Functional Connectivity between Layer 2/3 and Layer 5 Neurons in Prefrontal Cortex of Nonhuman Primates during a Delayed Match-to-Sample Task

Dong Song, *Member, IEEE*, Ioan Opris, Rosa H. M. Chan, *Student Member, IEEE*,
Vasilis Z. Marmarelis, *Fellow, IEEE*, Robert E. Hampson, *Member, IEEE*,
Sam A. Deadwyler, *Member, IEEE*, and Theodore W. Berger, *Fellow, IEEE*

Abstract—The prefrontal cortex (PFC) has been postulated to play critical roles in cognitive control and the formation of long-term memories. To gain insights into the neurobiological mechanism of such high-order cognitive functions, it is important to understand the input-output transformational properties of the PFC micro-circuitry. In this study, we identify the functional connectivity between the Layer 2/3 (input) neurons and the Layer 5 (output) neurons using a previously developed generalized Volterra model (GVM). Input-output spike trains are recorded from the PFCs of nonhuman primates performing a memory-dependent delayed match-to-sample task with a customized conformal ceramic multi-electrode array. The GVM describes how the input spike trains are transformed into the output spike trains by the PFC micro-circuitry and represents the transformation in the form of Volterra kernels. Results show that Layer 2/3 neurons have strong and transient facilitatory effects on the firings of Layer 5 neurons. The magnitude and temporal range of the input-output nonlinear dynamics are strikingly different from those of the hippocampal CA3-CA1. This form of functional connectivity may have important implications to understanding the computational principle of the PFC.

I. Introduction

THE prefrontal cortex (PFC) refers to the anterior region of the frontal lobe of the mammalian brain. It has been postulated to be critical for cognitive control, i.e., the top-down selection of appropriate behavior given multiple, possibly interfering sensory and motor inputs based on the internal goals [1].   Recent neuropsychological and neuroimaging studies have shown that the PFC also plays important roles in working memory and the formation of long-term memory [2].
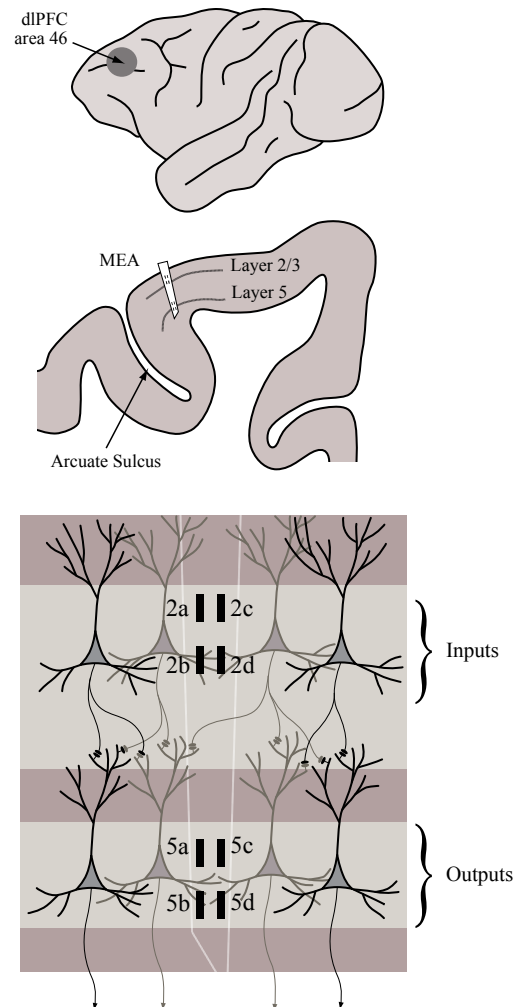

Figure 1. Prefrontal cortex (PFC) and the conformal ceramic multi-electrode array (MEA). dlPFC: dorsolateral PFC; Black rectangles: recording pads (2a-d, 5a-d) on the MEA. Spike trains recorded from layer 2/3 neurons and layer 5 neurons are the main input and output signals of the PFC circuitry, respectively.

