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The Neurochrome

An Identity Preserving Representation of Activity Patterns from Neural Populations

Willem Epping, Henk van den Boogaard, Ad Aertsen*, Jos Eggermont, and Peter Johannesma

Department of Medical Physics and Biophysics, University of Nijmegen, Geert Grooteplein Noord 21, NL-6525 EZ Nijmegen, The Netherlands

Abstract. Recording of simultaneous but separated activity of neural populations overwhelms the experimenter with a large amount of information. A clearly structured display technique the "*Neurochrome*" is introduced, usable on-line and real-time. It shows neural activity patterns while preserving neural identity by employing a color code. The Neurochrome assists the experimenter in generating and verifying hypotheses about neural correlations and stimulus-event relations already during the experiment. In auditory research single neurons are characterized by their spectro-temporal sensitivity to auditory stimuli. A straightforward generalization of this concept, applicable to neural populations, is proposed leading to a global indication of a populations' activity to stimuli: the *Multi-Unit Spectro-Temporal Sensitivity*. This approach is inversely related to the Neurochrome, the latter however containing more information. The combination of both approaches seems quite powerful in the investigation of neural assemblies. The procedures are illustrated with examples of extracellular multi-unit recordings from the auditory midbrain of the grassfrog (*Rana temporaria* L.).

1 Introduction

Nowadays in a number of laboratories extracellular recordings of simultaneous activity from small neural populations are being made routinely with a single microelectrode (Eggermont et al., 1983) or with multi-electrode configurations (e.g. Krueger and Bach, 1982). These experiments aim to measure correlations between firing activity of neurons in order to reveal possible underlying neural connectivity schemes. Usually correlations are estimated by means of cross-coincidence histograms (Perkel et al., 1967) or joint occurrence scattergrams (Gerstein and Perkel, 1972; Perkel et al., 1975). These procedures discard information originally present in the experimental data because of averaging or being sensitive to only very specific firing patterns. Recently a method was described (Dayhoff and Gerstein, 1983a, b) which enables the detection of complex activity patterns in single neuron spike trains; this method, however, appears rather time consuming.

In this paper a display-technique is presented the "*Neurochrome*", containing all original information by preserving both identity of and temporal relation between neural events. Neural identity is characterized by color. Use is made of the human color-processing capability (color contrast, pattern recognition) to note associations of colored events in the plane of representation, which points to coherent activity in small neural populations. This may be indicative for the existence of *neural assemblies* (Palm, 1982; Shaw et al., 1982) or *synfire chains* (Abeles, 1982). The procedure can be used on-line and real-time, thus enabling multi-unit experiments of high efficiency with respect to the search for populations exhibiting correlated activity as well as to presentation of adequate sensory stimuli.

Studying sensory systems often one or more stimulus parameters are varied, be it systematically or (pseudo-)randomly. By rearranging the original Neurochrome according to these parameters, e.g. tonal frequency in auditory research, a second representation emerges showing possible stimulus-dependencies of neural firing patterns. The second representation of the Neurochrome, with frequency as variable, is closely related to the concept of Spectro-Temporal Sensitivity: STS (Aertsen et al., 1980; Hermes et al., 1982), defined for single neurons. This is extended to multi-neuron networks, showing their combined sensitivity area in spectro-temporal space by

* *Present address:* Department of Physiology G4, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

superimposing individual STS's. The outcome is the *Multi-Unit Spectro-Temporal Sensitivity:* MUSTS. Whereas the Neurochrome is a *forward experimenter oriented* approach the MUSTS is a *backward* one, reflecting the *brain's view* of the outside world.

2 Methods

2.1 Preparation and Recording

Recordings were obtained from the auditory midbrain (torus semicircularis) in the immobilized (Buscopan) and locally anaesthetized grassfrog (*Rana temporaria* L.) using single metal microelectrodes (tungsten or stainless steel, Bak Electronics) with an exposed tip of about 10 µm and a 1 kHz impedance in the 1–3 MΩ range. The multi-unit spike-trains were separated into the component single-unit spike-trains on basis of spike waveform by using an on-line matched filter approach (e.g. Abeles and Goldstein, 1977) and interactive setting of ellipsoidal cluster boundaries. For details of physiological preparation, stimulus presentation, data registration and separation procedures the reader is referred to Eggermont et al. (1983).

