Odour encoding in olfactory neuronal networks beyond synchronization

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It has been suggested that odour encoding in olfactory systems occurs by synchronized firing in neuronal populations. Neurons correlated in terms of the Lempel–Ziv distance of spike trains and the sequential superparamagnetic clustering algorithm belong to the same cluster if they show similar, but not necessarily synchronous, firing patterns. Using multielectrode array recordings from the rat olfactory bulb, we have determined cluster incidence and stability in the neuronal network using both the Lempel–Ziv distance and a measure of synchronization. In the Lempel–Ziv paradigm, we found pronounced stabilization and destabilization effects in the neuronal network in response to odour presentation when compared with the synchronization paradigm. This suggests that synchronization alone may be insufficient for understanding olfactory coding. *NeuroReport* 17:1499–1502 © 2006 Lippincott Williams & Wilkins.

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Introduction

Olfaction is a chemical sense, differing largely from senses that process physical input like photon density (vision) or air pressure/particle velocity (hearing). A major distinction is the synthetic property of olfaction, that is, the ability to assign a specific identity to a great number of component mixtures [1]. In humans, there are approximately 350 different types of odour receptors present on the surfaces of the olfactory receptor neurons, while other species of mammal may have many more, allowing the discrimination of many thousands of different odours [2]. Therefore, it is assumed that odour identity is encoded in the activity of many cells in the output neurons of the olfactory system (in vertebrates it is the mitral cells of the olfactory bulb). Thus, olfactory coding is a typical example of a population code and requires the analysis of functional clustering within a neuronal network of an olfactory sensor when confronted by a specific odour. On the basis of studies in the invertebrate [3] and vertebrate [4] olfactory systems, the most frequently applied criterion determining membership of such a cluster for a specific neuron is whether the activity of that neuron is synchronized with the other neurons in the cluster. In invertebrate olfactory systems, for example in bees [5], it has been shown that synchronization is essential for discrimination between different odours. Although it has been proposed that this might be the case in the vertebrate brain, this has yet to be confirmed. Numerous theories of neuronal function rely on the assumption of synchronization in neuronal populations [6]. It has however been suggested that higher cortical areas may lack the necessary mechanisms for decoding synchronous spikes [7].

What properties in neuronal firing, other than synchronization, might enable identification of specific populations encoding for specific odours within an olfactory neural network? We address this question using alternative criteria for neuronal population identification on the basis of the Lempel–Ziv distance (LZ-distance) of spike trains [8] and the sequential superparamagnetic clustering paradigm [9]. In this approach, neurons belong to the same cluster if they show similar, but not necessarily synchronous, patterns in their firing. Here, we apply our criteria to electrophysiological data acquired from the mitral cell layer of the olfactory bulb of the rat using multielectrode array recordings, and compare the results with those obtained when using synchronization as the physiological resource for odour encoding in this neuronal population.

Methods

Experimental procedure

Neuronal activity of anaesthetised rats (25% urethane, intraperitoneal, 1.5 g/kg body weight) was sampled using a microelectrode array positioned in the olfactory bulb. The arrays comprised 30 electrodes (6×5 grid, 350 μm tip separation). An odour (cineole), carried as a saturated vapour in nitrogen gas (odourless), was mixed with dry air

(5.42×10^{-6} mol) and delivered for 10 s to the rat via a mask over the nose. Breathing was monitored, and the onset of odour delivery was timed to mid-expiration. Neuronal activity was sampled in the 10 s period before odour onset (the prestimulus period) and the 10 s period of odour presentation (the during-stimulus period). Spikes were detected when a triggering threshold was crossed by the recorded signal from an electrode. This threshold was set at $\geq 2 \times$ the background noise level. The activities of single or multiple neurons were detected in the activity sampled by each electrode. The activities of individual neurons were distinguished from multiple neuron activity using a machine learning algorithm to cluster spikes on the basis of waveform features acquired using principal component analysis combined with features describing the geometric shapes constituting a spike waveform [10], allowing discrimination of activity from multiple neurons at each active site. In this way, simultaneous recordings from more than 40 neurons were made per rat. Forty odour presentations were made, yielding a total of 80 spike trains per neuron. The experiments were performed on two different animals. All procedures used for data collection conformed to the Animals (Scientific Procedures) Act, 1986 (UK Home Office).

