Divergence and reconvergence: Multielectrode analysis of feedforward connections in the visual system

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Introduction

Multielectrode recording is rapidly becoming a standard tool in systems neurophysiology. Its main advantage over single electrode recordings is that temporal relations among the firing patterns of two or more neurons can be examined. The interpretation of these temporal relations, however, is notoriously difficult. The study of temporal relations between neurons can be serve two purposes: 1) to explore the connectivity or synaptic physiology of neurons in a circuit, or 2) to elucidate strategies of neural processing or encoding by an ensemble. These two aspects of multielectrode recording have been exemplified in numerous studies of the mammalian visual system. In some studies, correlations have been used to study the functional connectivity between visual neurons in retina, LGN and visual cortex (Alonso et al., 1996; Brivanlou et al., 1998; Cleland et al., 1971; Cleland and Lee, 1985; Mastronarde, 1983abc; Mastronarde, 1989; Reid and Alonso, 1995; Tanaka, 1983; Tanaka, 1985; Toyama et al., 1981; Ts'o et al., 1986; Usrey and Reid, 1999; Usrey et al., 1998; Usrey et al., 1999). In others, correlations have been examined as a potential means to encode visual information, such as with oscillations (Eckhorn et al., 1988; Frien et al., 1994; Gray et al., 1989; Jagadeesh et al., 1992; Kreiter and Singer, 1996; Livingstone, 1996; Neuenschwander and Singer, 1996) or correlated firing (Dan et al., 1998; Ghose et al., 1994; Meister, 1996; Meister et al., 1995). Of course, many studies have elements of both approaches. In this review, we will concentrate on studies that emphasize the first approach. In particular, we will summarize the strategy employed in our recent studies of the pathway from retina to thalamus (lateral geniculate nucleus or LGN) to visual cortex in the cat.

Temporal relations between neurons can be characterized in simple ways, such as with cross-correlation analysis of a pair of neurons, or the analysis of how several neurons can respond in a correlated fashion to a sensory input. They can also be characterized in far more complex ways, such as the clustering of ensembles of neurons into co-active groups (Gerstein et al., 1985). Here, we will concentrate on the use of first-order cross-correlations between pairs of cells to ask a simple question: are the two neurons monosynaptically connected? Higher-order correlations among three or more neurons can be used to analyze more complex questions. For instance, correlations among multiple neurons have been used to analyze patterns of firing and their relation to sensory stimuli (Vaadia et al., 1995). We have used similar techniques to address somewhat narrower questions: 1) In divergent feedforward systems—those in which one input neuron synapses on two output neurons—if a presynaptic spike drives one target to threshold, is it more or less effective in driving the second target? 2) In convergent feedforward systems—those in which two inputs synapse on a common output neuron—are nearly synchronous spikes more effective than asynchronous spikes in driving the common target?

Recording strategies

In our recent studies, we have recorded from two levels of the visual system at once in order to assess feedforward connections. In our simultaneous recordings in retina and LGN (Usrey et al., 1998; Usrey et al., 1999) we built upon the earlier work of Cleland, Dubin, Levick, and colleagues (Cleland et al., 1971; Cleland and Lee, 1985). In simultaneous recordings in LGN and visual cortex, we built upon the work of Tanaka (Tanaka, 1983; Tanaka, 1985). As noted in these earlier studies, and discussed below,

feedforward connections of this type are particularly well suited to the use of cross-correlation analysis

The novel feature of these experiments is that we have recorded from multiple neurons in the intermediate level of this pathway in order to analyze not only feedforward connections, but also the functional consequences of divergence and convergence. In the cat, retinal ganglion cells diverge to synapse on several target neurons in the LGN. An even larger number of geniculate neurons, in turn, converge onto each target neuron in visual cortex. Geniculocortical *divergence* is even more extensive, but will not be discussed here.

Numerous studies have described synchronous activity within a given neural ensemble (reviewed in Usrey and Reid, 1999). The study of the afferent connections to an ensemble allows for at least a partial analysis of the causes of synchrony. Conversely, the study of the efferent connections from an ensemble allows an analysis of the functional significance of correlated activity. Specifically, it allows one to ask the question: Are synchronous spikes from two neurons especially effective in driving a common postsynaptic target?

Interpretation of cross-correlations in feedforward systems

Cross-correlations between neurons in a strictly feedforward circuit can in many cases be quite easy to interpret. In order to discuss the topic, it is useful to define several terms so that they can be used unambiguously. Connections between two pools of neurons, **A** and **B**, will be called *strictly feedforward* if there are direct excitatory connections between neurons in the *presynaptic pool* (**A)** and neurons in the *postsynaptic*

pool (**B**), but no direct connections between **B** and **A**. By this definition, a system with *poly*synaptic feedback can still be called strictly feedforward. For instance, the connections from lateral geniculate nucleus to layer 4 of visual cortex are strictly feedforward, even though there is indirect feedback to the LGN via cortical layer 6. All correlation between neurons in the presynaptic pool and neurons in the postsynaptic pool will be called a *feedforward correlation.* This term will not be used to connote mechanism. Thus a slow correlation between a geniculate neuron and a cortical neuron will still be called a feedforward correlation, even though its ultimate cause may in fact be slow correlations in the retina (Mastronarde, 1983abc; Mastronarde, 1989). By contrast, the term *monosynaptic correlation* obviously connotes a mechanism and thus carries with it a significant burden of proof.

By "monosynaptic correlation," we mean a cross-correlation between two connected cells that is due to spikes in the presynaptic cell directly causing spikes in the postsynaptic cell (see figs. 2 and 3, below). It is well known that cross-correlation analysis can easily be misinterpreted—correlation need not imply causation—so great care must be taken in analyzing correlogram peaks even in this simplest case. One can safely call a peak monosynaptic if two criteria are met. First, the peak must appear at a reasonable delay between A and B. In a retinogeniculate correlation, the peak is usually at +3-5 msec, which is consistent with the conduction velocities of ganglion-cell axons, plus the synaptic delay (Henry et al., 1979). Second, and much more importantly, the rising phase of the correlogram must be fast. In most cases, the shape of a correlogram between two synaptically connected neurons is close to the first derivative of the excitatory postsynaptic potential (Fetz and Gustafsson, 1983), and therefore can be quite

fast. In many cases, it is this rate of rise that allows one to exclude potential sources of artifact, as discussed below.

