Information stored over many hours by sequences of neural activity in networks of cortical neurons

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Abstract

We hypothesized that a network of cortical neurons could store information over many hours in patterns of neural activity. To address this, slices of rat cortex were cultured and spontaneous extracellular field potentials were recorded using a 60 channel microelectrode array. Activity consisted of a wide variety of spatio-temporal patterns that recurred far more often than chance. These patterns were temporally precise, were stable over 10 hours, and were grouped into many statistically significant network states. Thus, patterns represent activation of unique network states that can store information. These patterns may form basic elements of memory at the network level.

Introduction

The physiology of the cortex has been the subject of intense interest for many decades. This interest is perhaps driven by the desire to understand the mechanisms of higher cognitive functions that are dependent upon the cortex. Previous approaches to cortical physiology have included single and multiple electrode recordings in vivo, intrinsic imaging, fMRI, and in vitro studies of cortical slices. Recent advances in technology have now made it possible to culture slices of cortex on multielectrode arrays (Gross et al, 1982; Novak and Wheeler, 1988; Maeda et al, 1995; Gahwiler et al, 1997; Potter, 2001). This new approach allows cortical networks to be recorded and stimulated in isolation from other brain areas (Maeda et al, 1995; Kamioka et al, 1996; Jimbo et al, 2000; Segev et al, 2001; Shahaf and Marom, 2001). Isolating a cortical network may simplify the analysis of complex network dynamics that are thought to be characteristic of the cortex (Jimbo et al, 2000).

While tremendous effort has been directed toward understanding cortical mechanisms of memory at the synaptic and systems levels, very little experimental work has been done to investigate how small networks of cortical neurons could store information. We hypothesized that a network of cortical neurons could store information over many hours, possibly in patterns of neural activity.

Methods

To address this issue, we cultured slices of rat frontal cortex for 4 weeks and then recorded their spontaneous activity for at least 10 hrs with an array of 60 microelectrodes. Cortical slices were co-cultured with slices of striatum and substantia nigra, but these

areas do not send projections back to the cortex, and only cortex was grown over the microelectrodes (Figure 1). Slice cultures were chosen to preserve the cortical circuitry that is largely disrupted in dissociated cultures. Local field potentials were low pass filtered at 50 Hz, then thresholded at –3 standard deviations. Their points of maximum excursion were then binned at 4 ms (Figure 1). Using this procedure, many sequences of continuous activity were extracted from the data (Figure 2). The pattern of suprathreshold activity on the electrode grid during one time bin was called a "frame." A sequence of consecutively active frames was called a "run." Runs of the same length were compared against each other for similarities, where similarity was measured by correlation values ranging from –1 to +1. Similar runs were grouped together using simulated annealing and greedy algorithms (Figure 3). To test for statistical significance, correlations produced by actual data were compared to correlations produced by 50 shuffled control data sets (Figure 4). Several different methods of shuffling were used, including electrode shuffling, frame shuffling, and event-count-matched shuffling (Oram et al, 1999). These shuffling methods allowed correlations expected by chance to be estimated. Temporal precision of the runs was probed by introducing various amounts of gaussian jitter to the time points of the original data. Correlations within runs were then plotted as a function of jitter size (Figure 5). The stability of runs over time was measured by comparing correlations within the first hour of recording to correlations produced up to ten hours later (Figures 6, 7). Information theory was also used to quantify the extent of overlap between distributions of correlations extracted from the first hour and subsequent hours (Figure 7).

Results

For years, spontaneous neuronal activity in dissociated cortex cultures has been described as synchronously bursting at random times (e.g. Maeda et al, 1995; Kamioka et al, 1996; Segev at al, 2001). At long time scales, the activity in our slice cultures also seemed to fit this pattern (Figure 1), but when local field potentials were binned at 4 ms, a dramatically different view of activity emerged (Figure 2). The cortical cultures produced spatial patterns of activity that appeared in distinct sequences (runs) that would reoccur spontaneously (Figure 3).

The cultures (n = 6) produced 3763 ± 1000 (mean ± s.d.) runs per hour. These runs had correlations with each other that far exceeded those produced by shuffled data (Figure 4). When these runs were grouped by clustering algorithms, the groups formed were larger and had higher average correlations than those produced by shuffled data (Figure 4). In one hour, the average culture produced runs that could be clustered into 34 ± 13 statistically significant network states. Average correlations of the runs with each other declined by 57% after a jitter of only 4 ms, indicating that the runs were temporally precise (Figure 5).

