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"Functional Analysis of Local Circuits in the Olfactory Bulb"

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8 Functional Analysis of Local Circuits in the Olfactory Bulb

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ABSTRACT An understanding of the functional organization of any region of the nervous system depends on the experimental methods used and the accessibility of the region and its constituent elements to the application of these methods. Any one method may provide essential clues or evidence about the functions of the region, but it can never be sufficient: for evidence gains creditability only through support from other independent methods, and its significance can only be judged within the fabric of all the information available about that region. Coherent concepts therefore require the correlation of results from as many approaches as possible. It is well to recognize this principle as a guide to experimental analysis and a means of qualifying the interpretation of results, particularly during a period when neuroscientific methods are multiplying rapidly in number and variety.

In this paper I shall outline briefly how such a multidisciplinary approach has evolved in the study of synaptic circuits in the vertebrate olfactory bulb. While describing this work I shall attempt to draw attention to some general considerations relevant to the analysis of local circuits and to some implications of local circuit organization for our understanding of the basic units for information processing in the nervous system.

Anatomical methods

GOLGI STUDIES have shown that there are three main neuronal types in the olfactory bulb (see Figure 1). The main output neuron is the *mitral cell*, the cell bodies of which are arranged in a thin layer. A subtype, the *tufted cell*, is scattered through the external plexiform and glomerular layers. There are two main types of intrinsic neuron: the *periglomerular cell* is confined to the glomerular layer, where the olfactory axons terminate; the *granule cell* has its cell body located in the deep granule layer and has central and peripheral dendrites, the latter ramifying extensively in the external plexiform layer. The granule layer also contains scattered short-axon cells.

The termination of olfactory axons among mitral-, tufted-, and periglomerular-cell dendrites implies synaptic connections onto those dendrites (Ramón y Cajal, 1911), and electron-microscopic (EM) studies have confirmed these axodendritic synapses (see insert, Figure 1). It has also been shown that the mitral- and periglomerular-cell dendrites are interconnected by numerous dendrodendritic synapses (Pinching and Powell, 1971). And there is evidence in some mammalian species that the axons may terminate on mitral-cell but not on periglomerular-cell dendrites (White, 1972, 1973). None of these details could have been deduced from the Golgi material itself; the Golgi method can indicate in what layer synapses are likely to occur, but the electron microscope is necessary to identify the synapses.

The intermingling of mitral secondary dendrites and granule-cell dendrites in the external plexiform layer similarly implies a connection between the two (Ramón y Cajal, 1911). Again the electron microscope has shown that the predominant connection is in the form of reciprocal synapses located side-by-side with opposite morphological polarities (Hirata, 1964; Andres, 1965; Rall et al., 1965; Reese and Shepherd, 1972). A modest proportion of the dendrodendritic synapses in the glomeruli have this arrangement, and nearly all the mitral and granule synapses are of this type.

EM studies of the bulb are greatly aided by the ease with which cell processes can be recognized. It has been found that mitral-to-granule synapses are characterized by spheroidal vesicles and asymmetric membrane thickenings, whereas granule-to-mitral synapses are characterized by flattened vesicles and symmetric membrane thickenings (Price and Powell, 1970). This correlates with the excitatory and inhibitory actions, respectively, of these synapses suggested by physiological studies (see below). A similar correlation has been suggested for synapses in other parts of the nervous system (Uchizono, 1966), and the evidence in the olfactory bulb is among the clearest for this morphophysiological correlation. Freeze-fracture

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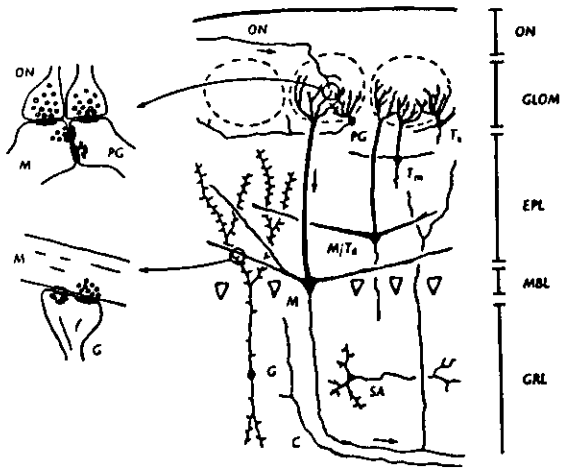


