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Change in pattern of ongoing cortical activity with auditory category learning

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Humans are able to classify novel items correctly by category^{1,2}; some other animals have also been shown to do this^{3–7}. During category learning, humans group perceptual stimuli by abstracting qualities from similarity relationships of their physical properties^{1,2,8}. Forming categories is fundamental to cognition⁹ and can be independent of a ‘memory store’ of information about the items or a prototype¹⁰. The neurophysiological mechanisms underlying the formation of categories are unknown. Using an animal model of category learning⁶, in which frequency-modulated tones are distinguished into the categories of ‘rising’ and ‘falling’ modulation, we demonstrate here that the sorting of stimuli into these categories emerges as a sudden change in an animal’s learning strategy. Electro-corticographical recording from the auditory cortex¹¹ shows that the transition is accompanied by a change in the dynamics of cortical stimulus representation. We suggest that this dynamic change represents a mechanism underlying the recognition of the abstract quality (or qualities) that defines the categories.

Mongolian gerbils (*Meriones unguiculatus*) can be trained with pairs of linearly rising and falling frequency-modulated tones (Fig. 1a) to establish the categories of ‘rising’ and ‘falling’ modula-

tion and transfer the concept of modulation direction to novel stimuli⁶. Transfer to novel stimuli is considered to be the most decisive behavioural index for category learning^{12,13}. The training consists of six sequential ‘training blocks’ (see Methods), in each of which a different pair of rising and falling frequency-modulated tones is presented (Fig. 1a, numbers). In the early blocks, discrimination performance gradually improves in each block as the animals learn to discriminate between the rising and falling tones over a series of daily training sessions (discrimination learning; Fig. 1b). A sudden transition in behaviour then occurs, after which novel stimuli are immediately identified as belonging to their correct category: discrimination performance is already high in the first session of a training block, even though the stimulus pair to be discriminated is novel (categorization; Figs 1c, 2). The point during the training when this transition occurs differs between animals (Fig. 2), although in the 10 animals studied it was always after the second training block.

In addition to the sudden emergence of concept transfer we find a sudden alteration in the perceptual scaling characteristics of the animals: measuring psychometric functions for frequency-modulation rate after each training block shows a generalization gradient for modulation rate during the discrimination-learning phase; that is, the greater the difference in modulation rates (rates of change of frequency) between the training stimuli used in the training block and the test stimuli, the smaller the conditioned response rate