To investigate whether these structured sequences were robust over time, recordings made in later hours were compared to recordings from the first hour. Remarkably, many of the runs were repeated with nearly exact similarity many hours later. In fact, the runs recorded 10 hrs later maintained 73 ± 5 % of their statistically significant correlations with those recorded during the first hour (Figures 6, 7). The distributions of statistically significant correlations between the first and eleventh hours

were also compared. After ten hours of recording, more than 95% of mutual information still remained between these distributions (Figure 7).

Discussion

The main finding of this report is that spontaneous activity in cortical slice cultures is intricately structured, containing a wide variety of spatio-temporal activity patterns that recur far more often than chance. These spatio-temporal patterns are diverse, and can be clustered into a large number of statistically significant groups. Each significant group can be thought of as a distinct network state. These patterns are also temporally precise, since jittering of activity times by as little as 4 ms reduces their correlations with each other by more than half. Finally, these patterns are stable over a period of 10 hours as indicated by both correlations and mutual information measures.

The fact that these activity sequences contain some information that does not degrade over time suggests that they may form basic elements of memory at the small network level.

References

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Figures

Figure 1. Multi-electrode recording of spontaneous activity in cortical slice culture. A, Photo of culture grown on multi-electrode array (MEA) dish taken at 2 days in vitro. Cx: cortex, CPu: caudate-putamen, SN: substantia nigra. Inter-electrode distance is 200 μm. B, Raw activity recorded from one electrode. C, Recording from all electrodes shows apparent synchronous activation (traces here downsampled by 8 for clarity). D, Same trace as in B, but low-pass filtered at 50 Hz. Dashed line shows threshold at –3 standard deviations. Size of dots represent magnitude of suprathreshold field potential. E, Raster plot shows periods of nearly synchronous activity separated by quiescent intervals of several seconds. F, Upper, inter-event interval histogram for this culture taken from one hour of activity (bin width: 0.5 s). Lower, average inter-event interval histogram.

Figure 2. Apparently synchronous activity is decomposed into runs. A, Raster plot of spontaneous activity (same as Fig. 1 E). B, Period of suprathreshold activity near 50 seconds is binned at 4 ms, showing that activity is not exactly synchronous. Activity here spans three bins and is preceded and terminated by bins with no activity. C, Activity shown in B is presented as a spatio-temporal sequence of activity on the MEA grid. In this case, a run of three frames is shown.

