

## Early and late patterns of stimulus-related activity in auditory cortex of trained animals

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**Abstract.** Epidural electrocorticograms over the right auditory cortex (field AI) were measured using implanted 18-channel (3 × 6) electrode arrays in four animals (Mongolian gerbil) trained to discriminate between a rising and a falling frequency modulated tone (frequency range 2–4 kHz). Using a previously introduced classification procedure, transient patterns of cortical activity suitable to discriminate between the rising and the falling modulation were identified. Early (locked to stimulus onset) and late (emerging at variable times poststimulus) patterns could be differentiated. Deletion of increasing numbers of randomly selected electrodes was used to determine a critical density of recording channels required to capture the discriminative power of the early and late patterns. Statistical analysis of the classification revealed a sigmoid dependence of the discriminative power from the number of remaining electrodes with an inflection point at 12 electrodes. The analysis of the minima of the classification statistic revealed that in the early patterns discriminative information was focal on regions corresponding to the tonotopic representation of the stimuli, whereas in late patterns this information seemed to be distributed nonfocally across larger cortical regions. This analysis supports the previous notion of the coexistence of topographically organized activity states related to the physical stimulus features and nontopographically organized states determined largely by intrinsic factors (Ohl et al. 2001).

### 1 Introduction

Under conditions of sensory stimulation paleocortical and neocortical activity have been shown to be influenced by factors determined to be attributable to the

stimulus and by factors of the intrinsic cortical activity (Freeman and Schneider 1982; Freeman and Grajski 1987; Arieli et al. 1996). Through the use of epidural electrocorticography in trained animals, spatial patterns of stimulus-related activity have been recorded not only time-locked to the stimulus onset but also emerging at apparently random times on the order of seconds poststimulus in somatosensory, auditory, and visual cortices (Barrie et al. 1996). (Relatedness to stimuli was assessed in these studies using a pattern classification procedure that indicated a pattern of spatial activity distribution as classifying “correctly” if it allowed to correctly predict the associated stimulus from the set of stimuli used in a particular experiment). For simplicity, patterns of the former stimulus-locked type will hereinafter be referred to as *early patterns* and patterns of the latter type as *late patterns*. Previous results (for review see Freeman 2000) have implied that the spontaneously emerging patterns reflect subjective aspects of an individual animal’s stimulus processing. By exploiting the experimental paradigm of category learning (*concept formation*) we have recently been able to focus on the subjective aspect of the late patterns (Ohl et al. 2001). This was possible because during category learning an individual imposes subjectively valid relational criteria between stimuli that can dominate over the naive perceptual scaling with respect to physical stimulus parameters. The formation of categories depends on the individual’s learning history and can be experimentally detected (Wetzel et al. 1998; Ohl et al. 2001). Specifically, it was shown that metric relations between late activity patterns across different stimulation trials correspond to perceptual relations after categorization training, namely, the parcellation of stimulus material into equivalence classes (*categories*) exhibited by the individual.

In the present study, we compared early and late patterns identified by the above-mentioned pattern classification approach. We studied how the classification decayed after removing randomly selected subsets of electrodes from the complete data set, i.e., after “diluting” the spatial activity pattern. From this analysis and the comparison with the spatial pattern of

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amplitude distribution of the averaged middle latency potential we drew inferences about the different spatial organization of stimulus-related information in early and late patterns.

## 2 Methods

### 2.1 Animal preparation, stimulation, training, and recording

Four Mongolian gerbils (*Meriones unguiculatus*) were implanted with  $3 \times 6$  arrays of stainless steel electrodes ( $600 \mu\text{m}$  interelectrode distance) centered epidurally over primary sensory field AI of the right auditory cortex as detailed elsewhere (Ohl et al. 2000a, 2001). Monopolar recordings of a 6-s time interval (including 2-s prestimulus time) in each trial against a frontoparietal reference were amplified ( $\times 10$  k), filtered (3-dB cutoff frequencies: 0.1 and 100 Hz; 6 dB/octave fall-off), and stored for offline analysis. Animals were trained in a (GO / NO-GO) avoidance paradigm (Ohl et al. 1999) to discriminate rising from falling linearly frequency-modulated (FM) tones (250-ms duration, rising or falling, respectively, between 2 kHz and 4 kHz) that were delivered via free-field loudspeakers at a mean sound pressure level of 70 dB SPL. Training was carried out in daily sessions á 60 trials (30 trials with rising FM tones as GO signal and 30 trials with falling FM tones as NO-GO signal in randomized order).

