

Correlated neuronal variability in monkey visual cortex revealed by a multi-microelectrode*

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Summary. Recordings from the visual cortex of anaesthetized monkeys taken with a 30-fold multi-microelectrode demonstrate that the neuronal variability, defined as the change in response strength over time spans of a few seconds to several minutes, is highly correlated within *groups of neurones*. Several such groups exhibiting *independent variability between groups*, coexist within the area recorded. This within-group covariance suggests that a major part of neuronal variability is due not to a noise process in the cells, but rather to additional inputs to the neurones, which are not under control of the experimenter.

Key words: Neuronal variability – Visual cortex – Monkey – Multi-microelectrode

Introduction

The striate cortex of the monkey receives its major neuronal input from the lateral geniculate nucleus. However, fibres originating in other structures should not be neglected: according to Doty (1980) they amount to about 30% of the total afferents. Signals from such sources would normally be interpreted as “neuronal variability”, since evoked responses are only in part reproducible. Genuine neuronal noise may be an additional source of response fluctuation.

It remains unclear to what extent these two sources contribute to neuronal response variability. The concept of the “unreliable neurone” (McCulloch

and Pitts 1943; Burns 1968) contradicts the view of other authors (Bullock 1970; Rosen 1973), who stressed the importance of additional inputs to the neurones which are not under experimental control. Theoretical models (Stein 1967; Sherry and Klemm 1980) and experimental studies (Werner and Mountcastle 1963; Schiller et al. 1976; Rose 1979; Holden and Ramadan 1980; Levine 1980) could not satisfactorily solve the issue.

In the framework of an investigation of a small cortical volume (roughly the size of a hypercolumn or “module”) in monkey striate cortex using a multi-microelectrode, we applied a variety of stimuli and data analysis techniques. Here we present those results which are relevant to the study of neuronal variability.

Methods

Eight adult vervet monkeys (*Cercopithecus aethiops*) were initially anaesthetized with Ketanest (1 ml i.m.; Parke-Davis, München), prospective wounds were infiltrated with Meaverin (2%, ICN, Eschwege), and the auditory canals were anaesthetized with Xylocain spray (Pharma-Stern, Wedel). After tracheotomy the animals respired a 75/25% N₂/O₂ mixture. This was supplemented by 0.5–1.5% Fluothane (ICI, Plankstadt) during surgery and, if necessary, by 0.1–0.5% during recording. Muscular relaxation was obtained by continuous infusion of 20 mg/h gallamine-triethiodide (Flaxedil, Specia, Paris) supplemented by 0.3 mg/h Alloferin (Hoffmann-La Roche, Grenzach) and 0.5 ml Laevulose. The level of expired CO₂, ECG, body temperature and EEG were continuously monitored. Corneas were protected with contact lenses of appropriate curvature and refraction. Artificial pupils of 1 mm diameter were used. A backprojection screen was located at a distance of 1.14 m.

The multi-microelectrode (Krüger and Bach 1981) consisted of 30 glass-coated (30 µm diameter) platinum-iridium wires (5 µm diameter); resistances ranged from 1 to 3 MΩ. The electrodes were positioned parallel to each other at a distance of 160 µm apart and formed a 5 × 6 array. The skull and dura were opened and the multi-microelectrode was introduced into area 17, at 3–10 mm caudally from the lunatic sulcus and about 5–10 mm above its lower

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tip. Spikes were inspected on oscilloscope screens and trigger levels of 30 independent conventional amplification channels were manually adjusted. Triggered events were recorded with a computer (Interdata) and stored on disc with 1 ms resolution.

During stimulation, spike activity on each channel was classified as follows: single units, mixtures with a dominant spike which could not be isolated with certainty, multiunit activity, and no activity. In the present study, all types of neuronal activity were used indiscriminately. Multielectrode recordings were taken at 5 (in 2 animals), 4 (in 5 animals) and 2 (in 1 animal) depth levels with roughly equal spacing in the cortical grey matter. Depth readings obtained from marks on one electrode, together with electrolytic lesions at the lowest level, permitted the histological reconstruction of recording loci.

When the multielectrode was inserted into the cortex, such receptive field properties as ocular dominance, orientation preference and colour specificity (Krüger and Bach 1981, 1982; Bach 1981; Krüger and Fischer 1983) could be recorded after an initial period of silence lasting about 15 min.