If a peak in the correlation between neuron A and B is consistent with a monosynaptic connection (fig. 1a), it is important to rule out other potential causes of this peak. Assuming that A and B are part of a strictly feedforward system, they could *falsely* appear to have a monosynaptic connection if there were:

1) Common neural input to A and B from one or more cells, C (fig. 1b),
2) Common external input, such as a sensory stimulus, to A and B (fig. 1c),
3) Correlation between A and A′, another member (or members) of the presynaptic group that is itself connected with B (fig. 1d), or
4) Correlation between B and B′, another member (or members) of the postsynaptic group that receives input from A (fig. 1e).

In many cases, such as in the retinogeniculate system, common neural input can be ruled out on anatomical grounds. In virtually all feedforward systems, however, the types of correlations in cases 2 through 4 are potentially present and therefore must be excluded. This is usually achieved by relying on arguments about timing. In general, it is necessary to demonstrate that monosynaptic correlations are too fast to be caused by other sources—either stimulus-dependent correlations or what can be called *lateral correlations:* correlations among neurons in either the presynaptic or the postsynaptic pool. It is important to emphasize that lateral correlations need not be caused by lateral connections but instead can be from any source, such as common input (neuron C in fig. 1d).

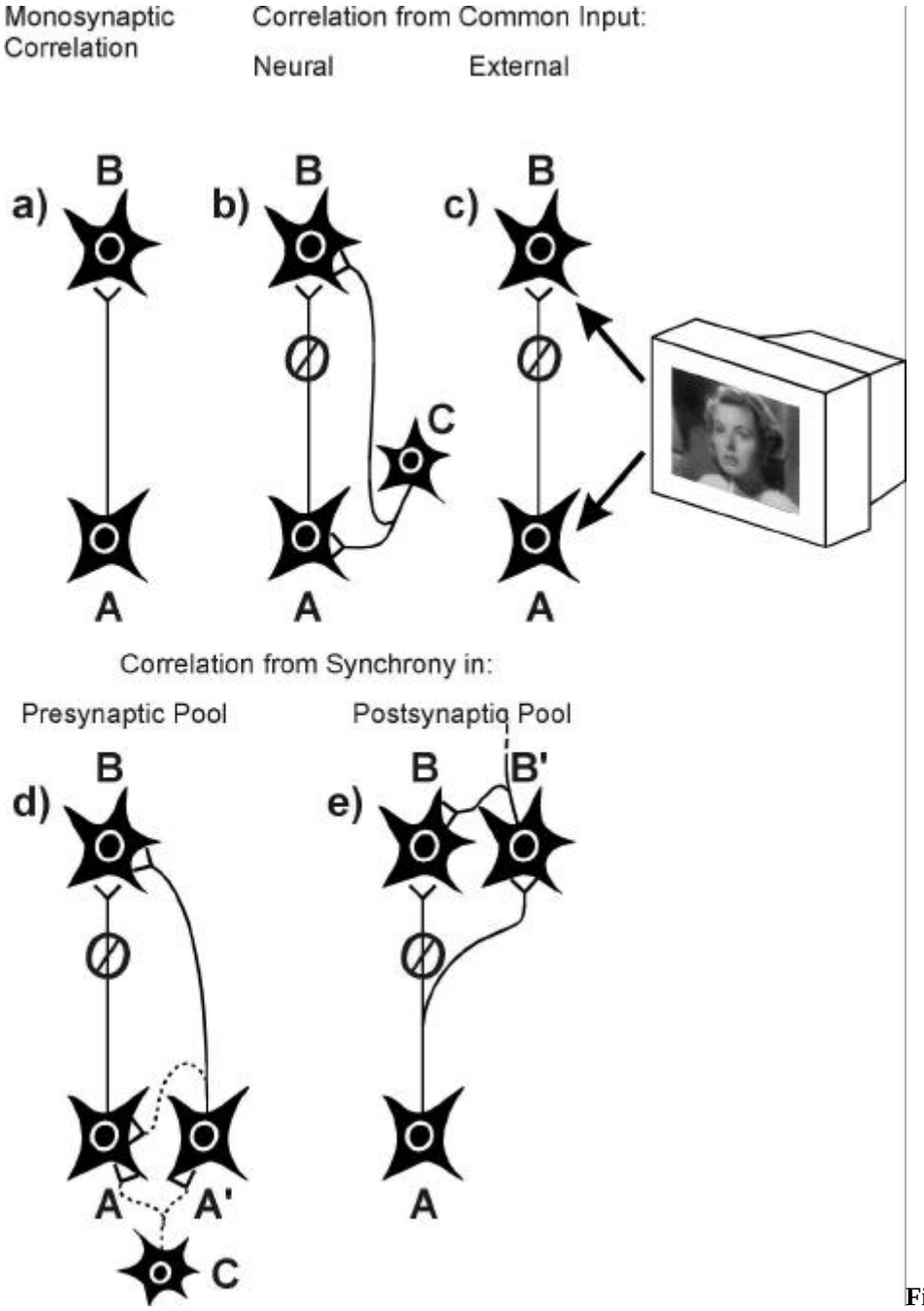

Figure 1. Five model circuits that could lead to correlation between neurons in a strictly feedforward pathway. In each example, neuron A is in the presynaptic pool, neuron B in the postsynaptic pool. (a) A and B are monosynaptically connected. (b, c) A and B are not connected, but they both receive common input. In one case (b), this common input comes directly from a neuron, C, in a different region. In another case (c), the common input comes indirectly from an external source, such as a sensory input. (d) A and B are not connected, but there is another neuron A', in the presynaptic pool, that both is correlated with A and is monosynaptically connected to B. (e) A and B are not connected, but there is another neuron B', in the postsynaptic pool, that both is correlated with B and receives monosynaptically input from A.

