Measuring Synaptic Interactions

A. P. Georgopoulos *et al.* present novel evidence which suggests that synaptic interactions between pairs of cortical neurons are directly related to the degree to which they fire together during directed limb movements (*1*). The cover for the issue of 2 April depicts synaptic interactions ranging from strongly excitatory (for cells with similar direction preference) to strongly inhibitory (for cells with opposite direction preference). The calculation used by Georgopoulos *et al.* to document synaptic interactions differs from the cross correlation traditionally used to measure the effects of synaptic connections on firing probability (*2*, *3*, *4*). Instead, they "estimated the strength of presumed interaction (synaptic weight) from the i^{th} to the j^{th} neuron in a pair using an analysis based on waiting time probability density function . . ." (*1*, p. 50). This waiting time method calculates the first recurrence times of spikes in a target cell relative to spikes of the reference cell (*2*, *5*, *6*). As applied in their study (*1*), the algorithm is subject to strong effects from response similarity, and the resultant measure reflects the degree to which the cells fire together, as well as possible synaptic interactions mediated by excitatory or inhibitory postsynaptic potentials (EPSP or IPSP, respectively).

The effect of covariation on the waiting time measure (Fig. 1) can be illustrated by representative spike trains for two units, U1 and U2, whose firing rates covary, but which need not be connected (Fig. 1A). The first recurrence times of spikes in U2 after spikes in U1 (upper arrows in Fig. 1A) are used to generate a probability density of "actual waiting times" (Fig. 1B). To determine whether the actual waiting times are affected by synaptic connections, control distributions are calculated in the same manner after U1 spikes are randomly shuffled (lower arrows in Fig. 1A). The normalized difference between the actual and the shuffled distributions (Fig. 1C) is integrated from 2 to 20 ms in a cumulative sum (CUSUM) (Fig. 1D); its value at 20 ms was taken to represent "synaptic strength" (*1*). When the firing rates of the two units covary, the actual waiting times contain more short intervals and fewer long intervals than are obtained after shuffling, which generates a positive CUSUM value. Conversely, if U1 and U2 fire reciprocally, more U1 spikes would occur during long intervals in U2, and the difference in the distributions would generate a negative CUSUM value. If the units fire independently, the CUSUM value is less likely to become significant, as found for pairs of cortical cells without directional preference (*1*).

To quantify the relative contributions of connections and covariation, we simulated this mechanism with a neural network model, using integrate-and-fire spiking units that integrated triangular EPSPs and IPSPs to a threshold for firing spikes, and