Receptive fields from all neurones were largely superimposed. They were located at about 1–3° from the centre of the fovea. Neurones were stimulated simultaneously by oriented slits, visual-noise patterns, vertical slits at variable binocular disparity, large coloured fields and small rectangular patterns displaced within the receptive fields. Stimuli were presented in cycles (trials) encompassing all relevant parameter values (e.g. orientations from 0° to 180°). The durations of trials ranged from 4 to 40 s; they were repeated 12–30 times. Stimuli were applied binocularly in a minority of cases; usually each eye was examined separately.

To gain insight into direct synaptic connections or common input of the recorded neurones (Perkel et al. 1967; Moore et al. 1970), short-term cross-correlograms with a binwidth of 1 ms and 11 ms between the spike trains of all possible pairs of neurones were computed and visually inspected for significant deviations from the expected value.

As a simple measure of variability the total number of spikes per trial ("SPT") was accumulated for every cell. The values of the SPT to repeated trials was defined as the "variability curve". The variability curve may be seen as an average orthogonally to the pst-histogram. Since each trial encompassed a complete range of the parameter measured, the SPT did not depend on stimulus specificity. After visual inspection, the variability curves were subjected to correlation analysis, factor (principal components) analysis (Überla 1968; Cooley and Lohnes 1971) and cluster analysis (Everitt 1974; Rollet and Bartram 1976).

Results

The central finding of this study is exemplified in Fig. 1: responses from four simultaneously recorded cells are depicted as dot rasters, post-stimulus-time (pst)-histograms and variability-curves (right). The neurones were stimulated simultaneously by white, oriented slits (0.11°×3°) moving at 2°/s sequentially back and forth at 8 different orientations in 22.5° steps. One horizontal chain of dots shows the resulting spike activity in one trial; subsequent trials are displaced vertically. The pst-histograms accentuate the responses which are also visible in the dot rasters. The top cell is broadly tuned to stimulus orientation, the two middle ones are more sharply tuned and the bottom one exhibits no orientation selectivity. On

the right, the spike sum per trial (SPT) is depicted (increasing to the right). Clearly, variability is a large effect. As can be seen in Fig. 1, the variability-curves are similar for the upper two and the bottom two cells respectively; the correlation coefficient between them exceeds the 1% significance level. The two pairs shown are representative for two different groups of neurones, each containing about 5 strongly covarying neurones (not shown in Fig. 1).

Contrarily, the majority of short-term cross-correlograms did not show any significant structure which would have been characteristic for synaptic interconnections involving few neurones, either for strongly covarying or for uncorrelated pairs of neurones. Neurones with strong common variability, however, displayed a slight enhancement of the cross-correlation coefficient above the expected value (shift-predictor corrected, see Perkel et al. 1967) over a time interval of about ± 30 ms. For further analysis, the correlation coefficients of variability-curves are inconvenient, because for each recording situation, 435 pairs of electrodes have to be considered. To reduce these data, the variability-curves were subjected to a factor-analysis: one tries to approximate the experimental data by a linear superposition of a minimal number of components (factors). The result of this operation can be presented in the common factor space which projects the activity at the 30 electrodes into a two-dimensional plane with distance as a measure of the difference of variability. In this plot, strongly covarying neurones will be immediate neighbours. In some cases however, this projection into a plane superimposes cells which are clearly segregated in higher dimensions. We found in all six experiments that the projection into the common factor space frequently gave rise to *segregated clusters of neurones*.

An example of these results is presented in the upper part of Fig. 2, which shows the common factor space (unrotated) of the data presented in Fig. 1 and also of the activity at the remaining 26 electrodes recorded at the same time. The two factors shown account for 71% of the total variance, which is high compared to 30–40% found in simulations where random variability curves were subjected to factor analysis. This means that the major part of the variability of the activities at 30 electrodes (17 of which showed single cell activity) can be accounted for by only two or three factors. Thus, the major part of the variability is due to *extrinsic* influences common to several neurones.

As an additional and unexpected finding, we frequently observed grouping or clustering in the plot of the common factor space (note the "bubbles" in the upper part of Fig. 2, "variability clusters"). This