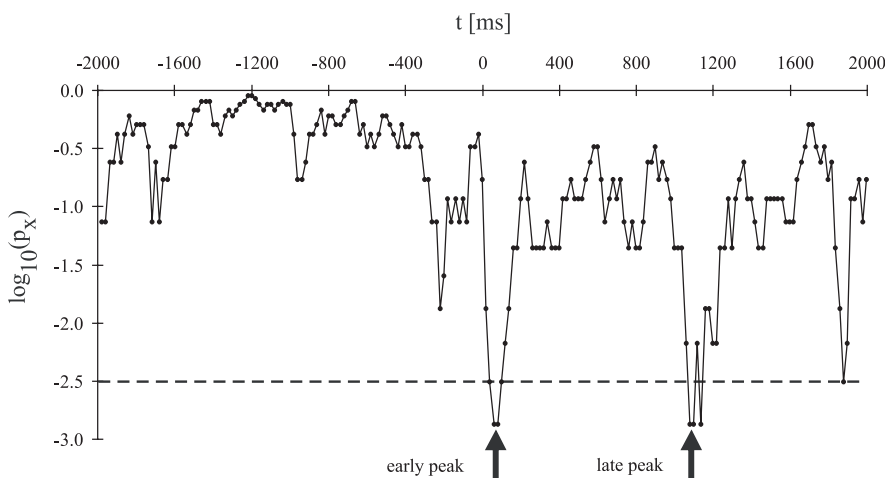
## 3 Data analysis

Spatial patterns of activity were obtained by determining the root mean squared (RMS) amplitude for each of the 18 channels in 120-ms time windows that were stepped through the 6-s recording in 20-ms steps. This yielded 18-dimensional state vectors that developed along trajectories as the RMS window was moved. Spatial patterns suited for discrimination between rising and falling FM tones were identified using a previously introduced classification procedure (Freeman and Baird

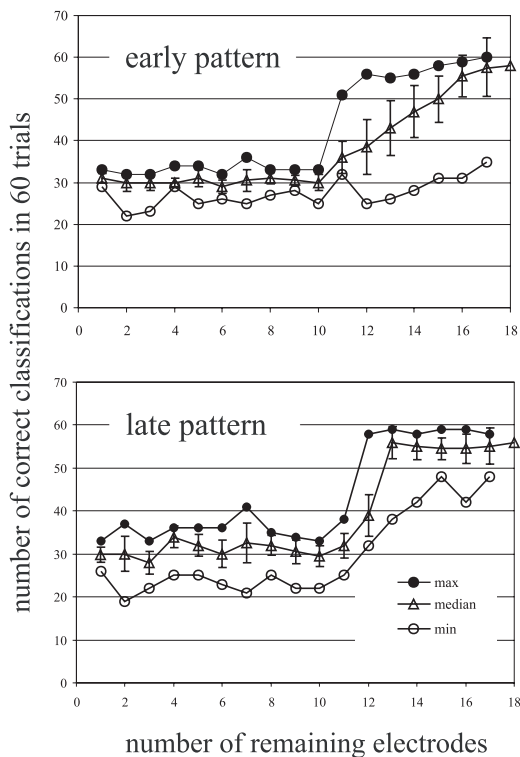
1987; Barrie et al. 1996). In short, the 30 trajectories recorded during presentation of rising FM tones (set A) and the 30 trajectories recorded during presentation of falling FM tones (set B) were each divided into two disjunct subsets á 15 trajectories (A1, A2 and B1, B2). For each subset a centroid trajectory was calculated. For each activity pattern in subset A1 (at a certain point in time on the trajectory) the question was asked whether its corresponding state vector was closer to the centroid of A2 or to the centroid of B2 at the given point in time. In the former case the pattern was said to classify correctly; in the latter case it was said to classify incorrectly. In the same manner, the patterns from set B1 were classified against the centroids of B2 and A2. Analogously, the patterns in A2 and B2 were classified against the centroids A1, B1 and B1, A1, respectively (cross classification). For each time window this yielded a whole number  $n \in [0, 60]$  of correct classifications. The probability  $p_n$  that in 60 trials a number of at least  $n$  correct classifications can be found by chance (the null hypothesis) is given by the cumulative binomial distribution