Consider the case of the retinogeniculate pathway. Correlations that provisionally could be called monosynaptic typically have a peak with a delay between retina and LGN of 3-5 msec and a rise time (from ½maximum) of less than 0.3 milliseconds (Usrey et al., 2000). These peaks can be seen under conditions in which stimulus-dependent correlations are on the order of hundreds of milliseconds (during slowly varying stimulation, such as drifting sinusoidal gratings) or 5-10 milliseconds (during spatiotemporal white noise stimulation (Reid et al., 1997). It should be noted that some stimuli—such as rapidly modulated, spatially diffuse flicker—can drive both retinal (Berry and Meister, 1998; Berry et al., 1997) and geniculate (Reich et al., 1997; Reinagel and Reid, 2000) neurons to respond reproducibly with sub-millisecond precision. Cross-correlations between retina and LGN would therefore be impossible to interpret under such conditions.

Lateral correlations

While it is possible to work under conditions such that stimulus-dependent correlations are far slower than monosynaptic correlations, it is not possible to remove lateral correlations. In many cases, however, lateral correlations are slower than feedforward correlations. The reasons why slower correlations cannot lead to a 'false positive' monosynaptic correlation are outlined below. In the following sections, illustrations will be drawn from retinogeniculate system and the geniculocortical system. For example, a peak in the correlation between retinal cell *A* and LGN cell *B* could appear in the absence of a monosynaptic connection if neuron *A* were correlated with another ganglion cell, *A′*, which in turn was monosynaptically connected with *B* (fig. 1d;

see fig. 2, below). In the absence of any other source of correlation between *A* and *B*, the first-order prediction of the cross-correlation between *A* and *B* will be the convolution of the transfer function between the neurons *A* and *A′* ($k_{A',A}$(t), discussed below) with the true monosynaptic correlation between neurons *A′* and *B*.

To simplify matters, the spike train *A*(t) can be considered as the sum of two processes, one which is independent of the spike train *A′*(t), $A_{\perp A'}$(t), and another which is linearly dependent upon *A′*(t): $A_{||A'}$(t). Given this formalism, the dependence between *A*' and *A* can be expressed with the transfer function, $k_{A\ell,A}$(t):

$$<A(t)> \quad = A_{\perp A'}(t) + \sum_{t''=0}^{T_k} A'(t-t'')k_{A',A}(t'') = A_{\perp A'}(t) + <A_{||A'}(t)> \qquad \text{(eq. 1)}$$

We can think of <*A*(*t*)> as the mean or expected value of the spike train *A*(*t*), taken over many samples of the spike train, *A*'(*t*). $k_{A\ell,A}$(t) is the temporal weighting function that gives the relationship between <*A*(t)> and *A′*(t). <*A*(t)> depends on the past history of *A′*(t) up to a relative delay of T_k.

Given this relationship between spike trains *A* and *A′*, it is possible to represent the cross-correlation between *A* and *B*,

$$XC_{A,B}(t) \quad = \lim_{T\to\infty} \frac{1}{T} \sum_{t'=0}^{T} A(t'+t)B(t') \qquad \text{(eq. 2)}$$

with the following series of equations:

$$XC_{A,B}(t) \quad = \lim_{T\to\infty} \frac{1}{T} \sum_{t'=0}^{T} <A(t'+t)> B(t') \qquad \text{(ergodicity of } A\text{)}$$

$$= \lim_{T \to \infty} \frac{1}{T} \sum_{t'=0}^{T} [A_{\perp A'}(t'+t) + \sum_{t''=0}^{T_k} A'(t'+t-t'')k_{A',A}(t'')]B(t') \quad \text{(from eq. 1)}$$

$$= \lim_{T \to \infty} \frac{1}{T} \sum_{t'=0}^{T} [A_{\perp A'}(t'+t)B(t') + \sum_{t''=0}^{T_k} A'(t'+t-t'')k_{A',A}(t'')B(t')]$$

$$= \langle A_{\perp A'} \rangle \langle B \rangle + \lim_{T \to \infty} \frac{1}{T} \sum_{t'=0}^{T} \sum_{t''=0}^{T_k} A'(t'+t-t'')k_{A',A}(t'')B(t')$$

(from assumed independence of $A_{\perp A'}$ and B)

$$= \langle A_{\perp A'} \rangle \langle B \rangle + \sum_{t''=0}^{T_k} k_{A',A}(t'') \lim_{T \to \infty} \frac{1}{T} \sum_{t'=0}^{T} A'(t'+t-t'')B(t')$$

$$= \langle A_{\perp A'} \rangle \langle B \rangle + \sum_{t''=0}^{T_k} k_{A',A}(t'')XC_{A',B}(t-t'') \quad \text{(eq. 3)}$$

Thus the cross-correlation between neurons *A* and *B* is equal to a constant plus the true monosynaptic cross-correlation between *A′* and *B*, convolved (or smoothed) by the transfer function between *A′* and *A*. The constants, $\langle A_{\perp A'} \rangle$ and $\langle B \rangle$, are respectively the mean firing rates of the portion of *A* that is independent of *A'*, and *B*.

It should be noted that correlations have been defined (eq. 2) in terms of the joint probability per unit time of two events, *A* and *B*, occurring with a relative delay, *t*. Various normalizations of this function can be used to express it in units more suited for a given application. Most normalizations involve N_A and N_B, the total number of spikes fired by *A* or *B*, and τ: the bin width at which time is quantized. If the correlogram is divided by $N_A\tau$, then the result can be thought of as the firing rate of neuron *B*, in spikes per second, with respect to the average spike from *A*. If it is normalized by N_A, then it

can be thought of as the conditional probability of B firing, per time bin, averaged over all occurrences of *A*.

A very similar calculation can describe the case illustrated in Figure 1e, in which neuron *A* projects only to postsynaptic neuron *B′*, which in turn is correlated with the 'postsynaptic' neuron *B*. The only difference in the calculation is that *B*', the postsynaptic neuron, must be decomposed into two dependent and independent terms: $B'_{\perp A}$ and $B'_{||A}$. In this case, the cross-correlation between the unconnected cells, *A* and *B*, is equal to a constant term plus the convolution of the lateral transfer function between *B*' and *B*, $k_{B',B}$, and the cross-correlation between *A* and *B*':

$$XC_{A,B}(t) = \langle A_{\perp B'} \rangle \langle B \rangle + \sum_{t''=0}^{T} k_{B,B'}(t'')\, XC_{A,B'}(t - t'') \,. \qquad \text{(eq. 4)}$$

In the retino-geniculo-cortical system, lateral correlations in all three levels could pose a problem in the interpretation of feedforward correlations. Discussion of the very fast correlations in the LGN will be postponed until the next section; here we will examine the potential confounding effect of intraretinal and intracortical correlations. To apply equations 3 and 4 to these cases, it would be best to know the transfer function between two neurons at a given level ($k_{A',A}$ in eq. 3 and $k_{B',B}$ in eq. 4). The cross-correlation functions between two neurons ($XC_{A',A}$ and $XC_{B',B}$), however, are very closely related and can be substituted to first approximation.

