APPENDIX IX

Response variability is correlated across multiple projection neurons in the antennal lobe of the locust

Odors appear to be represented in the antennal lobe of the locust by odor-specific, but overlapping, evolving assemblies of synchronously firing projection neurons. These projection neurons (PNs) fire in temporal patterns which are odor- and cell-specific. I have examined the variability of the responses of these cells across repeated stimulus presentations of the same odor. I found strong correlations ($p<0.001$) between the variability of spike trains of many, but not all, simultaneously recorded pairs of PNs. In some of these pairs, the firing of the neurons was negatively correlated: i.e. one neuron fired less than on average on trials when the other neuron fired more than on average. In other pairs, the firing was positively correlated. The correlation in some cell pairs was odor-evoked, and, interestingly, happened only at a particular epoche in the dynamically evolving response. I also observed significant correlations during periods of no odor stimulation, which disappeared at the onset of the odor responses. When the variability of the responses was analyzed as a function of time in the trial – assessed as the variance in the firing rate for short time windows – the variability appeared to vary systematically as the response evolved in time, so that a given cell would exhibit periods of high variability and periods of low variability. In summary, we found intertrial variability was not independent neuronal noise and that neurons are coupled at several timescales. These results suggest that the effective connectivity of the antennal lobe varies as the response to odors unfolds in time. From the standpoint of neural analysis, these results suggest that there is much to be gained in analyzing single trials rather than PSTH's

This work, which was presented in Bäcker, Wehr and Laurent, 1997, was carried out on pairs and triplets of PNs recorded simultaneously by Michael S. Wehr.

Methods

Electrophysiological recordings were done *in vivo* in immobilized adult locusts (*Schistocerca americana*).We simultaneously recorded extracellularly from two or three PN's with an equal number of