Spike train clustering

As a measure of similarity for neuronal clustering, we use the LZ-distance, a distance measure based on the Lempel-Ziv complexity [11]. The properties of the measure are outlined in [8], and we therefore only briefly review the main points here: the spike trains are translated into bitstrings X_n of length n using a bin size of 1 ms, such that the symbols '0' and '1' denote, respectively, the absence or presence of a spike. These bitstrings are coded using a parser that partitions the string into nonoverlapping substrings called phrases (LZ coding). The LZ-distance compares the set of phrases generated by LZ coding of two bitstrings originating from corresponding spike trains. A large number of similar patterns appearing in both spike trains leads to a large overlap of the sets of phrases. Thus, distances between spike trains with similar patterns are small, whereas distances between spike trains with different patterns are large. Tests have demonstrated that LZ-distances between spike trains with similar, but not necessarily synchronous, sequences of interspike intervals are considered small.

The application of the LZ-distance to a set of spike trains produces a distance matrix, which serves as an input for the sequential superparamagnetic clustering algorithm. Using this algorithm (see [9] for a detailed description of the algorithm), neither the number of clusters nor their size has to be predefined. The clustering algorithm comes equipped with an intrinsic measure for cluster stability s, in which $0 \leq s \leq 1$. It sequentially ranks clusters according to their stability, that is, the most stable cluster is detected first and the last cluster is the least stable one. The clustering parameters have been chosen as follows: minsize=2 (the minimum number of neurons in a cluster) and s_0=0.04 (the minimum required stability of a cluster). To compare the result obtained using the LZ-distance with a correlation measure, we applied the correlation distance (C-distance; the clustering procedure is analogous to that in [12]). For the C-distance, spike trains that share a large number of synchronous spikes are considered to be close. Therefore, the measure reflects the criterion of synchronization in neuronal group formation.

Our analysis was performed in a three-step procedure. First, we determined the number of clusters of neurons in each of the 80 periods of recording, using both the LZ-distance and the C-distance for the data obtained in both animals. Second, we quantified the interactions of each neuron with every other neuron in the during-stimulus period compared with the prestimulus period. This was accomplished by assigning each neuron a vector, whose components each indicate the number of times the specified neuron participated in a cluster with another neuron. Periods of recording were excluded if no clusters formed. Such periods occurred in both the prestimulus and during-stimulus periods using both distance measures. For example, for the ith neuron N_i, the vector has the form $\mathbf{N}_i=(x_1, \ldots, x_{max})$, where x_j indicates the number of times the neuron N_i is in the same group as the neuron N_j and max is the number of neurons measured in that animal. The distance between two such vectors $\mathbf{N}_i$ and $\mathbf{N}_j$ is simply

$$d(\mathbf{N}i, \mathbf{N}j) = 1 - \frac{\mathbf{N}i \cdot \mathbf{N}j}{|\mathbf{Ni}||\mathbf{Nj}|}. \quad (1)$$

Using this measure, proximity of two neurons indicates that they often participate in the same cluster. By clustering with this distance measure, averaged over all trials, the degree of interrelation of neurons within the network is derived ('clusters among partners'). We determined the dendrogram for s_0=0.1 such that only stable clusters are detected. Third, we identified those neurons that remain in the same cluster for both the prestimulus and the during-stimulus periods ('partners'). We found that almost all neurons, when associated with a partner, keep their partners in both periods. The stability of the clusters identified was then quantified as follows: we reduced the vector $\mathbf{N}_i$ of each neuron N_i generated in step two, to those components representing the partners of this neuron, leading to the vector $\mathbf{N}'_i$. For example, if N_i has four partners, then $\mathbf{N}'_i$ has five components. The mean of the lengths of the vectors of all neurons in a cluster reflects the stability of this cluster. This was determined for both prestimulus and during-stimulus periods. Note that in this analysis two different notions of clusters and stability are investigated. In the first step, the clusters reflect the degree of relatedness in a single period of recording and cluster stability is an intrinsic parameter of the clustering algorithm. In the third step, the clusters reflect the mean degree of relatedness between the neurons of the network and the stability is quantified by the number of times neurons of the same cluster are grouped together in each single trial.