FIGURE 1 Schematic diagram summarizing neuronal elements and synaptic connections in the rabbit olfactory bulb. Insets at left show main types of synaptic connections in the glomeruli (above) and external plexiform layer (below). *Abbreviations*, for this and following figures: ON, olfactory nerves; PG, periglomerular short-axon cell; T_s, superficial tufted cell; T_m, middle tufted cell; M/T_d, displaced mitral or deep tufted cell; M, mitral cell; G, granule cell; SA, short-axon cell of deep layer; C, centrifugal fibers. Histological layers shown at right: GLOM, glomerular layer; EPL, external plexiform layer; MBL, mitral body layer; GRL, granule layer. (From Getchell and Shepherd, 1975a.)

studies (Landis, Reese, and Raviola, 1974) showed that the mitral-to-granule synapse is characterized by accumulations of intramembranous particles on the postsynaptic side. This correlation with a presumed excitatory synapse is similar to the findings in the cerebellum (Landis and Reese, 1974). In both regions, particle accumulations are absent at presumed inhibitory synapses (e.g., granule-to-mitral in the bulb).

From these and other studies in the olfactory bulb it appears that individual synapses have a morphology similar to that of simple contacts seen in other regions of the nervous system. Thus the same basic type of junction can serve synaptic transmission from an axon to a dendrite or between two dendrites. Another important point is that dendrodendritic synapses are not specific for a given neuronal type or neuronal process. In the bulb, the long-axon (mitral) cell, the short-axon (periglomerular) cell, and the anaxonal (granule) cell all take part in dendrodendritic connections in which their processes occupy presynaptic as well as postsynaptic positions. This may be taken as evidence that functional circuits are organized in terms of patterns of synaptic connections rather than in terms of the particular geometry of the neuronal processes involved.

Physiological methods

By virtue of its distinct neuronal types and separation into layers, the olfactory bulb is a favorable site for electrophysiological analysis. The afferent and efferent pathways can be separately stimulated, and mitral cells can be identified by antidromic vollevs in the lateral olfactory tract. Numerous extracellular single-unit studies (reviewed in Shepherd, 1972a) have verified that mitral cells undergo a period of suppression after antidromic impulse invasion. Intracellular recordings have shown this period to be correlated with a long-lasting hyperpolarization of the mitral-cell membrane that has the properties of an inhibitory postsynaptic potential (IPSP). Typical recordings are shown in Figure 2A, B.

The interval between impulse invasion and the onset of the IPSP is dependent on the strength of the vollev and has a minimum duration of approximately 2 msec, which is sufficient for two synaptic delays. The evidence suggests that the granule cell acts as an interneuron in this pathway, in analogy with the Renshaw pathway for inhibition in the spinal cord. However, in contrast with Renshaw cells, in which inhibitory action is ascribed to high-frequency, long-lasting impulse discharges, impulse activity in the granule-