Potential artifacts caused by intraretinal and intracortical correlations

In the retina of the cat, there are three classes of correlations between ganglion cells (Mastronarde, 1983ac): the slow correlations between two X cells (peak width: 5-20 msec), the faster but weak correlations between X and Y cells (peak width: 2-3 msec), and yet faster and stronger correlations between Y cells (peak width: ~1-2 msec). The correlations that involve X cells are much too slow to confound the interpretation of monosynaptic peaks. The correlations between Y cells, however, have a time-scale that approaches that of retinogeniculate monosynaptic correlations.

The most dramatic evidence that Y cells in the retina are coupled is that electrical stimulation of one Y cell can evoke a strong response in another Y cell with a delay of ~1 msec and a peak width of <1 msec (Mastronarde, 1983a). The important factor to consider when applying equation 3, however, is not this one-directional effect of Y-cell coupling, but instead the actual cross-correlation between two Y cells. When examined at a fine time-scale, the cross-correlation between two Y cells has two peaks, one on each side of time zero, each with a width of ~1 millisecond (fig. 2a, upper right, $XC_{A',A} \cong k_{A',A}$, data from fig. 2 of Mastronarde, 1983a). When this lateral correlation ($k_{A',A}$, re-plotted in fig. 2b, middle) is convolved with what are presumed to be monosynaptic correlations between retina and LGN (fig. 2a, $XC_{A,B}$ and $XC_{A,B'}$, histograms; see eq. 3), the results are two-peaked correlations (fig. 2b, right and left plots, dark gray lines). These two-peaked correlations in no way resemble the actual monosynaptic correlation. Thus cross-correlations between retinal cells could not account for the extremely fast correlation peaks found between retinal and LGN cells. We can therefore conclude that the

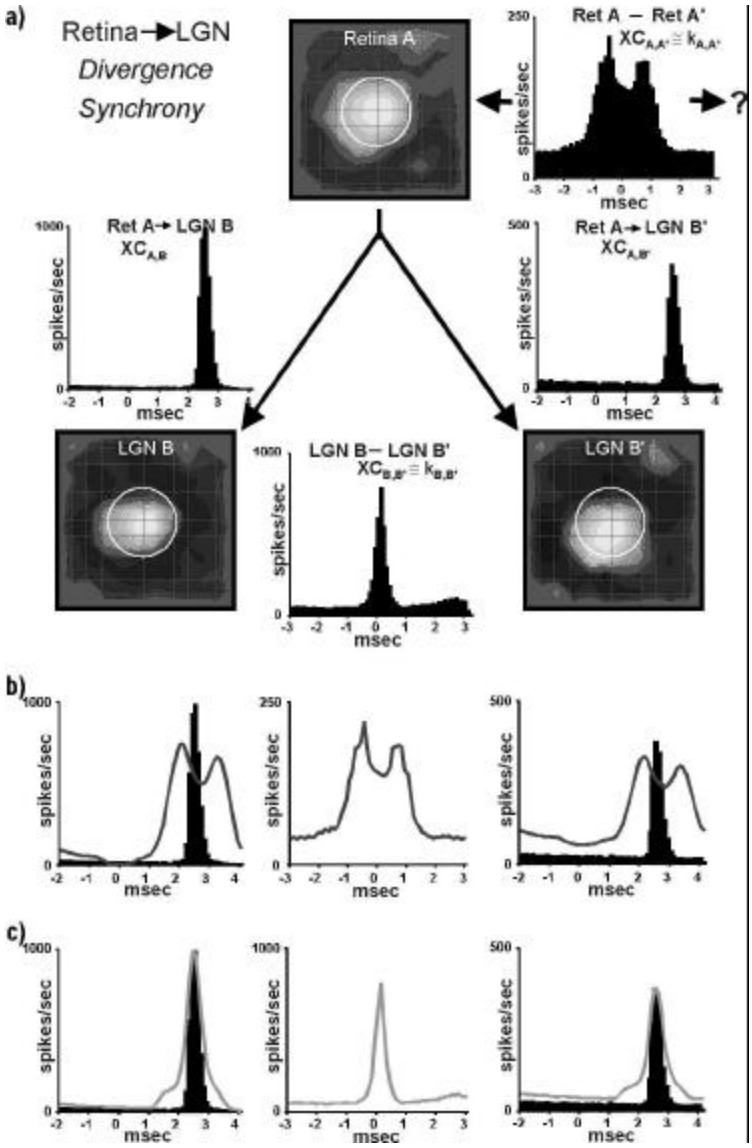

Figure 2. (a) An example of a triplet of simultaneously recorded neurons (adapted from Usrey and Reid, 1999; Usrey et al., 1998), one in the retina (A) and two in the LGN (B and B'). The three panels show receptive field maps of simultaneously recorded neurons (**Retina A, LGN B, LGN B';** each is an *on*-center Y cell; grid size: 0.6°). The circles shown over the receptive field centers correspond to the best fitting Gaussian of the retinal receptive-field center (radius: 2.5 σ_{ret}). The three histograms labeled $\mathbf{XC_{A,B}}$, $\mathbf{XC_{A,B'}}$ and $\mathbf{XC_{B,B'}}$ illustrate the cross-correlograms between each pair of neurons. The histogram $\mathbf{XC_{A,A'}}$, representing the expected correlation with another Y cell (not recorded), is re-plotted from another study (Mastronarde, 1983a, fig. 2). The retinogeniculate correlograms ($\mathbf{XC_{A,B}}$ and $\mathbf{XC_{A,B'}}$) both have the features indicative of a monosynaptic connection: a short latency and a very fast rise time. 84% of the synchronous events in correlogram $\mathbf{XC_{B,B'}}$ are accounted for by the peaks in correlograms $\mathbf{XC_{A,B}}$ and $\mathbf{XC_{A,B'}}$ (Usrey et al., 1998). Hence, divergence leads to synchrony. b) Illustration of potential artifacts caused by intraretinal synchrony. Middle: line plot of intraretinal correlation, from (a). Right and left: line plots superimposed on the retinogeniculate correlograms indicate the expected correlograms if the pair were not monosynaptically connected, but instead 'inherited' the correlation because of lateral correlations and a true monosynaptic connection (as in fig. 1d; eq. 3). Offset and gain are arbitrary. (c) Middle: line plot of intrageniculate correlation, from (a). Right and left: line plots superimposed on the retinogeniculate correlograms indicate the expected correlograms in absence of true connection (as in fig. 1e; eq. 4)

retinogeniculate correlations are in fact truly monosynaptic correlations (potential confounds due to intrageniculate synchrony are addressed below).