2.2 Chromatic Representation

The Neurochrome is displayed on a Ramtek RM 9300 colorgraphics system, coupled to a general purpose PDP 11/45 computer. The resolution is 640 pixels in horizontal direction and 512 pixels in vertical direction. Each pixel has 6 bits color information leading to 64 simultaneously displayable colors, selectable from a palet of 4096 colors. The color key was chosen to be of equal luminance and given this restriction the perceptual distances between the colors should be as large as possible. Therefore we selected the three principal colors: red, green, blue and their complements: magenta (purple), yellow and cyan (blue/green) located in between the former three in perceptual space, all colors being maximally saturated. For on-line assistance during experiments a Ramtek RM 6211 color-graphics terminal attached to a PDP 11/34 in use for data acquisition is available.

3 Representation of Multi-Unit Activity

3.1 The Neurochrome

The experimental datarecord, containing neural activity from a small population usually is divided into segments with duration T seconds. The segmentduration can be set equal to e.g. duration of period in case of a periodic stimulus, or the interval between successive tones. The *first representation* of the Neurochrome, useful under stimulus conditions as well as non-stimulus conditions, is a 2-dimensional image, color indicating neural origin. In this type of Neurochrome the k^{th} line displays the k^{th} data segment: a so called "lexicographic" dotdisplay, analogous to the lines on a page of text. In this way possible overall alterations of population characteristics during the experiment can be revealed.

In the *second representation* of the Neurochrome the lines are rearranged according to a varying stimulus parameter, expressing stimulus influences on e.g. latency, structure of neural firing patterns. In the case of an auditory stimulus consisting of tonepips of varying frequencies applied at regular intervals each line gives the population's response to a tonepip of a particular frequency, time after tonepip onset running from left to right.

3.2 Multi-Unit Spectro-Temporal Sensitivity

The Spectro-Temporal Sensitivity (STS) as defined for single neurons (Aertsen et al., 1980; Hermes et al., 1982) is an average functional on the *pre-event stimulus ensemble*, giving the combined spectro-temporal structure of stimuli preceding the occurrence of action potentials. A straightforward generalization of this concept, applicable to neural populations is the superposition of the individual STS's, showing the sensitivity of the entire population in spectro-temporal domain; the same color identification as in case of the Neurochrome is employed. No use is made of number of spikes nor of detailed structure in firing patterns: crosscorrelations among neural activity of the contributing neurons and autocorrelations within separate spike-trains are not considered. The name Multi-Unit Spectro-Temporal Sensitivity (MUSTS) is proposed.

4 Results

The procedures presented in this paper are illustrated with three multi-unit recordings from the auditory midbrain of the grassfrog. For reasons of paucity only experiments under identical stimulus conditions, apart from a different localization of sound source, are shown. The stimulus ensemble lasts 508 s and consists of tonepips with a duration of 48 ms, intervals of 1 s between onset of tonepips and intensity of 90 dB SPL. The frequency of the tonepips was varied in pseudo-random order and was drawn from a uniform distribution (on log-scale) between 125 and 2000 Hz. The first column of Fig. 1 shows the original Neurochromes, the second column the Neurochromes rearranged according to tonal frequency and the third column the MUSTS.

In the first row of Fig. 1 results are expressed of *recording 216-1*, which contains three units coded in red, green and blue, respectively. No trends in their firing activity throughout the experiment were observed (Fig. 1a). Unit 1 (red) has the shortest latency: 10 ms and is spontaneously active; unit 2 (green) has a latency of 32 ms; unit 3 (blue) with a latency of 26 ms shows a prolonged activation period of over 300 ms after a tonepip; units 2 and 3 are hardly spontaneously active. Unit 1 is broadly tuned over the entire frequency range (Fig. 1b); unit 2 is excited mainly in the 250–2000 Hz range like unit 3, the latter being particularly sensitive to frequencies above 900 Hz indicating a strong input from the basilar papillae. Note that

