Olfactory processing: **A time and place for everything**

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The behavioral effects of pharmacologically desynchronizing neuronal firing in the brain of the honeybee provide new evidence that the oscillatory synchronization of neuronal activity plays an important role in fine olfactory discrimination.

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To understand fully how neural systems work, we need to study how information is processed at many levels. In the visual system, for example, these levels range from the lowest level of the molecular events underlying primary visual transduction, to higher levels where many simultaneously occurring events in the pathway encode complex visual scenes, and eventually to the final output level of visually guided behavior. The challenge for neuroscientists is to follow the path(s) along which information is encoded and manipulated from one end of this continuum to the other, with the expectation that this will yield deep insight into how the brain works.

In studies of visual, auditory and somatosensory processing, a major strand of research has involved characterizing the spatial features of information mapping in the brain (see [1], for example). The various basic properties of a sensory stimulus — position in the visual field, color, auditory pitch, and so forth — are encoded by spatial maps which are in some cases relatively simple, but in others more complex. While space is important, so is time, and it is clear that any understanding of 'information processing' must involve characterizing not only which neurons are active, but also when and for how long they fire, and with what frequencies they respond. Until recently, less attention has been paid to the way that the firing patterns of spatially distributed neurons vary over time, and how such temporal variation might represent stimulus attributes. Many studies now suggest that temporal features of neuronal activity can be rich in information [2].

Studies of olfactory function have tended to take a rather different course. In even the earliest physiological studies [3–6], both spatial and temporal events were considered as potential ways of representing odor stimulus attributes in the brain. This early interest in time and place arose from the difficulties of defining basic properties of odors, and the lack, at that time, of obvious spatial maps that related to clear physico-chemical stimulus attributes. Significant progress has been made in understanding the primary reception [7] and signal transduction [8–11] mechanisms of olfaction, with tantalizing hints about some of the structural properties of odors that might be neurally encoded [12–13]. We have also gained insight into how odors might be encoded in space [14–17]. But still the general problem persists. Which odor molecule binds to which receptor, with what affinity, and for how long? We have even less information on how these prerequisite biochemical and physiological properties are represented at the higher levels of the pathway to generate output behavior.

A recent paper by Stopfer *et al.* [18] has provided new insight into the important relationship between the timing of odor-elicited physiological events and behavior in the honeybee. In previous studies, Laurent and colleagues [19] set the stage for these new experiments by recording from neurons in the antennal lobe of the locust. The antennal lobe, with functional and structural similarities to the olfactory bulb of vertebrates, is the first level where information processing takes place in synaptically connected circuits. These studies permitted comparisons to be made between intracellular responses from individual output neurons and local field potentials representing activity in cell populations. When cell populations fire synchronously, oscillations in local field potentials are recorded that represent the aggregate neuronal activity from many cells. The phase relationships between the firing patterns of single cells and the local field potentials show when single neurons are active with respect to their neighbors.

Such studies in the locust provided evidence that aspects of odor structure are encoded, not only by which antennal lobe neurons fire, but also by when they were active during the local-field-potential cycle [19]. A reasonable interpretation of these findings is that the encoding of an odorant in this animal involves the activation of many antennal lobe cells, firing as "a specific succession of synchronized assemblies" [18]. In one possible manifestation of this, odor-elicited activity in the antennal lobe, and perhaps also in higher olfactory centers, can be generally thought of as being similar to changes in the calm surface of a body of water perturbed by the impact of a handful of tossed pebbles. As a result of the pebbles hitting the surface (odor stimulation), groups of local events of oscillatory waves (local field potentials) are generated with individual particles of water (neurons) as components of these waves, changing their amplitude (firing) during different phases of the oscillation. Although not perfect, this analogy demonstrates that odor-encoding is an ensemble, spatio-temporal process.

Testing the spatio-temporal hypothesis has been difficult, although there have been experiments in which the spatial aspects of coding have been examined. For example, Slotnick *et al.* [20] have made local lesions in parts of the olfactory bulb in rats. Using behavioral assays, these studies have shown that the animals are still able to perform odor discriminations quite well with large amounts (up to 85%) of damage to the first synaptic relay. These data suggest that the encoding process, at least for general discriminations, is widely distributed and redundant. The effect of lesions on fine discrimination has not yet been examined. Until now, a similar test of how temporal relationships affect output behavior has been missing.

To investigate the contribution of timing relationships among olfactory neurons in odor discrimination, it was necessary to find a way of 'lesioning' or perturbing temporal events. To do this, Stopfer *et al.* [20] moved from the locust, which proved refractory to odor behavioral training, to the honeybee. After showing similar relationships between the behavior of individual neurons and local field potentials in this second insect species, they 'lesioned' the circuits pharmacologically with the γ-amino butyric acid type A ($GABA_A$) receptor blocker, picrotoxin. They had previously shown that picrotoxin, applied to the antennal lobe of the locust, perturbed the fast inhibitory synapse between local and projection neurons, with the effect of abolishing the synchrony between single-cell firing and population events; it did not, however, perturb the firing of individual cells, nor change their response patterns to odor stimulation. Application of this method to the honeybee showed that picrotoxin could similarly decouple the firing synchrony between single cells and the population.

The next experiment aimed to determine whether picrotoxin treatment could disturb performance on a behavioral test of odor discrimination — a proboscis-extension assay to test how finely the bees can discriminate among odors with related (the aliphatic alcohols 1-hexanol *versus* 1-octanol) or different (either alcohol *versus* geraniol) structures. Interestingly, picrotoxin treatment at the time of training impaired the animals' ability to make the finer discrimination between the structurally related odors but not between either of the alcohols and geraniol, without affecting learning *per se*. This suggests strongly that interference with oscillatory synchrony by picrotoxin, while leaving individual cell responsiveness intact, disrupts the ability to make fine odorant discriminations. This is similar to the lesion tests of spatial encoding, in that general discrimination was left intact. This is the first evidence that I am aware of where the temporal component of the spatial/temporal hypothesis has been tested directly.

With the experiments described here, and the newest information about spatial properties of the odorant-encoding process [16–19], we can look forward to the beginnings of a comprehensive view of how molecular attributes of odorant stimuli are represented by the nervous system. Significant progress has been made in recent years that confirms and greatly extends the earlier hypotheses that have consistently considered both time and place in the encoding process. The difficulties that have confounded analysis of the olfactory system for so long might just have pushed us to think about it in ways that can provide unexpected insights relevant to analysis of other regions of the brain.

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