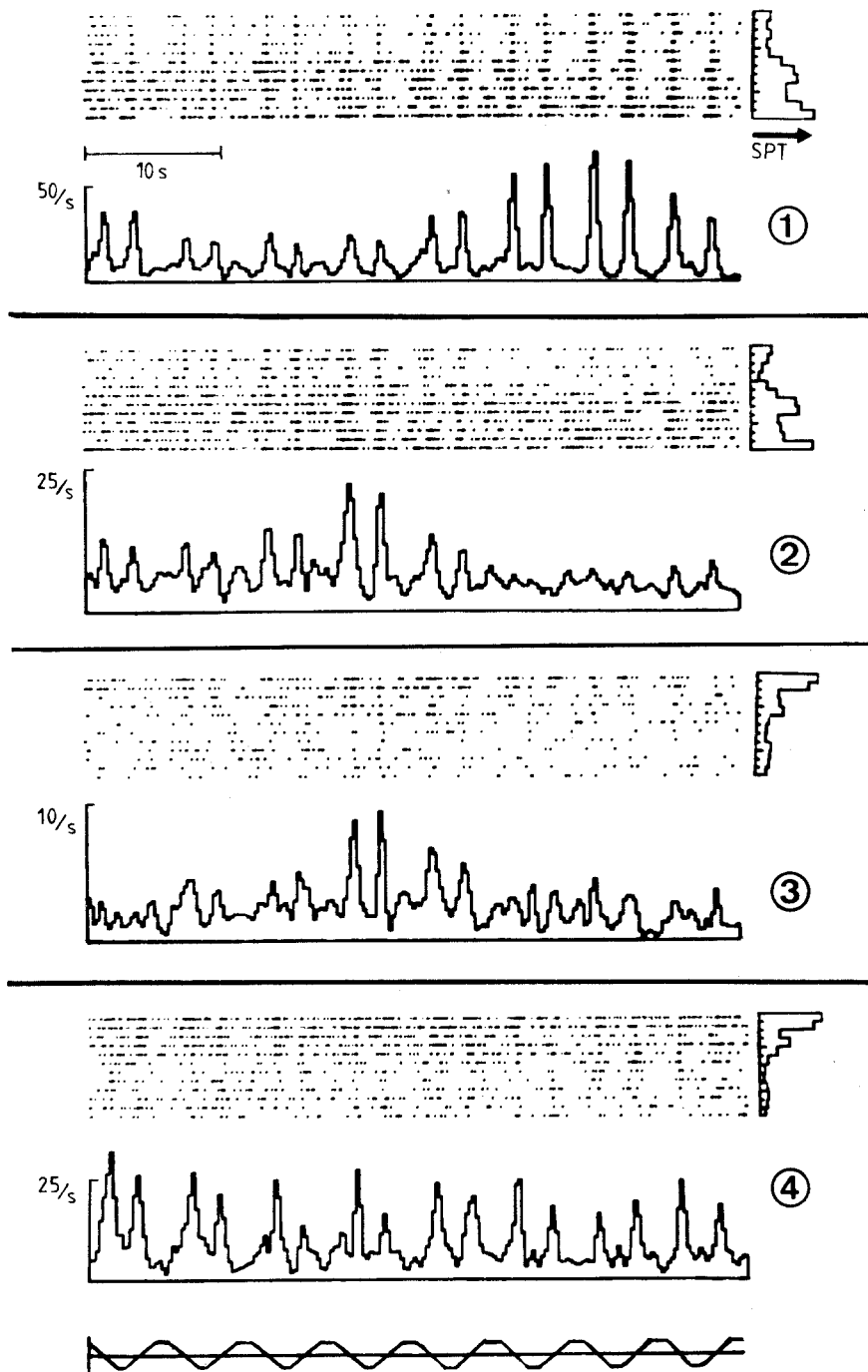


Fig. 1. Simultaneous recording from four cells in layer V/VI in A17. Bottom trace: Time course of the stimulus, an oriented slit ($0.11^\circ \times 3^\circ$) moving at $2^\circ/\text{s}$ sequentially forth and back at 8 different orientations in 22.5° increments. Above: Dot-rasters and pst-histograms show the resulting spike activity. The top three cells show orientation selectivity, which expresses itself most strongly in the pst-histograms with a broad tuning in the top cell and a sharper tuning in the middle row. Right of the dot rasters, the variability-curves depict the total spike count per trial (SPT), showing a change in response strength up to a factor of ten over a time course of 12 min. The top and bottom pair of variability-curves have very similar shapes, despite the different orientation selectivity properties

means that a similar mixture of components describes the variability of several neurones.

To interpret these variability clusters, it is important to note whether they change shape over time or show different spatial arrangements under different stimulus conditions. Thus, for an analysis of time and stimulus effects, the somewhat arbitrary assignment to different variability groups had to be replaced by a more objective method.