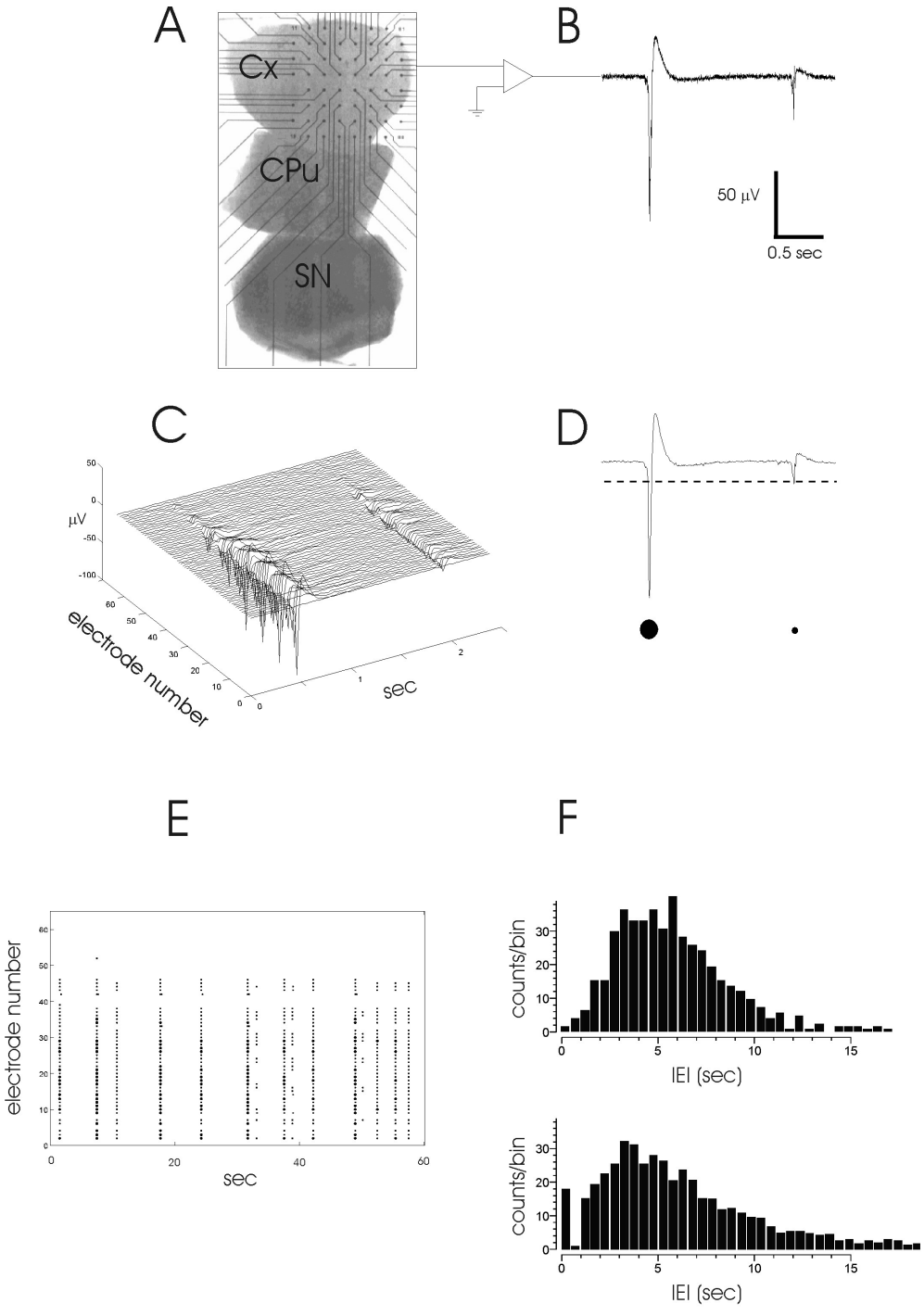
Figure 3. Sorted correlation matrix reveals similar runs. A, Example of how to construct a correlation matrix. Four hypothetical runs (a, b, c, d) of length two on a 2 x 2 electrode grid. Runs a and c have identical first frames, while runs a and b have identical last frames. Runs a and d are complements. Correlations between all possible pairs of these runs are taken and entered into a matrix. Color bar at right indicates blue for positive correlations, yellow for negative correlations. B, Unsorted correlation matrix of all runs of length 4 recorded in one hour from one culture (bin width 4 ms). Runs are numbered in order of appearance along the margins of the matrix. Diagonal elements have been removed for clarity. Note that blue (high correlation) and yellow (low correlation) regions are intermingled. C, Same correlation matrix as in B, now sorted in order of similarity by clustering algorithms. Note that blue regions are now concentrated along the diagonal, forming groups of runs with high mutual correlations. Red dots indicate regions of highly similar runs. D, Highly similar runs revealed by clustering. Each number with bracket shows a pair of runs that were clustered together at red dots in the sorted similarity matrix. Frames are shown as 8 by 8 grids, active electrodes are darkened, and darker squares indicate larger amplitudes. Differences in time of occurrence between runs is given by Δt. Note that runs show high spatial and temporal similarity despite occurring many minutes apart.

Figure 4. Significant structure within one hour. A, Sorted correlation matrix of all runs of length 4 from actual data. B, Sorted correlation matrix of all runs of length 4 from shuffled data. Note how actual data has larger blocks of high correlations along the diagonal (dark blue) than shuffled data. C, Sum of correlations plotted against threshold. Units of x-axis are standard deviations above the mean of shuffled data. As threshold is increased, fewer points in matrix exceed threshold, so sum decreases. Red line, actual data. Blue lines, 50 sets of shuffled data overplotted. Note that actual data far exceeds shuffled data for all thresholds tested. D, Clusters formed from actual data (red circles) have larger size and greater within-cluster correlations than clusters formed from 50 sets of shuffled data (blue circles).

Figure 5. Temporal precision of runs. Plot of average correlation (n = 6 cultures) against size of gaussian jitter. Original raster data was binned at 1 ms and all runs of length 5 were extracted and used to construct correlation matrices and to obtain average correlation value. The time points within the original rasters were then jittered, and average correlation values were again extracted. Note that average correlation declines sharply with jitter, losing 57% of its original value after a jitter of 4 ms. This demonstrates that the runs produced by spontaneous activity are temporally precise. Error bars indicate standard deviations.