$$p_n = \sum_{x=0}^n \binom{60}{x} p^x (1-p)^{60-x}$$

where the probability of correct classification of any single pattern was set to  $p = 0.5$ . Plots of  $p_n$  as a function of time display periods of significant classification invariably locked to the stimulus onset (early patterns) and at apparently randomly distributed later points in time (late patterns) (Barrie et al. 1996; Ohl et al. 2001) (Fig. 1). Inferences about the spatial localization of information relevant for classification can be obtained from the study of the above-described classification behavior after removing single recording channels or sets of channels (vector components) from the state vector (Freeman and Baird 1987; Barrie et al. 1996). Here we studied classification after removing  $n_r = 0, 1, 2, \dots, 17$  channels from the recording obtained after the animal showed significant stimulus discrimination (Ohl et al. 1999) in three consecutive training sessions (one session á 60 trials per day). This



**Fig. 1.** Plot of the  $\log_{10}$  of the probability  $p_n$  of finding at least the observed number of correct classifications of a spatial pattern by chance (null hypothesis). A spatial pattern was defined as the vector of RMS amplitudes of 18 recording channels in a 120-ms time window. This window was stepped in 20-ms steps through the recording. Time is referenced to stimulus onset at  $t = 0$ . The significance level of  $p = 1 \times 10E(-2.5)$  used for peak definition is indicated as a dashed line. Values below the dashed line mark periods of significant classifications. Arrows point to the early and late patterns selected as described in the Methods section



**Fig. 2.** Number of correct classifications of early and late patterns in 60 trials of a learning session in the plateau phase of the learning curve as a function of the number of remaining electrodes ( $18 - n_r$ ). Shown are the minimum number of correct classifications, the maximum number of correct classifications, and the median number of correct classifications (plus standard errors) after 40 (2 to 16 remaining electrodes) or 18 (1 and 17 remaining electrodes) repetitions of removing a given number of electrodes. For the cases of 18 remaining electrodes (the complete patterns) the *triangle* corresponds to the number of correct classifications observed in the experiment. Data were the pooled from four animals

characterized a phase in an animal's learning history when the individual had entered the plateau phase of its learning curve. Each removal of  $n_r$  channels was repeated 40 times, except for  $n_r = 0, 1, 17$ . (The case  $n_r = 0$  corresponds to the complete pattern, and for  $n_r = 1$  and 17 all possible 18 opportunities of removing electrodes were used). For each time window and each  $n_r$  the 40 numbers of correct classifications were accumulated from all four animals (yielding 160 numbers). Of these 160 numbers of correct classifications for each  $n_r$  the minimum number, the median number, and the maximum number were plotted (Fig. 2).