Similarly, correlations between cortical neurons, even the very fastest ones (Lampl et al., 1999, fig. 7b; re-plotted in fig. 3a, bottom right, $XC_{B,B'}$), are much too slow to confound the interpretation of monosynaptic inputs from LGN (eq. 4; fig. 3c). Geniculocortical correlations that pass our criteria for calling them monosynaptic correlations (Reid and Alonso, 1995; Alonso et al., 1996; Usrey et al., 2000), have a rise time on the order of one millisecond. When these same correlations are convolved with a cross-correlation between two cortical cells (fig 3c, middle) the results (fig 3c, right and left plots, dark gray lines) have rise times on the order of several milliseconds. These slow correlations would not pass our criteria for monosynaptic correlations.

Potential artifacts caused by intracortical correlations

The experiments illustrated in figures 2 and 3 were taken from studies (Alonso et al., 1996; Usrey et al., 1998, 1999) that were motivated by the original finding of correlations between neurons in the LGN (Alonso et al., 1996; as predicted in Cleland, 1986). These correlations are both quite fast, on the order of 1.0 millisecond, and strong: ranging from ~1% up to ~30% of the spikes in either spike train. These lateral correlations, while interesting as a phenomenon unto themselves, pose a potential problem in the interpretation of feedforward correlations, either between retina and LGN or between LGN and cortex. As discussed above, lateral correlations in the retina and cortex are too slow (and usually too weak) to influence the interpretation of feedforward

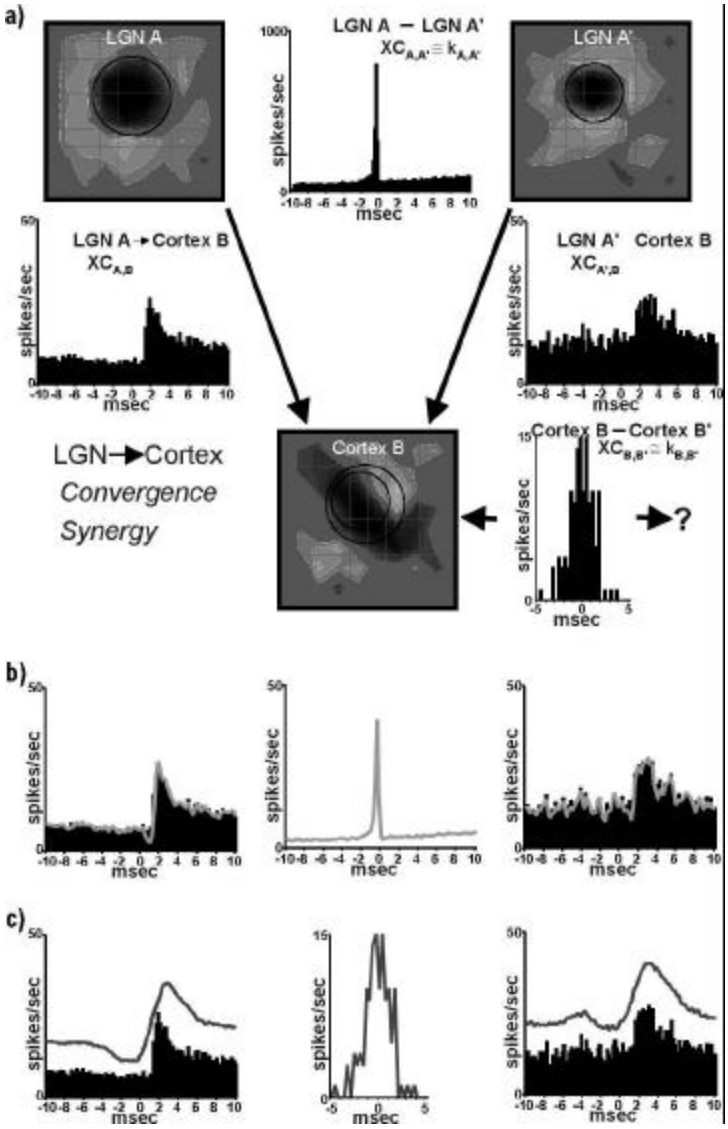

Figure 3. (a) An example of a triplet of simultaneously recorded neurons (adapted from (Alonso et al., 1996; Usrey and Reid, 1999), two in the LGN (A, A') and one in the visual cortex (B). Conventions as in fig. 2. The histogram $\mathbf{XC_{B,B'}}$, expected correlation with another cortical cell (not recorded), is re-plotted from another study (Lampl et al., 1999, fig. 7b). (b) Rigth and left: Light lines show expected correlations due to lateral correlations within the LGN (middle; as in fig. 1e; eq. 3). (c) Dark lines: expected correlations due to lateral correlations within the cortex (middle; as in fig. 1e; eq. 4).

correlations, but the fast and strong correlations in the LGN are precisely the sort that could pose a problem.