Figure 6. Significant structure across ten hours. A, Unsorted correlation matrices contain runs of length 4 from first and sixth hours. Black lines divide matrix into correlations within first hour (small square, lower left), correlations within sixth hour (large square, upper right), and interaction area that contains correlations between hours (two rectangular regions on either side of matrix diagonal). Leftmost matrix is an unshuffled merge where interaction area contains many high correlations (blue pixels). Middle matrix is a merge where data from the first hour had all electrodes randomly permuted the same way (done here merely to illustrate decoupling the hours – actual electrode shuffling was more complete, as described in Methods). This remapping preserved correlations within each hour, but reduced correlations between hours, indicated by reduced blue pixels in interaction area. Rightmost matrix is a merge where data from both hours has had shuffled electrodes and frames. This interaction area gives correlations between the first and sixth hours expected by chance. B, Actual data is significantly different from shuffled data. Sum of correlations in interaction area plotted against threshold for three time differences Δt (actual data: red, 50 shuffled data sets: blue). Note changes in vertical scale. Ratio of actual correlations to shuffled correlations is fairly constant for 10 hrs, and remains statistically significant.

Figure 7. Stability of patterns measured over time. A, Fraction of correlations in interaction area that were significant plotted against time for runs of length 5 (n = 6 cultures, matched shuffling). Here, the average retention for group was 81% after 5 hrs, 73% after 10 hrs. Values were normalized to the fraction obtained after one hour. B, Mutual information plotted against time shows less than 5% decline after 10 hours. Symbols represent individual cultures.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C
µV
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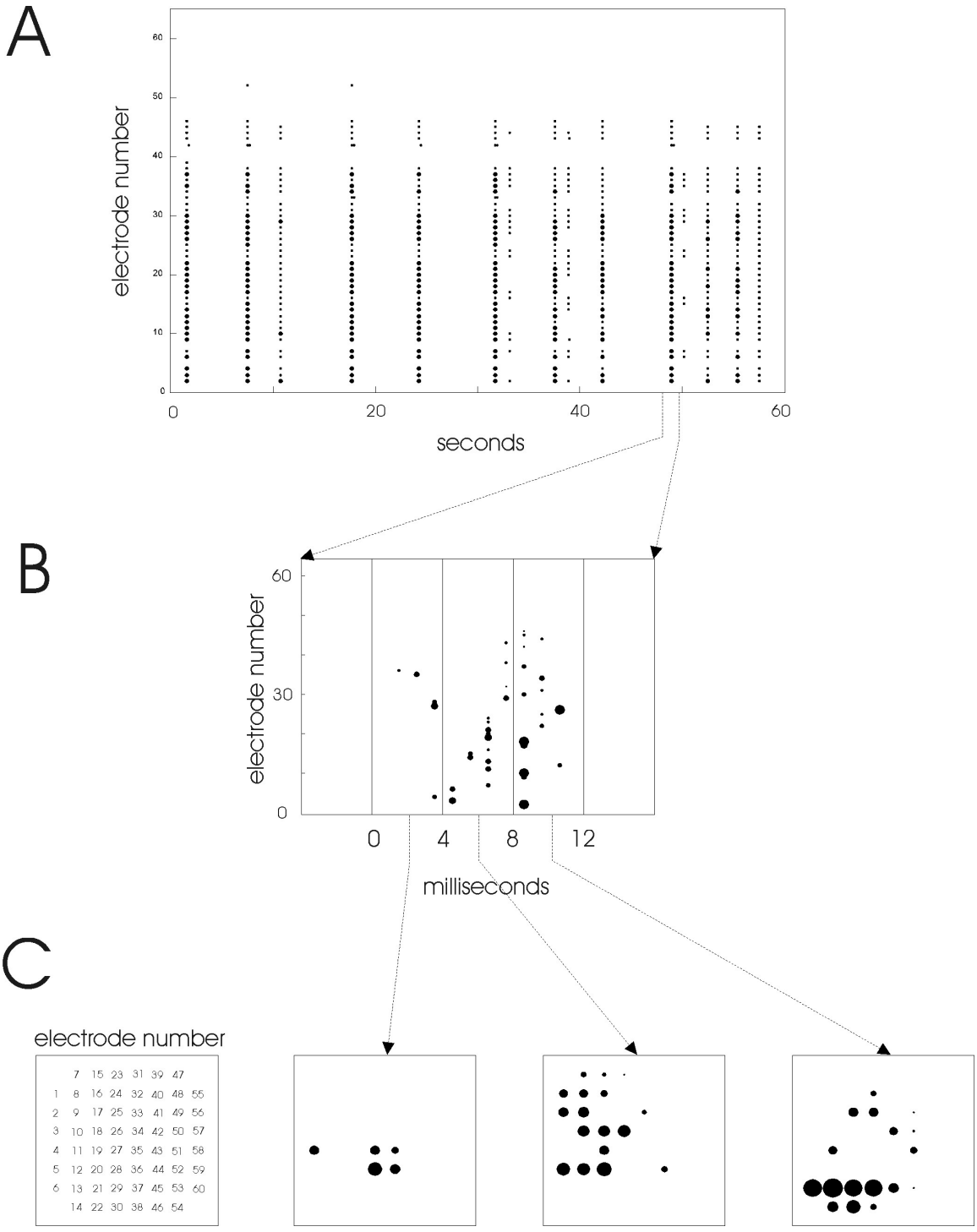
IEI (sec)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4
8
12
milliseconds
C
electrode number
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1 8 16 24 32 40 48 55
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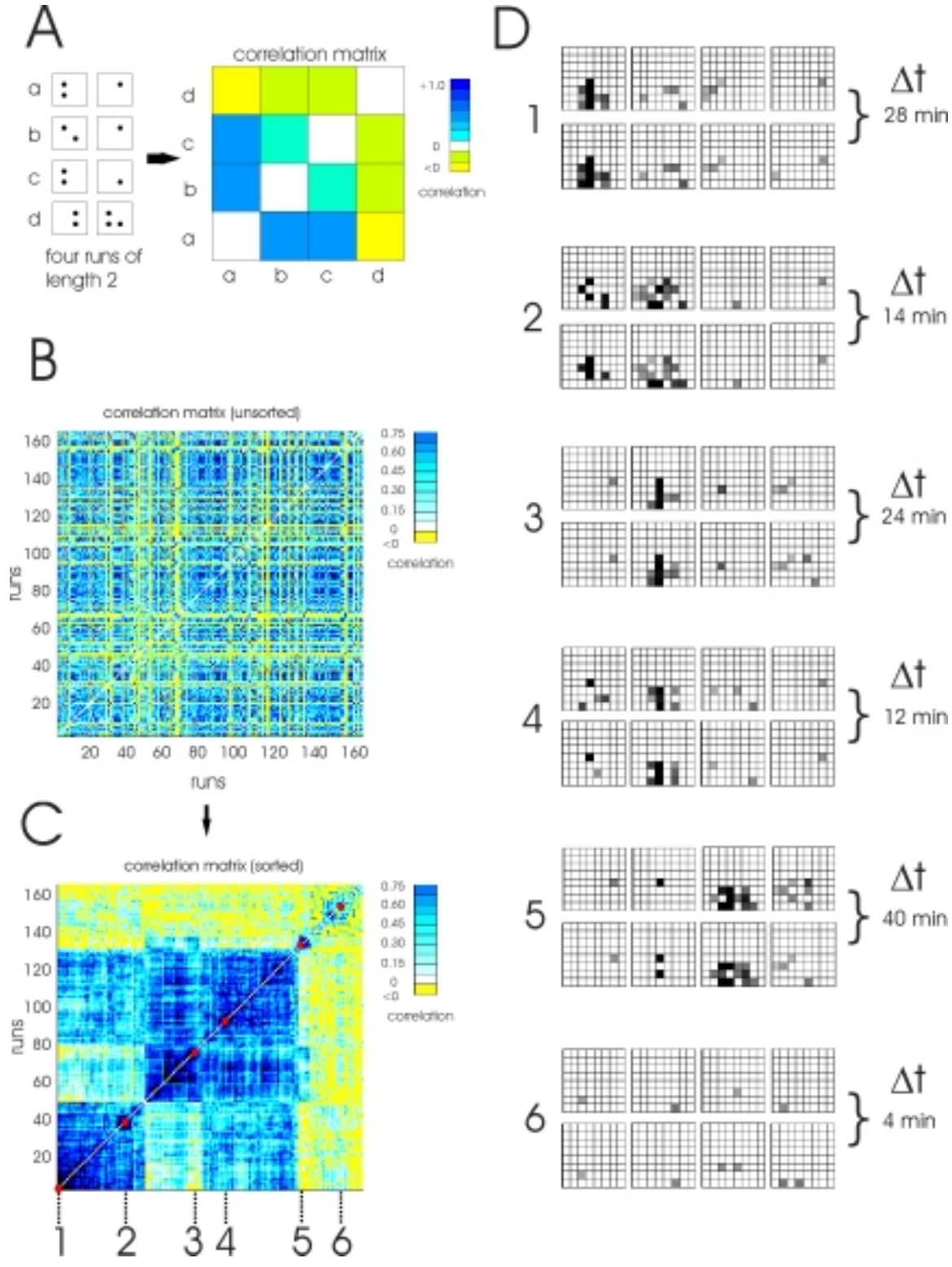
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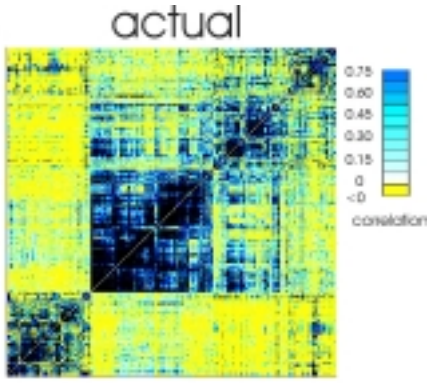
correlation matrix
a
b
c
d
four runs of length 2
+1.0
0
<0
correlation
B
correlation matrix (unsorted)
runs
C
correlation matrix (sorted)
1 2 3 4 5 6
D
1
2
3
4
5
6
Δt
28 min
14 min
24 min
12 min
40 min
4 min