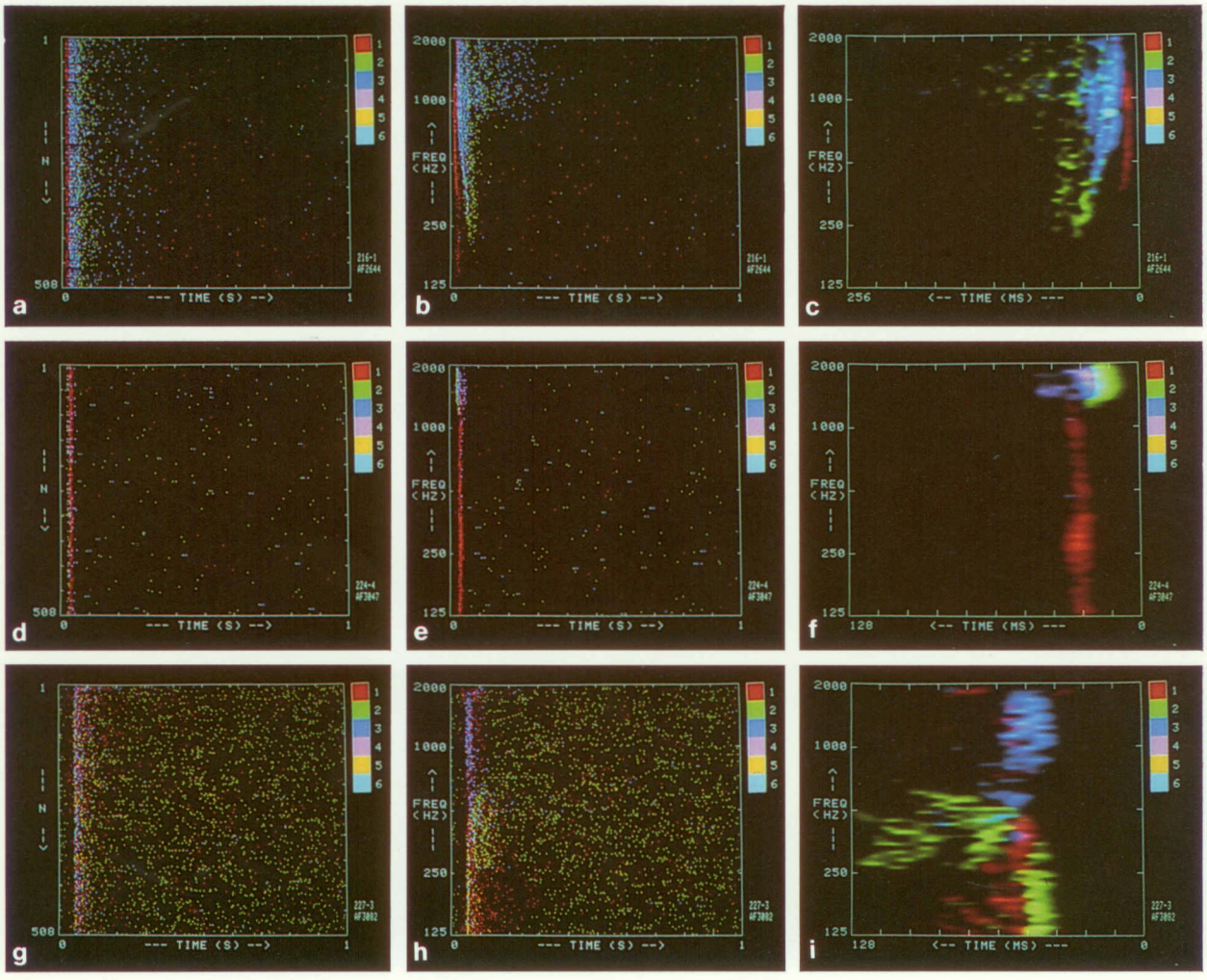

Fig. 1a–i. Representation of event-event and stimulus-event relations of multi-unit recordings. Units are identified according to the color key placed at the right of the figures. In the first column original Neurochromes are displayed, giving the exact timing relationships between spikes, apart from a segmentation in lines. The horizontal axis lasts 1 s, being the interval between onset of tonepips, and represents time after a tonepip running from left to right. The vertical axis gives the index of lines. Rearranged Neurochromes appear in the second column, lines are reordered according to frequency of tonepips; note the logarithmic frequency scale. In the third column Multi-Unit Spectro-Temporal Sensitivities are shown. The horizontal axis now represents time before a neural event running from right to left. The vertical axis is the same as in the second column. Results of multi-unit recording 216-1 containing three units are shown in the first row. Unit 1 has 528 spikes, unit 2 565 spikes and unit 3 933 spikes. Second row: recording 224-4 with four units; unit 1 519 spikes, unit 2 237 spikes, unit 3 88 spikes and unit 4 73 spikes. Third row: recording 227-3 with three units; unit 1 1119 spikes, unit 2 2244 spikes and unit 3 278 spikes. In case of recordings 216-1 and 227-3 the stimulus was applied to both ears, whereas recording 224-4 was made under contra-lateral stimulus presentation

latency of units 1 and 3 is frequency dependent. The spectro-temporal sensitivities are displayed in Fig. 1c: because of the chromatic display procedure only the more pronounced sensitivity areas can be discerned resulting in somewhat restricted areas as compared to Fig. 1b. The broadly tuned unit 1 is most effectively stimulated by tonepips in the midfrequency range 350–1200 Hz as could already be concluded from Fig. 1b, for unit 1 has the shortest latency in that frequency range. Moreover unit 1 expresses limited temporal spread as compared to the longer latency units 2 and 3, the latter having more diffused spectro-temporal sensitivities especially for frequencies above 1000 Hz.