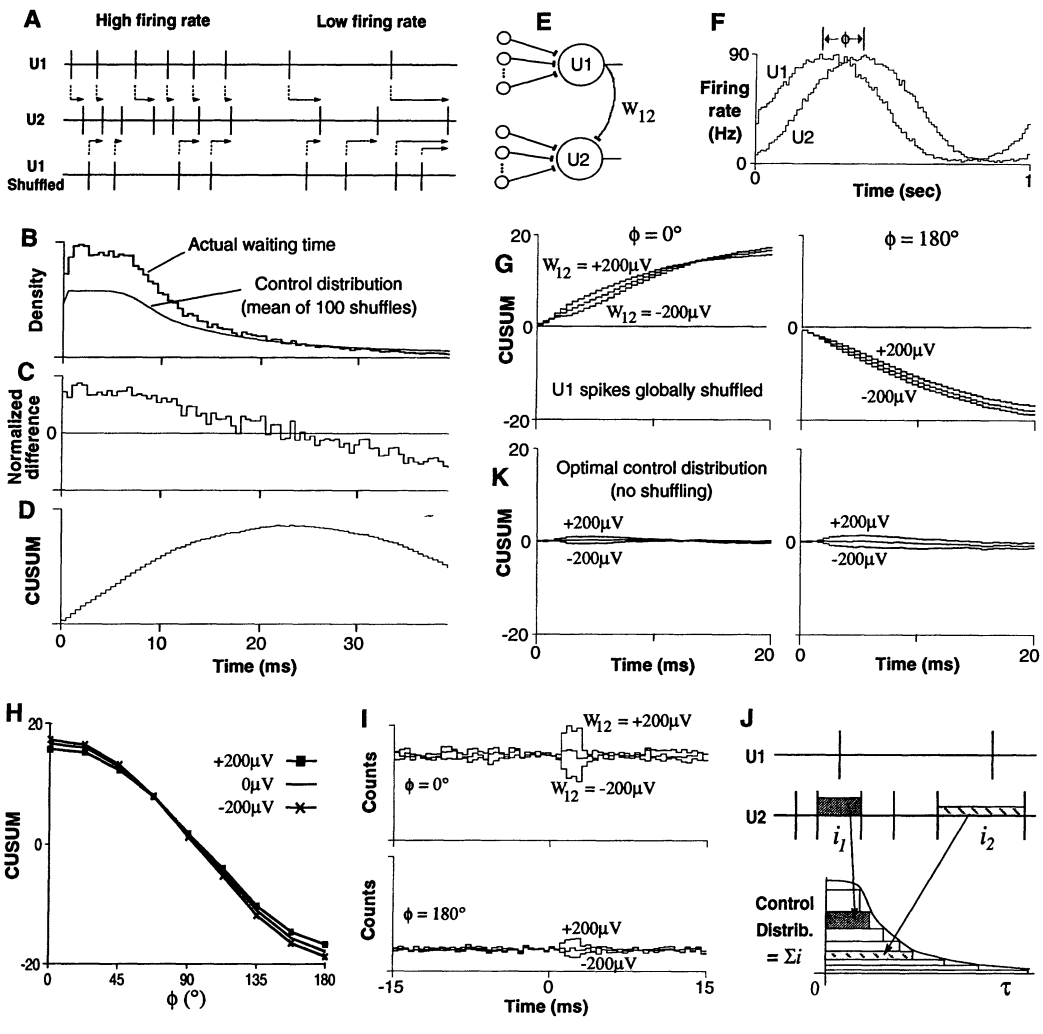

Fig. 1. Waiting time measures in covarying neurons. (**A**) Activity samples for two covarying units (U1 and U2) showing waiting times (arrows) for actual spike trains (top) and for randomly shuffled U1 spikes (bottom). (**B**) Probability density of waiting times of U2 relative to U1 for actual spike trains and for the shuffled control distribution, derived from the mean of 100 shuffles. (Data from Fig. 1G, for $\phi = 0°$, $W_{12} = 0$.) (**C**) Normalized difference, calculated as (actual density − shuffled density)/shuffled density. (**D**) CUSUM of the normalized difference. (**E**) Schematic of spiking unit network used in simulations; U1 and U2 were each driven by 17 units with spikes that produced 400-μV EPSPs (delay = 1 ms, rise time = 2 ms, decay = 10 ms, distance to threshold = 5 mV). W_{12} represents a synaptic connection between U1 and U2, which could be zero or could produce PSPs of amplitude ±200 μV. (**F**) Sinusoidal modulation of U1 and U2, firing rates (period = 1 s, 900 sweeps) showing phase shift ϕ. (**G**) CUSUMs of normalized differences for simulations with $W_{12} = 0$ and ±200 μV for global shuffling of spikes. (**H**) CUSUM values at 20 ms for simulations with different phase shifts between U1 and U2, and three values of W_{12}, using global shuffling controls. (**I**) Cross-correlation histograms between U1 and U2 for different W_{12} when units fired in phase ($\phi = 0°$) and out of phase ($\phi = 180°$). (**J**) Calculation of optimal control distribution showing contributions of two intervals (each contribution having unity area). (**K**) CUSUMs using optimal control distribution.

produced postsynaptic potentials (PSPs) in their target units. Two analyzed units (U1 and U2) each received EPSPs from separate populations of spiking units with activities that were generated by sinusoidally modulated Poisson processes (Fig. 1, E and F). When the two units were driven in phase, the waiting time algorithm produced a positive CUSUM measure (Fig. 1G, $\phi = 0°$). These three CUSUMs (Fig. 1G) represent simulations in which the synaptic connection (W_{12}) between U1 and U2 was zero (middle trace) or ± 200 μV. When U1 and U2 were driven out of phase, the CUSUMs reached negative values for each value of W_{12} (Fig. 1G, $\phi = 180°$). For intermediate phases, the CUSUM values at 20 ms varied systematically as a function of phase (Fig. 1H), much like the data shown in figure 7 of (*1*). Although we chose sinusoidal modulation for convenience, any form of covariation or countervariation in activity would produce the same effects. In the study by Georgopoulos *et al.* cells with similar directional preference would tend to fire together during trials and those with opposite directional preference would tend to fire inversely. In our simulations the plots of the CUSUM values for different values of W_{12} were quite similar (Fig. 1, G and H), indicating that the main determinant was the degree of covariation of the units' firing rates, not their synaptic interactions. In comparison, the classical cross-correlograms between the two units show that these connections can be resolved by cross-correlation techniques independently of their covariation (Fig. 1I).

