

Neural Representation of the Acoustic Biotope: On the Existence of Stimulus-Event Relations for Sensory Neurons

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Abstract. In the context of a behaviourally oriented investigation of neural information processing we have studied single unit responses from cochlear nucleus (12 units) and auditory cortex (23 units) of the cat. Attention was focused on two aspects: the composition of a wide stimulus ensemble and the influence of this ensemble on the single unit neural activity: action potentials or neural events. The stimulus ensemble was based on sounds occurring in the natural environment of the animal: the *Acoustic Biotope*. A realization of such an ensemble was constructed and used in the experiments. A systematic analysis of the effect of this stimulus ensemble on the neural activity comprises different questions with respect to a possible stimulus-event relation: existence and nature. In the present paper emphasis is given to the question of *existence* of a $s-e$ relation. The existence of such a relation has been investigated by presenting the stimulus ensemble twice. The degree of reproducibility of neural activity indicates to what extent a $s-e$ relation indeed exists. A formal measure of testing the reproducibility of two sequences of neural events is defined: the *Cross Coincidence Function*. Results are presented, interpretation and formal problems discussed. Results obtained so far indicate that on the whole the existence of a $s-e$ relation can be established more definitely for cochlear nucleus units than for auditory cortex units. Applications of the presented procedure arise in exploratory investigations in central regions of the sensory nervous system and in regions with presumed multimodal inputs.

1. Introduction

The study of sensory neural systems has in recent years attracted considerable attention from work inspired by

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nonlinear system theory as outlined by Volterra and Wiener. This theory, applicable to a wide class of nonlinear systems, states that such a system can be characterized by a set of different order kernels. The determination of these Wiener kernels forms a major problem in the Wiener theory. A relatively simple method of measuring these kernels was presented by Lee and Schetzen (1965).

This method, based on crosscorrelation techniques, is an extension of the crosscorrelation method as applied in linear system theory (Lee, 1960). In this context Gaussian wideband noise plays a central rôle in the composition of an adequate stimulus ensemble.

Although differing in actual terminology and methods, various studies on the visual system (Marmarelis and Naka, 1973; Marmarelis and McCann, 1973; Von Seelen and Hoffman, 1976) and the auditory system (De Boer and Kuyper, 1968; Johannesma, 1972; Grashuis, 1974; Van Gisbergen et al., 1975b; De Boer and De Jongh, 1978) all trace back to this same methodological approach.

In this same period the question emerged whether the full capacity of the sensory processor "may not be revealed except through study of its response to stimuli that pose analytical tasks of the kind that shaped its evolutionary development" (Worden and Galambos, 1972). This suggests that the criteria for an adequate composition of the stimulus ensemble may well be deduced from the study of animal behaviour in the natural environment i.e. ethology and ecology.

In the study of the auditory nervous system this leads to a stimulus ensemble based on natural sounds. Recent reviews of this line in auditory research are given by Worden and Galambos (1972) and Bullock (1977). Results obtained so far indicate that responses of peripheral neurons (up to the ventral cochlear nucleus) to complex, natural stimuli can be relatively simply predicted from responses to technical stimuli (Evans, 1975). This clearly does not hold for neurons in

more central regions like the auditory cortex (Goldstein and Abeles, 1975; Newman, 1977). This suggests that especially for central auditory regions the use of an ethologically based stimulus ensemble is indispensable in the investigation of neural functioning (Scheich, 1977; Newman, 1977).

The present work forms part of an investigation of the auditory nervous system of the cat. Extracellular single unit recordings are made from both a relatively peripheral and a central part in the auditory system: the cochlear nucleus and the auditory cortex. The stimulus ensemble consists of a wide selection of natural sounds together with technical sounds. The analysis of stimulus-response relations is, in its methodological aspects, inspired by nonlinear system theory as indicated before. The *Pre Event Stimulus Ensemble* (PESE) is defined as the set of stimuli preceding a neural event: the occurrence of an action potential. As a consequence the PESE forms a subset of the *Stimulus Ensemble* (SE). The basic idea is that the characteristics of the PESE, as compared to those of the SE provide a functional description of the neuron's rôle in the representation of acoustic stimuli (Johannesma, 1972; Grashuis, 1974).

The analysis of a stimulus-response or stimulus-event relation logically starts with the question whether such a relation exists for the particular neuron from which a recording is made. In a number of cases the existence of a $s-e$ relation is fairly easily demonstrated by a significant change in the neuronal firing pattern, related to the stimulus presentation. However, in case a wide, ethologically based stimulus ensemble is used it often proves difficult or even impossible, just employing simple means (visual or aural inspection of the data), to demonstrate this change unambiguously. Several reasons may account for this problem, which arises in the case of cochlear nucleus units as well as auditory cortex units, be it that it occurs more frequently in the latter case. It may be due to the complex structure of the stimulus ensemble, which holds for both peripheral and central neurons. Furthermore the neural functional properties are involved. These are known to be more complex in central regions than in the periphery (Newman, 1977).

Thus the question of the existence of a $s-e$ relation proves to be a problem, especially when neural activity is recorded from central brain areas which are involved in a complex mode of sensory information processing. As already pointed out, particularly these regions appear to be the natural candidates for an investigation with an ethologically inspired stimulus ensemble. Therefore the question of existence of a stimulus-event relation deserves some special attention. The elaboration of this question forms the principal objective of this paper.

A behaviourally oriented stimulus ensemble comprises many different stimuli and as a consequence has a long duration. The stochastic nature of the neural activity suggests many repetitions of the same experiment. On the other hand the recording time from one neuron is limited ($\sim 10-100$ min). This constraint implies an optimization to be made between both diversity and precision of information to be gained. In this paper the attention is concentrated on the analysis of neural function using a wide ensemble of ethological stimuli, where the number of presentations is limited to the minimum of two.

