

4. **Communication within a Neural Network**

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Abstract. Recent observations of complex sequences of neuronal activity, at the level of both synaptic transmission and action potential generation amongst simultaneously sampled multiple neurons, have demonstrated that such patterns can occur spontaneously in the brain. Using multielectrode array (MEA) technology we have sampled simultaneously from a large number of neurons (typically >100 neurons), across a large area (>2mm²) of the olfactory bulb in anaesthetised rats. Here also we find complex sequences in neuronal firing. Connections in these sequences may traverse the entire area populated by the sampled neurons. Most notably, these studies have demonstrated functional significance in such sequences of neuronal activity; in the olfactory bulb precise sequences of discharge are related both to ventilatory activity and to odour information. Indeed, both the number of occurrences and the variety of neuronal sequences appear to reflect stimulus quality.

Keywords: Olfactory bulb; electrophysiology; neuron; neuronal sequence; encoding; odour.

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4.1 Introduction

It is more than 40 years since the first observations on temporal sequencing of the activities of simultaneously sampled multiple neurons were published [1]. Since then such interactions have been linked to perceptual cognition, namely “the binding problem” [2], whereby the combined features of a complex stimulus come to be associated by the synchronisation of the activities of neurons responsive to one or more of those features. The most widely accepted theory of the physiology of memory formation, that of Donald Hebb [3], is also based upon the occurrence of such interactions in a memory system – “*When an axon of cell A is near enough to excite B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased*”. Through this process, Hebb postulated that a cell assembly would be formed (the *Hebbian* cell assembly) which would constitute “*the simplest instance of a representative process... the firing of the efferent cell is more likely to follow the lead of the afferent cell*”. It is interesting to note that James [4] proposed similar principles underlying memory formation – “*When two elementary brain-processes have been active together or in immediate succession, one of them, on recurring, tends to propagate its excitement into the other... The tendency is proportionate to the intensity of the excitement and to the number of times it has occurred*”. Even according to Aristotle [5], “*Acts of recollection, as they occur in experience, are due to the fact that one thought has by nature another that succeeds it in regular order*”.

Certain experimental models provide evidence that memory formation may adhere to hebbian principles. Long-term potentiation (LTP) is a physiological process that has been studied extensively since it was first described by Bliss & Lomo [6]. In LTP, pre- and postsynaptic elements in a neural pathway are driven into synchronised activity by repeated electrical stimulation of the pre-synaptic elements, thereby fulfilling the first of Hebb’s principles – neuron A repeatedly activates neuron B through the synaptic connection between the two elements. Subsequently, the efficiency of A in firing B is increased, and thus 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 manifests in the increased amplitude of the field potential generated when a single pulse is delivered to the pre-synaptic elements. However, evidence for such a process occurring in a functioning memory system remains elusive. Often comparisons between the experimental model (LTP) and memory formation in the functioning system assume that activation of a postsynaptic neuron may be reliably predicted by activation of an afferent pre-synaptic neuron. However, in a system where a postsynaptic neuron may receive many inputs from many afferent neurons, activation by the input from a single afferent neuron may have little impact relative to ongoing activation by the large number of other neurons. For this reason, and because spike-timing is of such theoretical importance, analysis of temporal sequencing in multiple neuronal activity, using such techniques as T-pattern analysis, may be of great value in understanding the mechanisms whereby neuronal networks encode sensory information.

4.2 Neuronal encoding of odour information in the olfactory bulb

Behavioural paradigms underpinning studies of the neurobiology of olfactory learning and memory are considered particularly robust [7] and considerable progress has been made in establishing the neural substrates and pathways involved [8-10]. 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 given over to processing this information [11]. The area has been confirmed as playing an important role in olfactory memory formation and for this reason understanding the processes involved in encoding olfactory information is of great importance to understanding the 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 [11]. Optical imaging studies demonstrate that different odors elicit spatially defined patterns of glomerular activity in the olfactory bulb [12, 13]. The quality of an olfactory stimulus is encoded by the specific combination of glomeruli activated by a given odorant. Gaining access to the olfactory bulb with a multiple electrode array (MEA) allows in vivo electrophysiological monitoring of neurons over a relatively large area of cortex. This makes possible the study of spatiotemporal patterns of activation across, and interactions between a large number of simultaneously sampled and widely dispersed neurons in this area.