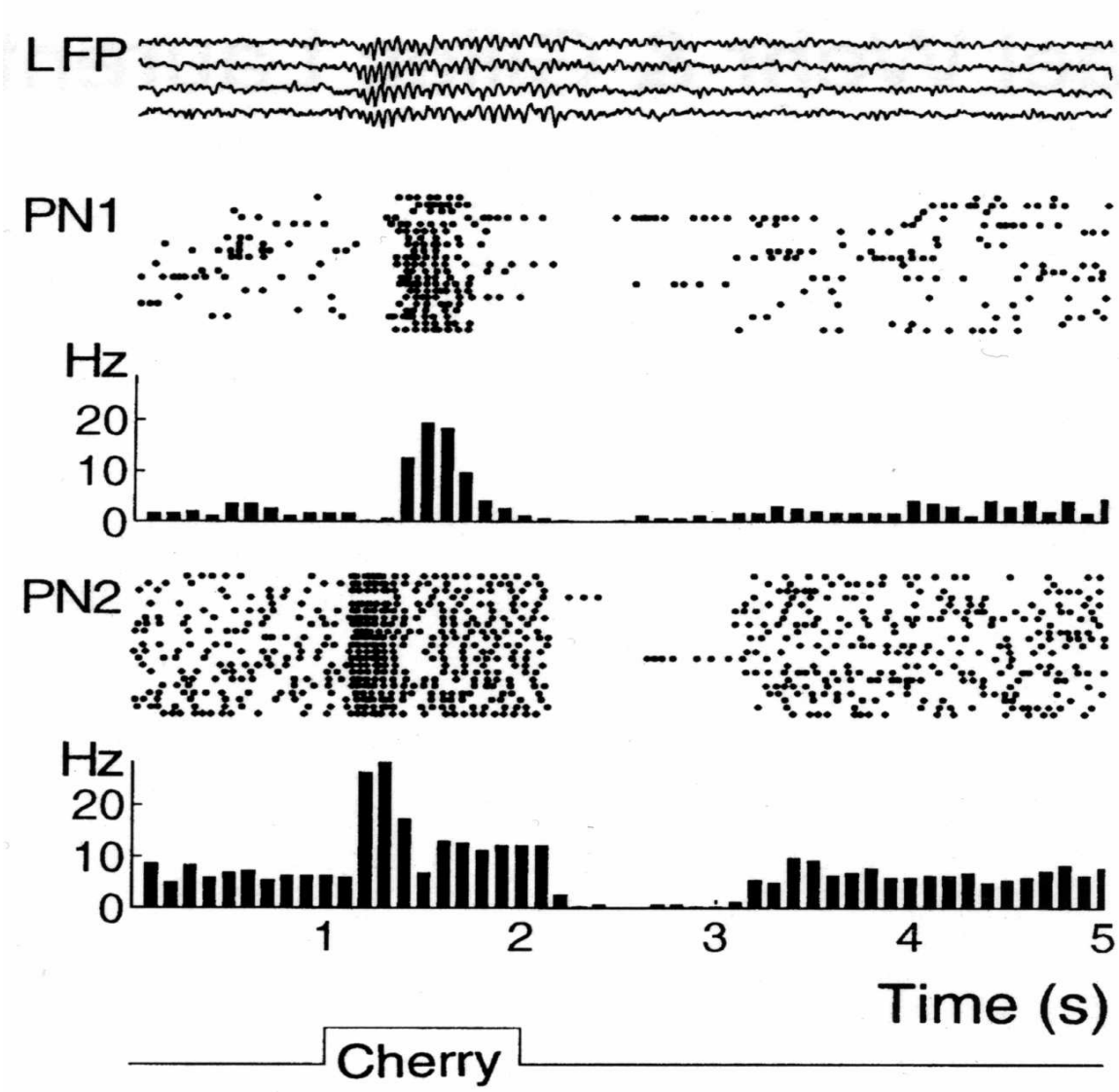

Figure IX.1. Some spikes are reliable; others are not. Odors evoke temporal response patterns of variable reliability in projection neurons (PNs). Notice the extreme degree of repeatability across trials in the first burst in the left diagram, and the much more irregular nature of firing patterns in the second burst or in the diagram on the right. Trials are aligned on the odor delivery pulse onset. Note also the atypical response pattern after odor onset in trial 4 for both PNs. Odor delivery lasts 1 sec, marked 1-2 sec. From Wehr & Laurent (1996), *Nature* 384: 162-166.

glass micropipettes while one-second long puffs of odor were applied to one antenna at regular intervals. We later assessed the independence of the variability of two neurons' responses, i.e. the