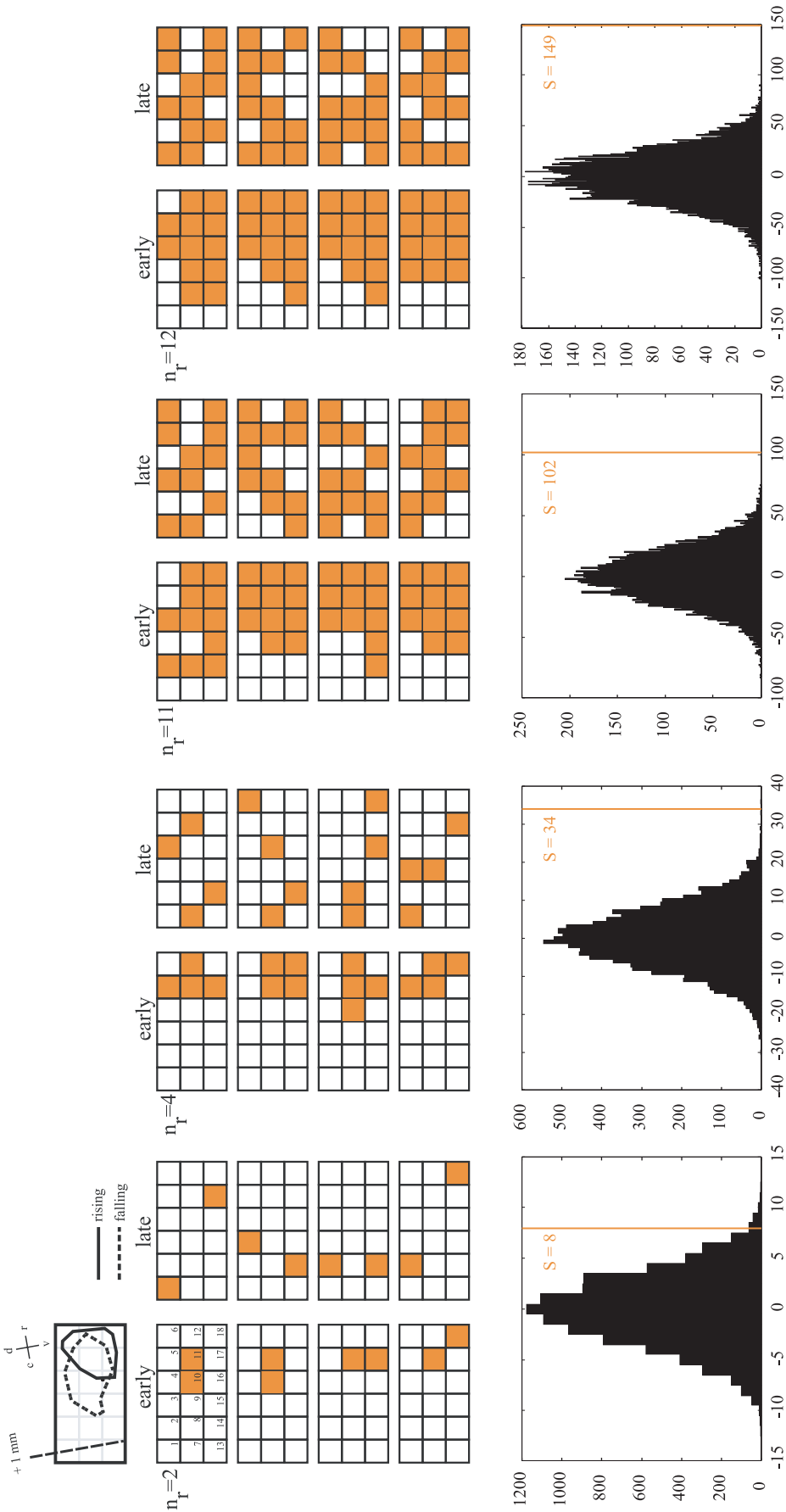
To identify the vector components (recording channels or electrodes in the recording array) contributing maximally to the significantly classifying spatial activity pattern, for each animal and each number  $n_r$  of removed electrodes the particular set of removed electrodes was determined that was associated with the minimum number of correct classifications. This set of electrodes was marked on the electrode grid of the recording array (Fig. 3). In order to compare the "compactness" of this set of  $n_r$  electrodes between early and late patterns we calculated for each pattern the  $n_{\text{dist}} = n_r(n_r - 1)/2$

distances between each electrode pair within a pattern based on the Moore neighborhood, i.e., electrodes adjacent both in the horizontal-vertical and in the diagonal direction have a distance of 1 to the considered electrode. We constructed a test statistic  $S = L - E$ , where  $L$  is the sum over all distances between marked electrodes in a late pattern in all animals and  $E$  is the sum over all distances between marked electrodes in an early pattern in all animals. Note that  $S$ ,  $L$ , and  $E$  are sums over  $4 \times n_{\text{dist}}$  elements, 4 being the number of experimental animals. A higher compactness of the marked electrodes in the early patterns as compared to the late patterns should yield  $S > 0$ . To estimate the significance of the empirically observed  $S$  values, we compared for each  $n_r$  the observed  $S$  value with a distribution of  $S$  values obtained from 10,000 sets of  $4 \times n_{\text{dist}}$  distances randomly selected from the set of all calculated distances for a given  $n_r$  (Good 2000).

## 4 Results

Gerbils were able to acquire significant discrimination performance for the discrimination between the rising and the falling frequency-modulated (FM) tone within 5–12 days, as has been repeatedly reported in various experimental contexts (Wetzel et al. 1998; Ohl et al. 1999; Ohl et al. 2001). Patterns of spatial activity were recorded using an epidural  $3 \times 6$  electrode array and investigated with respect to their classification potential, as described in Sect. 2.2. Figure 1 plots for each time window the probability of obtaining at least the observed number of correct classifications across the 60 trials of a training session by chance (under the assumption of a binomial distribution of correct and incorrect classifications as detailed above) in one animal. A small probability (indicated by a negative logarithm in Fig. 1) would indicate that the observed number of correct classifications is significant, i.e., unlikely to expect under the null hypothesis. A significance level of  $p < 10^{-2.5}$  was used to define a peak. In correspondence to previous reports (e.g., Barrie et al. 1996) it can be seen that significant classification is reached directly after stimulus onset and for a number of time intervals after it. For the further analysis we selected the two spatial patterns that corresponded to the first peak (early pattern) and to the largest of the subsequent peaks (late pattern) as previously suggested (Ohl et al. 2001). If the maximum value was reached by more than one peak (as in the example shown in Fig. 1), the peak with the greater number of data points above the significance level was chosen. It never occurred that peaks sharing the maximum value also had the same number of data points above the significance level. Considering duration and overlap of the analysis window the duration of the early peak was expectedly of the same order of magnitude as that of the evoked potential (Ohl et al. 2000a,b).