Data currently being analysed by T-pattern analysis [14, 15] with the THEME software (see www.patternvision.com & www.noldus.com) have been collected from the olfactory bulb of anaesthetised rats (25% urethane, intraperitoneal, 1.5g per kg body weight). Using an MEA of either 30 or 48 electrodes advanced laterally into the OB (see Fig. 4.1A), action potentials (spikes) were sampled from mitral layer OB neurons across an area of approximately 2.3mm² using a 100 channel laboratory interface (Cyberkinetics Inc., USA). Typically, spikes were sampled from approximately 60 – 70% of electrodes. After completion of recordings, off-line discrimination of spikes from individual neurons was performed using a PCA (principle components analysis) sorting algorithm developed specifically for these data allowing discrimination of activity from multiple neurons at each active electrode (Horton PM, <http://www.sussex.ac.uk/Users/pmh20>). Typically, spikes were sampled simultaneously from > 100 neurons across the entire MEA. Times of occurrence of spikes generated by individual neurons were stored as events coded with the identity of the neuron and its location on the MEA. Also stored were events marking onset of expiration and onset of inspiration in the breathing cycle to allow this data to be related to patterns identified in the neural data. The data stored in this way are suitable for analysis by THEME. Separate data files were generated for activity preceding and during presentation of odour stimuli to the rat over a number of trials using different odours at various concentrations.

4.3 Spatiotemporal patterns in olfactory bulb neuronal activity

Event types entered into the T-pattern analysis were times of occurrence of spikes from individual neurons, and times of onset of inhalation and exhalation in the breathing cycle. A minimum of 15 repetitions with a significance level of $p < .005$ was required for recognition of a pattern.

Many patterns were identified in all of the data, both pre- and during odour presentation (see Fig. 4.2): as many as 3949 different patterns of neuronal firing sequence were identified in a single 10s period of sampling, with patterns repeating as frequently as 480 times during that period. Patterns included as many as nine event types. In all data sets, the number of patterns detected greatly exceeded that found when the data were randomised ($p < .001$), i.e. when all events occurring in the data were located randomly in the observation period. This was so across all pattern lengths. By plotting temporal sequences of neuronal firing in two dimensions, it can be seen that an individual pattern may span a large part of the area covered by the MEA (an example plot of such a sequence is shown in Fig 4.1A, another is presented in chapter 1 of this book).

