

Supplementary Methods:

Notes for the clustering analysis of excitatory synaptic connections

A heterogeneity of excitatory synapses between PCs is a major new finding in this study. Such heterogeneity always evokes the difficult issue of classification and we therefore tried to address this as comprehensively as possible. The finding that there are 3 *E*-types with 2 subtypes each, requires thoroughness to make a convincing case.

The reasons we used kernel smoothing were (1) because the number of connections studied differed by a factor of 6 between cortical areas, (2) the relative graininess of the visual cortex data made visual comparison difficult, (3) they cannot be overlaid for direct contrast, and, (3) the shapes differed markedly for *D*, *F*, *U*, *A*, so that raw data normalization “squashes” the left-weighted distribution of *F*. The advantages of the kernel smoother is that it emphasizes the gradient of the shape of the histogram on similar scales (and autoscales using the sample-specific standard deviations), and permits overlay and comparison across patterns. We thought this was the most compact way to inspect the distributions. Therefore, both the kernel smoothing distributions of the *DFUA* were shown together with the raw histograms in **Fig. 3**. In order to address the issue of multidimensional clusters of the *DFUA* variables, we used the very standard Wilcoxon rank-sum and Kolmogorov-Smirnov tests (as reported in right panel of **Fig. 3**) to test distributional differences. These approaches merely provide the background. It is mainly in the QTC (below) that the number of multidimensional clusters is best tested.

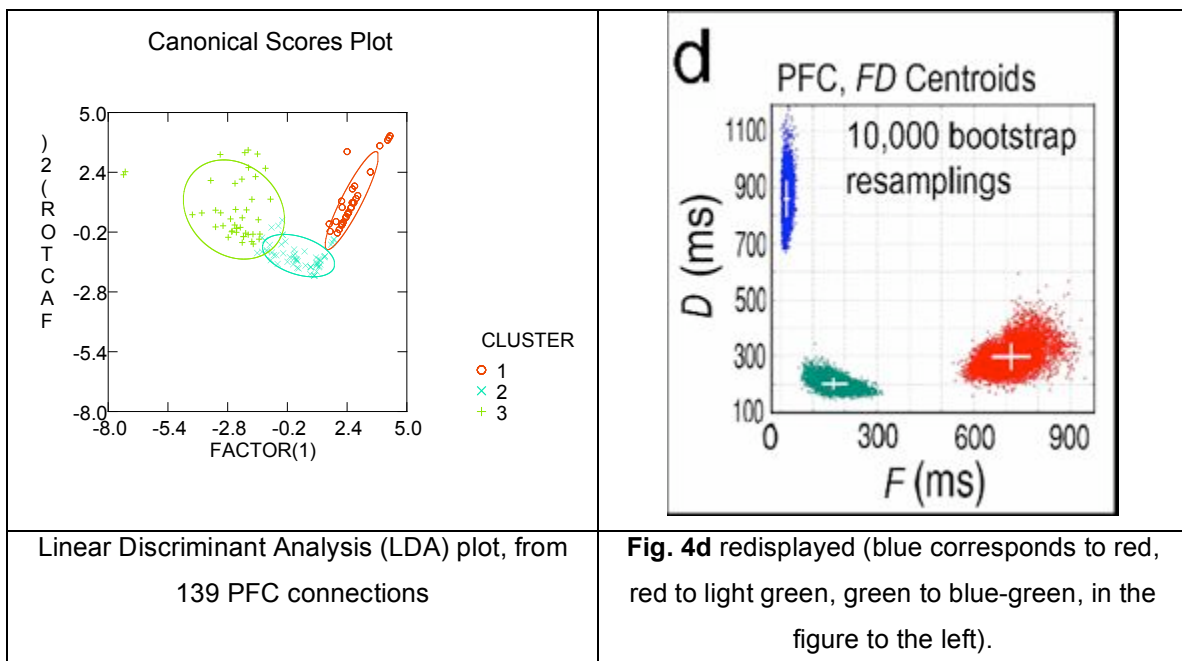
In order to objectively do analysis based on the modeling parameters of *DFUA*, QTC was used to define the clusters, which is a widely accepted unsupervised clustering technique. QTC arrived on the bioinformatics scene just 6 years ago [Heyer, L.J., S. Kruglyak, and S. Yooseph. 1999. *Exploring expression data: identification and analysis of coexpressed genes. Genome Res. 9:1106-1115.*] and has rapidly been accepted by the bioinformatics community as indicated by its incorporation into the most widely used genomic/proteomic statistical packages, **GeneSpring** (Silicon Genetics) and **Multiexperiment Viewer** (J. Craig Venter's Institute for Genomics Research). QTC considers all possible vectors as initial cluster seeds, and an internal jackknife procedure to reduce bias from outliers. It uses the standard Pearson correlation as a similarity measure between any two vectors. It does not require the user to pre-specify the number of clusters. *All* possible clusterings are considered. Another strength of QTC is that it has only one free parameter, the maximum cluster diameter the algorithm is allowed to fit. (Another setting, the minimal cluster size requirement, is used only as a stopping criterion). The diameter of a cluster is just the sum of squares of the vector components (here, z-normalized *DFUA* vectors). It is thus straightforward to perform a sensitivity analysis of the QTC as a function of the maximum