2. The Acoustic Biotope: An Ethologically Based Stimulus Ensemble

The natural environment in which an animal lives, as far as functionally related to the animal, is defined as its habitat or biotope (Dahl, 1921). The biotope encompasses both the biotic and abiotic environment (Odum, 1971). Important characteristics of the biotope are its geophysical properties, climate regime and variety and numbers of occurring animals and plants. The *Sensory Biotope* may be defined as the total of sensory aspects of the biotope. It consists of different components, each one corresponding to a specific sensory modality of the animal under investigation (visual, auditory, tactile etc.). Since our work is concerned with the auditory nervous system the attention is focused on the acoustical component of the sensory biotope: the *Acoustic Biotope* (Smolders et al., in preparation). A more formal definition of the Acoustic Biotope as a probabilistic generator of stimulus ensembles as particular realizations, together with possible ways to characterize the Acoustic Biotope will be presented elsewhere (Aertsen et al., in preparation).

For the present investigation a realization of a wide stimulus ensemble, inspired by ethological and ecological observations was made.

This realization was not restricted to (species specific) vocalizations nor was it designed in view of remarkable behavioural functions and/or neural structures. The stimulus ensemble was composed of a wide selection of sounds recorded from the natural environment of the cat. Furthermore it included technical sounds, in order to be able to relate results obtained with this stimulus ensemble to the outcome of previous investigations (Grashuis, 1974; Van Gisbergen et al., 1975a-c). The realization was composed according to the scheme as shown in Diagram 1 (Smolders et al., in preparation).

The stimulus ensemble consisted of 64 different sound segments, each one with a duration of 8-9 s. The figures in Diagram 1 specify the number of sound segments taken from different categories. This classifi-

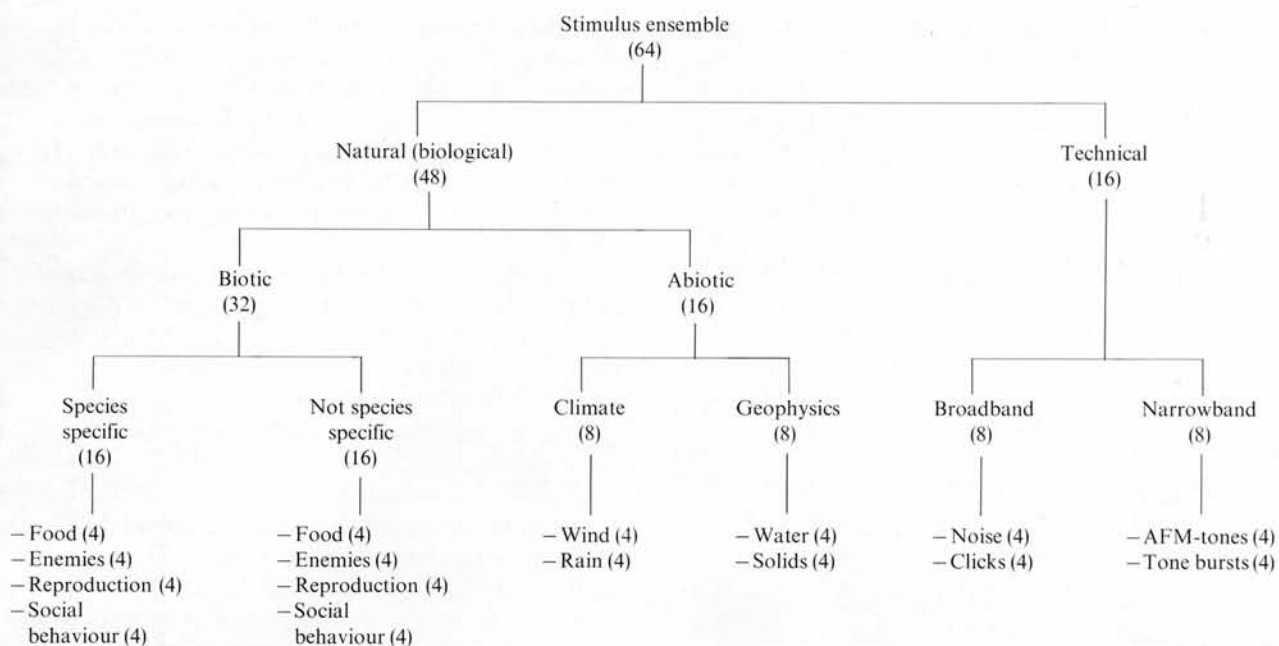


Diagram 1. Construction scheme for realization of a stimulus ensemble inspired by the Acoustic Biotope of the cat. (From Smolders et al., in preparation)

cation of sounds was based on biological or technical characteristics, depending on the nature (biotopic or synthetic) of these sounds. The 64 sound segments were concatenated in a random order on analog tape, thus constituting a stimulus ensemble lasting about 9 min, its bandwidth being 20 kHz. In the following this realization will be denoted briefly as "stimulus ensemble" or "acoustic biotope". It should be born in mind, however, that it is a particular realization of the Acoustic Biotope, with additional technical sounds included in it.

3. Methods

Preparation, Recording, and Histological Procedures

Nine adult cats weighing 1.8–2.5 kg were used. Extracellular single unit recordings were made in the cochlear nucleus and the auditory cortex. The cochlear nucleus results were obtained from cats under nembutal anaesthesia. The methods used for the preparation of the animal, recording of single unit activity and histological control of the anatomical location of neurons were the same as have been described in detail by Van Gisbergen et al. (1975a). Auditory cortex experiments were performed on semichronic preparations. Only a brief outline of the preparation is given here.