Fast correlations in the LGN could be seen as a potential problem for the interpretation of retinogeniculate correlations. If the lateral correlations in the LGN were intrageniculate in origin, that is if they were caused by excitatory local interactions between neurons, then it would be possible to have a retinogeniculate correlation that appeared as a monosynaptic correlation, even in the absence of a direct connection (fig. 1c). This is unlikely for two reasons. First, even though the intrageniculate correlations are extremely fast, on the order of 0.2-0.3 msec half-width at half-maximum, they tend to be symmetrical, while retinogeniculate correlations have faster rise times than decays (Usrey et al., 1999). The correlations between retina and LGN that would be predicted in the absence of a connection—calculated by convolving the correlation between two LGN cells (fig. 2c, middle) with the actual retinogeniculate correlations (fig. 2c, see eq. 4)—are therefore slightly slower than the actual retinogeniculate correlations. In particular, they have a symmetric tail, before the peak as well as after, which is not present in the actual correlations. Second, in admittedly anecdotal simultaneous recordings from sets of three neurons, two in the LGN and one in the retina (as in fig. 2a), we have found that that virtually all correlated firing in a pair of LGN cells can be derived from the common retinal input. Specifically, the vast majority of synchronous spikes in the correlated pair of neurons are preceded with a 'monosynaptic latency' by spikes from a specific retinal input (Usrey et al. 1998).

A much more problematic consequence of the fast correlations in the LGN is that they complicate the interpretation of correlations between LGN and visual cortex.

Geniculocortical correlations, with a rise-time of ~1 msec, are slower than intrageniculate correlations. Therefore, it would be possible for the scenario illustrated in Figure 1d to yield false positives: cross-correlations with the characteristics of monosynaptic correlations in the absence of a true connection (fig. 3b, right and left plots, gray lines). To examine the issue of false positives, we therefore recorded simultaneously from groups of three neurons: two geniculate neurons (*A* and *A*') along with a cortical neuron (*B*) that exhibited apparently monosynaptic correlations with both inputs (Alonso et al., 1996).

Second-order analysis: interactions between inputs

In order to analyze triplets of neurons—highly correlated neurons in the LGN and a postsynaptic neuron in visual cortex—we split the presynaptic spike trains, *A* and *A*', into three derived spike trains: *A*&*A*', *A** and *A*'*. *A*&*A*' is the spike train of synchronous spikes in the two LGN cells (arriving within 1.0 msec of each other). The remaining, non-synchronous events are put in the two spike trains: *A** and *A*'*. In this analysis, it was clear that isolated spikes from both LGN cells (*A** and *A*'*) affected the firing of cortical neurons with a monosynaptic latency (Alonso et al., 1996; Usrey et al., 2000). Of course, this scenario could be complicated by other LGN cells (*A*") that are correlated with both *A* and *A*'. Given that highly correlated cells in the X-cell system probably occur in groups not much greater than three neurons (two of which we have recorded; see Cleland, 1986), this sort of argument ensures that at least two cells in such an ensemble are connected to the postsynaptic neuron.

Functional role of divergence and reconvergence: synchrony and synergy

Recording from triplets of correlated cells, two in the LGN and one in the visual cortex, would seem an unreasonable amount of work if the only benefit were to prove that 'monosynaptic correlations' corresponded in fact to true connections. A more interesting result of these experiments, however, is an analysis of the potential effect of synchronous input to visual cortex (Usrey and Reid, 1999).

Given the phenomenon that neurons in the lateral geniculate nucleus fire near-synchronous action potentials, the question naturally arises: are synchronous spikes arriving at a cortical neuron treated differently than isolated spikes? Of course, the two inputs will sum together in some fashion, so the question can be reformulated: do synchronous spikes sum in a fashion that is different from a reasonable null hypothesis? What might this null hypothesis be?

A number of studies, both theoretical and experimental, have addressed the question of whether two excitatory postsynaptic potentials (EPSPs) to a cortical neuron sum linearly. It should be noted that this limited question is distinct from the more global question: do the combined effects of the thousands of inputs to a neuron sum linearly (Borg-Graham et al., 1998; Ferster, 1994; Hirsch et al., 1998; reviewed in Wandell, 1993)? Summation could be locally roughly linear but globally nonlinear, or even vice versa. Various models would predict that two inputs would add together in a linear fashion (Rall, 1964, for small, distant inputs; see also Bernander et al., 1994), a sublinear fashion (Rall, 1964), for larger, nearby inputs), or even a supralinear fashion (Mel, 1993;

Softky, 1994). Most experimental studies have found that linear summation tends to be the rule: (Cash and Yuste, 1999; Langmoen and Andersen, 1983). All of these studies have examined how two EPSPs interact. Our question, however, is somewhat different. Given that we have no access to the intracellular potential, we are asking: what is the expected probability of a postsynaptic spike given near-simultaneous spikes from both presynaptic cells. In other words, what are the combination rules for cross-correlograms from two feedforward connections onto a common target? Do they simply add or is the interaction more complex?

In addressing these questions, the first thing is to define what is meant by simple addition of inputs. The linear summation of EPSPs is a well-defined concept. Before the concept of summation of correlograms can be considered, however, there must be some interpretation of what the correlogram represents. The most natural interpretation of a correlogram in this regard is to consider it as the differential probability, above chance, that the post-synaptic cell, *B*, fires at a certain time after the presynaptic cell, *A*. To make a correlogram (as defined in eq. 2) correspond to this interpretation, one must first subtract the baseline activity of neuron *B*, or the portion of *B* that is independent of A: $<B_{\perp A}>$. Next, the cross-correlogram must be divided by N_A, the total number of spikes fired by *A*. This baseline-subtracted, normalized correlogram corresponds to the differential probability that *B* fires a spike following *A*. Finally, the integral of the 'monosynaptic peak' in this function yields what is known as the *efficacy* of *A* (Levick et al., 1972). Very loosely, the efficacy can be thought of as the percentage of *A*'s spikes that caused a spike in *B* for a given duration after *A* fired. In our studies of connections between LGN and visual cortex, we set the duration of the monosynaptic peak equal to

3.0 msec to compute efficacy (Alonso et al., 1996; Reid and Alonso, 1995; Usrey et al., 2000).

In this interpretation of cross-correlograms, in which the efficacy represents a marginal increase in probability that the postsynaptic cell fires a spike, approximate linear summation is well-defined and would be expected, at least for extremely small inputs. In virtually any probabilistic model, even a grossly nonlinear one, infinitesimal marginal probabilities combine linearly. Given the fact that thalamic inputs to cortical neurons have an efficacy of several percent (small, but certainly not infinitesimal), one might expect some deviation from linearity. The question remains, therefore, what would be the expected deviation from linearity given a reasonable model of synaptic integration and neuronal spiking?