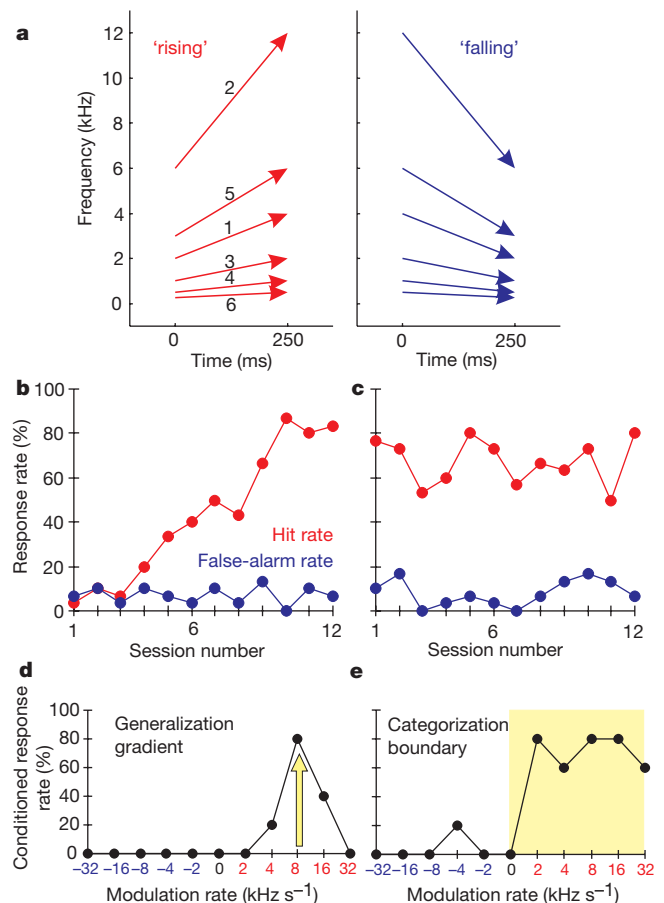


Figure 1 Stimuli and behavioural measures of category learning. **a**, Rising (red) and falling (blue) frequency-modulated tones used in the six sequential training blocks (numbers). **b**, Sample learning curve of gerbil 3 before transition to categorization. **c**, Sample learning curve of same animal after transition to categorization. **d**, Psychometric function for modulation rate obtained after training block shown in **b**. Peak modulation rate of 8 kHz s⁻¹ (arrow) corresponds to modulation from 2 kHz to 4 kHz in 250 ms used in this block. **e**, Sigmoid psychometric function obtained after training block shown in **c**.

(Fig. 1d). After the transition, the psychometric function becomes sigmoid (categorical perception, Fig. 1e), indicating that the modulation rate is being perceived as a member either of the 'rising' or the 'falling' category.

The development of spatiotemporal activity patterns in primary auditory cortex was studied in four animals using high-resolution surface electrocorticography¹¹ during the training, including the transition from discrimination learning to categorization. Record-

ings from four initially naive gerbils were made with an 18-electrode array on the surface of the right primary auditory cortex (Fig. 3a), which contains neurons performing the high degree of spectral integration^{14,15} that is essential for the discrimination of frequency-modulated tones¹⁶. Sample recordings are shown in Fig. 3b. This recording technique provides sufficient resolution to detect differences in spatial activity patterns elicited by pure tones in a frequency range comparable to that traversed by the frequency-modulated tones¹¹.

Electrophysiological correlates of category learning do not necessarily occur at times locked to the presentation of a stimulus, so