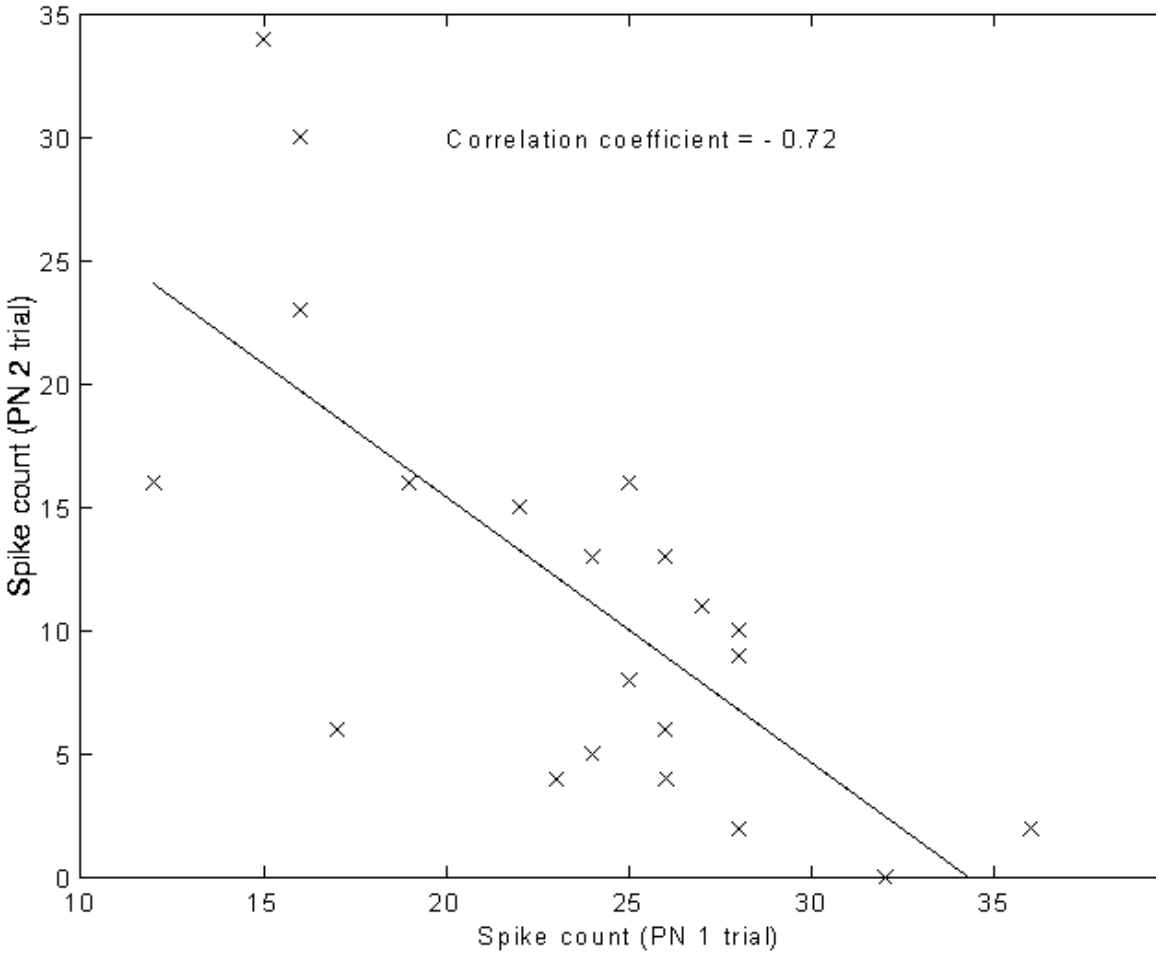

Figure IX.2. There is a tight coupling between two PN's spike count *on a trial by trial basis*. Spike count of each cell during a 3- second time window following odor presentation. Each data point represents the activity of two cells recorded simultaneously during one trial. The line shows the best linear interpolation of the data. The correlation coefficient is –0.72, indicative of a strong negative correlation between the firing of the two cells. Note that it is impossible to derive this information by using only responses averaged over trials.

deviation away from their respective average responses, by computing the correlation coefficient (r) between the number of spikes in each cell for a specified time window in the odor response. This correlation coefficient yields 1 for perfect correlation, 0 for independent variables, and –1 for perfect anticorrelation. We then plotted this correlation coefficient both as a function of time in the trial and of lag between the time windows for both cells. The significance of the numbers thus obtained was