In each of the four animals, the early and late peaks of the function shown in Fig. 1 were studied after removing a number of  $n_r = 0, 1, 2, \dots, 17$  electrodes from



**Fig. 3.** Spatial arrangement of sets of most discriminating electrodes. The top left *inset* shows the topography of the 50% isopotential contour of the middle latency component P1 evoked by linearly frequency-modulated tones rising and falling, respectively, between 2 and 4 kHz, superposed on a rectangle representing the  $3 \times 6$  array of recording electrodes (cf. Ohl et al. 2000b). The *dashed line* marks the intersection of the parasagittal plane embedding the array surface with a frontal plane 1.0 mm rostral to landmark  $\lambda$ . The *orientation cross* specifies the directions rostral (r), dorsal (d), caudal (c), and ventral (v) with reference to the animal. The positioning of the array was such that it spanned the tonotopic representation of the five octaves from 250 hz to 8 kHz in field AI in all animals (cf. Ohl et al. 2000a). The *diagrams* below show the sets of removed electrodes (for  $n_r = 2, 4, 11, 12$ ) in the early and late patterns in the four animals (*rows of diagrams*). The histograms show the distribution of the test statistic  $S$  (see Methods) in 10,000 random samples together with the experimentally observed value indicated by a *red line*

the recording. Figure 2 plots for each number of remaining electrodes ( $18 - n_r$ ) the minimum number of correct classifications, the maximum number of correct classifications, and the median values of the number of correct classifications separately for the early peaks (top) and the late peaks (bottom). Since the set of channels to be removed was randomly selected, the median values convey information about the “density” of electrodes required to capture the discriminant information about the two stimuli in the spatial activity pattern. It can be seen that a number of 15 remaining electrodes in the diluted pattern was sufficient to retain the classification potential of the complete pattern (all 18 electrodes) in both early and late patterns. For smaller numbers of remaining electrodes a gradual decline of the classification potential was observed for the early peaks, and a sigmoid decline with a point of inflection at 12 remaining electrodes was observed for the late patterns.

For numbers of remaining electrodes less than or equal to 11, the classification potential decayed to chance level. A similar behavior was shown by the maximum number of correct classifications in 40 repetitions of the removal of  $n_r$  electrodes. For both early and late patterns this number sigmoidally decreased to chance level for numbers of remaining electrodes of 11 and less. The minimum number of correct classifications showed a different dependence on the number of remaining electrodes in early and late patterns. In late patterns this number increased sigmoidally with an increasing number of electrodes in a fashion similar to the median and maximum numbers of correct classification. In the early patterns, however, the minimum number of correct classifications did not exceed the chance level for higher numbers of remaining electrodes, not even for the complete pattern (18 remaining electrodes).

This indicates that the early patterns differ from the late patterns in that even a small  $n_r$  (number of electrodes to be removed) can be found that, when properly selected, can destroy the pattern’s classification potential. We have therefore studied the sets of removed electrodes associated with the minimum number of correct classifications in early and late patterns. The upper part of Fig. 3 shows these sets for the four animals (rows of panels), for  $n_r = 2, 4, 11,$  and  $12$  (blocks of panels), and for the early and late patterns (left and right columns in each block of panels). The upper left inset in the figure shows for one representative animal the shape of the recording array (rectangle) including the 50% isopotential contours of the early evoked activity (peak P1 of the middle latency potential, cf. Ohl et al. 2000b) for the rising and falling FM tone together with an anatomical landmark, ensuring proper positioning of the recording array over the primary auditory cortical field AI and the cross of orientations. The remaining panels show the arrangement of the 18 recording electrodes with the electrode indices given in the top left panel.