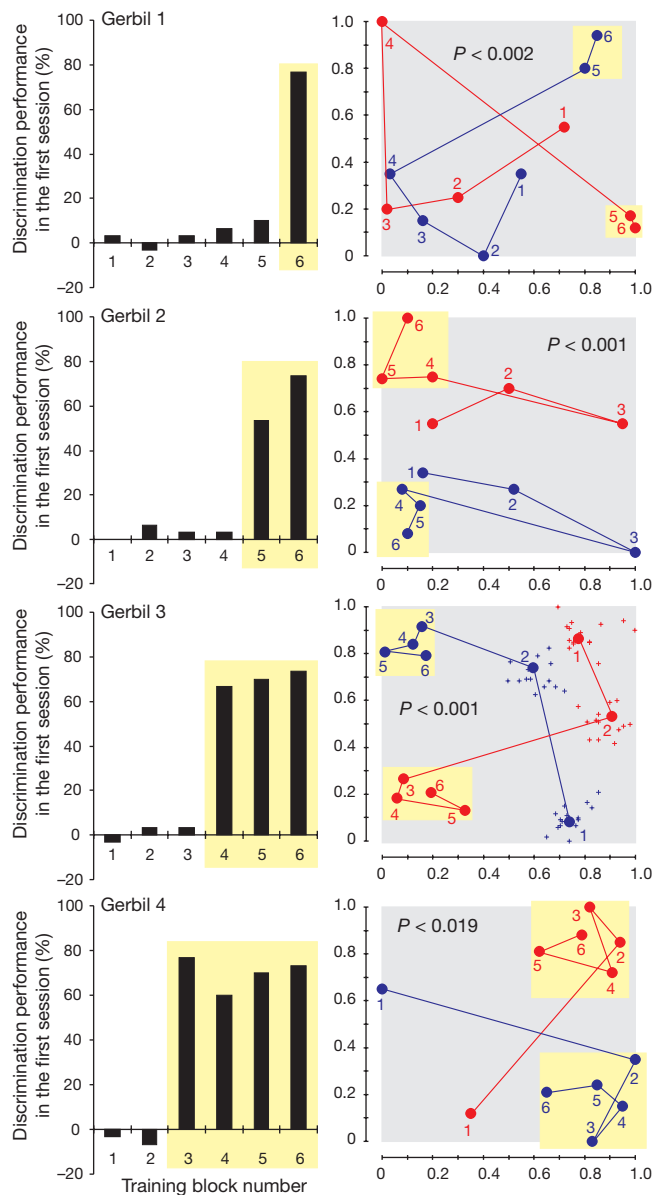


Figure 2 Behavioural transition to categorization (left column) parallels development of cortical spatial activity patterns (right column). Left column: discrimination performance in the first session of each of the six training blocks. Yellow areas indicate categorization phase (Wilcoxon's test, $P < 0.05$). Right column: similarity relations between spatial activity patterns during the marked states (compare with Fig. 3d). Transition to category learning in the behavioural data correlates with clustering (P -values of resampling test given) of the marked states 'within category' (yellow areas). Only the activity pattern during the marked state that gave rise to the maximum peak value of the dissimilarity function for each category is plotted for each training block (numbers). For gerbil 3, marked states of later sessions in blocks 1 and 2 have been included (+) to demonstrate that these point clouds do not fall into the clusters found after the transition to categorization. Absolute coordinates of points have no particular meaning other than scaling relative distances between any pair of points.

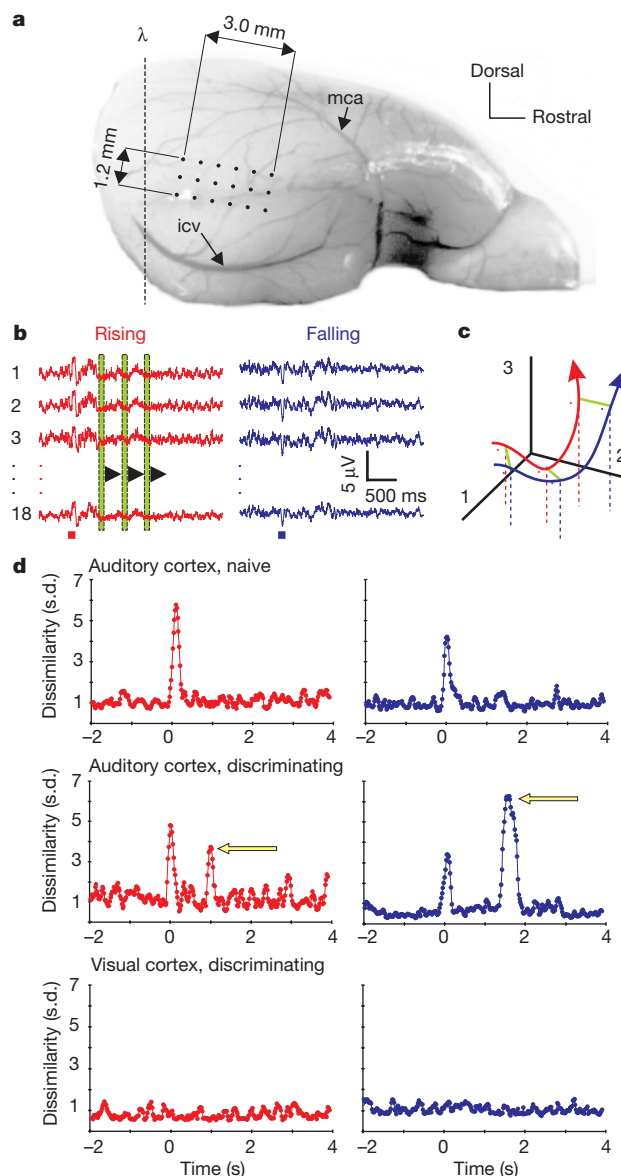


Figure 3 Measurement and analysis of ongoing spatiotemporal activity. **a**, Positioning of electrode array over right auditory cortex. λ , caudal fusion point of parietal bones; icv, inferior cerebral vein; mca, middle cerebral artery. **b**, Sample surface electrocorticograms from a single trial using a rising (red) and a falling (blue) frequency-modulated tone. Stimulus markers (squares) are indicated below bottom traces. Green bars indicate the sample time windows used to calculate the r.m.s. amplitudes. **c**, Mapping spatial patterns of 18 r.m.s. amplitudes to points in 18-dimensional state space (three dimensions shown). Dissimilarity between spatial patterns can be quantified by euclidean distances between corresponding trajectory points (green lines). **d**, Sample 'dissimilarity functions' calculated between a single trajectory and the average over all trajectories of the opposite stimulus category. Marked states are indicated with arrows.