The anatomy of the PFC is well suited for performing such high-order, complex cognitive functions. The PFC receives inputs from and sends outputs to virtually all other cortical areas and many subcortical structures. Within the PFC, the subregions are also heavily interconnected.   However, similar to other neocortical areas, the PFC has a rather uniform structure at the micro-level: it consists of a large

number of 6-layer minicolumns that operate as the elementary computational units (Fig. 1). Specifically, within a minicolumn, Layer 2/3 neurons receive sensory and motor inputs from other cortical areas, and Layer 5 neurons generate local circuit outputs to subcortical structures that mediate planned behaviors [3]. To gain insights into the neurobiological mechanism of the PFC functions, it is important to understand the input-output transformational properties of the PFC micro-circuitry.

The goal of the research reported here is to identify the functional connectivity between PFC Layer 2/3 neurons and Layer 5 neurons during behavior using a generalized Volterra modeling approach. Generalized Volterra model (GVM) was previously developed by our group for studying the hippocampal CA3-CA1 dynamics in the context of building a hippocampal prosthesis [4, 5]. It seeks to solve the problem of indentifying the nonlinear dynamics underlying multi-input-multi-output (MIMO) point-process signals and can be used as a general framework for modeling the spike trains to spike trains transformations performed by brain regions.

II. METHODOLOGY

A. Behavioral Task

Four nonhuman primates (*Macaca mulatta*) were trained to criterion on a Delayed Match-to-Sample (DMS) task (Fig. 2) [6]. During the task, animals sat in primate chairs in front of a display screen and moved a cursor with their right arms. A trial began with a ring tone and a visual cue at the center of the screen. The animal moved the cursor to the cue to start the trial. An image was then presented (Sample Presentation) and the animal had to move the cursor to the image to form a Sample Response. The image then disappeared for a randomly occurring variable delay interval (1 – 30 sec). After the delay, a number of (2 – 6) different images including the sample image were presented on the monitor (Match Presentation). The animal had to move the cursor to the sample image (Match Response) to receive a juice reward. The task is memory-dependent since the animal needed to form a memory of the sample image and retain it during the delay period.
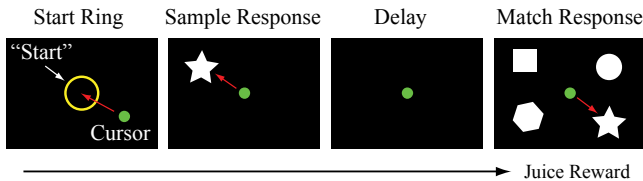

Figure 2. Delayed match-to-sample task (DMS) for NHPs. During the DMS, the NHP needs to match a sample image after a delay.

### B. Recording and Preprocessing of Spike Trains

Spike trains were recorded from the PFCs of nonhuman primates (NHPs) performing the DMS task using a conformal ceramic multi-electrode array (MEA). The MEA has eight recording pads (area: 20 × 150 μm/pad; 40 μm horizontal

distance and 100 μm vertical distance between adjacent pads, e.g., 2a to 2c and 2a to 2b; 1350 μm distance between higher pads and lower pads, e.g., 2b and 5a). The geometry of the MEA is designed in such a way so that pads 2a-d are in Layer 2/3 and pads 5a-d are in Layer 5. Figure 1 shows the arrangement of the ceramic MEA recording sites relative to the orientations of the pyramidal cell bodies and dendrites in Layer 2/3 and Layer 5. Spikes were sorted and timestamped with a MAP Spike Sorter (Plexon, Inc.) and NeuroExplorer software (Nex Technologies), and further discretized with a 2 ms bin size with Matlab (MathWorks). Spike trains recorded from Layer 2/3 neurons and Layer 5 neurons are the input and output signals of the model, respectively.

C. Multi-Input-Multi-Output Nonlinear Dynamical Model

To identify the functional connectivity between Layer 2/3 and Layer 5 neurons in PFC, we use a GVM approach previously developed for the modeling of hippocampal CA3-CA1 nonlinear dynamics [4].
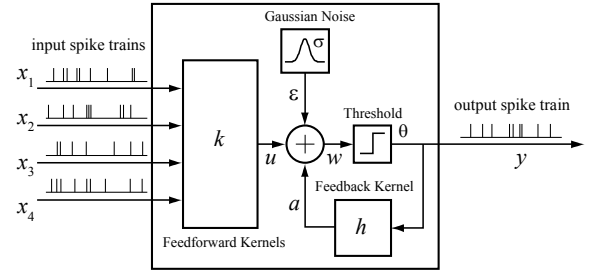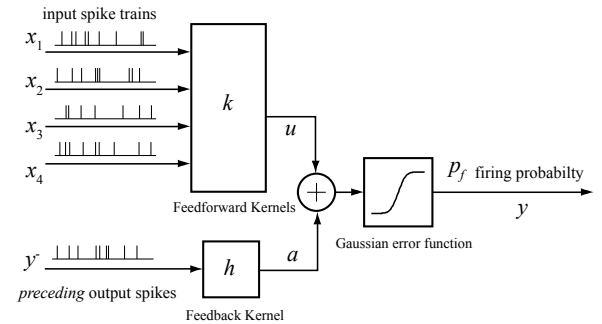