A few weeks before the actual recordings began the cat was anaesthetized and a hole was drilled into the skull under stereotaxic control. A ring-shaped chamber

was screwed and cemented onto the skull around the exposed brain area. The dura remained intact. The chamber could be closed. A small holder was screwed onto the skull overlying the frontal sinus, it served for later pain-free fixation of the head to the stereotaxic apparatus in the actual experiment. The preparation was performed aseptically. The animal was then allowed to recover during a period of at least one week. In the actual experiment the cat was premedicated with atropine and immobilized. (Flaxedil was administered intramuscularly in an initial dose of 5 mg/kg and subsequent injections of 2.5 mg/kg at the first sign of muscular contractions.) Xylocain ointment was applied to the eyes to prevent them from drying.

Artificial respiration was performed using a 30% O₂–70% N₂O gas mixture to obtain an end tidal CO₂-percentage of about 3.8. Body temperature was kept at 37–38 °C.

By means of a remotely controlled microstepdriver tungsten microelectrodes (typical impedance 3 MΩ at 1000 Hz) were driven into the brain.

In order to improve recording stability the chamber on the skull was filled with an agar solution.

Stimulation and Data Collection

Experiments were performed with the cat in an electrically shielded acoustic chamber (IAC type 1202 A). The main part of the stimulus ensemble consisted of natural sounds, recorded on analog tape (recorder Uher 4200, tape speed 19 cm/s, microphones

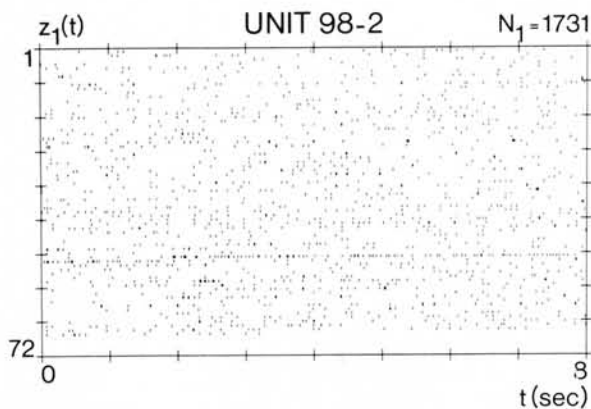


Fig. 1. Dot display representing neural activity $z_1(t)$ recorded from unit 98-2 (auditory cortex) during presentation of the acoustic biotope

Sennheiser MD 211 N). Technical sounds were generated by standard electronic equipment.

A stimulus tape, composed of these sounds, was played back to the animal from an instrumentation recorder (Honeywell 7600, FM, tape speed 60 inch/s, bandwidth 20 kHz). The stimulus was amplified (Luxman 507 X) and transduced by two microphones (Sennheiser MD 211 N) mounted near the cat's pinnae (open sound system). Sound pressure levels were measured using a half inch condenser microphone (Bruel and Kjaer 4134) mounted near the cat's pinnae. Average intensities of the stimuli near the cat's ears were in the order of 50–70 dB SPL with considerable loss at low frequencies (up to some 20 dB loss at 500 Hz) due to the sound system being open.

Neural activity was amplified (Grass P 16) and recorded on the stimulus delivering instrumentation recorder. Furthermore it was transformed into unitary pulses by means of a level discriminator and fed, together with a synchronizing clock signal from the stimulus recorder, to the laboratory's computer system (time resolution 100 μ s).

The calculations, involved in the analysis as will be described, were carried out on the following configuration: central processor PDP 11/45, additional floating point processor, operating system RSX 11-D, memory 160 Kbytes core, disks RK 05, software Fortran IV+. The analysis of two neural recordings both containing some 2000 events (example discussed in Sect. 4) takes about 100 s.

4. Existence of Stimulus-Event Relations

Because of the all-or-none character of action potentials, single unit activity as measured during presentation of the stimulus ensemble can be modelled as

a stochastic point process:

$$z_1(t) = \sum_{i=1}^{N_1} \delta(t-t_i) \quad (0 \leq t \leq T) \quad (1)$$

with $\delta(t)$ = Dirac delta function, $\{t_i\}$ = moments of occurrence of action potentials, N_1 = total number of action potentials, measured during the experiment (duration T).

A graphical representation of $z_1(t)$ for an auditory cortex unit is shown in the dot display in Fig. 1. Each dot represents a neural event. The dot display is composed analogously to a page of text. Each line corresponds to a time interval of 8 s.

The acoustic biotope forms a long and complex stimulus ensemble. The neuron under investigation produces at the same time a long and usually complex sequence of neural events. In this situation the characteristics of the stimulus-event relation, portraying the influence of the sensory stimulus on the neural activity, cannot be found from direct visual inspection of the experimental data as shown in the dot display. A systematic analysis of the stimulus-event relation comprises different questions:

1. *Existence*: to what extent does a relation exist between the acoustic stimulus and simultaneously occurring action potentials?

2. *Nature*: if such a relation exists, how can it be characterized? Which stimulus properties determine the occurrence of action potentials?

As already mentioned, the nature of the stimulus-event relation is reflected in the characteristics of the Pre Event Stimulus Ensemble as compared to those of the Stimulus Ensemble. The analysis of these characteristics may have to take into account both first and second order aspects. Results from this analysis for an ethologically based stimulus ensemble will be published (Aertsen et al., in preparation).

The question regarding the existence of a stimulus-event relation for a particular neuron, when stated in more neurophysiological terms, turns into the question whether the present stimulus ensemble influences the neural activity as recorded from that neuron in an appreciable way. Basic to the notion of existence of a $s-e$ relation is the requirement of *reproducibility*: for a $s-e$ relation to exist in the context of a wide stimulus ensemble as discussed before, it is necessary and sufficient that presentation of this stimulus ensemble has a reproducible effect on the simultaneously recorded sequence of neural events.