In one analysis of this question, Abeles (Abeles, 1982; Abeles, 1991) examined the relation between the asynchronous attenuation (A_A)—equal to the inverse of the efficacy—and the synchronous attenuation (A_S): the number of synchronous inputs needed to create a spike 50% of the time. In order to quantify this idea, he defines the term *coincidence advantage, CA,* given by:

$$CA = 0.5\, A_A / A_S \qquad \text{(eq. 5).}$$

The coincidence advantage can be thought of as the ratio: the half the number of identical asynchronous spikes needed to evoke an incremental spike in the postsynaptic spike train ($0.5\, A_A$), divided by the number of synchronous spikes needed to evoke one spike 50% of the time: A_S. A_A is easily measurable (it is the inverse of the efficacy of a single input), but A_S is a high number (Abeles estimates that 37 is a reasonable number for cortico-cortical synapses), so the coincidence advantage would be virtually impossible to

measure experimentally. We have asked a slightly different question (Alonso et al., 1996): how much better are *two* synchronous inputs than the sum of the same inputs arriving asynchronously? This number, which we term the *synergy ratio*, will almost certainly be less than the coincidence advantage for many inputs, but it is one that can be measured experimentally.

Considering no more than the threshold for firing, it might be expected that both quantities—the coincidence advantage and synergy ratio—would tend to be greater than one. Small inputs are unlikely to reach threshold and are therefore ineffective, while synchronous inputs are more likely push the neuron over threshold. It is worthwhile to examine this simple idea in more detail. As outlined by Abeles (Abeles, 1982), it is relatively straightforward to analyze the behavior of a neuron that adds together many small synaptic inputs in a roughly linear fashion and that has a fixed threshold.

Abeles made the following simplifying assumptions. First, a neuron can be characterized in terms of the probability distribution of its instantaneous membrane potentials (fig. 4a). Second, the probability that a neuron fires is linearly related to the probability that the membrane potential is above threshold (a premise that can be proven by simple statistical arguments). Further, a typical cortical neuron tends to fire at rates far below the upper limit set by its refractory period. It is therefore reasonable to assume that most of the time it is below the spiking threshold. Given these assumptions, the neuron will have a coincidence advantage greater than one if: "the function describing the probability of the (instantaneous) transmembrane potential being above the threshold vs. the threshold level is concave." This complicated statement is best illustrated when the instantaneous membrane potential, *V*, has a Gaussian probability density (fig. 4a),

$$p(V) = \frac{1}{\sqrt{2p}\,s} e^{-V^2/2s^2}, \qquad \text{(eq. 6)}$$

where V is measured relative to V_{rest}, and s is the standard deviation of V. At any given time, the probability that V is above threshold, T, is given by.

$$\mathrm{P}(V>T) = \mathrm{P}(x>T/s) = \frac{1}{\sqrt{2p}} \int_{T/s}^{\infty} e^{-x^2/2} dx = erfc(T/\sqrt{2}s)/2 \qquad \text{(eq. 7)}$$

where $x = V/s$, and *erfc* is the complementary error function (equivalent to the dark shaded are in fig. 4a; plotted in fig. 4b and 4c). As argued by Abeles, the mean firing of a neuron under these conditions is approximately $\mathrm{P}(V>T)/\Delta t$, where Δt is the interval over which the neuron will tend to fire exactly one spike if V is above threshold. That is, Δt is somewhere between the absolute refractory period and the relative refractory period. For our purposes, we can take Δt = 3 msec. Although this is clearly an approximation, it allows T/s to be estimated as the unique value that satisfies:

$$\mathrm{P}(x>T/s) = erfc(T/\sqrt{2}s)/2 = l\Delta t, \qquad \text{(eq. 8)}$$

where λ is the mean firing rate.

Given this scenario, if a spike from presynaptic neuron A depolarizes the postsynaptic neuron by an amount V_A, this shifts the distribution of V to the right by V_A. For our purposes, this is equivalent on average to shifting the threshold to $T - V_A$. This increases the probability of firing by the marginal increase in area under the Gaussian distribution (fig. 4a, light shaded region). This increase in area is clearly not a linear function of V_A, as further illustrated in figures 4b and 4c. Although we cannot determine V_A from an extracellular recording, we can determine the average efficacy of the synapse