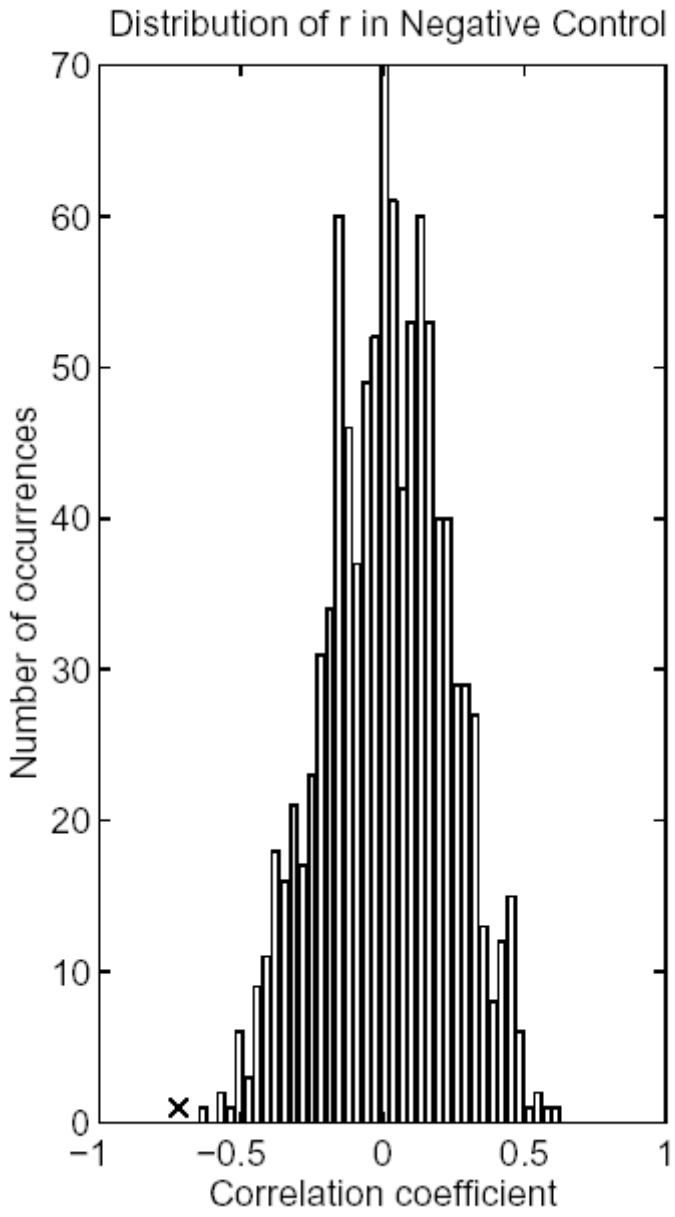

Figure IX.3. The covariation in firing rates across cell pairs is highly significant. Histogram of correlation coefficients between the two cells in Fig. IV.2 obtained with 100,000 random rearrangements of trial order in one of those cells, showing that the probability of obtaining by chance a correlation magnitude as large as or greater than that in Fig. IV.2 is less than 0.0005.

assessed by performing the same computation for a large number of data sets in which the order of the trials had been randomly shuffled. This manipulation preserves the average responses (PSTH)

intact, but eliminates all simultaneity between the records of the two cells.

$$r = \frac{\langle (\#\text{ spikes cell 1}) \cdot (\#\text{ spikes cell 2}) \rangle - \langle \#\text{ spikes cell 1} \rangle \cdot \langle \#\text{ spikes cell 2} \rangle}{\left[\langle (\#\text{ spikes cell 1})^2 \rangle - \langle \#\text{ spikes cell 1} \rangle^2 \cdot \left(\langle (\#\text{ spikes cell 2})^2 \rangle - \langle \#\text{ spikes cell 2} \rangle^2 \right] \right]^{1/2}}$$

Significance of a measurement among multiple comparisons

Determining the statistical signficance of the correlation timecourses shown in the figures in this appendix is not trivial, because each time series presents multiple measurements. This is explained in the general discussion below, together with some proposed solutions for different cases.

Let us assume we are making an experiment in which we are trying to decide if any of a set of measurements under experimental condition A is different from the corresponding set of measurements made under the negative control condition B. For example, we might be trying to tell whether a spike train in response to a stimulus is different from spike trains under a control condition where there is no stimulus; one measurement might be the # of spikes within a time window, the set might be given by a series of successive windows. The null hypothesis is that both sets are indistinguishable: that the response to A is no different from that to B.

If making many measurements and reporting *any* deviation from the value expected given the null hypothesis, the probability of finding *a* value equal or greater than X given the null hypothesis is *not* given by the probability of finding that value if one were performing a single measurement --even if one uses the probability for the measurement that actually gave the deviation.

$$P = p(\text{any of N measurements} >= X) = 1 - p(\text{all N measurements} < X)$$

If all N measurements are independent, we can write

$$P = 1 - p(M_1<X).p(M_2<X)\ldots(p(M_N>=X)$$

Furthermore, if all N measurements are drawn from the same distribution and thus have the same p-values

$$P = 1 - [1-p(\text{one measurement} >= X)]^N$$

Thus if we are making two independent measurements, and we wish to be as strict as if we were doing a single measurement and using a p-value of 0.05, we must make P above equal to 0.05:

$$0.05=1-(1-\text{p value from a single comparison to be reported as significant given multiple comparisons})^N$$

p value from a single comparison to be reported as significant given multiple comparisons = Nth root of 0.05 , and thus report any measurement where p<1-(1-0.05)^2.

But what if we do not know if the measurements are independent, or if we suspect they are not? There are at least two possible empirical solutions:

A. If one has plenty of experiments under the experimental condition (condition A): one can use a subset of the experiments (e.g. half of them) to identify measurements that one believes may be significant, and then formulate a specific hypothesis that those are significant, that one can then test with the rest of the experiments and for which one can obtain a p-value without accounting for multiple comparisons, since there is only one hypothesis being tested.

