

Temporal coding of repeated stimuli in the olfactory pathway

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Abstract

We performed single-unit recording from olfactory bulb and hippocampus of anesthetized, freely breathing rats. The stimulus was 7 to 30 cycles of 10-second pulses of odorant followed by a fixed air period. We performed runs with air periods ranging from 10 to 150 seconds. Both bulbar and hippocampal neurons respond to odor with changes in firing rate and respiratory phase-locking. Olfactory bulb units respond to most air periods. Unexpectedly, most hippocampal units were tuned to only one of the air periods. We developed an analytical formulation that may describe respiratory phase-locked temporal responses of mitral/tufted neurons.

Introduction

Olfactory responses are strongly modulated by temporal course of the stimulus, both in terms of the stimulus periodicity and in terms of habituation. In the locust there is evidence for field-potential clocking as a means of odorant classification (Wehr and Laurent, 1996). Mammalian olfactory responses are complicated by the presence of the respiratory cycle, which can vary over wide intervals and which leads to repetitive, semi-periodic sampling at the receptors. We designed a stimulus protocol to study details of olfactory temporal responses in the rat using cyclical presentation of odorants interspersed with air periods. Olfactory bulb responses are known to be affected by respiration (Bhalla and Bower, 1997; Chaput et al., 1992), behavioral state (Kay and Laurent, 1999) and habituation (Harrison and Scott, 1986). The hippocampus has also been implicated in aspects of olfactory recognition (Eichenbaum et al., 1987) so we simultaneously recorded activity from the hippocampus as a possible central substrate for habituation (Deshmukh and Bhalla, in press).

Methods

Female Wistar rats (National Institute of Nutrition, Hyderabad) were acutely anesthetized (Ketamine/Xylazine + thiopental sodium) and surgery was performed to expose olfactory bulb and cortex over the hippocampus. All procedures were in accordance with NCBS and Govt. of India guidelines. Tetrodes (25 μ M nichrome, gold-plated) were simultaneously inserted into the olfactory bulb and hippocampus using independent micromanipulators. Signals were amplified (total 5000X gain, 300-6000 Hz bandpass) and digitized using a DAP3200e card (Microstar laboratories) sampling at 20KHz -30KHz per channel. Data were stored to disk and analyzed using custom-written cluster cutting software and MClust (D. Redish). The same computer controlled odor stimuli through an air-dilution olfactometer. Odors were diluted 30-fold from saturation. Isoamyl acetate and cineole were used for the periodic stimuli. In each run, 10-second odor pulses were followed by 2-second purge periods and a fixed air period in a cyclical manner for 7 to 30 repetitions. Successive runs of were separated by at least 15 minutes. The sequence of air intervals

used in the runs was 150 seconds, 100 seconds, 50 seconds, 20 seconds, and 10 seconds (air + purge durations). Recent experiments are designed to extend these recordings to awake, freely moving rats.

Results and discussion

Odor responses.

Bulbar mitral/tufted cell responses were much as described previously (Bhalla and Bower, 1997; Harrison and Scott, 1986). Mitral/tufted cell responses involved firing rate as well as changes in respiratory phase-locking. Interestingly, hippocampal pyramidal neurons also showed both kinds of responses, with the respiratory-locked responses accounting for approx 40% of the responses. It was unexpected to find such similar activity patterns both in the olfactory bulb and a higher processing center such as the hippocampus.

Interval tuning.

Bulbar units typically responded to multiple contiguous air intervals. This could either be interpreted as coarse tuning or as habituation-modulated responses. Hippocampal units were surprisingly tightly tuned, and most responded only to a single air interval. Others responded to a small number of air intervals, not necessarily contiguous. Further, there did not appear to be any selectivity for short or long air intervals (Figure 1). We postulate that this form of tuning may be related to odor-guided navigation. Due to the duration of the experiments it was not possible in the anesthetized recordings to determine if this tuning was consistent over long periods of time. Chronically implanted recordings are in progress to resolve this.

Temporal responses of mitral/tufted cells.

Our protocol provides repeated episodes of odor stimulation at a given air interval. This makes it possible to perform statistical analyses of individual respiratory cycle responses following odor onset. This is important because behavioural studies suggest that odor recognition occurs within one sniff (Mainen, personal communication). We devised a smoothed representation of firing rate as a function of respiratory phase and time. We then analyzed various analytical formulations for firing probabilities. There are several features of these responses that these formulations must account for, including initial responses, changes in phase dependence, rebound and habituation effects. Relatively simple formulations appear to capture most of these features and we propose that this may be a good description of individual mitral-tufted cell temporal responses. This formulation may be important to relate to the spatial/population patterns of mitral/tufted cell response to describe odor representation. It may also be useful as a basis for analyzing departures from baseline patterns of activity when the system performs different kinds of processing or retrieval of odorant information. We are currently examining if these formulations are also applicable to freely breathing animals, especially when sniffing.