classical averaging techniques cannot be applied in the analysis of the electrocorticograms. Instead, we developed an analysis that can identify neural activity patterns of putative relevance to the coding of category-specific information in single trials independent of their locking to the stimulus. The spatiotemporal activity pattern in each 'rising' trial was compared to a spatiotemporal reference pattern given by the mean across all 'falling' trials within a training session; the reverse comparison was made for all 'falling' trials. The dissimilarity in spatial pattern between the investigated trial and the reference pattern, termed the 'dissimilarity function', was determined as a function of time for each comparison (Fig. 3c).

Analysis of the 2 s before stimulus presentation revealed a baseline to which post-stimulus responses can be related. After stimulus onset, the dissimilarity function peaked from baseline, indicating a transient activity state in which the dissimilarity function was locally maximum compared with the reference pattern. This peak, which reflects the stimulus perturbing the ongoing activity^{17,18}, is found in all trials even in naive animals (Fig. 3d, top row), but not in animals aroused by presentation of the unconditioned stimulus only. Additional peaks (1–5) emerged in each dissimilarity function (mean of 3.5 ± 1.3 peaks per trial) with continued training but not in the sensitization control (0.3 ± 0.5 peaks per trial) (Fig. 3d). The peaks were randomly distributed during the 4-s observation interval between stimulus onset and conditioned response, without apparent correlation to the response latency (coefficient of determination, $R^2 = 0.13$). These additional peaks tag transient states in the ongoing cortical activity, here referred to as 'marked states'. To further rule out contribution to the emergence of the marked states by factors unspecific to the auditory stimulus (for example, movement, response preparation), we implanted three gerbils with the same recording array over the visual cortex and trained them in the auditory model. We found neither the early peak nor any later marked states (0.3 ± 0.6 per trial) emerging in the activity of the visual cortex (Fig. 3d).

We tested whether category-specific information is processed during these marked states by analysing the similarities among the spatial activity patterns during the marked states in single trials. This showed that the behavioural transition to categorization (Fig. 2, left column) was reflected physiologically by changes in the cortical spatial activity patterns (Fig. 2, right column). Specifically, with categorization, the patterns reflected the 'belongingness' to the formed category rather than the physical characteristics of the stimuli. The similarity relations between spatial activity patterns during the marked states is depicted in two-dimensional plots (Fig. 2, right column), in which the dissimilarity between two patterns is proportional to the distance between the corresponding points. During discrimination learning, dissimilarities within and between categories were similar in magnitude. In contrast, the dissimilarities after the transition to categorization were significantly smaller within a category than between categories in all animals. For example, for Gerbil 2, which made the transition after training block 4, the points representing marked states in blocks 5 and 6 (after the transition) are much closer to that of block 4 in same category (yellow areas in plot) than to points 5 and 6 in the other category. In comparison, in blocks 1–4 (before the transition), points within a category are about as far apart as those between categories. For all four animals, we found such clustering of marked states within categories emerging with the behavioural transition to categorization (Fig. 2, right column).

Thus, two types of transient activity state discriminating between rising and falling frequency-modulated tones coexist in the same neuronal substrate. First, independent of the training state, an activity state occurred during a 150–250-ms time interval locked to stimulus onset, during which rising and falling frequency-modulated tones give rise to spatially distinct patterns in the tonotopic map, as was expected from previous frequency-modulated tone studies^{19,20}. The stimulus specificity of these activity states

therefore reflects the tonotopic organization of the early evoked cortical activity¹¹. Second, the transient states that emerge later in the 4-s interval between stimulus onset and reinforcement, which did not occur in the naive animal, are likely to be related to learning, particularly as their pattern reflected the learned category structure.