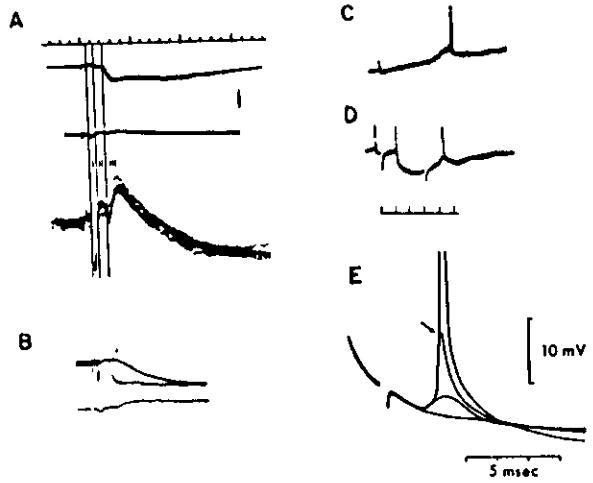


FIGURE 2 Intracellular recordings from mitral cells. (A, B) Responses to LOT volley. (A) Upper trace shows long-lasting IPSP, middle trace the field potential, lower trace the field potential at higher gain with time components 1–3. Time scale in 2 msec divisions; voltage calibration 10 mV (upper traces), 2mV (lower traces). (Modified from Shepherd, 1970.) (B) Response straddling threshold for axon of this cell, with field potential trace shown below. Arrows show onset of spike and onset of inhibition. (From Nicoll, 1969.) (C, D, E) Responses to ON volley. (C) Spike respon arising from EPSP. (From Yamamoto, Yamamoto, a Iwama, 1963.) (D) Succession of spontaneous impulse, fi ON shock artifact, spike response followed by IPSP; secor ON shock artifact, spike response arising from small EPS Time in 5 msec divisions. (From Getchell and Shepher 1975). (E) Response during hyperpolarization of cell mer brane by applied current, showing EPSP, fast prepotenti and full spike. (From Mori and Takagi, 1975.)

cell layer is rare, and discharges are brief. Long-lasting synaptic inhibition is, in fact, common in neurons of the brain. The possibility that such inhibition might be mediated wholly or in large part without concurrent impulse activity was recognized early in the bulb (Phillips, Powell, and Shepherd, 1963; Shepherd, 1963).

Orthodromic vollevs in the olfactory nerves also set up a sequence of impulse generation followed by inhibition in mitral cells. In some cases the impulse can be seen to arise from a depolarizing EPSP (Figure 2C); in other cases it arises more abruptly from the baseline (Figure 2D). Note in Figure 2D that in the response to the second volley, a small EPSP is revealed. This is consistent with the idea that hyperpolarization following the first volley moves the membrane potential away from the equilibrium potential for the EPSP and toward that for the IPSP. It appears that depolarization due to electrode entry into this cell is a complication in the activity recorded. This complication requires continual assessment in intracellular recordings, particularly the smaller neurons of the CNS.

In Figure 2E, the cell has been hyperpolarized l injected current, and a fast prepotential is reveale Similar potentials have been seen in several oth sites; for example, in chromatolytic motoneuro (Eccles, Libet, and Young, 1958), hippocampal p ramidal cells (Spencer and Kandel, 1961), and Pu kinje cells (Llinás and Nicholson, 1971). In analo with those cells, Mori and Takagi (1977) have su gested that the fast prepotential in the mitral c represents "booster" activity in the distal part of t primary dendrite. EM studies have shown that th part of the dendrite is surrounded by glial mer branes; in the monkey these appear as a thin myel sheath (Pinching, 1971). The latter finding shows th myelination is not an exclusive property of axor whether it is associated with active or passive pro erties in the mitral dendrite has not yet be established.

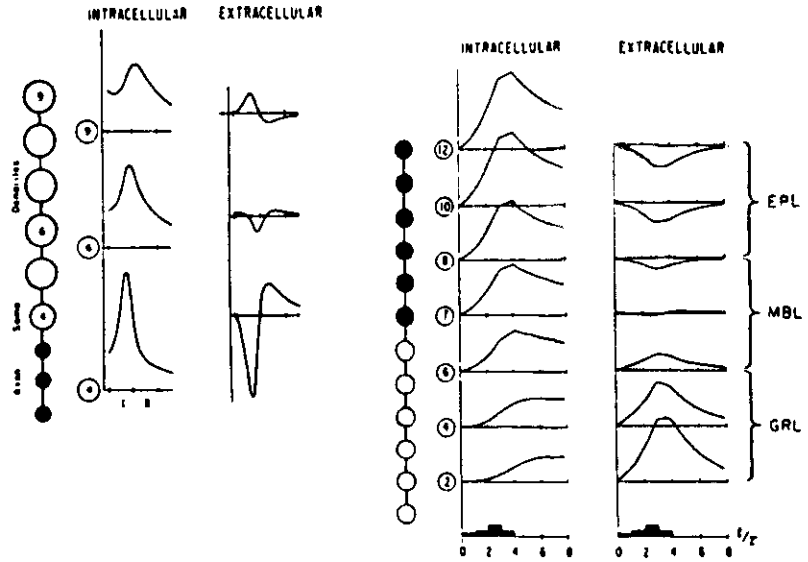
Physiological identification of mitral cells by an dromic criteria and by correlation with evoked p tentials can be carried out relatively easily. Ident cation by means of intracellular dyes has not yet be reported, although in vertebrates, intracellular sta