Conclusions

We have examined long and short-term aspects of temporal coding in the mammalian olfactory system. Mammalian olfaction is complicated by receptor as well as central habituation, and by the presence of a respiratory cycle that constrains stimulus sampling. We find evidence for long-term

temporal coding in the form of surprisingly sharp representation of air intervals between odor pulses in the hippocampus (Deshmukh and Bhalla, in press). We also find that respiratory cycle and short-term habituation effects may be possible to express in an analytical form which may facilitate future analysis of departures from baseline activity.

References

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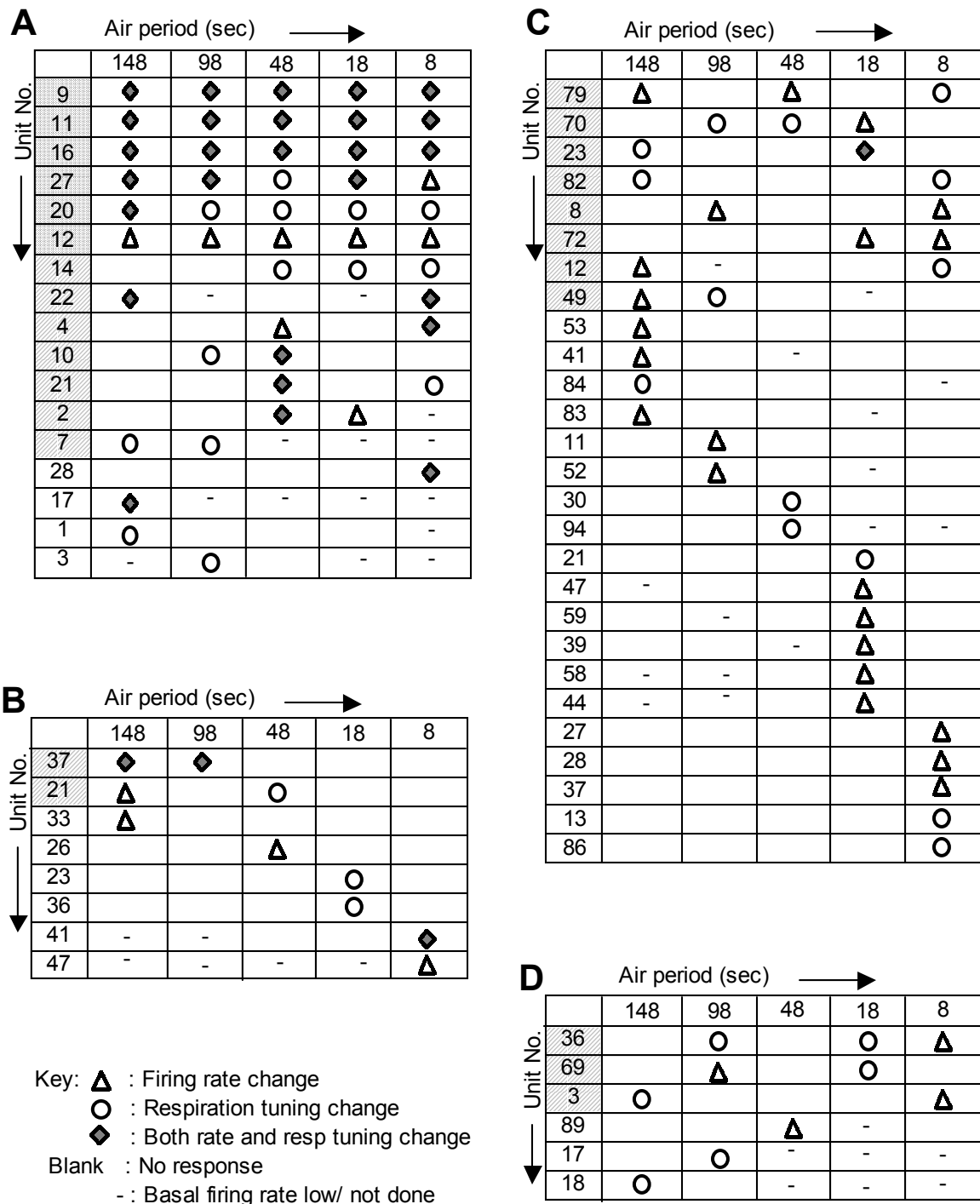


Figure 1. Matrix of unit responses against air period for odor responsive olfactory bulb mitral/tufted cells (A), hippocampal CA1 pyramidal cells (B) hippocampal CA3 pyramidal cells (C), and CA1 interneurons (D). Only the neurons that responded to at least one air period are included. Units are sorted by number of responses. Units that responded to odors delivered at all air periods are highlighted with a dotted background. Units responding to more than one air period are highlighted with a diagonal line background. Air periods were preceded by 2 seconds purge, so the interval between odors was the air period + 2 seconds. From Deshmukh and Bhalla, in press.