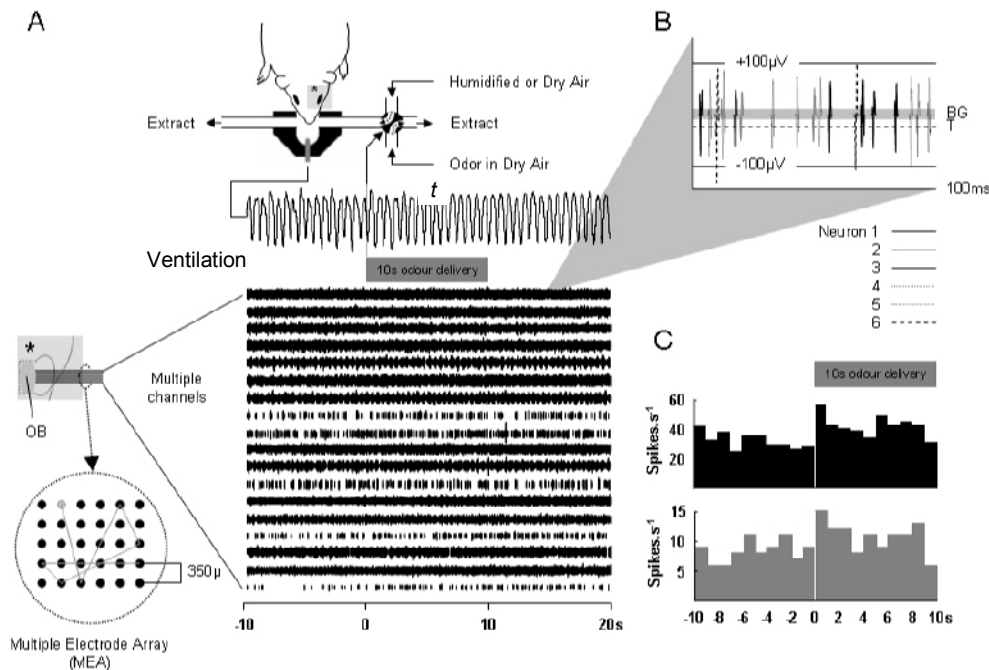


Figure 4.1 Recordings from OB during odour presentations. (A) Neuronal activity (action potentials, shown, and local field potentials) was sampled from electrodes in a microelectrode array (MEA) positioned in the olfactory bulb (OB). Microelectrode arrays comprised either 30 electrodes (6×5, 350µm separation, illustrated) or 48 electrodes (6×8, 250µm separation). An example two-dimensional plot of a sequence of neuronal discharges (grey line) is superimposed on the diagram of a 30 electrode MEA, commencing at the electrode denoted by the grey circle. Craniotomy and removal of the left eye permitted lateral access to the OB through the left orbit (inset*). During surgery and recordings, humidified air was supplied to the rat through a mask over the nose. A thermistor was used to record air temperature in the mask and so monitor breathing. Shortly (~30s) before delivery of an odour stimulus, the air to the mask was switched to dry air. Odours, carried as saturated vapours in nitrogen gas (odourless), were mixed in various concentrations with dry air, and were delivered for 10s to the rat via the mask. Onset of odour delivery (t) was precisely timed to mid-expiration. Neuronal activity was recorded for a period spanning 10s before odour onset to 10s after odour offset. In the recordings shown, spikes were detected in 18 of 30 channels. (B) Spikes were detected when a triggering threshold (T) was crossed by the recorded signal from each electrode. The threshold was set at $\geq 2 \times$ the background noise level (BG). A 100ms section of the upper spike train has been expanded to show times of occurrence of discriminated spikes. Two of the neurons, identified by the solid black and solid grey spikes, responded to the presentation of 5.4×10^{-4} M amyl acetate. The responses of these neurons to the presentation of this odour are represented (C) by the black and grey histograms respectively, representing the firing rates of the two neurons over a period spanning 10s before to 10s after stimulus onset.

The activities of approximately 25% of the neurons were involved in patterns that included breathing-related events. Patterns were categorised in terms of their being associated with one or other, or both breathing-related events and analysed using *ANOVA*. The distribution of patterns between pre-stimulus activity and activity during stimulus presentation varied significantly across the three categories of breathing-related pattern, both in terms of the number of different patterns detected ($F_{2,29} = 7.25, p < .05$) and the number of occurrences of patterns ($F_{2,29} = 7.12, p < .05$). The number of patterns involving one or other of the breathing-related events (onset of inhalation or onset of exhalation) was greater during odour presentation than before (59% : 41%). The difference between pre-stimulus activity and that during stimulus presentation was most marked for patterns involving both event types (62% : 38%) or those involving the onset of inhalation but not onset of exhalation (67% : 33%) (Fig. 4.3). The number of patterns detected similarly reflected stimulus presence or absence. The numbers of patterns involving both onset of inhalation and onset of exhalation, and those involving onset of inhalation but not onset of exhalation were greater during stimulus presentation than in pre-stimulus activity (66% :

34%). For patterns involving onset of exhalation but not onset of inhalation, there was little difference between pre-stimulus activity and activity during stimulus presentation either in terms of number of different patterns or number of occurrences of patterns (see Fig. 4.3). The implication of these findings is that sequences of neuronal firing are associated with inhalation, particularly when the inhaled air is carrying odour information.

