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THE OLFACTORY ADVENTURE*

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I began graduate studies under the direction of Warren S. McCulloch in 1955. At that time there was intense interest among the neuroscience community in discovering the properties of the individual cells which constitute the nervous system. The larger scientific community was also newly curious about the workings of the brain, largely as a result of Wiener's Cybernetics (1948) and von Neumann's interests in intelligent machines (1956). The McCulloch laboratory, more than any other, cared about the relations between cellular events and the processes of mind. The spirit of the laboratory was well characterized by the sign on the door. It read "Experimental Epistemology".

The range of research interests was broad. At the time I started, a seminal contribution to analysis of central nervous system function was being prepared for publication (Howland et al., 1955). Investigations were in progress which revolutionized experimental approaches to sensory perception (Lettvin et al., 1955). McCulloch had begun the exploration of logical symbolism to represent complex neural processing (McCulloch, 1956). The investigations of spinal afferents which matured into the gate control pain theory had begun (Wall, 1961). Low noise amplifiers and new electrodes were being developed which allowed investigations of activity of the smallest fibers in the nervous system. My first participation was concerned with these (Gesteland, 1957; Lettvin et al., 1958; Gesteland & Howland, 1959; Gesteland et al., 1959).

The work on electrodes ultimately led to our study of the olfactory receptor organ. I spent the 1957 summer in Steven Kuffler's Nerve-Muscle Training Program at the Woods Hole

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Marine Biological Laboratory. David Ottoson from Sweden was working with Kuffler on the crustacean stretch receptor at the time. His important study on olfaction in the frog had just been published (Ottoson, 1956) and we talked about noses quite often. He described the frustration of trying to record from single receptor neurons in the nose. Conventional micropipettes killed cells upon penetration. Their noise obscured unit field potentials when extracellularly located.

Back at MIT that fall, we set out to see if the electrodes which had been so spectacular in recording from unmyelinated axons of the frog optic nerve would work as well on the small, unmyelinated axons which constitute the olfactory nerve. Walter Pitts liked the project because of his interest in molecular kinetics and because this was a sensory cell in which a chemical stimulus acted directly on the receptor membrane. (In vision, hearing and touch, the stimulus is transduced first into an unknown chemical intermediate which then causes changes in membrane conductance.) Jerry Lettvin was encouraging, partly because of his curiosity about any nervous system mystery and partly because of his current involvement in another chemosensory receptor study (Hodgson et al, 1945). Warren had been interested in the olfactory sense since his early days in psychiatry. I was intrigued at the prospect of research on a sensory organ whose biophysics had been little studied.

The olfactory receptor organ is unique among sensory systems in its simple anatomical organization. The receptor neuron soma lies outside of the brain in the olfactory epithelium. It is a bipolar neuron with a single dendrite extending to the surface of the epithelium. From the dendrite apex, cilia project into the mucus layer. Inhaled odorous vapors dissolve into the watery mucus and diffuse to the ciliary surface. Schultze (1862), Hopkins (1926) and Ottoson (1956) all argued that the cilium was the part of the cell on which odors exerted their effect.

From the opposite pole of the receptor cell soma an axon projects, passing through the cribriform plate to the olfactory bulb of the brain. Here, in glomerular structures, about 50 thousand second-order neurons receive synaptic inputs from about 50

million receptor cells. There are no collaterals from the receptor axons. There are no lateral interactions between receptor neurons. There are no efferent fibers from the central nervous system to the neurons of the olfactory epithelium. This simplicity of connectivity is accompanied by a simplistic geometry. The receptor neurons lie in an orderly array. Each dendrite lies within an enveloping invagination of the glial-like supporting cells. Tight junctions between receptor dendrites and the supporting cells just beneath the mucus serve as a diffusion barrier between the mucus and the extracellular space of the sensory epithelium.

We saw that the nose presented a unique opportunity to study receptor processes uninfluenced by feedback or by concurrent events in cellular neighbors.

Our first recording of single unit action potentials was obtained in 1959. In the experiments which followed we found chaos where we expected order and complexity where we expected simplicity. We learned almost nothing about coding of odor information (Gesteland, 1961; Gesteland et al., 1963; Gesteland et al., 1965; Lettvin & Gesteland, 1965). The harvest of confusion which we reaped 20 years ago has only now begun to be sorted. What follows is a summary of the early results and their recent unravelling.

The extracellular unit potentials were of unusually long duration, typically 5 ms, and their shapes were complex. Often we could identify 3 to 5 components. We know now that the abrupt velocity change which occurs when the action potential in a 0.2 micron diameter axon antidromically invades a large soma is the cause. In the absence of stimulation the cells fired spontaneously. The firing rate was less than once each second and the periods between spikes were highly variable. When stimulated, the firing rate usually increased but the intervals remained irregular. Sometimes a stimulus would inhibit activity.