A cluster-analysis was therefore used to select 6 different groups of covarying neurones. The number 6 was chosen after several trials as it gave the best consistency between the intuitively judged grouping in the common factor space and the results of the cluster analysis. For the data presented in Fig. 2, the two most coherent clusters (as calculated by the cluster analysis) were obvious. The topographic distribution in the cortex of the loci corresponding to

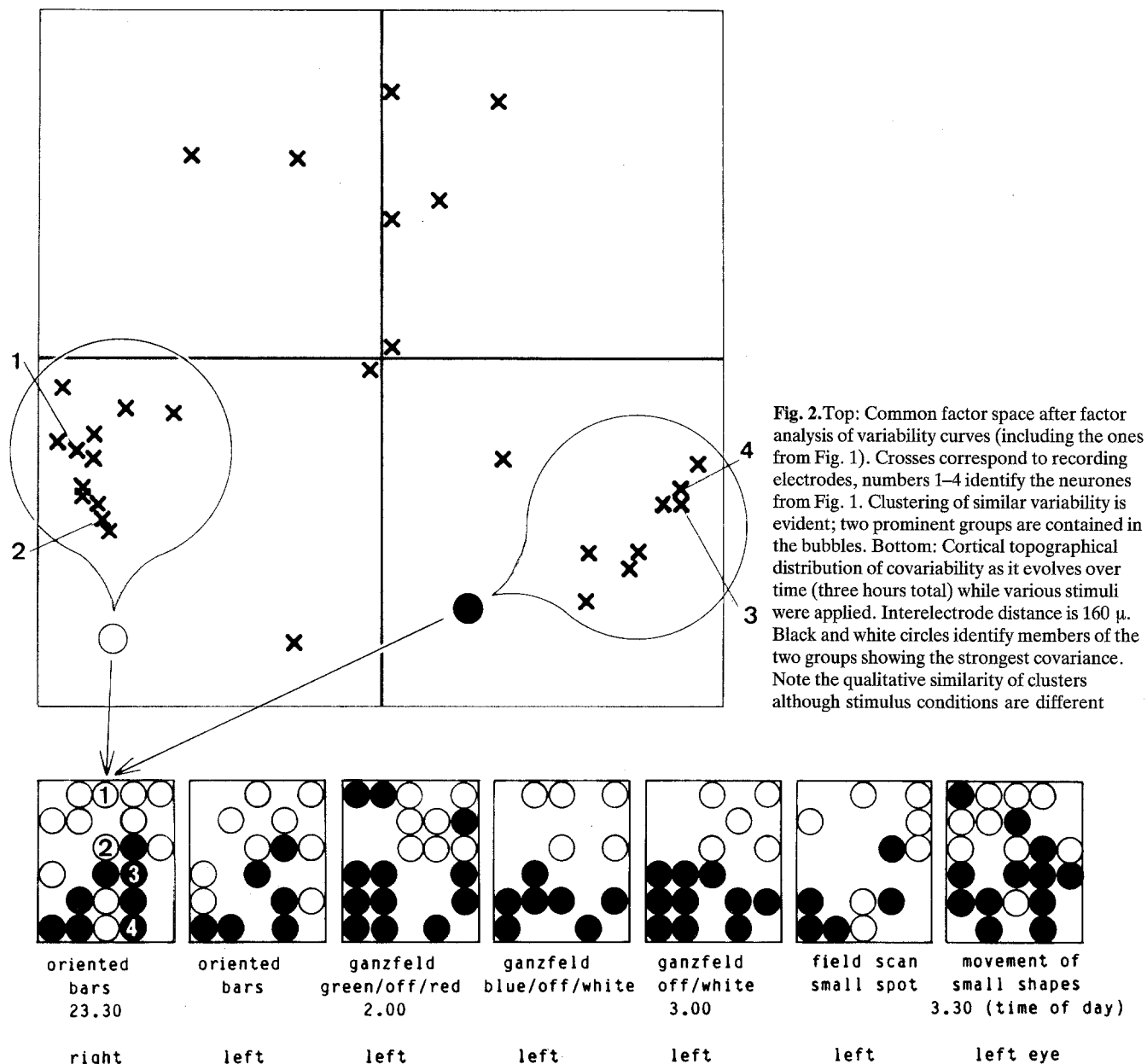


Fig. 2. Top: Common factor space after factor analysis of variability curves (including the ones from Fig. 1). Crosses correspond to recording electrodes, numbers 1-4 identify the neurones from Fig. 1. Clustering of similar variability is evident; two prominent groups are contained in the bubbles. Bottom: Cortical topographical distribution of covariability as it evolves over time (three hours total) while various stimuli were applied. Interelectrode distance is 160 μ . Black and white circles identify members of the two groups showing the strongest covariance. Note the qualitative similarity of clusters although stimulus conditions are different

the two most coherent clusters is shown in the lower left corner of the figure. The four neurones from Fig. 1 are identified by the numbers 1 to 4. As can be seen in Fig. 2, neurones exhibiting common variability are often, but not necessarily, spatially adjacent.

