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SYNERGISTIC ACTIVITY BETWEEN PRIMARY VISUAL NEURONS

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Abstract—Cortical microcircuitry plays a pivotal role in encoding sensory information reaching the cortex. However, the fundamental knowledge concerning the mechanisms that govern feature-encoding by these sub-networks is still sparse. Here, we show through multi-electrode recordings in V1 of conventionally prepared anesthetized cats, that an avalanche of synergistic neural activity occurs between functionally connected neurons in a cell-assembly in response to the presented stimulus. The results specifically show that once the reference neuron spikes in a connected neuron-pair, it facilitates the response of its companion (target) neuron for 50 ms and, thereafter, the excitability of the target neuron declines. On the other hand, the functionally unconnected neurons do not facilitate each other's activity within the 50-ms time-window. The added excitation (facilitation) of connected neurons is almost four times the responsiveness of unconnected neurons. This suggests that connectedness confers the added excitability to neurons; consequently leading to feature-encoding within the emergent 50-ms-period. Furthermore, the facilitation significantly decreases as a function of orientation selectivity spread.

Key words: inter-neural relationships, primary visual cortex, functional network, microcircuit, synergy.

INTRODUCTION

Cortical neurons propagate information about the experience (input) through the complex temporal relationships of their action potentials (Alloway and Roy, 2002; Barthó et al., 2004; Kohn and Smith, 2005; Yoshimura et al., 2005; Samonds et al., 2006; Fujisawa et al., 2008; Ghisovan et al., 2008). Neurons principally coordinate in local sub-networks termed 'cell-assemblies' to represent distinct cognitive entities (Hebb, 1949; Buzsáki, 2010; Kampa et al., 2011; Ko et al., 2011, 2013; Perin et al., 2011). Visual cortex is perhaps the most studied area of the brain; yet, how local circuits emerge to encode specific information in response to a visual input, is a question to be deeply explored—that is, how the intriguing interplay between the stimulus and a microcircuit is engineered and modulated?

Rate coding and temporal coding seem to dominate the literature on population coding in response to a stimulating feature (Perkel and Bullock, 1968; Ainsworth et al., 2012; Ratté et al., 2013), and both appear to generate a code either as independent or mutually inclusive events (Ainsworth et al., 2012; Ratté et al., 2013). However, recently, authors (Ainsworth et al., 2012) have suggested a bias toward a precise temporal code (stimulus-driven-synchrony) that is a reflection of the anatomical architecture and synaptic biophysical properties of neurons (Ainsworth et al., 2012; Ratté et al., 2013). Synchronized neural activity implying specific functional connectivity has already been associated to the generation of coherent percepts (Gray and Singer, 1989; Barthó et al., 2004; Duret et al., 2006; Fujisawa et al., 2008; Ghisovan et al., 2008).

Cross-correlogram function is an efficient tool to study synchrony between the neural spike trains (Perkel et al., 1967; König et al., 1995; Barthó et al., 2004; Fujisawa et al., 2008). Employing cross-correlograms, we have already shown that co-active neurons in a V1 (layer II/III) cell-assembly frame a specific functional connectome in relation to a stimulus (Bretzner et al., 2001; Duret et al., 2006; Bharmauria et al., 2012). In other words, how a cell-assembly waxes and wanes as the visual stimulus varies. It is to be mentioned that these functional connections were revealed within a ±5-ms quasi-synchrony window in the cross-correlogram.

Building on the previous work, the goal of our current investigation is to observe and compare the most likely avalanche of neural activity between functionally connected, unconnected and untuned neuron-pairs in an assembly on a broader time-scale. That is, for how long, in relation to the spiking of one neuron, the discharge of the target neuron is modulated. We report that in V1 (layer II/III) of conventionally prepared anesthetized cats, the target neuron's discharge augments for 50 ms after reference neuron's spiking, and thereafter, the firing rate decreases. This is applicable to neurons (paired) that share orientation preference, as well as pairs exhibiting a large difference in orientation tunings. On the other hand, no significant increase was noticed for unconnected and untuned cell-pairs within the same

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Abbreviations: OSI, orientation selectivity index; PSTH, peristimulus time histograms; SEM, standard errors of the mean.

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time-window. Thus, our results indicate that a functional network in V1 is set within 50 ms as a result of maximal synergistic activity, after the onset of the activity of companion cells in the assembly.

EXPERIMENTAL PROCEDURES

Ethical approval

Nine cats (*Felis catus*) were prepared in a conventional fashion for electrophysiological recordings in the primary visual cortex, as per the guidelines of Canadian Council on Animal care and approved by the Institutional Animal care and Use committee of the Université de Montreal. The experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals of the National Institutes of Health (USA).