Figure 3. Multi-input-single-output (MISO) model of spike train transformations. This model is equivalent to a generalized Volterra model with a *probit* link function.

In this approach, the identification of spatio-temporal pattern transformations from the input region to the output region is formulated as the estimation of a MIMO model that can be decomposed into a series of multi-input, single-output (MISO) models with a physiologically plausible structure that can be expressed by the following equations (Fig. 3A):

$$w = u(k,x) + a(h,y) + \varepsilon(\sigma) , \quad y = \begin{cases} 0 & \text{when } w < \theta \\ 1 & \text{when } w \geq \theta \end{cases}$$

The variable x represents input (Layer 2/3) spike trains; y

represents output (Layer 5) spike trains. The hidden variable w represents the pre-threshold membrane potential of the output neurons. It is equal to the summation of the post-synaptic potential u caused by input spike trains, the output spike-triggered after-potential a, and a Gaussian white noise ε with standard deviation σ. When w exceeds threshold, θ, an output spike is generated and a feedback after-potential (a) is triggered and then added to w. The transformation from x to u is expressed as a MISO Volterra series with a set of feedforward kernels k. The transformation from y to a is expressed as a single-input, single-output Volterra series with a feedback kernel h.

To reduce the total number of model coefficients and thus facilitate model estimation, Laguerre expansion of Volterra kernel technique is used. The Volterra kernels are reconstructed with the Laguerre basis functions and the estimated Laguerre coefficients.

Due to the Gaussian noise term and the threshold, this model is a special case of the GVM, which employs a *probit* link function (Fig. 3B). All model parameters, i.e., k, h, σ, and θ, can be estimated simultaneously with an iterative re-weighted least-squares method and a simple normalization procedure [4]. In the final model representation, resting membrane potential is 0; threshold value is 1; first order kernel k_1 can be interpreted as the post-synaptic potential (PSP) elicited by a single input spike; second order self-kernel k_{2s} can be interpreted as the paired-pulse facilitation/depression function (See [4, 5] for more details. To simplify the notation, k is used to represent the response function r in [5]).

To further reduce model complexity and avoid overfitting, a statistical procedure is used to select the significant inputs and model terms [5]. The resulted sparse GVM achieves maximal out-of-sample likelihood [7].

III. RESULTS

A. GVMs of the PFC Layer 2/3-Layer 5 Dynamics

We estimate second order GVMs of the PFC using the spike trains recorded during the DMS task. A typical dataset contains 2-4 Layer 2/3 neurons and 2-4 Layer 5 neurons. To focus on the most relevant neuronal activity and alleviate computation burden, only spikes recorded between the Sample Presentations and Sample Responses are considered. MISO models are first individually estimated and then concatenated to form the MIMO models for each dataset.

Figure 4 illustrates the first order (k_1), second order self (k_{2s}) and feedback (h) kernels of a 3-input-2-output model. For both output neuron 1 and 2, input neuron 3 does not have significant effect so is not plotted.

The most significant characteristics of the kernels are their large amplitudes. In k_1, the peak amplitudes are 0.24, 0.39, 0.62, and 0.22, respectively. Considering the estimated noise standard deviation σ of these two output neurons (0.33 and 0.41), the corresponding firing probabilities within a 2 ms time window can be calculated with the Gaussian error function (erf) as the follows:

$$p_1(\tau) = 0.5 - 0.5 \times \text{erf}\left(\frac{1 - k_1(\tau)}{\sqrt{2}\sigma}\right)$$

The peak firing probabilities calculate to be 0.01, 0.03, 0.18, and 0.03, respectively.

The second significant characteristics are the short durations of the kernels, i.e., all kernels expand to approximately 20 ms with larger values happening in the first 5 ms. This indicates that the causal relations from Layer 2/3 neurons to Layer 5 neurons are not only strong, but also fast.