The spatial arrangement of the grouping was found to be fairly stationary over some hours, independent of the different stimuli applied. This can be seen by comparing the seven boxes corresponding to the topographical distribution of covarying neurones during different stimulus conditions (over a time of three hours). To assess this result quantitatively, two procedures were employed (Bach 1981):

1. The probability for any two randomly varying cells to reappear in a common group over repeated stimuli was calculated and compared with the experimental results.

2. To estimate chance effects, a Monte-Carlo simulation (Hammersley 1979) was performed, using the same procedure as applied to the experimental data, but with variability curves consisting of random data.

Both methods showed that, in spite of some fluctuations, the underlying grouping of neurones was topographically stationary in the cortex, with a probability exceeding 99%.

The topographical distribution of the neuronal clusters exhibiting common variability was compared with the spatial arrangement of such neuronal properties as ocular dominance columns and orientation columns (Krüger and Bach 1981, 1982). No obvious relationship could be found. Additionally, the variability clusters were very similar when analysis was based on evoked responses or the interleaved spontaneous activity (Bach 1981). No consistent similarity in the position of the variability-clusters was observed between sequential recordings in different cortical layers.

Discussion

We interpret the observed correlations of the neuronal variability in simultaneously recorded neurones as evidence that neuronal variability *does not result from intraneuronal noise processes*, but rather is due to some influence affecting groups of cells in common.

We believe that this is not an artifactual result on the following grounds: as the sum of the diameters of the 30 electrodes amounts to only 2.8% of the area covered by the array, we expect the damage to the cortical tissue to be not much greater than with single microelectrodes. This is supported by histological examination performed after each experiment and by the observation of well known receptive field properties. Also, there were no signs of mechanical influences, such as heartbeat, which would not give rise to several segregated clusters anyway. Generally, recordings with our multielectrode are more stable than single-electrode recording, presumably due to increased tissue adhesion.

With single electrode recording, variability is often thought to be due to a general state change of the animal, especially in acute preparations. With the multielectrode, however, the often observed grouping into several coexisting variability-clusters without a common trend towards higher or lower activity suggests, that the variability cannot be accounted for by a global factor such as the level of anaesthesia or arousal. At least such factors do not act homogeneously on the neurones from which we recorded.

Thus it is most likely, that the variabilities observed are due to neuronal input and probably reflect some ongoing processing. The entry point of this nonvisual input to the visual pathway could be the lateral geniculate nucleus or the striate cortex itself, but it cannot be in the eye (even if it were generated there), because the variability clusters frequently crossed eye-dominance boundaries.

The factor analysis showed that the activity at 30 electrodes could be explained by the linear combina-

tion of a few components. One could identify each such component with an unknown neurone branching to the observed neurones, with a synaptic efficiency proportional to the factor weights. This implies strong common monosynaptic input to co-varying neurones. However, the corresponding cross-correlogram peaks a few milliseconds wide contain only about 1% or less of the spikes recorded. Such connections are far too weak to entrain another neurone to the extent observed as variability. Instead, there is a slight enhancement of the cross-correlation above the expected value (shift-predictor-corrected) over a time span of ± 30 ms. This can be explained in two ways:

Either the recording area is innervated by a few monoaminergic fibres from the brain stem. These are supposed to deliver their transmitter to the extracellular space at loci where there is no juxtaposed pair of specialized membranes forming a synaptic cleft, but rather the transmitter reaches several postsynaptic processes by diffusion (see Bloom 1981). Strong covariability of small groups of neurones and loose temporally synchronisation among them would then be expected.

Or, on the other hand, slow activity changes in neuronal networks could give rise to the variability phenomena observed. It is known from theoretical work (e.g. Dammasch and Wagner 1984), that weakly coupled networks with many participating neurones can show complicated, spatially structured oscillations. The mainly indirect interaction between any two neurones would lead to broad synchronisation peaks in the cross-correlograms.

In any case, the spatial stability of the variability-clusters suggests an underlying anatomical structure. A mixture of several interleaved arborisation trees of modulating inputs would account for their shape and their stability – in so far as the terminal cluster maps on the variability cluster – as well as for the partial lack of stability when the arborisation trees are differently active.

We conclude, that neuronal variability is a sign of highly organized central influences on afferent visual processing. Under our artificial experimental conditions they may not be properly guided, but probably they reflect part of what the visual cortex can do under natural conditions.

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