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Complex spike patterns in olfactory bulb neuronal networks☆

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HIGHLIGHTS

- Complex patterns of spikes can be detected in the mitral cell layer of the olfactory bulb.
- These patterns can be detected by utilizing a multivariate approach known as T-pattern analysis.
- The incidence of sequences is much greater in real data than when those data are randomized.
- The incidence of sequences may reflect physiological condition.

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ABSTRACT

Background: T-pattern analysis is a procedure developed for detecting non-randomly recurring hierarchical and multiordinal real-time sequential patterns (T-patterns).
New method: We have inquired whether such patterns of action potentials (spikes) can be extracted from extracellular activity sampled simultaneously from many neurons across the mitral cell layer of the olfactory bulb (OB). Spikes were sampled from urethane-anaesthetized rats over a 6 h recording session, or a period lasting as long as permitted by the physiological condition of the animal. Breathing was recorded to mark peak inhalation and exhalation.
Results: Complex T-patterns of up to ~20 elements were identified with functional connections often spanning the full extent of the array. A considerable proportion of these sequences incorporated breathing.
Comparison with existing methods: In contrast to sequence detection by synfire, the incidence of sequences detected in our real data is very much greater than in the same data when randomized either by shuffling, or an alternative procedure preserving the interval structure of each spike train, and so more conservative. Further, when recordings were terminated before completion of the full recording session, the relative pattern detection in real and randomized data was a strong indicator of physiological condition—in recordings leading up to the preparation becoming physiologically unstable, the number of patterns detected in real data approached that in the randomized data.
Conclusions: We conclude that such sequences are an important physiological property of the neural system studied, and suggest that they may form a basis for encoding sensory information.

1. Introduction

Many studies have detailed the responses of single neurons to sensory stimuli. However, such studies cannot address the issue of interaction patterns within large ensembles of neurons. Yet there is little doubt that much of the processing capacity of the brain resides in the activities of cooperating and competing networks of neurons. Temporal sequencing between simultaneously sampled neurons in an in vivo preparation was first reported by Griffith and Horn (1963) and has long been recognized as theoretically important in cognitive neuroscience. Synchronized activity amongst neurons has been linked to perceptual cognition, namely "the binding problem" (Engel et al., 1997), whereby the combined features of a complex stimulus are associated by the synchronization of the activities of neurons responsive to one or more of those features. The most widely accepted theory of the physiology of memory formation,

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proposed by Hebb (1949), is also based upon the occurrence of such interactions in memory systems—"When an axon of cell A is near enough to excite B and repeatedly or persistently takes part in firing it... A's efficiency, as one of the cells firing B, is increased". Certain experimental models provide evidence that memory formation may adhere to so-called "*Hebbian*" principles.

Long-term potentiation (LTP) is a physiological process that has been studied extensively since it was first described by Bliss and Lomo (1973). In LTP, pre- and postsynaptic elements in a neural pathway are simultaneously activated by repeated electrical stimulation of the presynaptic elements, thereby fulfilling the first of Hebb's principles (Hebb, 1949)—neuron A repeatedly activates neuron B through the synaptic connection between the two elements. Subsequently, the efficiency of neuron A in activating neuron B is increased, and so is formed a simple hebbian assembly. In this paradigm, a large number of neuron A's activate a large number of neuron B's (i.e. there is little noise in the system), and the enhanced efficiency of transmission from one to the other is evident in the increased amplitude of the field potential generated when a single pulse is delivered to the presynaptic elements. However, evidence for such a process in a functioning system remains elusive.

Attempts have been made to discover spike patterns within populations of neurons, but so far these have not produced the desired kinds of results. Thus, Abeles and Gerstein (1988) proposed a search algorithm for the detection of multi-neuron patterns called "synfire". While numerous patterns were detected, doubt remains regarding the statistical significance of the findings (Oram et al., 2001; Baker and Lemon, 2000). Here, a more flexible pattern model, called a T-pattern, is applied. T-pattern detection uses an evolution algorithm for the detection of the repeated hierarchical and multi-ordinal real-time patterns in data sets consisting of a number of time point series all occurring within the same observation period (Magnusson, 2000, 2004; Bonasera et al., 2008; Casarrubea et al., 2013, submitted). The large number of T-patterns detected frequently far exceeds those found in randomized data thus the complex patterns discovered through T-pattern analysis provide a dynamic view of neuronal interaction which may be invaluable in understanding the mechanisms of neuronal networks and the way they encode sensory information.