B. If one has access to plenty of negative controls, but few under the experimental condition (A) so that partitioning the set of condition A experiments is impractical:

1. Calculate the p-value, without accounting for multiple comparisons, for the mea-

surement whose multiple comparisons (MC)-corrected p-value we wish to obtain.

2. For each of the other measurements, calculate, without accounting for multiple comparisons, the level of the measurement that constitutes the same p-value calculated in (1).

3. By analyzing a large number of trials for the negative control, compute the probability that a *set* of measurements (one of each type, e.g. one in each time window) in the negative control yields *any* value more extreme than the corresponding levels calculated in (2). This will be the multiple-comparisons-corrected p-value for the result obtained in the original measurement used in (1).

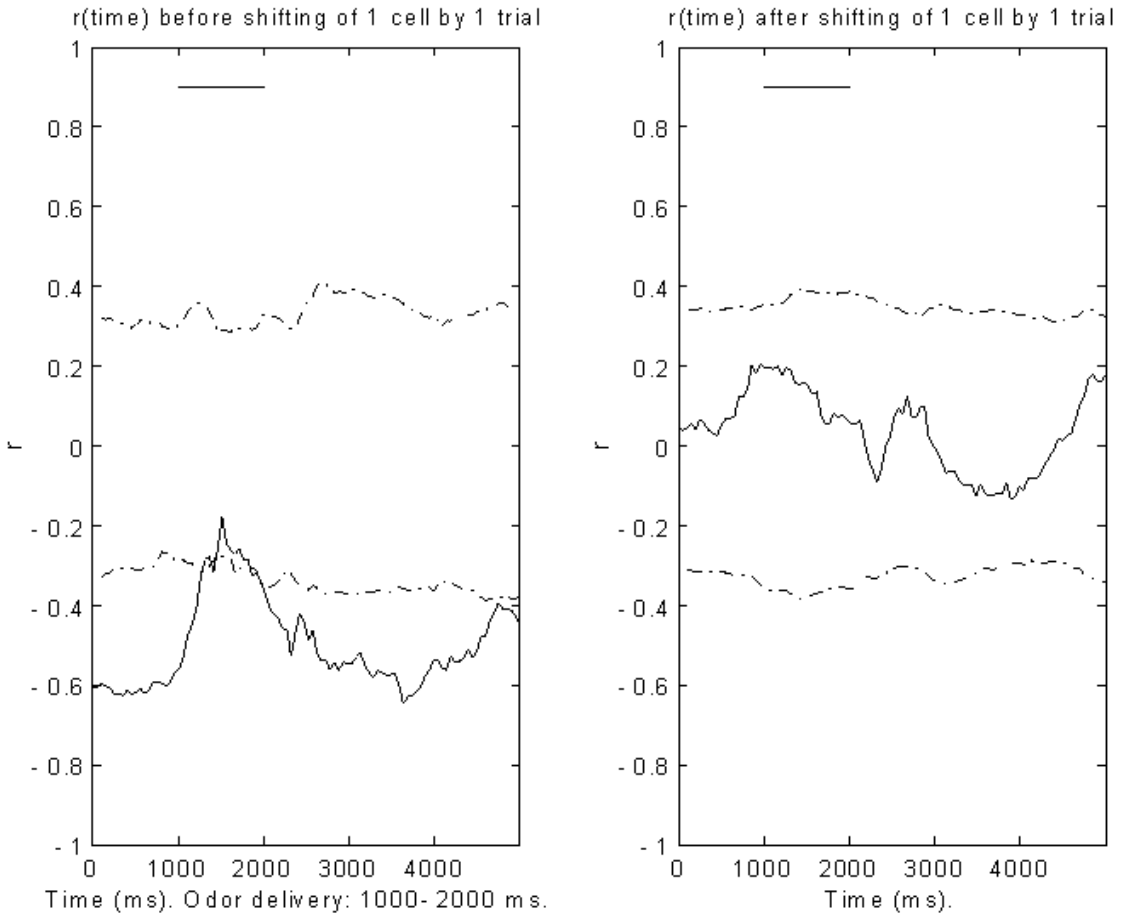

Figure IX.4. There is no correlation between successive trials of a pair of cells. The plot on the right was obtained by shifting all trials of one cell by one trial position. The disappearance of the correlation found on the left-hand plot illustrates the transient nature of thesecorrelations. The dashed lines represent the p=0.05 significance value, measured from the variance of r for sets of randomly shuffled trials (see above).