diameter parameter. Sensitivity analysis strongly supported the 3-*DF* cluster model (the full *DFUA* vectors were used, leading to a total of 6 clusters, 2 variations of each *DF* pattern). Thus it was the QTC (**Fig. 4a-b**), not the subsequent *D:F* plot (**Fig. 4c**) or KMEANS analysis (**Fig. 4d**) which revealed a 3-cluster model for *D:F*. KMEANS just provides a means of testing the validity of this clustering possibility revealed by the QTC. The bootstrapping of the 3-cluster KMEANS was not meant as a test to *confirm* the assumption of 3 clusters (the QTC was used to discover the number of clusters supported by the data); rather, under the *assumption* of 3 clusters, to what degree would 3 enforced (but data-dependent) centroid means remain distinct in future samples obtained under similar experimental conditions. This is more a test of how sub-sampling errors could have influenced the claim. **Figure 4d** clearly shows the likely reproducibility of a 3-mean model.

While cluster analysis uses no a priori knowledge of group membership, discriminant analysis were used as the following to show how well clustering on the basis of *DFUA*.

Linear discriminant analysis, or LDA (SYSTAT)

In it's first application, we tested the coherence of LDA upon the raw *D* and *F* parameters, with the cluster assignment generated by Kmeans (k=3 based on QTC) among the 139 PFC connections:



Classification matrix (cases in row categories classified into columns)

CLUSTER	1	2	3	%correct
1	34	1	0	97
2	0	61	0	100
3	0	1	42	98
Total	34	63	42	99

Jackknifed classification matrix (Leave-one-out Cross-validation)

CLUSTER	1	2	3	%correct
1	34	1	0	97
2	0	61	0	100
3	0	1	42	98
Total	34	63	42	99

LDA findings:

1) The LDA factor plot strongly reproduces the geometry of the 10,000-bootstrap raw-scaled Kmeans (note that the colors assigned are arbitrary). This shows that factor 1 loads mainly on *F* and factor 2 on *D*.

2) The three K-mean clusters almost perfectly (99%) separate the raw data, and leave-one-out cross-validation does not affect any of the classifications (i.e., not sensitive to individual data points in determining the discriminant function).

With respect to performing LDA using the Kmeans-assigned cluster membership, using “intrinsic spiking pattern, morphology, plasticity, etc as predictors:” these are properties of the *neurons*, rather than the *connections* between neurons. An alternate idea would be to extract physiological parameters from the EPSP response over the longer time window (**Fig. 5b**) in addition to short term (*DFUA*). Unfortunately, for only a subset of the 139 connections do we have the long-term physiological measures. Further, this would be using *DFUA* to predict longer-term dynamics (e.g., SA), which was not an objective of the study.