Recording 224-4 containing four units is represented in the second row of Fig. 1. From Fig. 1d a strong locking to the stimulus appears for all units with latencies in the 20–30 ms range, albeit that unit 1 (red) obscures spikes of the other units in the initial activity band because of its strong responsiveness. The units are spontaneously active in varying degrees. Furthermore a clear multi-unit activity pattern can be discerned notably in spontaneous activity: green-blue, green-blue-blue and green-blue-purple, pointing to coherent activity of units 2, 3, and 4. The rearranged Neurochrome (Fig. 1e) reveals a large similarity of properties of units 2, 3, and 4 being excited in the 1200–2000 Hz range, in which the green-blue-purple pattern can also be distinguished now. Moreover the activity of units 2, 3, and 4 is suppressed after the initial activity band for frequencies above 500 Hz, including a frequency band where no initial excitation occurred. Correlation analysis corroborates the relationship between units 2, 3, and 4, and establishes that the activity pattern is not entirely due to stimulus influences. As an example crosscoincidence histograms between units 2 and 3 are shown in Fig. 2. A preference for unit 3 firing about 6 ms after unit 2 can be discerned. The difference between both histograms points to the existence of a neural pathway, be it direct or common, between units 2 and 3 (for details see Perkel et al., 1967; Eggermont et al., 1983). Unit 1 is complementary to the other units in the sense that its frequency sensitivity lies in the 125–1200 Hz range. Correlation analysis does not indicate a relation of unit 1 with the other units apart from common stimulus influences. Spectro-temporal sensitivities of the units are shown in Fig. 1f, substantiating former observations.

In the third row of Fig. 1 *recording 227-3* containing three units is represented. From Fig. 1g it appears that the units have latencies greater than 50 ms and that spontaneous activity of units 1 and 2 is substantial. Occasionally purple dots can be observed due to coincidence of spikes of units 1 (red) and 3 (blue) (see discussion). Unit 1 is broadly tuned (Fig. 1h) with a