This statement, in fact, defines the notion of "auditory neuron" in operational terms: each neuron for which, upon stimulation with a wide acoustic stimulus ensemble, a $s-e$ relation is shown to exist is considered to be an "auditory neuron". This interpretation will receive further consideration in the discussion.

In view of the stochastic nature of neural activity the use of the term "reproducibility" suggests repetition of experiments: the stimulus ensemble is presented at least twice and the resulting sequences of neural events have to be tested for similarity. Since the stimulus ensemble has a long duration and the recording time is limited, the stimulus ensemble is presented only twice.

Quite analogously to (1) the neural activity $z_2(t)$ recorded during second presentation of the stimulus ensemble is given by

$$z_2(t) = \sum_{j=1}^{N_2} \delta(t-t'_j). \quad (2)$$

Figure 2 shows $z_2(t)$ for unit 98-2 (i.e. the same unit as shown in Fig. 1).

Due to the complex structure of the sequences of action potentials it appears not to be possible in general to decide on the similarity by mere visual inspection of the dot displays. The notion of similarity needs a more formal elaboration.

The (non-normalized) *crosscorrelation function* $R_{z_1 z_2}(\tau)$ of $z_1(t)$ and $z_2(t)$ is defined as

$$R_{z_1 z_2}(\tau) = \frac{1}{T} \int_0^T dt z_1(t) z_2(t+\tau) \quad (3)$$

with T =duration of the experiment (i.e. of the stimulus ensemble). In order to avoid difficulties at the boundaries ($t=0$ and $t=T$) both $z_1(t)$ and $z_2(t)$ are continued in a cyclic way.

Substitution of the expressions (1) and (2) gives

$$R_{z_1 z_2}(\tau) = \frac{1}{T} \int_0^T dt \sum_{i=1}^{N_1} \delta(t-t_i) \sum_{j=1}^{N_2} \delta(t-t'_j + \tau).$$

Using the convolution of two impulses (Papoulis, 1962)

$$\delta(t-\sigma) * \delta(t-\nu) = \delta[t - (\sigma + \nu)] \quad (4)$$

we obtain

$$R_{z_1 z_2}(\tau) = \frac{1}{T} \sum_{i=1}^{N_1} \sum_{j=1}^{N_2} \delta[\tau - (t'_j - t_i)]. \quad (5)$$

It is clear that some type of smoothing has to be applied to $R_{z_1 z_2}(\tau)$ in order to obtain a practically useful estimate.

To this end we define the *Cross Coincidence Function*

$$C_{z_1 z_2}(\tau, \Delta) = \frac{1}{\Delta} \int_{\tau}^{\tau+\Delta} d\sigma R_{z_1 z_2}(\sigma) \quad (\Delta > 0). \quad (6)$$

$C_{z_1 z_2}(\tau, \Delta)$ counts the number of coinciding events from the spike sequence $z_1(t)$ and the set of time-shifted sequences $\{z_2(t+\sigma)\}$ with $\tau < \sigma \leq \tau + \Delta$.

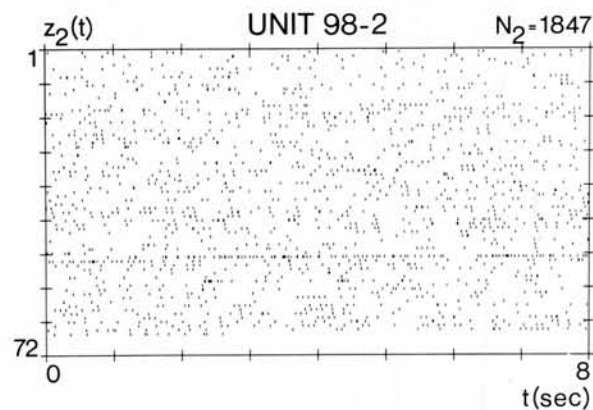


Fig. 2. Dot display representing neural activity $z_2(t)$ recorded from unit 98-2 during second presentation of the acoustic biotope. This dot display should be compared to Fig. 1

It is easily verified that $C_{z_1 z_2}(\tau, \Delta)$ formally equals $R_{z_1 z_2}(\tau)$ with the smoothed sequence $Z_2(t) = (z_2 * h)(t)$ and the weight function

$$h(t) = \frac{1}{\Delta} (0 < t \leq \Delta) \quad 0 \text{ (otherwise)}. \quad (7)$$

Furthermore we note that (for $\tau \neq 0$) it holds that

$$\lim_{\Delta \rightarrow 0} C_{z_1 z_2}(\tau, \Delta) = \frac{N_1}{T} I_{z_1 z_2}(\tau) \quad (8)$$

with $I_{z_1 z_2}(\tau)$ the *Cross Intensity Function* (Cox and Lewis, 1966), for $\tau \neq 0$ defined as

$$I_{z_1 z_2}(\tau) = \lim_{\Delta \rightarrow 0} \frac{1}{\Delta} \text{prob} \left(\begin{array}{l} \text{event in sequence } z_2 \\ \text{between } t + \tau, t + \tau + \Delta \end{array} \middle| \begin{array}{l} \text{event in} \\ \text{sequence } z_1 \text{ at } t \end{array} \right). \quad (9)$$

Inspired by these results we propose an approach to the question of reproducibility, based on the cross coincidence function $C_{z_1 z_2}(\tau, \Delta)$ as an estimate of the cross intensity function $I_{z_1 z_2}(\tau)$.