We found that the waiting time method can generate measures of synaptic interaction that are less affected by covariation if the intervals are locally shuffled (for example, by interchanging successive pairs of reference interspike intervals), which preserves the effects of firing rate in the control distribution. A more exact control distribution can be derived by calculating all possible waiting times for the U2 intervals in which each reference spike falls (Fig. 1J). The contribution of each U1 reference spike to this control distribution is a uniform distribution with unit area, extending from zero to the duration of its associated U2 interval; adding these unit distributions generates a control distribution that appropriately blurs the relative timing, but preserves the sampling effect of covariation. This procedure produces a smoother, more complete control distribution than does multiple shuffling of intervals and it requires less computation. Subtracting this optimal control distribution from the actual waiting times revealed the effect of the synaptic connection (Fig. 1K). In this case the CUSUM reached maximum at the peak of the PSP, typically at 3 ms, rather than at 20 ms (*1*); in fact, the CUSUM resembles the underlying PSP more closely than the cross-correlogram, one purported advantage of the waiting time method (6). However, the CUSUM still reflects some influence of covariation (for example, curves for $\phi = 0°$ and 180° in Fig. 1K). In contrast, the peaks and troughs in the standard cross-correlation histogram are less distorted by covariation (although their amplitudes depend on the number of associated spikes). The cross-correlogram has the additional advantages of counting multiple firings for a given EPSP and of representing events before and after the trigger, thus detecting effects of synaptic connections in either direction.

Maintaining the distinction between a synaptic interaction and simple covariation of cell pairs is essential for analyzing the causal mechanisms in neural circuits. For example, sensory cortex cells with similar receptive fields can be coactivated, but still have inhibitory connections that mediate subtle differences in their response properties. Conversely, many motor cortex cells that fire reciprocally during wrist movements exhibit positive correlogram peaks, revealing an unexpected source of common excitatory input. In both of these examples [described in (*4*)] the actual synaptic interactions would have been obscured by a measure reflecting response similarity. Thus, to investigate how the synaptic connections between neurons shape their response properties, it is necessary to use a measure of synaptic interactions that is unaffected by covariation in their activity.

E. E. Fetz
L. E. Shupe
Department of Physiology and Biophysics and
Regional Primate Research Center,
University of Washington,
Seattle, WA 98195, USA

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Response: In our article (*1*) we used the waiting time method because we were interested in detecting the immediate synaptic effect of one cell on another. We found that (i) the prevalence of synaptic interaction was significantly higher in pairs of directionally tuned cells, as compared with pairs of nontuned cells, and (ii) the strength of the signed synaptic interaction was negatively correlated with the angle between the preferred directions of the two cells in a pair. We show here that our findings hold equally well when the data are analyzed with the cross-correlation method; therefore, these findings are firmly established. We also show that in our data the similarity in directional preference was dissociated from the similarity of time courses of neural activity; therefore, the concerns of the comment do not apply to our study.

We analyzed our data using the cross-correlation method in order to validate our results with a different technique. The data consisted of two sets of cell pairs: 1126 pairs in which both cells in a pair were directionally tuned, and 602 pairs in which none of the two cells in a pair were tuned. In our previous analysis (*1*) using the waiting time method, we found, "first, significant interactions were 2.25 times more frequent in the directionally tuned (203 of 1126 cells or 18%) than in the nontuned (48 of 602 or 8%) group" ($\chi^2 = 31.9$; $P < 10^{-5}$) and second, that the mean synpatic strength "was negatively correlated with the angle (0° to 180°) between the preferred directions of the two neurons [correlation coefficient (r) = −0.815; $P < 0.004$]" (*1*, p. 50) (*2*). In our present analysis, using the cross-correlation method (*3*), we found first, that significant interactions were 2.12 times more frequent in the directionally tuned (256 of 1126 cells or 22.7%) than in the nontuned (64 of 602 or 10.6%) group ($\chi^2 = 38.1$; $P < 10^{-5}$) and second, that the mean synaptic strength was negatively correlated with the angle between the preferred directions of the two neurons ($r = -0.863$; $P < 0.001$). These results validate our previous findings (*1*) with the cross-correlation method.