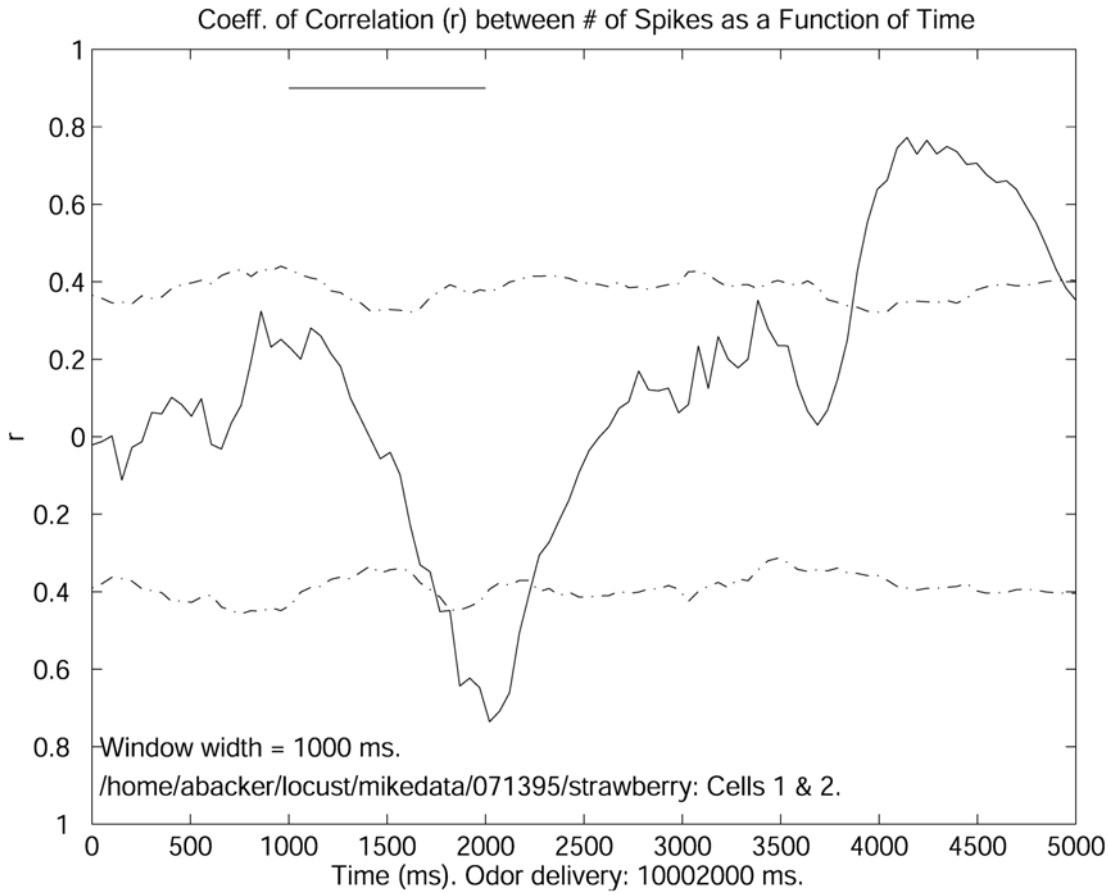

Figure IX.5. Correlations can be positive or negative and occur during specific periods of the odor response. Plot of the correlation coefficient (r) of the spikes in two PN's as a function of time in the trial. Each point represents r for a single time window centered around the point.