A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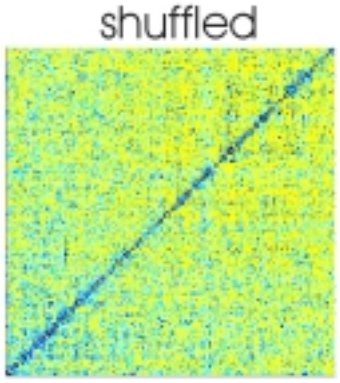
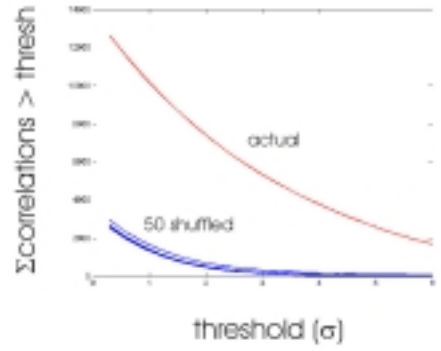
correlation

B


shuffled

C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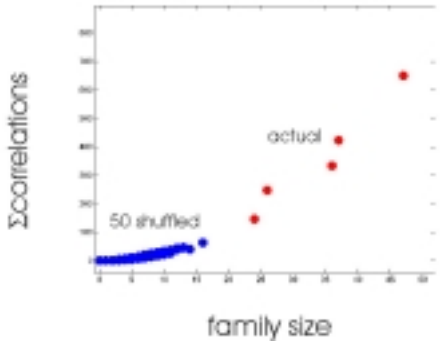
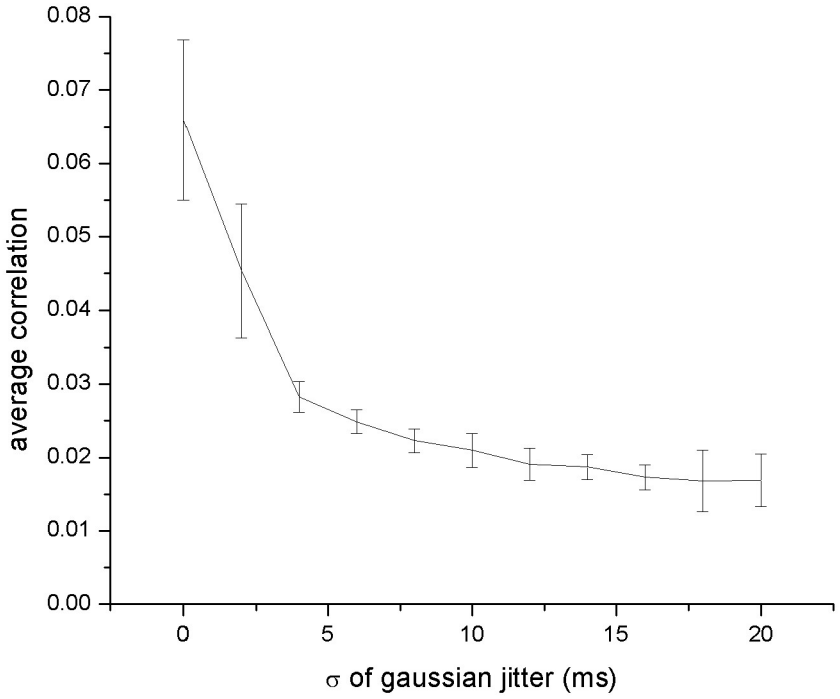
actual
50 shuffled
threshold (σ)