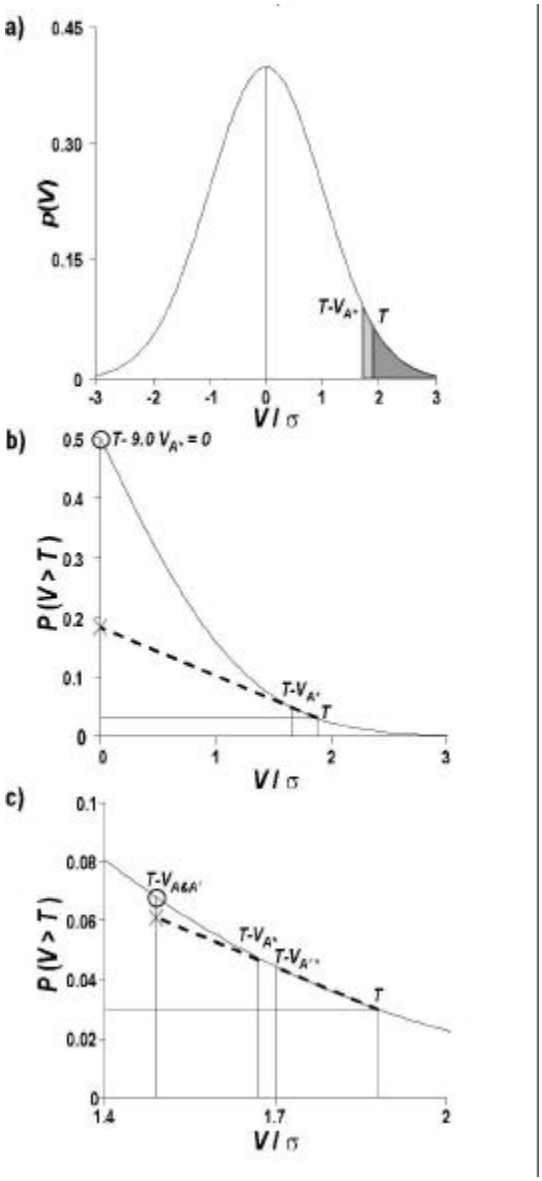

Figure 4. Analysis of the firing probability of a neuron with a threshold, T; a membrane voltage, V, with standard deviation, σ; and EPSPs of asynchronous inputs, V_{A*} and $V_{A'*}$. (a) Probability density function of membrane voltage. Abscissas: normalized membrane voltage, V/σ, in dimensionless units. The area of the dark shaded region, where V > T, is proportional to the firing rate of the neuron. The area of the light shaded region, where T- V_{A*} < V < T, is proportional to the average efficacy of asynchronous inputs, A*, in driving the neuron. (b) The probability that the membrane voltage is greater than threshold *versus* normalized voltage, V/σ. This is equivalent to the probability that one spike fires in any given 3 msec period (see text). Graph illustrates the calculation yielding coincidence advantage (Abeles, 1982). Horizontal line from T: baseline probability of firing. O: probability of firing (0.5) given 9 synchronous EPSPs of size V_{A*}. X: cumulative additional probability of firing (above baseline), for 9 asynchronous EPSPs of size V_{A*} . The coincidence advantage is the ratio: (O minus baseline) /(X minus baseline). (c) Same plot as (b), but over a narrower range. Graph illustrates calculation yielding synergy ratio (see text). O: probability of firing given 2 synchronous EPSPs of sizes V_{A*} and $V_{A'*}$. X: cumulative additional probability of firing (above baseline), for two asynchronous EPSPs of sizes V_{A*} and $V_{A'*}$. Synergy ratio is the ratio: (O minus baseline) /(X minus baseline).

by cross-correlation. Again, the efficacy is the marginal increase in firing probability during the monosynaptic peak (again, defined as 3 msec for the thalamocortical synapse, Alonso et al., 1996; Reid and Alonso, 1995). Therefore, V_A/s is the unique value such that:

$$P(x>T/s - V_A/s) = erfc(T/\sqrt{2}s - V_A/\sqrt{2}s)/2 = l\Delta t + \text{efficacy}. \quad \text{(eq. 9)}$$

For the cortical neuron, *B*, illustrated in figure 3, the baseline firing rate was ~10 spikes/sec and the efficacies of the non-synchronous inputs, *A** and *A*′* (defined above: *Second-order analysis: interactions between inputs*), were 1.71% and 1.41%, respectively (Alonso et al., 1996). Substituting these values into equations 8 and 9 yields:

$$T/s = 1.88,\ V_{A*}/s = 0.21,\ \text{and}\ V_{A'*}/s = 0.18. \quad \text{(eqs. 10)}$$

By assumption, the synchronous activation of both inputs yields a depolarization that is the sum of the two individual depolarizations:

$$V_{A\&A'}/s = 0.39. \quad \text{(eq. 11)}$$

Finally, substituting this value into equation 9 yields an efficacy of 3.8%. Under the simple model of a Gaussian distribution of membrane potential and linear summation of synaptic inputs, therefore, one would have predicted a synergy ratio of:

$$Syn_{model} = 3.8\% / (1.71\% + 1.41\%) = 1.22. \quad \text{(eq. 12)}$$

This point can be made graphically. The lines tangent to the curves in figure 4b and 4c indicate the expected efficacy of two or more inputs if efficacy added linearly. The

concavity of the curve (defined in eq. 7) assures that the actual efficacy is greater than the linear prediction.

Given this relatively modest synergy ratio for two synchronous inputs, it is instructive to use the same model to examine the coincidence advantage, as defined by Abeles (Abeles, 1982), for a larger number of synchronous inputs. The coincidence advantage is defined in terms of the number of identical synaptic inputs required to overcome the threshold, T (i.e., equivalent to shifting threshold all the way to zero in fig. 4a). For the stronger of the two asynchronous inputs to *B*, *A**, this number, termed the synchronous attenuation, is given by:

$$A_S = T/V_{A^*} = 9.0. \quad \text{(eq. 13)}$$

Substituting this value into equation 9, we get a coincidence advantage, *CA*, equal to:

$$CA_{model} = 0.5 \ (1/1.7\%) \ / 9.0 = 3.27 \quad \text{(eq. 14)}$$

This means that the net effect of nine synchronous inputs is 3.27 times stronger than if the inputs arrived asynchronously. For the weaker asynchronous input, *A*'*, the coincidence advantage is slightly higher: $CA_{model} = 3.40$.

In summary, given the assumptions of linear summation of EPSPs and a simple threshold model of firing, the effect of many synchronous thalamic inputs would strongly synergistic, but two inputs would only be slightly synergistic. From a past study (Alonso et al., 1996), however, we have found that two thalamic inputs sum *more* synergistically than would be expected from this simple model: the synergy ratio had a mean value of 1.71 and a median of 1.50. In this study, an important control was that the synergy ratio was high not only for pairs of synchronized inputs (as in fig. 3), but also for uncorrelated

inputs, which fired synchronous spikes at a chance level. Therefore, the higher synergy ratio was almost certainly due to postsynaptic summation, and not caused by a larger ensemble of synchronous inputs.

As reviewed elsewhere (Usrey and Reid, 1999), the synergistic integration of synchronous thalamic inputs has several potential consequences. First and most simply, it is a means of increasing the effect of thalamic input on cortical firing. In particular, when retinal cells are firing at a high rate, spikes are transmitted through the LGN at a higher relative rate (Usrey et al., 1998, see Mastronarde 1987, fig. 12; Kaplan et al., 1987, fig. 9). Because of this, not only are relatively more thalamic spikes reaching the cortex, but also relatively far more synchronous spikes (Usrey et al., 1998). This mechanism might be seen as increasing transmission particularly when a visual stimulus is strong and thus driving the retina to a high rate. Second, several groups have suggested that the preferential transmission of synchronous thalamic inputs might help in the cortical processing of certain classes of visual stimuli (König et al., 1996; Neuenschwander and Singer, 1996; Sillito et al., 1994). Finally, preferential cortical responses to synchronous inputs may help in the transmission of visual information. For pairs of synchronized LGN cells, if synchronous spikes are considered as a separate spike train (A&A', see above), information-theoretic analysis with the stimulus reconstruction technique (Rieke et al., 1997) yields more information about the stimulus than if synchrony were ignored. Thus the ability to 'read-off' synchronous spikes might help in transmitting information from retina to cortex, through the potential bottleneck of the thalamus.

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