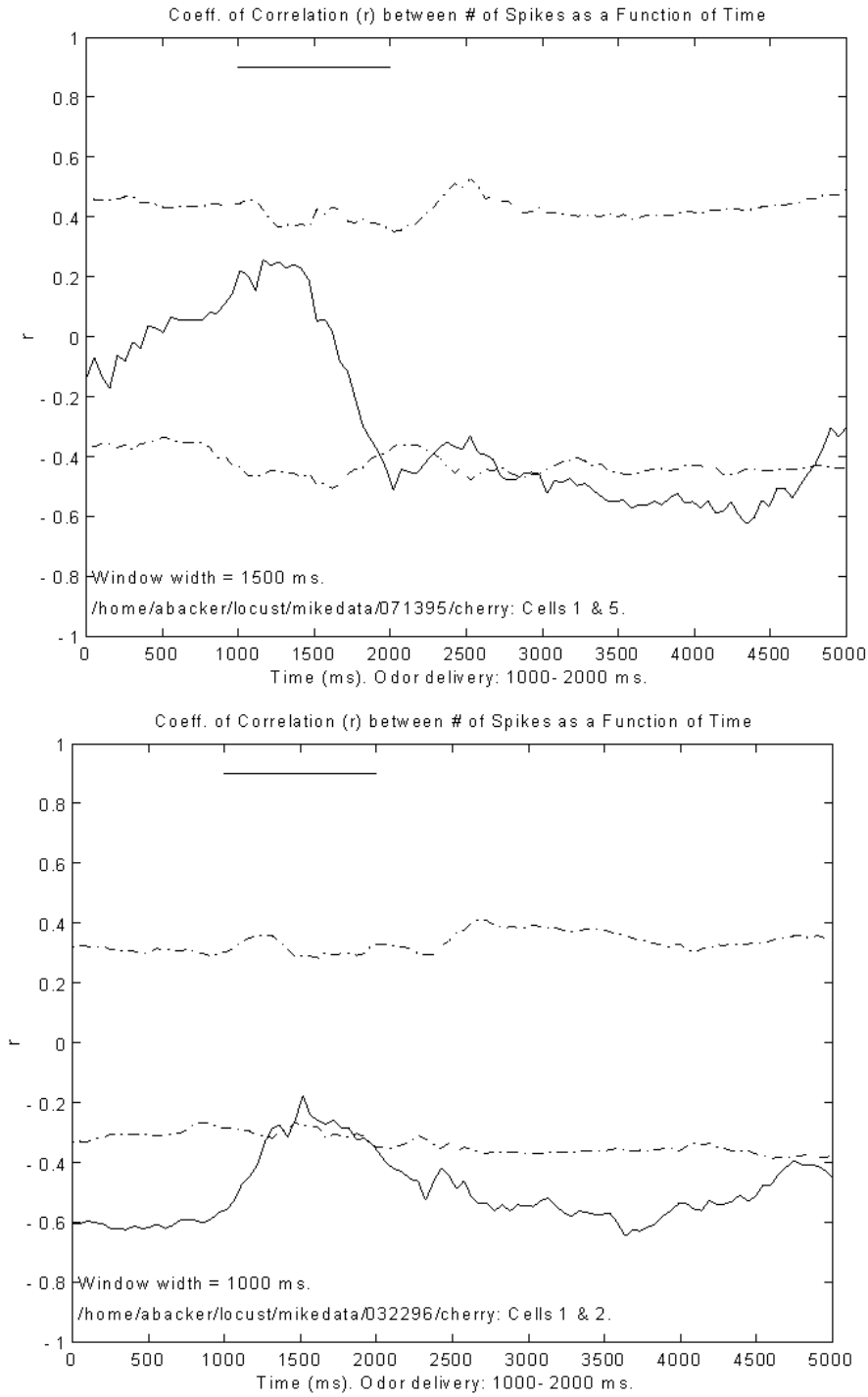

Figure IX.6. Cell pairs can correlate (top) or decorrelate (bottom) in response to an odor. A decorrelation is a change from coordinated variability to independent responses. The two neurons in the bottom plot had highly correlated activity before and after odor presentation, but suffered a marked decrease in their correlation during the duration of the odor pulse.