When we attempted to classify cells according to the subgroups of stimulus substances to which they responded, we failed. No two cells responded in the same way to the odors in our stimulus set. Chemically similar or perceptually similar substances

often evoked very different activity patterns. Responses of a particular cell were quite repeatable. However, this information was not sufficient to predict how a different cell would respond to the same stimuli. We could not identify categories of substances which produced similar effects. When we knew how a cell responded to each of two stimuli, we could not guess the response when they were delivered as a mixture.

In experiments on the receptor population, in which the voltage and impedance changes evoked by odors were measured with large electrodes, we found two kinds of processes. Odors evoked an excitatory event which we inferred to be a conductance increase to ions whose equilibrium potential was positive with respect to the membrane potential. They simultaneously evoked another process which appeared to be a shunt, decreasing the excitation process. Rise times and durations of the two processes were independent of each other and were dependent upon the stimulus substance.

These results provided an explanation for our finding that responses recorded from the olfactory epithelium are not simple functions of stimulus intensity. The ionic mechanisms remain to be determined. It was easy to say that all would be resolved when reliable intracellular recordings were achieved. This has not yet been accomplished. Nor is such a method likely to provide answers to the important questions. There is indirect evidence for the existence of multiple active processes occurring in different regions of the same cell (Adamek & Gesteland, 1983).

As it turned out, I didn't heed the most important lesson taught by my mentors. If you ask the wrong questions, the answers will not make sense. About a decade after the olfactory work at MIT, it was found that olfactory receptors are continually generated (Moulton et al., 1970; Graziadei & Metcalf, 1971; Thornhill, 1970). We had assumed that olfactory receptor neurons were like other neurons of the vertebrate nervous system, i.e., elaborated early in life and never replaced.

Olfactory receptor neurons, unlike all other nerve cells in vertebrates, have a lifetime of only 6 to 7 weeks. They are continually

replaced by new ones which differentiate from basal cells in the receptor epithelium. We recently studied the response properties of olfactory receptors at different stages of development (Gesteland et al., 1982; Mair et al., 1982; Adamek et al., 1983). They have quite different response properties at different stages of the life cycle. It requires about a week after cell differentiation for the dendrite to reach the epithelial surface and sprout cilia. As soon as this happens, the cell is responsive to odorous substances. Most surprisingly, each cell is excited by all the substances we use. It can make no distinctions among different odors. Two weeks after this time, the axon has extended far enough to reach the second-order neurons at the olfactory bulb. At this time, the cell properties change abruptly. Any single cell responds henceforth to only a fraction of substances in the stimulus set. It is these mature neurons that provide the messages to the brain.

Our original attempts to determine classes of cell selectivity types were certain to fail because of the inclusion of many cells which are generally irritable and not selective. Any study done on a normal adult receptor epithelium includes two populations of quite different physiological properties, a situation totally unexpected.

The selective, mature neurons have another property which distinguishes them from most other neurons. For any effective stimulus there is a particular stimulus intensity (concentration) which produces a maximum response. At intensities significantly above the optimum, the cell responds with a single action potential followed by a period of inactivity due to depolarization block. Thus what we had called inhibition was often, if not always, extreme excitation. Since different cells have different "best intensities", the response properties can only be defined by a series of different concentrations of each test stimulus. It is seldom possible to hold a cell long enough to accomplish this.

There is another problem in determining cell sensitivity. Spontaneous activity in many cells waxes and wanes quasiperiodically over a period of several minutes. A given stimulus concentration produces a greater response if delivered during a time of maximum spontaneous activity than it does during a minimum. If

stimulated with a brief odor during a period of low spontaneous firing, the response begins a minute or more later. Responses so poorly synchronized with stimulus timing were generally ignored as artifactual in past studies.

Finally, extracellular action potential amplitudes vary by a factor of 2 or 3, due to the profound sensitivity to depolarization block. Much of the activity recorded in earlier studies was rejected on the basis that differing spike amplitudes indicated that the activity of more than one neuron was responsible.

These observations coupled with some powerful new experimental methods suggest that some of the mystery will be resolved in the near future. By studying response properties at fixed states of development, signalling should be separable from responses of unconnected immature cells. By isolating single cells and investigating them in vitro, cellular biophysical processes ought to be resolvable. Patch studies on tiny membrane areas will certainly define the properties of the molecular receptors. Analysis of cellular membrane conductance changes during stimulation will reveal the properties of the transducer and allow its properties to be separated from the generator events. Desorption dilution promises to provide simple and precise stimulus control which will simplify study of response-concentration functions.

Each of these methods has been developed within the past 3 years. We expect some more surprises as they are applied.

Warren McCulloch's enthusiasm and his support for his people in pursuit of their own science are legendary. It was in startling contrast to the attitude which prevails in most laboratories now. He didn't produce clones of data collectors performing slight variations on their mentor's ideas. Much of the learning was from one's own errors. The studies on olfaction are a particularly clear example. We do not know much about the physiology of olfactory reception. Most of what we thought we knew is either out-and-out wrong because it was based on invalid assumptions or is partial truth with no basis for separating truth from falsehood. The delight is that we now have some clues to what the questions

are which we should be asking. These studies should provide joyous days for a generation of young investigators.

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