In each panel, the one set of  $n_r$  removed electrodes is marked that was associated with the minimum number of correct classifications in 40 repetitions of removing this number of electrodes from the recording. By implication, this set of electrodes has the maximal contri-

bution to the discriminant power of the pattern. It was found that for the early patterns the set of maximally discriminating electrodes was always a connected set (“compact pattern”) and colocalized with the area of maximal average activation by the incoming stimulus, as exemplified by the 50% isopotential contour in the inset to the figure. As previously shown, this area corresponds grossly to the tonotopic representation of the frequencies traversed by the FM tones (Ohl et al. 2000a), including a tonotopic shift of the spatial representation due to the modulation direction (“rising” or “falling”) (Ohl et al. 2000b). Conversely, for the late patterns the set of maximally discriminating electrodes was disconnected, apparently randomly distributed over the recording array, and showed no correlation with the area of maximal average activation by the incoming stimulus.

Moreover, the variability of the set across the four animals seems to be larger for the late patterns. We quantified the compactness of the early and late patterns by calculating the sums over all animals and interelectrode distances between all pairs of marked electrodes in a pattern. We tested the difference between the corresponding sums in early and late patterns using a permutation test (Good 2000) (see Methods). The lower part of Fig. 3 shows the distribution of the distance of sums  $S$  together with the observed  $S$  value (see Methods) for  $n_r = 2, 4, 11,$  and  $12$ , corresponding to attainable significance levels of 0.012,  $10^{-4}$ , 0.0, and 0.0. (An attainable significance level of 0.0 in the permutation test means that in no one of the  $10^4$  permutations was the corresponding  $S$  larger than or equal to the experimentally observed one). Significantly higher compactness in early patterns compared to the late patterns was found for all  $n_r$ .

## 5 Discussion

In this study, we investigated stimulus-related activity patterns in the auditory cortex of Mongolian gerbils trained to discriminate between a rising and a falling linearly frequency-modulated tone. The auditory cortex has previously been shown to be relevant for the discrimination of the modulation direction but not for many other types of auditory discrimination (e.g., pitch discrimination of pure tones) (Ohl et al. 1999). In the present study, we discerned early patterns of stimulus-related activity, locked in time to the stimulus onset, from late patterns that emerge at later points in time from the ongoing cortical activity. Such early and late patterns have been reported from the auditory cortex of the rabbit (Barrie et al. 1996) as well as from other paleocortices and neocortices (for review see Freeman 2000). Relatedness to stimuli was assessed using a classification procedure. A previous study (Ohl et al. 2001) suggested that the early patterns reflect spatial aspects of the neuronal processing of physical stimulus features and later patterns reflect subjectively valid perceptual features of the stimuli by showing a correspondence between metric properties of activity patterns and the associated perceptual categorization.

The present analysis suggests that the discriminative potential of the early pattern reflects the stimulus-specific input in a topographically organized region of cortex. As indicated in the inset of Fig. 3, rising and falling frequency-modulated tones elicit discernible spatial patterns of activity in a tonotopic map despite the fact that the same range of frequencies is traversed by the stimulus. As has been discussed in detail elsewhere (Ohl et al. 2000b), this can be understood on the basis of spectrotemporal tuning properties of single neurons. That it is this distinction of spatially organized input activity in the cortex that is responsible for the early peaks in the classification potential (Fig. 1) is supported by the correspondence between these input-activated areas on the one hand and the spatial distribution of electrodes maximally contributing to the classification potential on the other (Fig. 3).

In contrast, no such focal organization could be determined for the later patterns where the set of most discriminating electrodes is distributed across larger areas of the recording area. The inflection point of the curve of the median number of correct classifications at  $n_r = 12$  in Fig. 2 shows that at least two thirds of the recording area had to be covered to extract sufficient information from the distributed spatial activity pattern for correct classification of the modulation direction of the FM tone. Since the recording area spanned the five octaves from 0.25 kHz to 8 kHz (Ohl et al. 2000a), we conclude that the discriminative information is distributed across larger areas of at least the primary auditory cortex field AI and does not reflect the topographic input into this area of cortex.

In summary, the present analysis shows that spatial activity patterns in the primary auditory cortex field AI, on the basis of which a classification of the modulation direction of an FM tone traversing the frequency range between 2 and 4 kHz is possible, occur as transient patterns time-locked to stimulus presentation (early patterns) and transient patterns with variable higher latencies (cf. Ohl et al. 2001). While the former are

spatially focal and topographically organized, the latter are nonfocal and nontopographically organized.

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