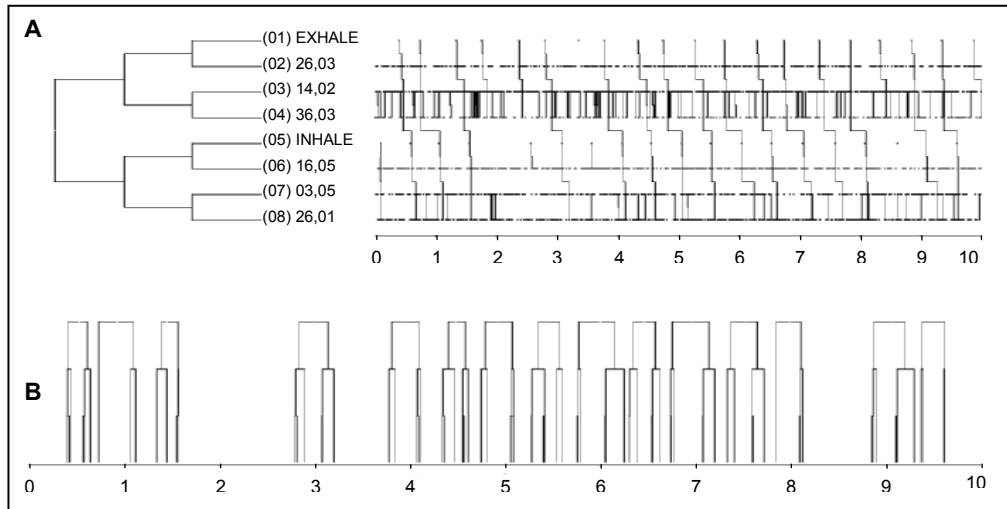


Figure 4.2 *Breathing-related sequence of neuronal discharge.* This sequence shown in A, detected in activity recorded during odour presentation, involved eight event types, including both markers of breathing – onset of exhale and onset of inhale (events 01 and 05 in the sequence). The first of the two comma-separated numbers annotating the neuronal events (spikes) indicates the position of the neuron sampled using a 5×6 electrode array (i.e. 03 = row 0, column 3). The second number indicates the identity of an individual neuron sampled on the given electrode. Sequences involving both breathing markers, and those involving onset of inhalation but not onset of exhalation, were more numerous during odour presentation than in pre-stimulus activity. Occurrences of events associated with this T-pattern, and connections between them, are plotted to the right of their labels over the 10s period of recording. Complete occurrences of the sequence shown in A are plotted in B.

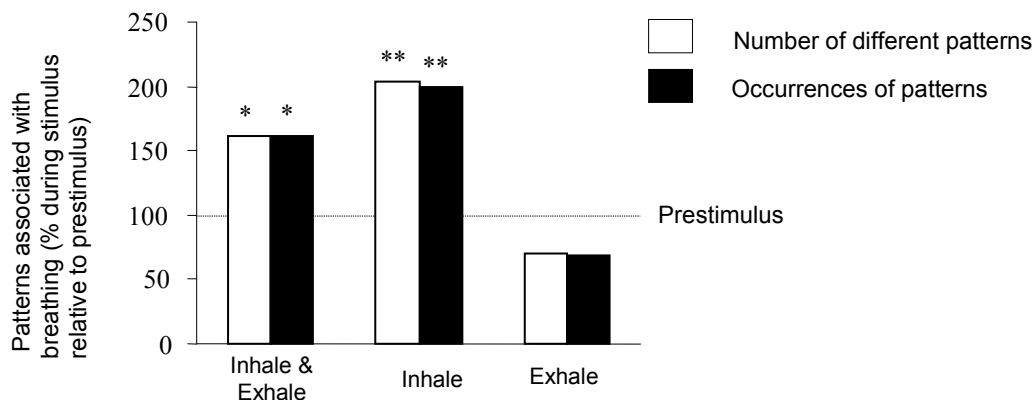


Figure 4.3 *Odour-related change in neural sequences associated with breathing.* The number of different patterns associated with both the start of inhalation and the start of exhalation, and also the number of patterns associated exclusively with the start of inhalation increased significantly between pre-stimulus activity and activity during stimulus presentation (*ANOVA*: * $p < .05$, ** $p < .01$). This was the case also for the number of occurrences of patterns. There was no such distinction in either measure between pre-stimulus activity and activity during stimulus presentation for patterns involving onset of exhalation but not onset of inhalation. Here the number of different patterns and the number of occurrences of patterns detected in activity during stimulus presentation are expressed relative to the corresponding numbers in pre-stimulus activity (100% representing no change).

4.4 Discussion

Precise sequences in spontaneous cortical neuronal activity were recently identified both in an isolated tissue preparation and *in vivo* [16]. These patterns were found both in intracellularly recorded postsynaptic potentials, reflecting release of discrete quantities of neurotransmitter, and in extracellularly recorded neuronal action potentials (spikes). Here we show that such sequences occur across large areas of the two-dimensional network of mitral neurons in the olfactory bulb, with functional connections spanning in some cases the entire area sampled by the MEA (>2mm).

This perhaps is less remarkable given a recent account of anatomical connections (“short axons”) spanning many mitral cells across the olfactory bulb [17]. Most notable in our findings is that these sequences, whilst present in spontaneous neuronal activity, have functional relevance. Presentation of an odour stimulus increases both the variety of sequences detected, and the number of patterns generated amongst the neurons. These increases are selective for sequences of neuronal firing that are associated with the onset of inhalation. Included amongst these sequences are those associated with both onset of inhalation and onset of exhalation, but not those associated exclusively with onset of exhalation. Thus stimulus quality is represented in the richness of neuronal sequencing in mitral cell activity.

These findings are consistent with other recent observations of neuronal activation in phase with breathing using *in vivo* optical imaging techniques [13] or electrophysiological techniques [18], the latter study demonstrating that mitral cell membrane potential fluctuations, and therefore likelihood of discharge, occur in phase with ventilatory rhythm. However, here we have shown that neuronal activity involves precise sequences of discharge that are related both to ventilatory activity and to odour information.

4.5 References

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