D


Σcorrelations
actual
50 shuffled
family size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0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
average correlation
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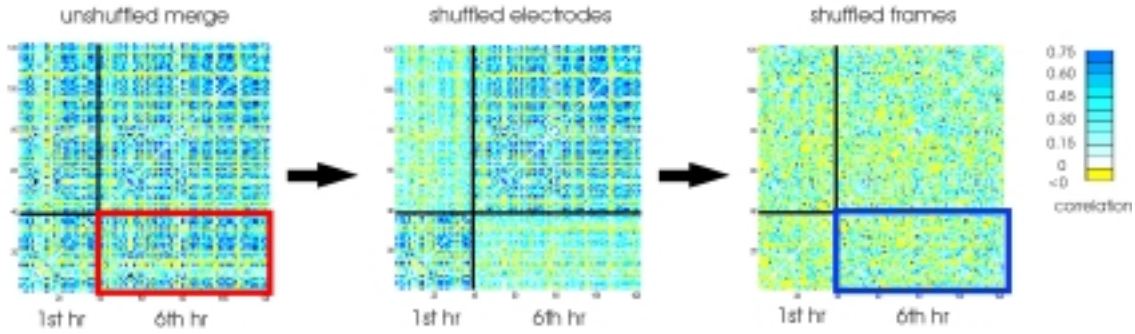
5
10
15
20
σ of gaussian jitter (ms)

A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shuffled electrodes
shuffled frames
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6th hr
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6th hr
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6th hr
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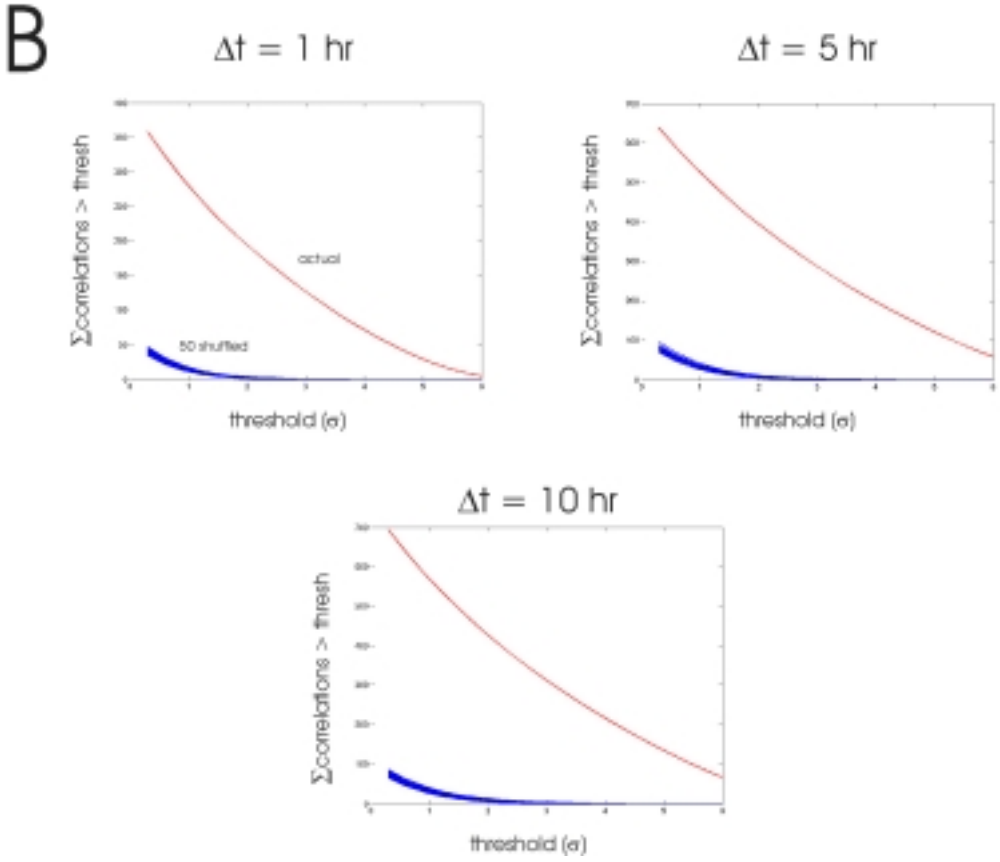
0.30
0.15
0
<0
correlation