is been effective in identifying cells in several
l regions (retina, spinal cord, cerebellum, cor-
However, for both recording and staining, the
cells and processes have offered the best tar-
small cells and small processes present a severe
nge in the vertebrate nervous system and also
ertebrate neuropil. Information at this level is
ial for analysis of local circuits, but assessment
effects of electrode penetration on physiolog-
operties will be of critical importance.
dence for the properties of periglomerular
axon cells will be discussed after consideration
nputer simulation studies of mitral and granule

retical models

ophysiologists traditionally have been hesitant
ply theoretical methods to the interpretation of
imental data relating to neuronal organization.
reluctance derives in part from a healthy skep-
that the theoretical assumptions can adequately
porate the complexities of the regions and sys-
under study. The methods of Rall for describing
electrotonic properties of dendritic trees have
n that theory can in fact be applied to neurons
to provide the essential basis for interpreting exper-
imental results (Rall, 1977; see also Jack, this volume).
Rall has emphasized the need for an ongoing inter-
play between experiments on the living system and
experiments on realistic models of that system.

In the olfactory bulb we first attempted to recon-
struct intracellular and extracellular potentials with
computational models of the mitral- and grade-cell
populations, as they are activated by an antidromic
volley in the lateral olfactory tract (Rall et al., 1966;
Rall and Shepherd, 1968). This work has been re-
viewed before (Shepherd, 1972a); Figure 3 displays
representative computations of antidromic impulse
spread in the mitral cells and synaptic excitation of
the granule cells. The computations in the mitral-cell
model explored the effects on impulse propagation
of the geometrical hurdle from axon to cell body to
dendritic equivalent cylinder. Goldstein and Rall
(1974) subsequently analyzed this interesting theo-
retical problem in detail, and computer simulations
of the effects of similar changes in nerve-process di-
ameters on impulse propagation have now been car-
ried out for several types of neuron (see the chapters
by Parnas and by Pellionisz, this volume). Our com-
putations also explored the alternatives of active ver-
sus passive dendritic properties, and we concluded



RE 3 Computed intracellular and extracellular poten-
generated by mitral-cell (left) and granule-cell (right)
partmental models in response to a LOT volley. Filled
compartments indicate sites of active impulse generation
(left) and EPSP (right); open compartments have only pas-
sive electrical properties. (From Rall and Shepherd, 1968.)

that active properties might be present in some of the mitral-cell dendrites but could be ruled out to any significant extent in granule-cell dendritic branches if the computed extracellular transients were to agree with the recorded field potentials.

The extracellular transients were computed on the basis of a potential divider model for describing the relation of the recording electrodes to primary and secondary extracellular current flows. Subsequent studies showed that partial activation of the bulb produces potentials in active and inactive regions as predicted by the model (Shepherd and Haberly, 1970). Recently the theoretical basis for the potential divider model has been described by Klee and Rall (1977) (See Figure 4.)

The sequence of activity in the computer simulations, together with reasonable assumptions about the laminar locus and timing relative to the mitral-cell EPSP, led to the postulate of mitral-to-granule excitation followed by granule-to-mitral inhibition through the dendrites of these two cell types. The reciprocal synapses in the external plexiform layer, as described above, provide a morphological basis for this pathway (Rall et al., 1965; Rall and Shepherd, 1968).