Animal preparation

Briefly, animals were sedated with acepromazine maleate (Atravet, Wyeth-Ayerst, Guelph, ON, Canada; 1 mg kg^{-1}, intramuscular) and atropine sulfate (ATRO-SA, Rafter, Calgary, AB, Canada; 0.04 mg kg^{-1}, intramuscular), and anesthetized with ketamine hydrochloride (Rogarsetic, Pfizer, Kirkland, QC, Canada; 25 mg kg^{-1}, intramuscular). The cats were then paralyzed with 40 mg and maintained with 10-mg kg^{-1} h^{-1} gallamine triethiodide (Flaxedil, Sigma Chemical, St. Louis, MO, USA; intravenous) administered in 5% dextrose lactated Ringer's nutritive solution. General anesthesia was maintained by artificial ventilation with a mixture of N_2O/O_2 (70:30) supplemented with 0.5% isoflurane (AErrane, Baxter, Toronto, ON, Canada). Electroencephalogram, electrocardiogram, rectal temperature and end-tidal CO_2 partial pressure were monitored throughout the experiment, and kept in physiological ranges. Pupils were dilated with atropine, and Plano lenses with artificial pupils (5 mm diameter) were fixed. The electroencephalogram pattern ensured that the animals were properly anesthetized. The loci of the area centrales were inferred from the position of the blind spots. At the end of the experiment, the cats were euthanized intravenously with Sodium Pentobarbital (CEVA, Sante Animale). The injecting dose was 1 ml/2 kg of animal weight.

Visual stimulation

Monocular stimulation was done. The multi-unit receptive fields (RF) were mapped as the minimum response field (Barlow et al., 1967) by using a hand-held ophthalmoscope after clearly detectable activity was obtained. These preliminary tests revealed qualitative properties such as dimensions, velocity, orientation and direction selectivity. Visual stimuli were generated with a VSG 2/5 graphic board (Cambridge Research Systems, Rochester, England) and displayed on a 21-inch monitor (Sony GDM-F520 Trinitron, Tokyo, Japan) placed 57 cm from the cat's eyes, with 1024 × 768 pixels, running at a 100-Hz frame refresh rate. Stimuli were sine-wave drifting gratings covering the excitatory RF of neurons at both electrode tips. Contrast was set at 80%. Mean luminance was 40 Cd m^{-2}. Optimal spatial and temporal frequencies were set at 0.24 cycles deg^{-1} and 1.0–2.0 Hz range respectively, where V1 neurons are driven maximally by sine-wave drifting gratings (Bardy et al., 2006).

Electrophysiological recording and single-unit selection from multi-unit activity

Multi-unit activity in the primary visual cortex was recorded at 410–820 μ apart by a tungsten multi-electrode (Frederick Haer & Co., Matrix Electrode). Recordings were performed in the supragranular layers (cortical depth < 1000 μm; mean = 650 μm). The signal from the microelectrodes was amplified, band-pass filtered (300 Hz–3 kHz), digitized and recorded with a 0.05-ms temporal resolution (Spike2, CED, Cambridge, England). All cells were discriminated on the basis of three criteria: (1) the spike-waveform difference, (2) principal component analysis (PCA) showing well-dissociated clusters, and, (3) auto-correlograms showing no events at central point (Csicsvari et al., 1998; Barthó et al., 2004; Fujisawa et al., 2008; Harris et al., 2001). The stability of each cell's activity across conditions was verified qualitatively by visual control of the disposition of the clusters and the shapes of the waveforms.

The cluster analysis was performed using Spike2, CED, Cambridge, England in a 3-dimensional plot. The isolation distance was calculated as the Mahalanobis distance. Mahalanobis distance is the distance from the center of the cluster within which as many events belong to the other clusters as many belong to the specified cluster (Harris et al., 2001). In other words, given multivariate data values for which the values in each variable are normally distributed around a mean, this measure allows us to define boundaries of constant probability around the multi-dimensional center of the distribution. This estimation allows the separation of a cluster from the nearest cluster. Units within Mahalanobis distance of 2.5 were considered for analysis.

Fig. 1 illustrates the protocol and isolation of neurons from a single electrode tip. Fig. 1A exemplifies the experimental protocol. Each drifting grating was presented 25 times lasting 180 s, with varying inter-stimulus (1–3 s) intervals. Each trial lasted 4 s. Orientations of gratings were presented in a random order. Fig. 1B depicts the separated and averaged waveforms of four units recorded simultaneously. Each waveform is distinct, well separated, and corroborated by Principal Component Analysis. Fig. 1C shows the respective auto-correlograms of the four separated neurons, and no event at zero (corresponding to the refractory period of neuron) ascertains the individuality of the neuron. Fig. 1D illustrates the cluster analysis as explained above.

Data analysis

Once single cells were sorted out off-line from multi-unit spike trains, orientation tuning curves of cells were obtained from raw data and fitted with the von Mises function (Swindale, 1998).