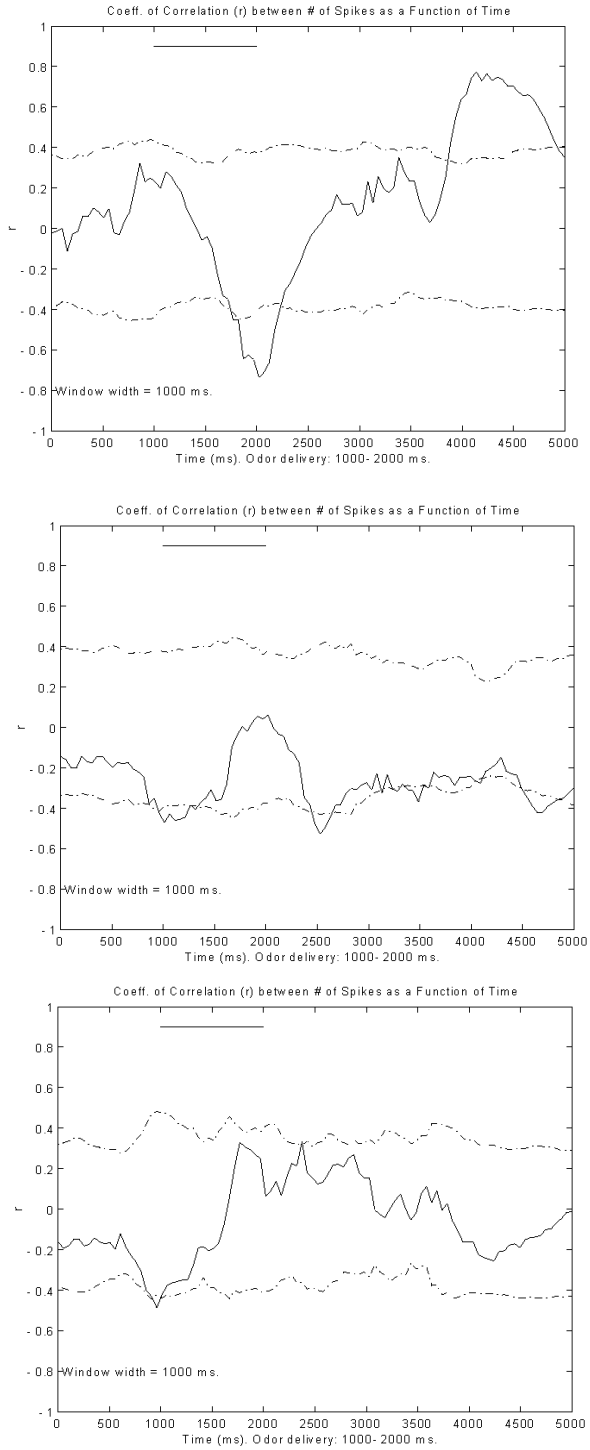

Figure IX.7. Correlations occur within specific neuronal populations. Recordings from triplets of neurons show that correlations and their timecourses are specific to a particular neuronal pair. The triplet above exhibits 3 different correlation patterns in the 3 pairs that compose it (top, center and bottom).

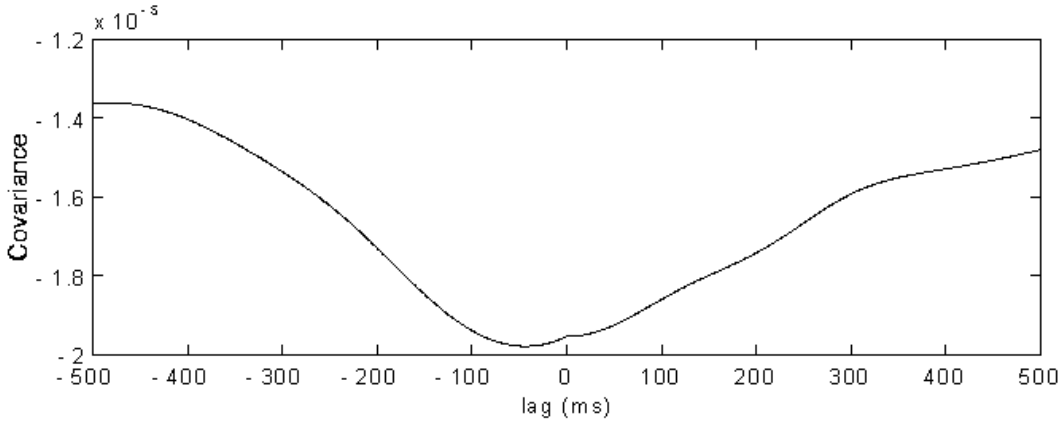

Figure IX.8 The cross-correlation for this pair of PNs exhibits a timeconstant of several hundred milliseconds and a peak at roughly 50 ms lag (i.e. 1 oscillation cycle). The plot above shows the covariance between the number of spikes in one time window for each cell, as a function of the lag of one cell's window with respect to the other cell's.