Results

We performed the procedure described in the spike train clustering section using both the LZ-distance and C-distance as similarity measures for clustering on the data obtained in both animals. The result of the first step of our analysis is shown in Fig. 1. We find that the number of clusters emerging on stimulus presentation does not seem to indicate whether the network is in a prestimulus or a during-stimulus condition. The standard deviations reflect a considerable intertrial-variability of the network behaviour. A special case emerged in animal B using the C-distance

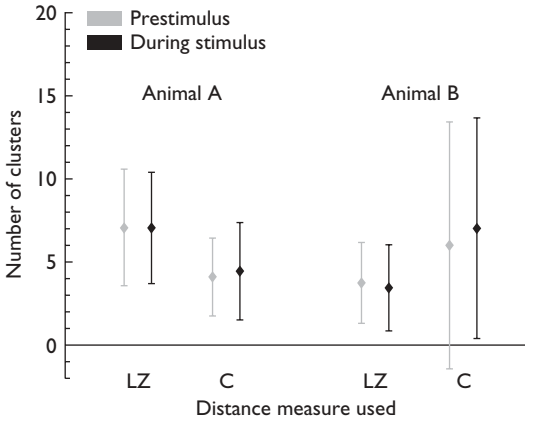

Fig. 1 Olfactory neural network clustering, measured by the Lempel–Ziv (LZ) distance and the C-distance: the mean (±SEM) number of clusters in the prestimulus and during-stimulus condition for both animals investigated.

paradigm. Here, for s_θ=0.04, no clustering was observed. Clusters emerged in some cases only at s_θ=0.02, and were almost entirely of sizes 2 and 3, leading to the large standard deviation shown. This result is typical for situations where no natural clusters are present in the dataset [9], that is, indicating that no significant synchronization effect emerged in the olfactory neuronal network of animal B. We thus excluded the results obtained in the C-distance paradigm of animal B from steps 2 and 3 of the analysis.

The results of the second and third steps of our analysis are shown in Fig. 2. Here, we find clear differences between the prestimulus and during-stimulus periods for some clusters of partners. In animal A, using the LZ-distance paradigm, seven clusters were identified among partners. Among them, cluster 1 shows a clear stabilization effect (i.e. the mean vector length is significantly increased), whereas cluster 3 shows a destabilization effect (the mean vector length decreases). The other clusters do not show significant changes. Using the C-distance paradigm, four clusters among partners are identified. Here, only cluster 2 shows a significant effect in consequence of stimulus presentation (a destabilisation), although the magnitude of the effect is smaller than in the LZ-paradigm. In animal B, using only the LZ-distance paradigm as explained above, four clusters were identified among partners, two of them changing in response to stimulus presentation: destabilization for cluster 3, and stabilization for cluster 4.