The angle between two preferred directions and the "phase angle" between two time courses (see figure 1F of the comment by Fetz and Shupe) are entirely different measures. We calculated the preferred direction as follows. Our data consisted of 40 trials corresponding to five reaching movements in each of eight directions in space (*4*); each trial was ~1 s in duration, and different trials were recorded at different times, separated by a ~2-s inter-trial interval. In order to calculate the preferred direction of a cell, the average frequency of discharge in each of the 40 trials was computed and analyzed as a function of the direction of movement: the peak of that

function was the preferred direction of the cell, namely the direction of movement for which the cell would discharge at the highest average frequency. Given two directionally tuned cells, the angle between their preferred directions denoted the similarity between their directional preference. (i) In order to calculate the preferred direction, data from all trials for all movement directions are needed, and (ii) because the average frequency of discharge in individual trials is employed for these calculations, the information about the time course of cell activity during a trial is not used. In contrast, the "phase angle" of the comment (i) directly relates to the time course of neural activity, for it is a direct measure of the relative time shift between two time courses, and (ii) is not related to the average frequency of discharge, for two cells can have the same average discharge frequency in a trial and, at the same time, any "phase angle" from 0° to 180°, as exemplified in figure 1F of the comment. Thus, the similarity between two preferred directions is not obligatorily connected to any particular "phase angle." Therefore, the statement by Fetz and Shupe that, in our study (*1*), "cells with similar directional preference would tend to fire together during trials and those with opposite directional preference would tend to fire inversely" is unwarranted.

The supposition above is also unwarranted in our study, for in our data the similarity of preferred directions was dissociated from possible similarity in the time course of cell activity. We estimated the latter by calculating the correlation coefficient between the two time courses in neural activity of two cells in a pair (counts in 20-ms binned spike trains) over the 40 trials used to determine the preferred direction of each cell and the synaptic interaction between the two cells (using the waiting time analysis). We analyzed data for two groups of cells drawn from cell pairs in which both cells in a pair were directionally tuned and in which synaptic interactions were detected (*1*). The first group (n = 25 cell pairs) consisted of cells with very similar preferred directions (angle θ between preferred directions of two cells in a pair = 0° to 18°), whereas the second group (n = 14) consisted of cells with very different preferred directions (θ = 162° to 180°). We found that the correlation coefficient between pairs of time courses did not differ significantly between the two groups (t test on the z-transformed r: t = 0.967, df = 37; P = 0.34). These results demonstrate that the similarity in directional preference was dissociated from the similarity of time courses of neural activity. At the same time, as we described (*1*), the mean CUSUM (i) differed significantly between the two groups (t = 2.64, df = 37, P = 0.01) and (ii) was positive in the first group and negative in the second (5).

We conclude that the associations we described (*1*) between the prevalence of synaptic interaction and directional tuning, and between the strength of the signed synaptic interaction and preferred direction, are valid and that, therefore, the cover graphics of our article (*1*) does portray a correct model.

A. P. Georgopoulos
Brain Sciences Center,
Veterans Affairs Medical Center,
Minneapolis, MN 55417, USA

M. Taira
First Department of Physiology,
Nihon University School of Medicine,
Tokyo, Japan
A. V. Lukashin
Brain Sciences Center,
Veterans Affairs Medical Center,
Minneapolis, MN 55417, USA

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2. The spike train was shuffled separately for each trial; "global shuffling" could not even be performed by stringing all trials together in time because they were recorded at separate times. The peak signed value of the CUSUM was taken as the estimate of the strength of the interaction [note 34 in (*1*)]. This could have occurred at any time during the period of 2 to 20 ms analyzed. It is stated incorrectly in the comment by Fetz and Shupe that we used only the CUSUM "value at 20 ms."
3. In order to properly compare the two methods, the same time period (2 to 20 ms) and statistical techniques (CUSUM) were used to analyze the cross-correlation function as were used to analyze the waiting time function in note 34 of (*1*).
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5. These data were presented (*1*) as the first and last point in figure 7A. We see no connection between our figure 7A (*1*) depicting synaptic interaction as a function of the angle between preferred directions and figure 1J of the comment by Fetz and Shupe depicting CUSUM value (biased because it was derived from "global shuffling") as a function of the "phase angle" characterizing the relative time shift.
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