trials during pre-learning (*STD1*) block, having only with colors that DO NOT appear in the learning epoch (*naïve-col*).

(C) Trials during post-learning (*STD2*) block, having only with colors that appear in the learning epoch (*learned-col*).

(D) Trials during post-learning (*STD2*) block, having only with colors that DO NOT appear in the learning epoch (*naïve-col*).
Supplementary Figure 3 Correlations between pairs of cells with one cell having learning-induced-color-sensitivity, and the other cell not sensitive (One). All pairs are from different electrodes, recorded from monkey K. Trials aligned around Movement Onset (MO, ranges [-2.5 2.5]). (A-D) In each subplot the central image is the JPETH (color-bar codes for the noise-correlation (correlation-coefficient (cc))); Marginal histograms are the PETHs of the cells (spikes/second (=Hz) as a function of time in the trial); Inset is the CCH constructed from the diagonals of the JPETH (correlation (cc) as a function of time lag (sec)).

(A) Trials during pre-learning (STD1) block, having only with colors that appear in the learning epoch (learned-col).
(B) Trials during pre-learning (STD1) block, having only with colors that DO NOT appear in the learning epoch (naïve-col).
(C) Trials during post-learning (STD2) block, having only with colors that appear in the learning epoch (learned-col).
(D) Trials during post-learning (STD2) block, having only with colors that DO NOT appear in the learning epoch (naïve-col).
Supplementary Figure 4 Correlations between pairs of cells with both cells having learning-induced-color-sensitivity (Both). All pairs are from different electrodes, recorded from monkey K. Trials aligned around Movement Onset (MO, ranges [-2.5 2.5]). (A-D) In each subplot the central image is the JPETH (color-bar codes for the noise-correlation (correlation-coefficient (cc))); Marginal histograms are the PETHs of the cells (spikes/second (=Hz) as a function of time in the trial); Inset is the CCH constructed from the diagonals of the JPETH (correlation (cc) as a function of time lag (sec)).

(A) Trials during pre-learning (STD1) block, having only with colors that appear in the learning epoch (learned-col).
(B) Trials during pre-learning (STD1) block, having only with colors that DO NOT appear in the learning epoch (naïve-col).
(C) Trials during post-learning (STD2) block, having only with colors that appear in the learning epoch (learned-col).
(D) Trials during post-learning (STD2) block, having only with colors that DO NOT appear in the learning epoch (naïve-col).
Supplementary Figure 5 Multiple comparison analysis of ANOVA in Table 3, for two factors: (A) Block and Sensitivity; (B) Block and Colors; (C) Sensitivity and Colors.
Supplementary Figure 6 Multiple comparison analysis of ANOVA in Table 3, for three factors: Block, Sensitivity and Colors.
The processing of information in the brain is performed by interconnected neurons at a high density. Previous studies have shown that neurons encode information not only individually, but also as components in larger networks. Therefore, to study the activity of the brain, we need to consider the individual properties of the neurons, but we also need to examine their collective activity in the network.

Despite the coordinated activity between cortical neurons being weak statistically, previous studies have suggested that the quality of information can be high in networks with a low level of coordination.

In this study, we looked at the shared activity of neuron pairs and the relationship between this activity and the individual properties of the neurons composing the pairs. To do this, we recorded the individual activity of neurons in the motor areas of the monkey's cortex, using arrays of micro-electrodes. The monkeys executed a sensorimotor task of moving their hand from a central point.

Our main findings indicate a relationship between the similarity of the preferred directions of neurons in a pair, and the degree of coordination between them: pairs with similar preferred directions have a higher correlation of noise compared to pairs with opposite preferred directions. In addition, relationships develop as a result of mapping of a muscular system between color and movement direction. During the learning process, new features emerge in the individual properties of the neurons. We found that the coordination of neuron pairs with two neurons sensitive to color after learning was higher than the coordination between the other neuron pairs.

However, a detailed analysis of the results revealed a surprising range of time dynamics and levels of average coordination, and in some pairs, the dynamics of time showed increases or decreases in the correlation level. These modulations were associated with (A) behavioral changes; (B) differences in preferred directions of neurons. Other neurons...
מרアイים תأهل המתחבר לאורכר התורגלו התנגונים עם דינמיות גבוהה, ואילו תנגוניה, זוהמה, בכמה, בתנאי
הunft פורייה, וולפック- חומת הפוריה בולע דינמי מודפס קרובי הבソ על-ידי דינמי מודפס הפוכים
בוגר תاهل הוורכ, וולפック- חומת הפוריה בולע דינמי מודפס קרובי הבソ על-ידי דינמי מודפס הפוכים
וה-ft מודפסים. מונטיני לינט, שבני התאים השוכנים (שנerdale מואצות האלקטרודה) ואין מגוון תأهل שליל,
הוא רק בת-האולקטרודה של הוורכ בולע דינמי מודפסים הדופק המ죴 בולע דינמי מודפסים
תקאות, ישנה קבוצה פוגעת של תأهل עם תأهل שליל.
לפיו, תוצאות המחקר מצביעות על שבבי שבו מתואר נוירון בוגר תأهل חלוקת הזרימה הקוטקה
ה當您在コーペר. אני מביא את שניות של הזרימה של הוורכ בוגר תأهل חלוקת הזרימה הקוטקה
הتعاerator. אני מתרשם שניורון מתאimité שוגני גורמים להבטחת פעילות של הזרימה. שני גורמים
עיניים של אנרגיה רישתי מתחדשים מת僭ת הדינמיות בוגר: ראשית, יש על-פי מראות דינמיותAPPED,
שמ蹩ייזפואה בודגמיות בוגר של התاهل אשר Threads ירי המגנטים להתנגשים. שני, יש על-פי婴幼儿
לביצי, שמ seriizp אצטאס מתנשך שיאגרי Threads ירי המגנטים להתנגשים.
עבודת זו נעשתה בהדרכתו של פרופ' אילון ועדיה:
פרופ' אילון ועדיה
דינמיקה של פעילות עצבית

אנטראקציה בין אזורי קורטיקליים קדミים

בת אור קריסים סנסאומוטוריים הסטגלאטים

ה보호 על שם קבלת תואר דוקטור לפילוסופיה

מאת

דורית ענבר

הוגש לסמינר האוניברסיטה העברית, בירושלים

טבת, תשע"א
דינמיקה של פעילות עצבית
ואנרגטרקציות באוררים קורטייקליים קדמיים
בצאת קירוריים סטטוסטרטיריים הסתגלותיים

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