For reasons of symmetry we prefer to use a modified cross coincidence function $\tilde{C}_{z_1 z_2}(\tau, \Delta)$, defined as

$$\tilde{C}_{z_1 z_2}(\tau, \Delta) = C_{z_1 z_2}(\tau - \Delta/2, \Delta) \quad (10)$$

for which it holds that

$$\tilde{C}_{z_1 z_2}(\tau, \Delta) = \tilde{C}_{z_2 z_1}(-\tau, \Delta). \quad (11)$$

This implies that the (modified) autocoincidence function is an even function of τ .

The behaviour of $\tilde{C}_{z_1 z_2}(\tau, \Delta)$ in the region around $\tau=0$, as compared to its behaviour for larger values of $|\tau|$, indicates to what extent the event sequences $z_1(t)$ and $z_2(t)$ are correlated (synchronized).

A detailed correspondence in the instants of events in both sequences, as revealed by a relatively sharp

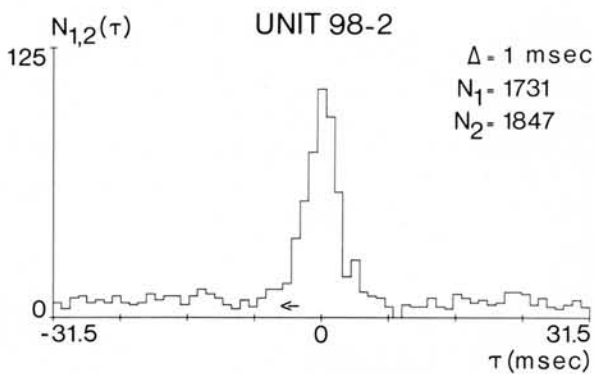


Fig. 3. Cross Coincidence Histogram of neural event sequences $z_1(t)$ and $z_2(t)$, recorded from unit 98-2 during subsequent presentations of the acoustic biotope

peak in the cross coincidence function around $\tau=0$ is attributed to the effect on neural activity exercised by effective (i.e. acoustic) stimulation. In that case the existence of a stimulus-event relation has been established.

The actual analysis of experimental data is performed by means of the *Cross Coincidence Histogram* $\{C_n(A)\}$, with

$$C_n(A) = \tilde{C}_{z_1 z_2}(nA, A) \quad n=0, \pm 1, \pm 2, \dots \quad (12)$$

as a sampled representation of the cross coincidence function.

Figure 3 shows the form of the cross coincidence histogram as calculated for the spike sequences recorded from unit 98-2 (Figs. 1 and 2). In this case Δ has been taken 1 ms. Note that the result has not been divided by T or Δ ; ordinate values $N_{1,2}(\tau)$ refer to numbers of coincidences in one bin Δ . Numbers of action potentials in sequences $z_1(t)$ and $z_2(t)$ (N_1, N_2) are given for reference.

The arrow in Fig. 3 indicates the expectation $E(N_{1,2}) = N_1 N_2 \Delta / T$ for independent stationary point processes (Cox and Lewis, 1966).

It is clear from Fig. 3 that $z_1(t)$ and $z_2(t)$ are highly correlated. The distinct peak around $\tau=0$ (width some 5 ms) against the flattened background for larger values of $|\tau|$ implies a detailed correspondence between both recordings. From this we infer that the firing pattern of unit 98-2 is influenced in a reproducible way by presentation of a wide acoustic stimulus ensemble. For this neuron the existence of a stimulus-event relation has now been established.

5. Results

Experiments were performed on 9 cats, recordings were made from 23 auditory cortex neurons and 12 cochlear nucleus neurons. As in a number of cases

some doubt existed about the quality of the recordings, the analysis as described in this paper was applied to 9 auditory cortex units and 9 cochlear nucleus units.

Figures 4 and 5 show the results for four neurons, two of them from the cochlear nucleus (Fig. 4) and two from the auditory cortex (Fig. 5). Note the different time scales in the histograms. As in Fig. 3 the arrow in the histogram indicates the expectation $E(N_{1,2})$ for independent stationary point processes.

Results for all cochlear nucleus units proved to be very similar to the examples shown in Fig. 4: a distinct peak around $\tau=0$, its width some 5-10 ms. A conclusion regarding the existence of a $s-e$ relation for these neurons is clearly positive.

Results for neurons recorded from the auditory cortex can be divided into two groups. One group (5 neurons) showed results which lead to a positive conclusion regarding the existence of a $s-e$ relation. Compared to cochlear nucleus neurons peaks around $\tau=0$ tend to be less distinct: width up to some 40 ms (Fig. 5). Unit 98-2 (Fig. 3) forms an exception to this.

For the second group (4 neurons) the cross coincidence histograms did not show a clear structure, which implies that on the basis of these results no conclusion can be drawn regarding their possible $s-e$ relation. For one of these neurons the average firing rate under zero-stimulus conditions appeared to be considerably lower than during presentation of the stimulus ensemble, which suggests that a $s-e$ relation existed nevertheless. For the remaining 3 neurons (from different cats) in the second group no additional evidence permitted to draw a positive conclusion about a possible $s-e$ relation.

For the cochlear nucleus experiments histological analysis revealed that in all cases considered, the neuron indeed was located within the target area. For the auditory cortex experiments histological data were not available.

6. Discussion

In the context of a behaviourally oriented investigation of neural information processing we have studied single unit responses from different auditory regions of the cat: cochlear nucleus and auditory cortex. To this end a wide stimulus ensemble was constructed, based on arguments adopted from ethology and ecology. The Acoustic Biotope is intended to create a natural stimulus environment for the animal; an environment to which the auditory nervous system, in its phylogenetical and ontogenetical development structurally and functionally has adapted.