Olfactory encoding is of specific interest as behavioural paradigms underpinning studies of the neurobiology of olfactory learning and memory are considered particularly robust (Bolhuis and MacPhail, 2001) and considerable progress has been made in establishing the neural substrates and pathways involved (Kendrick et al., 1992, 1997; Da Costa et al., 1997). Much of the encoding takes place at the level of the olfactory bulb (OB), the primary cortical projection area for olfactory input, and an area that is entirely committed to processing this information. Structurally, the OB is widely conserved across vertebrate taxa. The area has been confirmed as playing an important role in olfactory memory formation. Thus, understanding the processes involved in encoding olfactory information is of great importance to understanding the fundamental neuronal mechanisms of learning and memory. Olfactory receptor neurons in the olfactory epithelium in the nasal cavity project to mitral cells in glomeruli in the OB (Mori et al., 1999). Optical imaging studies demonstrate that different odorants elicit spatially defined spatial patterns of glomerular activity in the olfactory bulb (Johnson et al., 2002). The quality of an olfactory stimulus is thus encoded by the combined specific activation of glomeruli by a given odorant. Gaining access to the olfactory bulb with a microelectrode array (MEA) allows in vivo electrophysiological sampling of neuronal activity over a relatively large area of cortex (>2 mm^2). We have applied T-pattern analysis to spike data collected simultaneously across many neurons, using microelectrode arrays, to establish that recurring complex sequences of spikes can be detected in extracellular activity sampled simultaneously from many neurons across the mitral cell layer of the OB. We characterize these patterns in light of their putative role in processing sensory information.

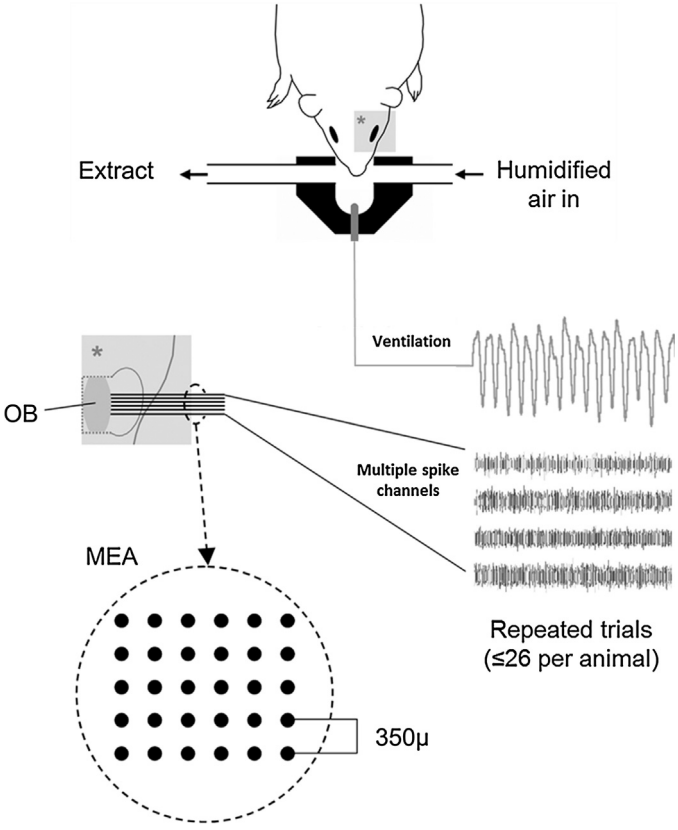

Fig. 1. *Recordings.* Action potentials (spikes) from individual neurons were sampled using a microelectrode array positioned laterally in the mitral cell layer of the left olfactory bulb of urethane-anaesthetized rats. Typically, each electrode in the array could sample spikes from multiple neurons. Spikes from individual neurons were discriminated using software developed for this task (Horton et al., 2007), and these are coded in varied greyscale in the four traces displayed. Throughout the recordings, the rats were supplied with humidified air via a mask over the nose. Breathing was recorded using a thermistor embedded in the mask.

2. Materials & methods

The present data (see Figs. 1 and 2) were collected from the olfactory bulb of six male Wistar rats (4–9 months) under urethane anaesthesia (1 mg/kg body weight, i.p.). Throughout surgical and experimental procedures, humidified air was supplied through a mask over the nose, and breathing was monitored and recorded using a thermistor in the mask. Data were sampled in 10 s periods (trials) at 5 min intervals through a recording session of 6 h, or for as long as the animal remained physiologically stable as judged by its breathing. If animals reached a point when irregular breathing clearly indicated degrading physiological condition, recordings were terminated, and degrading condition noted as an experimental variable. Here we compare one such animal with others which remained physiologically viable throughout the full sampling period.

Microelectrode arrays of sharpened tungsten electrodes, arranged in a 6×5 array with 350μ spacing, were advanced laterally into the OB (for one animal a 6×8 electrode array, with 250μ spacing was used). Action potentials (spikes) were sampled from mitral layer OB neurons across an area of ~2.2 mm^2 using a 100 channel laboratory interface (Bionic Technologies Inc./Cyberkinetics Inc., USA). Spikes sampled in the mitral cell layer