Similar learning-related spatial activity patterns have been reported from various cortical areas but their interpretation as sensory representations has posed a problem, because they typically do not show an invariant relationship to the trained stimuli^{21–24}. Our discovery that these states possess an internal metric, specific to the individual, that reflects the category structure rather than the physical features of the stimulus may resolve this issue. This relationship cannot be detected in a pure discrimination experiment or a contingency-reversal control. The category-learning experiment, however, provides an objective way to observe a subjectively generated cognitive structure. Furthermore, category-specific deficits observed clinically after circumscribed cortical lesions^{25–28} fits with our demonstration that category formation relies on a spatial representation in the cortex. □

Methods

Subjects and electrophysiological recording

Four male Mongolian gerbils (85–115 g) were chronically implanted with a 3×6 array of stainless steel microwires (100 μm diameter, 600 μm interelectrode distance) placed on the epidural surface over the right auditory cortex and centred over the primary field AI as judged by a preceding analysis of evoked potential topography¹¹. For control purposes three animals were implanted with the array over the right visual cortex. During the behavioural experiments, monopolar recordings against a fronto-parietal reference were made from the each of the 18 electrodes, amplified (10,000 \times), filtered (fall-off: 6 dB octave⁻¹, 3-dB cutoff frequencies: 0.1 Hz, 100 Hz), and stored on a computer for off-line analysis.

Stimuli and behavioural procedures

Stimuli consisted of linearly frequency-modulated tones (250 ms duration, 5 ms linear onset and offset ramps, modulation ranges see Fig. 1a) delivered in free field through calibrated loudspeakers at 70 dB sound pressure level. Using a previously designed model, we trained animals to form the concept of modulation direction and establish the categories of 'rising' and 'falling' frequency-modulated tones⁶. Experiments took place in a two-compartment shuttle box (E10–15, Coulbourn) positioned in a sound-attenuating Faraday cage. In each trial, the animals received either a rising or a falling tone and were trained to discriminate between them in a GO/(NO-GO) procedure motivated by negative reinforcement (150–300 μA , electrodermal stimulation administered through a metal floor grid) after misses (failing to GO) or false alarms (mistaken GO). The GO-conditioned response, that is, moving to the other compartment of the shuttle box, had to be accomplished within 4 s after tone onset to count as a hit trial. Training was given in six sequential blocks with daily sessions of 30 trials using rising frequency-modulated tones and 30 trials using falling frequency-modulated tones in a randomized sequence. In each block, one of the frequency-modulated tone pairs (Fig. 1a) was used and training continued until significant discrimination was observed for at least four consecutive sessions. When each training block was completed, psychometric functions for modulation rate were measured following a standard procedure²⁹ (see Supplementary Information). Modulation rates varied in steps of ± 2 octaves s^{-1} with respect to the modulation rate of the stimulus pair used in the training block. These measurements yielded generalization gradients in the discrimination phase and categorical psychometric functions in the categorization phase.

Discrimination performance in each session was quantified by the difference between hit rate and false-alarm rate, expressed as a percentage, and tested by Wilcoxon's test against the null hypothesis of equal rates, using a criterion of $P < 0.05$ for significant discrimination. Transition from discrimination learning to categorization was indicated by the instantaneous transfer of the concept of modulation direction to a novel frequency-modulated tone pair, which showed as a high-discrimination performance for the novel stimuli early in the first training session of a block (see Fig. 2). To test the specificity of electrophysiological correlates of learning, we carried out an arousal control (stimulation with the unconditioned stimulus alone and a sensitization control (60 trials of uncorrelated presentations of conditioned stimulus and unconditioned stimulus with each animal before onset of the described categorization training).