Biological and physical characterization of sounds from the Acoustic Biotope can reveal which properties of sound signals are behaviourally important. Results

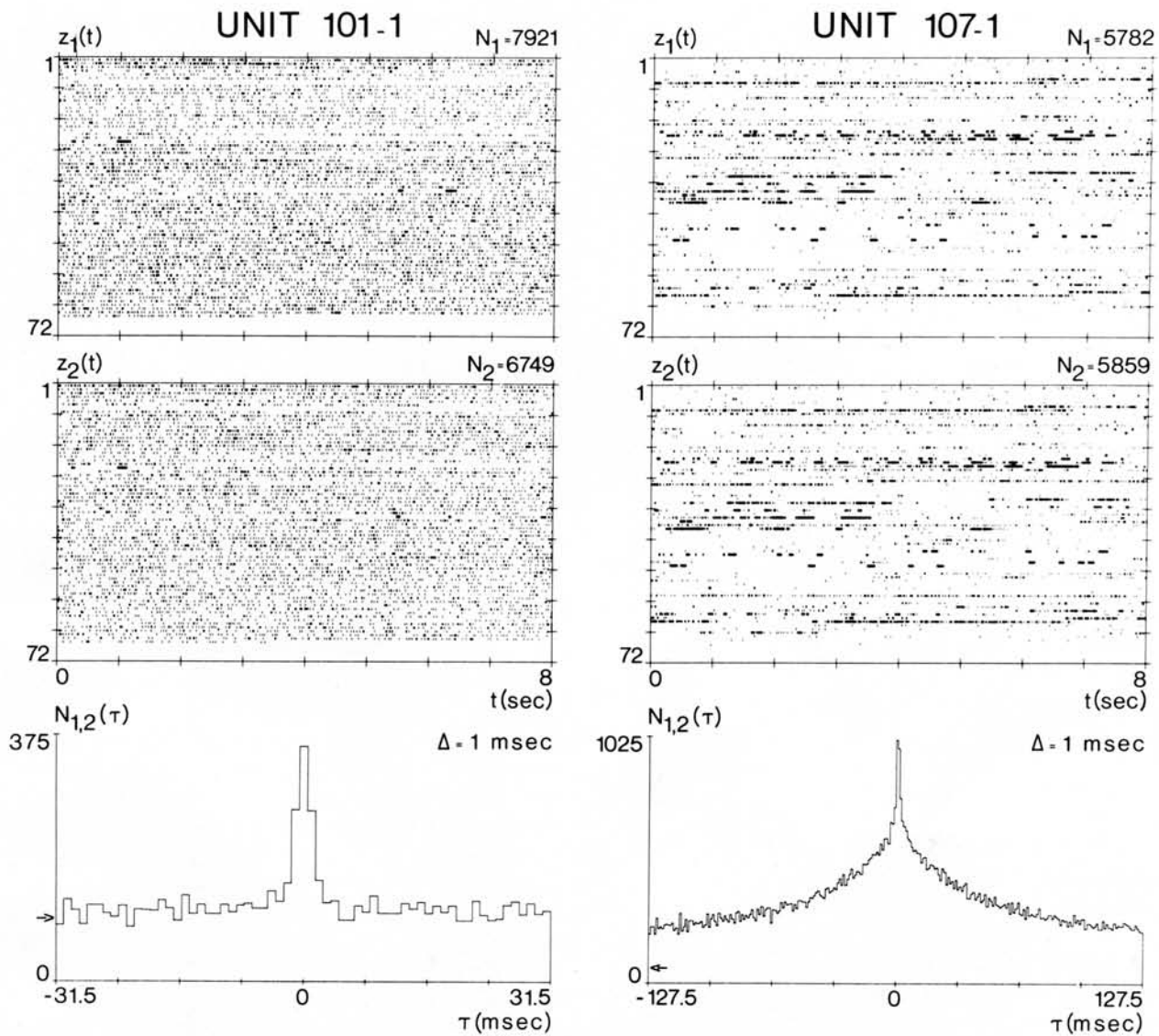


Fig. 4. Dot displays and cross coincidence histograms of neural activity recorded from two cochlear nucleus units during subsequent presentations of the acoustic biotope. Left column: unit 101-1, right column: unit 107-1

from this analysis have to be related to the neural representation of (natural) sounds as deduced from single unit studies, thus providing a possible frame of reference for meaningful interpretation of neuronal functional properties. Moreover such an analysis of the Acoustic Biotope may give clues to the problem of a suitable parametrization of complex natural sounds, ultimately resulting in a stimulus grammar, capable of generating a synthesized acoustic pseudo-biotope. By systematic variation of the stimulus parameters, preferably on an interactive basis, we would be able to study the auditory system in a quasi-natural acoustic environment without having to give up stimulus control.

During presentation of a wide, mainly natural, partly technical stimulus ensemble a neuron produces a long and complex sequence of action potentials. Aspects of a possible stimulus-event relation then have to be subjected to systematic analysis: does it exist anyhow and, if so, what is the nature of it. In this paper we have concentrated on the problem of existence, the analysis of its nature will be the subject of a forthcoming paper (Aertsen et al., in preparation).