The computations using the potential divider model were for the case of synchronous activation of large populations of mitral and granule cells. Recently Robert Brayton and I focused on the situation at the other extreme—the minimum parts of individual mitral and granule dendritic trees involved in mediating self-inhibition and lateral inhibition

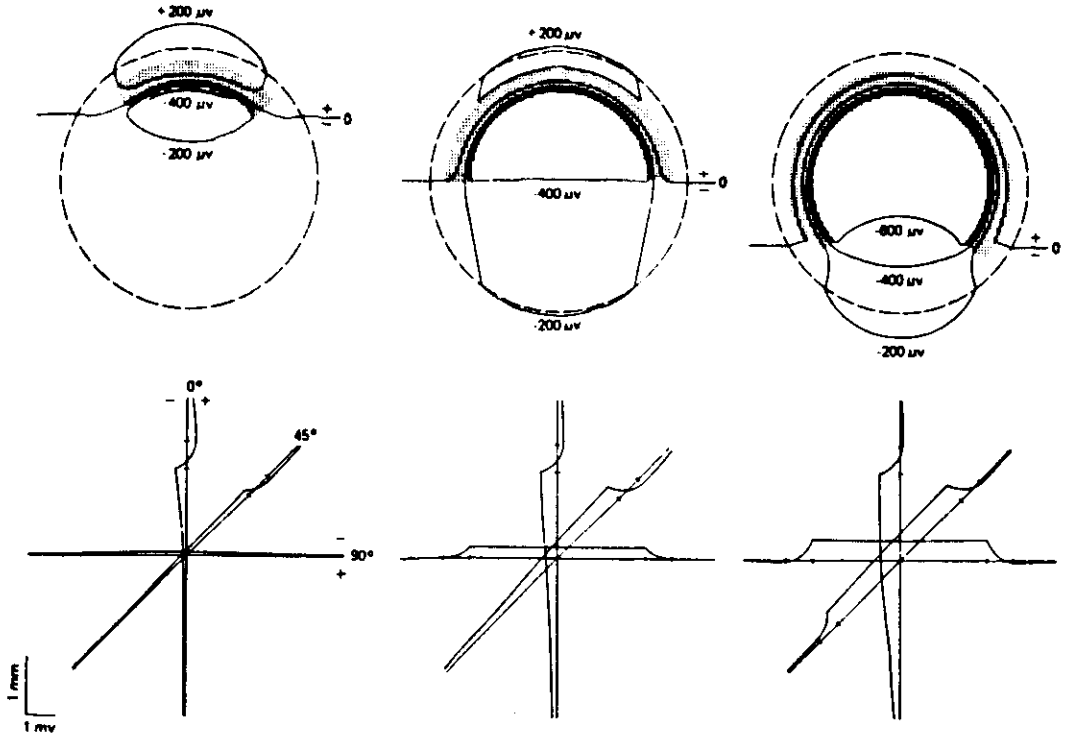


FIGURE 4 Computed field potentials generated by populations of synchronously active cells of differing cortical extents. Extents of active populations are indicated by shaded areas. Activity consists of depolarization of 10 mV in cell somata, which are arranged along the inner border with dendrites extending radially outward. Isopotential contours are indicated in upper diagrams, and depth profiles through the entire region (dashed sphere) are shown below. Resistivity within the region is 250 Ω-cm, outside is 2,500 Ω-cm. Note superficial positivity and deep negativity for recording tracks through active zones, in agreement with experiments on olfactory bulb. (From Klee and Rall, 1977.)