While all k_1 are mostly positive going (i.e., facilitatory), the second order kernels are mostly negative going indicating paired-pulse depression effects. Similar to k_1, k_{2s} are also large and transient.
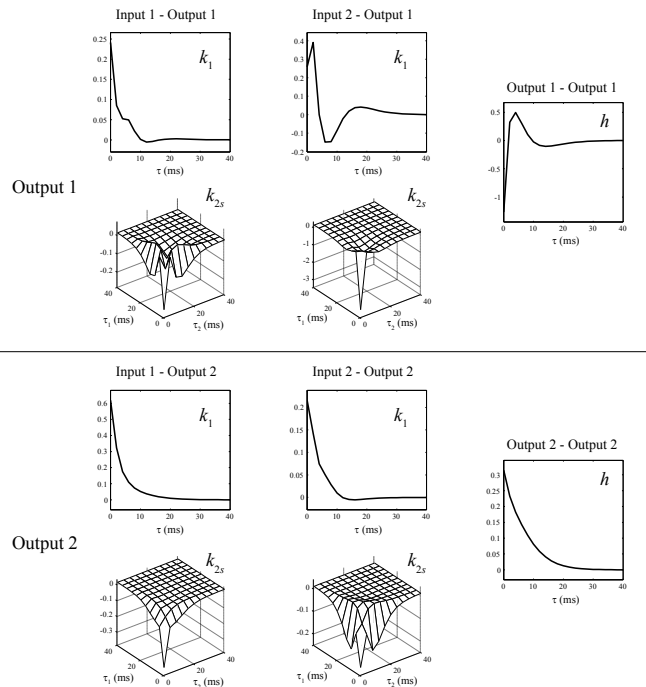

Figure 4. A second order GLVM of PFC nonlinear dynamics. This specific model has two Layer 2/3 inputs and two Layer 5 outputs. First order (k_1) and second self (k_{2s}) kernels are shown for each input-output pair. Feedback kernels h are shown for each output.

B. Model Goodness of Fit

We further evaluate the model goodness of fit using a Kolmogorov-Smirnov (KS) test [7]. According to the time-rescaling theorem, an accurate model should generate a conditional firing intensity function that can rescale the recorded output spike train into a Poisson process with unit rate. By further variable conversion, inter-spike intervals should be rescaled into independent uniform random variables on the interval (0, 1). The model goodness-of-fit then can be assessed with a KS test, in which the rescaled intervals are ordered from the smallest to the largest and plotted against the cumulative distribution function of the

uniform density. If the model is accurate, all points should be close to the 45-degree line of the KS plot. Confidence bounds (e.g., 95%) can be used to determine the statistical significance [8].
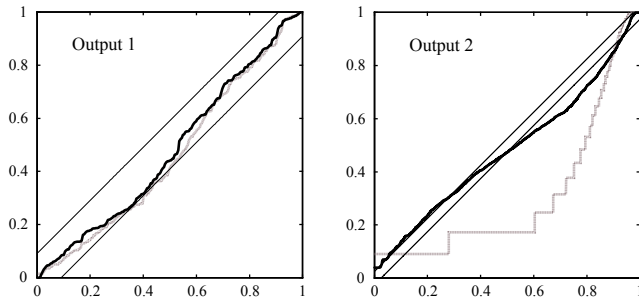

Figure 5. Model goodness of fit evaluated with the Kolmogorov-Smirnov test based on the time-rescaling theorem. Thick black lines: KS plots with the GVM; dashed gray lines: KS plots without applying the GVM; thin black lines: 95% confidence bounds.

Figure 5 shows that KS plots of the two Layer 5 output neurons. The gray dashed lines show the KS plots without applying the GVMs and the black lines show the KS plots with the GVMs. It is evident that the GVM can fairly accurately predict the output spike training, i.e., the KS plots are within (output 1) or close to (output 2) the 95% confidence bounds with the model, especially compared with the large deviation of the KS plots without applying the model.