The question regarding the existence of a $s-e$ relation was reformulated into the question whether presentation of a wide stimulus ensemble has a reproducible effect on the recorded single unit activity. The stimulus ensemble therefore is presented twice and an

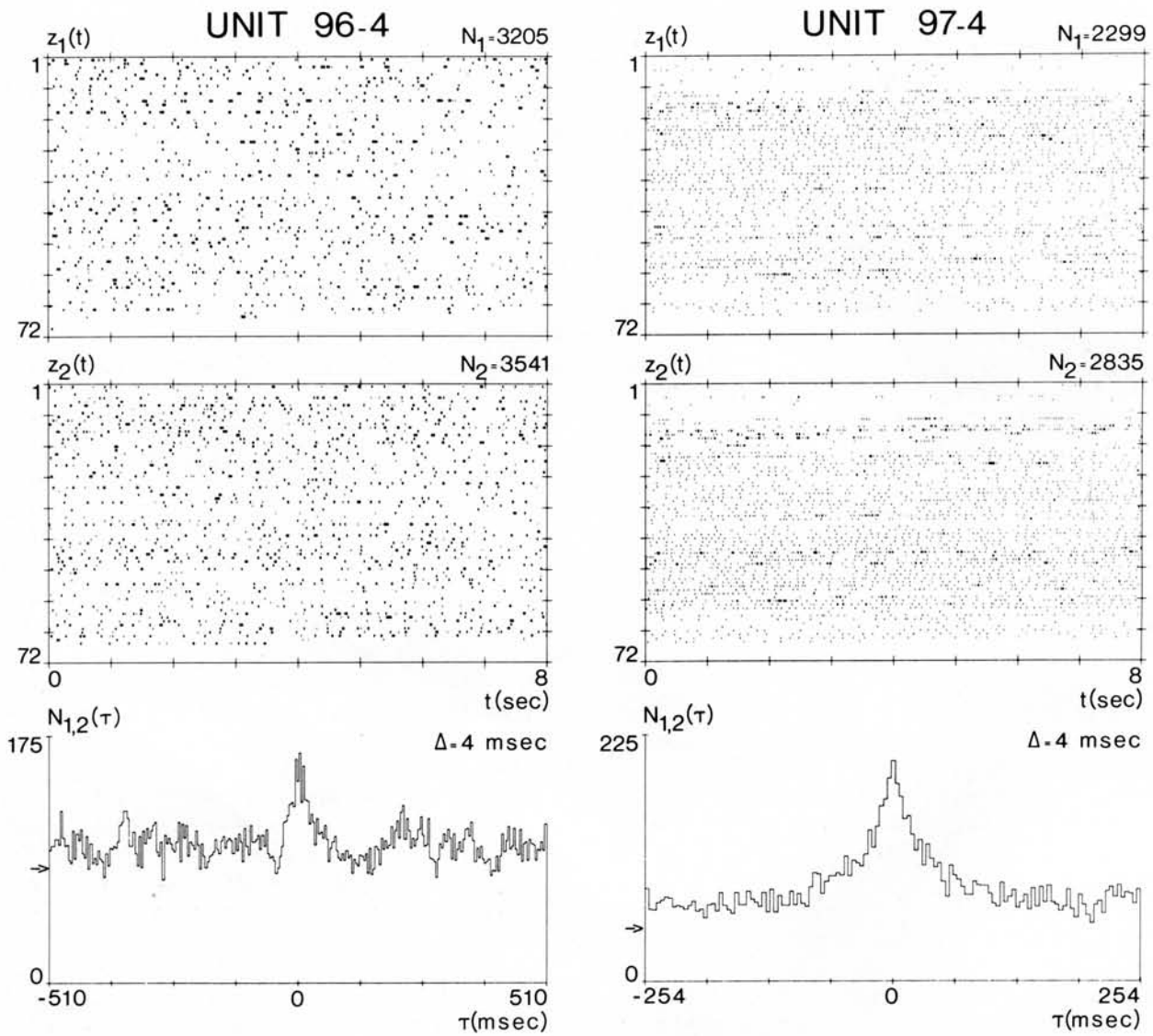


Fig. 5. Dot displays and cross coincidence histograms of neural activity recorded from two auditory cortex units during subsequent presentations of the acoustic biotope. Left column: unit 96-4, right column: unit 97-4.

analysis of the two resulting sequences of neural events has to reveal whether the hypothesis of independent firings during subsequent presentations of the stimulus ensemble is to be rejected. The rejection of independentness is considered to prove the existence of a $s-e$ relation.

We have used an analysis which is based on the cross correlation function: the cross coincidence function (or -histogram). This approach was inspired by, amongst others, a similar approach used in the evaluation of synaptic interactions between different neurons from which simultaneous recordings were made (Griffith and Horn, 1963; Perkel et al., 1967; Moore et al., 1970; Gerstein, 1970; Bryant et al., 1972; Dickson

and Gerstein, 1974). Some important distinctions, however, have to be made. The first one concerns the neuronal data, subjected to the analysis. In the multi-unit studies the correlation analysis is applied to stationary spike sequences, recorded under zero-stimulus conditions and on sequences, recorded during repetitive presentation of a stimulus, the stimulus necessarily being short because the generation of post stimulus time histograms is involved. The present paper describes the application of correlation methods to two, in general nonstationary, spike sequences recorded under identical complex stimulus conditions. A second distinction which is to be made, concerns the actual form of the coincidence histogram and the

information which is derived from it. Apart from being used in testing the hypothesis of independent firings, the auto- and cross coincidence histograms, supplemented with results from detailed model studies, are used in multi-unit investigations to give information about the possible nature of synaptic interaction between the investigated neurons. The detailed structure of the histograms is essential in that case. It is obvious that in our case the shape of the coincidence histogram reflects properties of the stimulus ensemble as well as characteristics of the $s-e$ relation of the investigated neuron. (Note for instance the wide "tails" in the histogram for unit 107-1 in Fig. 4.) These effects, however, are interwoven in a complicated way. In the present stage the coincidence histogram is primarily intended to test the existence of a possible $s-e$ relation, additional evidence and hypotheses regarding the nature of the $s-e$ relation are necessary to be able to draw more specific conclusions from the detailed structure of the histogram.

Results obtained up till now suggest that on the whole cochlear nucleus units tend to have a narrower peak in the cross coincidence histogram than auditory cortex units have.

Some formal problems associated with the analysis as presented here have to be considered: the choice of binwidth Δ and the problem of *statistical significance* of features in the cross coincidence histogram.