Analysis of electrophysiological data

In each trial the data from the 18-channel electrocorticogram was analysed in a 6-s time interval (2 s before stimulus + 4 s after stimulus). For each recording channel a 120-ms time window was moved through the data in steps of 20 ms and the mean signal power in each window was determined by calculating the root-mean-square (r.m.s.) amplitude. The power values obtained from the 18 recording channels in each window were summarized into an 18-dimensional state vector that characterized the spatial distribution of activity in that window (Fig. 3b). Each vector was z-transformed to remove activity common to the channels. The temporal development of the spatial activity pattern corresponded to a trajectory described by the state vector in an abstract 18-dimensional configuration space

as the window was stepped along the time axis (Fig. 3c). We calculated the distance in configuration space between the trajectory for each trial (the presentation of either a rising or a falling frequency-modulated tone) and a trajectory formed by averaging over all trajectories for the other stimulus in that session; that is, a single trial with a rising frequency-modulated tone was compared with the mean over all trials with falling frequency-modulated tones and vice versa. The distance is a dissimilarity function, a measure of the dissimilarity between the compared spatial activity patterns expressed in standard deviations (s.d.) of the mean dissimilarity obtained over the entire trajectory (Fig. 3d). Marked states are defined as peaks in the dissimilarity function with amplitudes at least three s.d. over the baseline of the amplitude distribution. We used a nonlinear projection algorithm³⁰ to display the combinatorial multitude of similarity relations between marked states, resulting in a map of the corresponding 18-dimensional state vectors in the two-dimensional plane that preserves all mutual distances between projected states. This is achieved by minimizing the relative mapping error

$$E(k) = \frac{1}{\Delta} \sum_{i \neq j}^N \frac{(d_{ij}^* - d_{ij}(k))^2}{d_{ij}^*}$$

where

$$\Delta = \sum_{i \neq j}^N d_{ij}^*$$

and d_{ij}^* is the euclidean distance between marked state vectors i and j in the original 18-dimensional state space, $d_{ij}(k)$ is the distance between the corresponding vectors projected into the two-dimensional plane after the k th step, and N is the number of vectors projected. The mapping error was minimized with a step-wise steepest-gradient procedure starting with a two-dimensional uniformly random vector distribution in the unit square. The mapping was accepted when the criterion

$$E(k \rightarrow \infty) \leq 0.5\%$$

was reached; when this was not achieved, another random starting condition for the steepest-gradient procedure was used. Clustering of the spatial patterns during the marked states after categorization (Fig. 2, right column) was tested using a resampling approach (see Supplementary Information).

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Supplementary information is available on Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from the London editorial office of Nature.

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Purification of a pluripotent neural stem cell from the adult mouse brain

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The adult mammalian central nervous system (CNS) contains a population of neural stem cells (NSCs)^{1–4} with properties said to include the generation of non-neural progeny^{5–7}. However, the precise identity, location and potential of the NSC *in situ* remain unclear. We purified NSCs from the adult mouse brain by flow cytometry, and directly examined the cells' properties. Here we show that one type of NSC, which expresses the protein nestin but only low levels of PNA-binding and HSA proteins, is found in both ependymal and subventricular zones and accounts for about 63% of the total NSC activity. Furthermore, the selective depletion of the population of this stem cell in *querkopf*⁸ mutant mice (which are deficient in production of olfactory neurons) suggests that it acts as a major functional stem cell *in vivo*. Most freshly isolated NSCs, when co-cultured with a muscle cell line, rapidly differentiated *in vitro* into myocytes that contain myosin heavy chain (MyHC). This demonstrates that a predominant, functional type of stem cell exists in the periventricular region of the adult brain with the intrinsic ability to generate neural and non-neural cells.

As the precise location of NSCs in the adult brain remains controversial^{9–11}, cells were gathered from the two areas with the reported highest NSC content: the ependymal and subventricular zones of the lateral ventricular walls. To identify the putative NSCs within these populations, we examined single-cell suspensions for the expression of a variety of cell surface markers and characteristics with a fluorescence-activated cell sorter (FACS). The frequency of NSCs in sorted sub-populations was determined by the ability of individual cells to generate multipotent neurospheres^{1,12,13}.