B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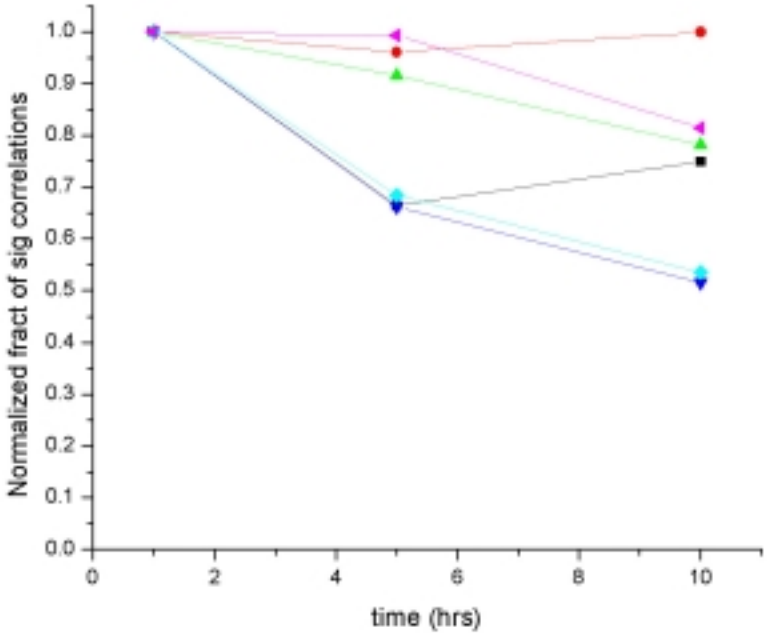
Δt = 5 hr
Δt = 10 hr
Σcorrelations > thresh
threshold (σ)
actual
50 shuffled

A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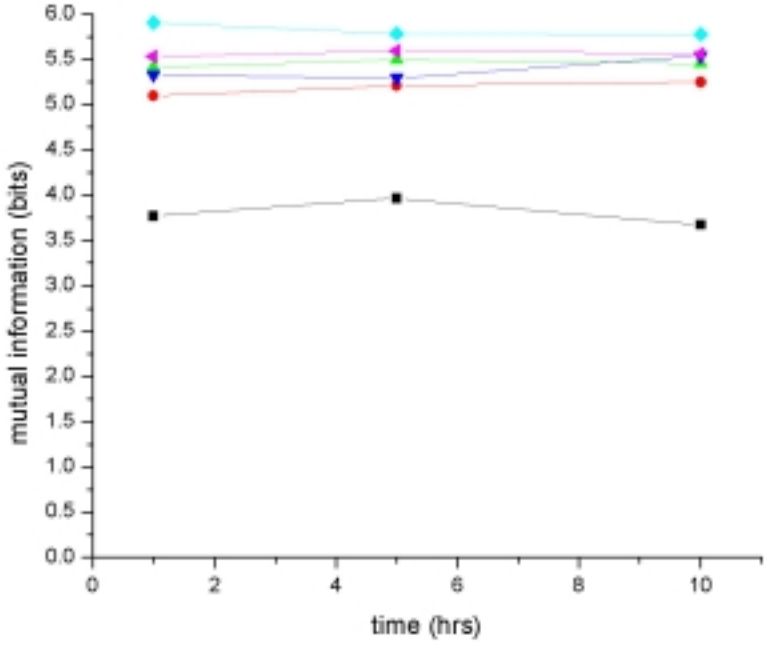
0
2
4
6
8
10
time (hrs)

B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0.5
0.0
0
2
4
6
8
10
time (hrs)