Purely formal arguments for a particular choice of Δ cannot be derived from the theory. It is possible however to restrict the range of possible values of Δ on the basis of knowledge about the physiological mechanisms associated with the point processes to be analyzed.

On the one hand it appears to be rather pointless to take a value for Δ which is smaller than some 0.1 ms, since such a value suggests a time resolution in the cat's auditory system which contradicts the experimentally found loss of phase-lock at frequencies above a few kHz in the auditory nerve (Kiang et al., 1965) and the cochlear nucleus (Lavine, 1971). There appears to be no evidence for phase-locking by auditory cortex units (Goldstein and Abeles, 1975). On the other hand the available data about integration times for neurons in the cat's cochlear nucleus (some 10–20 ms; Van Gisbergen et al., 1975c) and auditory cortex (up to 200 ms; Abeles and Goldstein, 1972; De Ribaupierre et al., 1972) suggest an upper limit for Δ . Both the lower and upper limit thus appear to vary with the level in the auditory system, with smaller values for the periphery. For various neurons we have varied Δ in the range from 0.2 to 10 ms: the global structure of the cross coincidence histograms (a "peak" around $\tau=0$) was not seriously affected by this. In case the histogram did not show a clear structure, variation of Δ was

not able to improve this. For various reasons a value for Δ of about 1–4 ms seems to be a reasonable choice.

We have not been able to develop satisfactory statistical tests of the null hypothesis of independent firings. This problem is also mentioned in the case of stationary spike sequences as studied in multi-unit investigations (Moore et al., 1966; Perkel et al., 1967). Theoretical problems in this respect are aggravated by the nonstationary character of the point processes to be analyzed here.

In practice this implies that the histograms were judged by the – quite arbitrary – criterion whether a peak around $\tau=0$ "clearly" stood out against a flattening background for larger values of $|\tau|$. In this respect unit 96-4 (Fig. 5) may be considered as a limiting case, only just passing the test.

We realize that the problems of significance and of the choice of binwidth Δ are critical ones as they are of fundamental importance for a judgement regarding the reliability of results of this analysis. Further theoretical analysis on these points as well as on related problems is currently in progress (Barndorff-Nielsen and Pedersen, Mathematical Institute, University of Århus, Denmark; in collaboration with the authors of this paper). This theoretical analysis explicitly takes into account the *nonstationary character* of the point processes to be analyzed here.

Results obtained so far suggest that the procedure as described in this paper is capable of demonstrating the existence of a stimulus-event relation, including a number of cases in which this was hard or even impossible to demonstrate by mere (visual or aural) inspection of the experimental data.

For a number of neurons in the auditory cortex the existence of a $s-e$ relation could not be demonstrated. Apart from a possible insensitivity of the procedure several types of shortcomings of the stimulus ensemble may very well be the cause of this. For instance it should be realized that the stimulus ensemble as it was used in this investigation did not contain frequencies exceeding 20 kHz, whereas neurons in the cat's auditory cortex with characteristic frequencies as high as 45 kHz have been reported (Evans and Whitfield, 1964).

It is clear that more theoretical insight as well as experimental evidence to be gained in further investigations will help to obtain a more complete evaluation of the procedure as proposed.

A fundamental problem, often met in single cell sensory electrophysiology, is the question whether a particular neuron should, on the basis of physiological data, be considered to be an "auditory" neuron (or a visual one in another context). The notion of "auditory" commonly is operationalized by requiring that the neuron's firing pattern should be affected repro-

ducibly (within statistical limits) by presentation of a proper acoustic stimulus: "auditory" in these terms means "liable to be influenced by adequate acoustic stimulation".

This functional definition works both ways. Any neuron, the firing pattern of which is influenced reproducibly by acoustic stimulation, is considered to be an "auditory" one. It is obvious that these findings, on the basis of physiological evidence, should be confronted with other, i.e. anatomical, evidence. Furthermore, it should be realized, that an answer to the question whether a particular neuron is an "auditory" one, is valid only within the reach of the presented stimulus ensemble, i.e. within the range of questions posed to the neuron. It may very well be possible that for different stimulus ensembles the question of the neuron being "auditory" is answered in different ways. This is related to the notion of "adequate" stimulation. Especially in areas of the central nervous system, involved in a more complex mode of sensory information processing, this may form a serious problem, the more so as available recording times are finite. The approach of using a stimulus ensemble containing only a small selection of biologically significant sounds (usually from the animal's vocal repertoire), presented many times, may very well, wrongly and unintentionally, give rise to the idea of highly specific neurons (Scheich, 1977).

Therefore we have used a *wide stimulus ensemble*, inspired by ethological and ecological observations, not restricted to species-specific vocalizations but containing all sorts of sounds from the natural environment of the animal (cat). Consequently, a neuron is considered to be "auditory" if for this wide stimulus ensemble a stimulus-event relation is shown to exist. If such a relation cannot be demonstrated, we have no arguments to call the neuron an "auditory" neuron in a functional sense. This point has been stressed somewhat amply because, to our opinion, it is of fundamental importance to define the subject of investigation as precise as possible. Once a stimulus-event relation has been shown to exist, the nature of it has to be clarified, which may not in all cases be easy or even possible with the available methods and present-day understanding of neural functioning. This, however, does not detract anything from the existence of the stimulus-event relation.

The procedure as it is described should be able to demonstrate the influence of external factors on a possible $s-e$ relation. In this respect one might think of influences from other modalities (e.g. visual, tactile) or external factors like attention, level and type of anaesthesia, hormonal condition etc. Therefore this approach may be a useful tool in an exploratory investigation of complex regions in the central nervous

system, which are known to receive inputs from different modalities or the function of which is not clear yet.

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