IV. DISCUSSION

The differences between the PFC Layer 2/3-Layer 5 and hippocampal CA3-CA1 dynamics are striking. In the hippocampal CA3-CA1, typical amplitudes of first order kernels are below 0.1, corresponding to firing probabilities approximately below 0.005, which means a single input spike almost never can drive an output neuron to fire. In the PFC, the firing probability in a 2 ms time window can reach as high as 0.18 in the dataset we show. This indicates a much stronger and less stochastic causal effect from Layer 2/3 to Layer 5. On the other hand, the hippocampal CA3-CA1 dynamics have a much longer duration. The typical memory lengths of the kernels are hundreds of milliseconds as opposed to less than 20 milliseconds in the PFC. It implies that the hippocampal CA1 neurons integrates "weak" information from possibly larger number of input neurons during a longer time span; while in contrast, the PFC Layer 5 neurons receive "strong" information from possibly smaller number of input neurons during a shorter time span. These different characteristics of the nonlinear dynamics may have important implications to the fundamental computational principles of those two brain regions.

The KS results show that the model can capture the majorities of the nonlinear dynamics underlying the transformations from the Layer 2/3 spike trains to the Layer 5 spike trains. However, significant deviations from the actual output spike distribution still exist in the predictions (Fig. 5, especially in output 2). This is not surprising since the current MEA technology only allows recording from a small number of neurons. Even located in the same minicolumn, it is still unlikely that a Layer 5 neuron receives inputs from only two adjacent Layer 2/3 neurons. More accurate model prediction might be achieved by recording signals from more input neurons with a multi-probe array and including them into the model.

One must keep in mind that the functional connectivities are estimated based on the (nonlinear dynamical) correlations between the input and output signals and thus cannot be interpreted directly as synaptic connectivities. For example, the near-zero delays between Layer 2/3 and Layer 5 neurons observed in this study are very unlikely caused solely by synaptic connections considering the synaptic transmission delays. The underlying mechanism of this peculiarly tight coupling is unknown. One possible explanation is that the Layer 2/3 and Layer 5 neuron are receiving common inputs and respond with high temporal precision. Indeed, the cortical minicolumn consists of a highly interconnection neuronal network instead of the much simplified two-region feed-forward configuration used here in the MIMO modeling. Network-level modeling with realistic connectivity and multi-site recording in all six layers of the minicolumn might provide answers to such a mystery.

REFERENCES

[1] E. K. Miller, and J. D. Cohen, "An Integrative Theory of Prefrontal Cortex Function," *Annual Review of Neuroscience*, vol. 24, pp. 167-202, 2001.

[2] R. S. Blumenfeld, and C. Ranganath, "Prefrontal Cortex and Long-Term Memory Encoding: An Integrative Review of Findings from Neuropsychology and Neuroimaging," *The Neuroscientist*, vol. 13(3), pp. 280-291.

[3] I. Opris, R. E. Hampson, T. R. Stanford, G. A. Gerhardt, and S. A. Deadwyler, "Neural Activity in Frontal Cortical Cell Layers: Evidence for Columnar Sensorimotor Processing," *Journal of Cognitive Neuroscience*, vol. 23(6), pp. 1507-21, 2011.

[4] D. Song, R. H. M. Chan, V. Z. Marmarelis, R. E. Hampson, S. A. Deadwyler, and T. W. Berger "Nonlinear dynamic modeling of spike train transformations for hippocampal-cortical prostheses," *IEEE Transactions on Biomedical Engineering*, vol. 54, 2007.

[5] D. Song, R. H. M. Chan, V. Z. Marmarelis, R. E. Hampson, S. A. Deadwyler, and T. W. Berger, "Nonlinear Modeling of Neural Population Dynamics for Hippocampal Prostheses," *Neural Networks*, vol. 22, pp. 1340-1351, 2009.

[6] R. E. Hampson, T. P. Pons, T. R. Stanford, and S. A. Deadwyler, "Categorization in the monkey hippocampus: a possible mechanism for encoding information into memory," *Proc Natl Acad Sci U S A*, vol. 101(9), pp. 3184-3189, 2004.

[7] D. Song, R. H. M. Chan, V. Z. Marmarelis, R. E. Hampson, S. A. Deadwyler, and T. W. Berger, "Sparse Generalized Laguerre-Volterra Model of Neural Population Dynamic," *Proceedings of the 31st Annual International Conference of the IEEE EMBS*, pp. 4555-4558, 2009.

[8] E. N. Brown, R. Barbieri, V. Ventura, R. E. Kass, and L. M. Frank, "The time-rescaling theorem and its application to neural spike train data analysis," *Neural Computation*, vol. 14, pp. 325-46, 2002.