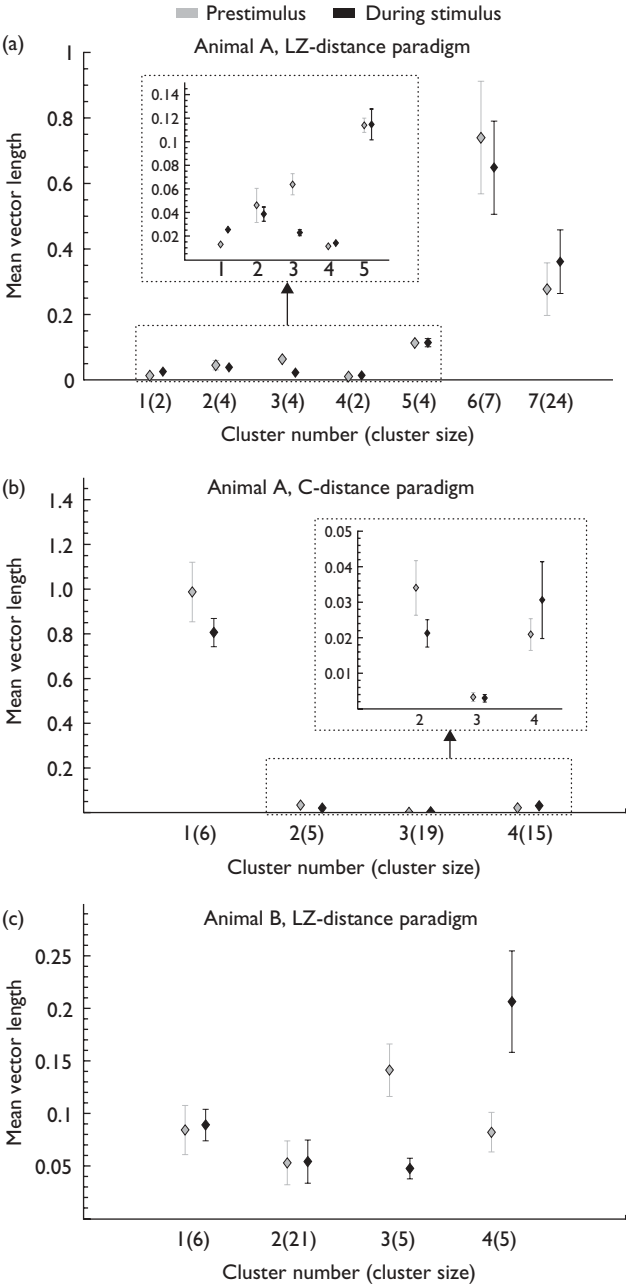

Fig. 2 Stability of clusters among partners (see the text): (a) animal A, LZ paradigm; (b) animal A, C-paradigm; (c) animal B, LZ paradigm. In (a) and (b), the upper inset (dotted line) reproduces the area of the lower inset on an expanded vertical axis.

Discussion

In recent times, the dynamic aspects of olfactory network computation have become an important topic in olfaction research (e.g. [13]). We have investigated this issue by focussing on functional clustering in olfactory neuronal networks using two different paradigms of similarity in neuronal firing. We find that, for both paradigms, the number of clusters formed does not reflect the presence or absence of a stimulus and that, on the basis of our 'partner analysis', neurons indeed keep their partners in both prestimulus and during-stimulus conditions. This probably reflects the underlying neuronal network structure that does not change over the relatively short time scale used in our experiment. Rather, odour presentation alters the degree of interrelation between the partners in these clusters. This manifests in the effects of stabilization and destabilization in some of the clusters identified. These effects might reflect the computation performed by the network: those clusters that are involved in olfactory encoding stabilize their firing patterns, whereas others remain unaffected or even destabilize – the latter might also have a functional role. For example, in the context of signal reproduction with a limited set of resources, negative correlation may result in systems with lower noise, and more accurate representation of information (S. Durrant, K.M. Kendrick, J.F. Feng, in

preparation; [14]). Evidence for negative correlation in neuronal firing in the olfactory bulb as a component of the response to odour presentation has been presented elsewhere [15]. We find that the effect of stabilization and destabilization is more prominent in the LZ-paradigm than in the C-paradigm, indicating that synchronization alone may be insufficient for understanding olfactory coding. An open question remains, however: how are spike trains that are similar under the LZ-paradigm decoded by higher cortical areas?

Conclusion

Our multielectrode array recordings from the mitral cell layer of the rat olfactory bulb reveal significant effects of stabilization and destabilization in neuronal clustering expressed by the LZ distance as a result of odour presentation. These effects are much more pronounced than those for neuronal clustering using the synchronization paradigm, indicating that the effect of synchronization is of less importance in understanding population coding in the vertebrate olfactory system than has been theoretically predicted.

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