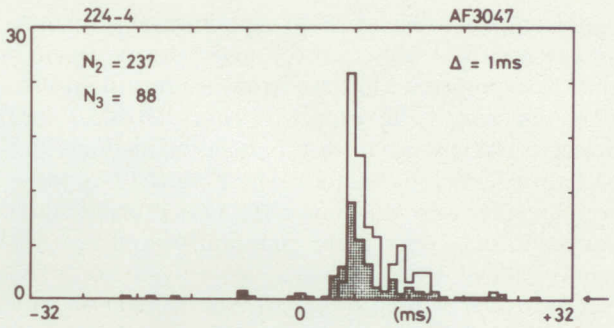

Fig. 2. Simultaneous and nonsimultaneous crosscoincidence histograms of unit 2 (green) and unit 3 (blue) of multi-unit recording 224-4. The nonsimultaneous (shaded) histogram is obtained by shifting one spike-train over one or more stimulus periods with respect to the other spiketrain. The vertical axis represents number of coincidences per bin, binwidth Δ is 1 ms. The arrow indicates the expected number of coincidences in case of uncorrelated spike-trains

preference to frequencies below 600 Hz. The same holds for unit 2, note that its activity is prolonged in the 300–600 Hz range but suppressed in the 600–2000 Hz range and after an initial activation band also in the 125–300 Hz frequency range. Unit 3 is only sensitive to frequencies above 500 Hz. Correlation analysis points to weak excitatory influence of units 2 and 3 on unit 1. Spectro-temporal properties are clearly expressed in Fig. 1i; whereas unit 2 leads with respect to unit 1 in the 125–300 Hz range, their roles are changed for frequencies above 300 Hz.

5 Discussion

In previous studies of the auditory midbrain of the grassfrog it was demonstrated that this center can hardly be understood on basis of the single-unit approach. A substantial fraction of neurons exhibits STS's under various stimulus conditions, which cannot be brought in line with one another (Aertsen and Johannesma, 1981; Johannesma and Eggermont, 1983). Likewise crosscoincidence histograms of simultaneously recorded activity from pairs of neurons differ under altered stimulus conditions (Eggermont et al., 1983). It is hypothetized that simultaneous recording of activity of a neural population, subsequent separation into single-unit spike-trains, correlating the spike-trains with one another as well as with stimulus can clarify underlying neural connectivity schemes and will contribute to an understanding of the brain. The minimum size of the population, that has to be recorded from, will depend on specific questions posed and on the part of the brain under investigation; this is still a matter of debate (e.g. Shaw et al., 1982).

Both representations of the Neurochrome are constructed real-time during the experiment, offering the

opportunity of influencing the experimental procedure interactively and thus to investigate possible significance of hypotheses while the neurons are still on-line. This can hardly be expected from statistical and timeconsuming procedures as correlation methods and pattern detecting techniques. These procedures, however, together with the Neurochrome as possible starting point, may offer more quantitative and detailed results allowing conclusions to be stricter. The Neurochrome as used by us permits the analysis of a population of six neurons at most. This number can be extended by the addition of extra colors, however, at the expense of reduced discrimination of events from different neural origin. The applicability of the Neurochrome is limited by spike-density: when in the 2-dimensional image dots overlap additive colors, which may hard to be distinguished from the original colors, are generated and perceived. This problem can be circumvented by displaying the Neurochrome in parts.

To investigate spectro-temporal properties of neural populations the MUSTS was proposed as a first attempt, showing global sensitivity areas. Taking seriously the possibility that the brain codes and transforms at least a part of incoming and outgoing information by highly structured firing patterns of cooperating cell assemblies [this type of coding is called ensemble correlation (Sejnowski, 1976)], the information content of the original datarecords should be much richer than expressed in the MUSTS. One way to exploit this information more exhaustively is through detection of significant neural activity patterns, e.g. on basis of the Neurochrome, and using these as "superevents" in the construction of a higher level pre-event stimulus ensemble in order to come to a more coherent spectro-temporal measure. By applying these ideas, in combination with a rich stimulus repertoire, to multi-unit experiments it may be concluded whether firing patterns of populations are more robust to stimulus and/or environmental alterations than single-neuron activity.

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W. J. M. Epping
Dept. of Medical Physics and Biophysics
University of Nijmegen
Geert Grooteplein Noord 21
NL-6525 EZ Nijmegen
The Netherlands