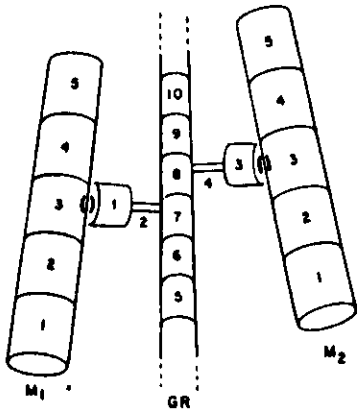


FIGURE 5 Compartmental model of dendrodendritic synaptic circuit, comprised of two mitral secondary dendrites (M_1 and M_2) and a branch of a granule-cell dendrite (GR) with two spines. Reciprocal synapses connect the spines to the mitral cells. The mitral dendrites are 4 μm in diameter; each compartment is 100 μm in length, with specific membrane resistance of 2,000 Ω-cm² and specific cytoplasmic resistance of 80 Ω-cm. For the granule-cell dendrite, the corresponding values are 4,000 Ω-cm² and 80 Ω-cm; compartments 5–10 are 1 μm in diameter and 50 μm in length; the spine necks (2 and 4) are 0.2 μm in diameter and 3 μm in length; the spine heads (1 and 3) are 1 μm in diameter and 3 μm in length.

through the reciprocal synapses. Figure 5 shows these parts, which consist of secondary dendrites of two mitral cells and a part of a granule dendrite with two spines connecting to the two mitral dendrites. The model is in compartmental form. An action potential is generated in the first compartment of mitral cell M_1, simulating antidromic invasion. The subsequent events are passive impulse spread into the M_1 dendrite, activation of the excitatory synapse from compartment 3 to the spine head of the granule dendrite, and activation of the inhibitory synapse back onto the mitral dendrite, producing self-inhibition of the mitral cell. This sequence is illustrated in Figure 6. During this same period, the EPSP in the first spine head spreads electrotonically through the granule-cell tree and into the second spine head. The depolarization activates the inhibitory synapse of the second spine head onto the second mitral dendrite, producing lateral inhibition of the second mitral cell.

The computer simulation thus reproduces the basic properties of the responses recorded from the mitral cells (cf. Figure 2) by the postulated dendrodendritic pathway. A key point is that the pathway involves only certain parts of the dendritic trees of the neurons involved. These parts define the functional unit (Shepherd, 1972b, 1977) for self-inhibition and lateral inhibition under these conditions. The functional unit is in fact a dendritic synaptic network with this specific morphological locus and these specific physiological properties. Two levels of local circuits are included in this unit: the reciprocal synapse and the dendritic circuits in which they are embedded. We will return to the question of levels of organization in the discussion section.

Previous attempts to model neuronal networks have generally treated the nerve cell as a single node or integrating locus and described the network as being built up by axonal connections between the nodal points. The present approach begins with Rall's recognition of the extended dendritic nature of the neuronal integrative surface and builds the relevant functional network within that context. This, of course, requires specific estimates of dendritic and synaptic properties. The present results represent a first step toward analyzing the dynamic relations between synaptic inputs and outputs in individual parts of dendrodendritic networks.

Short-axon cells

The granule cell is unusual among local interneurons in that it combines a large population with a parallel orientation of extended dendritic trees. These factors were, in fact, necessary morphological preconditions for modeling the field potentials described above.

The other interneuron in the olfactory bulb—the periglomerular (PG), short-axon cell—operates under very different conditions. PG cells are confined within a narrow layer; their dendritic trees are short and have different orientations as they enter and ramify within the glomeruli. Thus field-potential analysis has limited value in unraveling the functional properties of these cells; unfortunately the same limitation applies to many other kinds of short-axon cells in other parts of the nervous system. Fortunately, in the case of the periglomerular cell, there are counterbalancing advantages.

Single unit recordings have provided some understanding of PG cell properties (Shepherd, 1963, 1971; Getchell and Shepherd, 1975b). PG cells characteristically respond to a strong volley in the olfactory nerves with a brief burst of impulses. The impulse frequency tends to increase with increasing volley strength, although the amount of increase varies in different cells. The results indicate that in the rabbit the PG cells, like the mitral cells, are excited monosynaptically by the olfactory-nerve terminals in

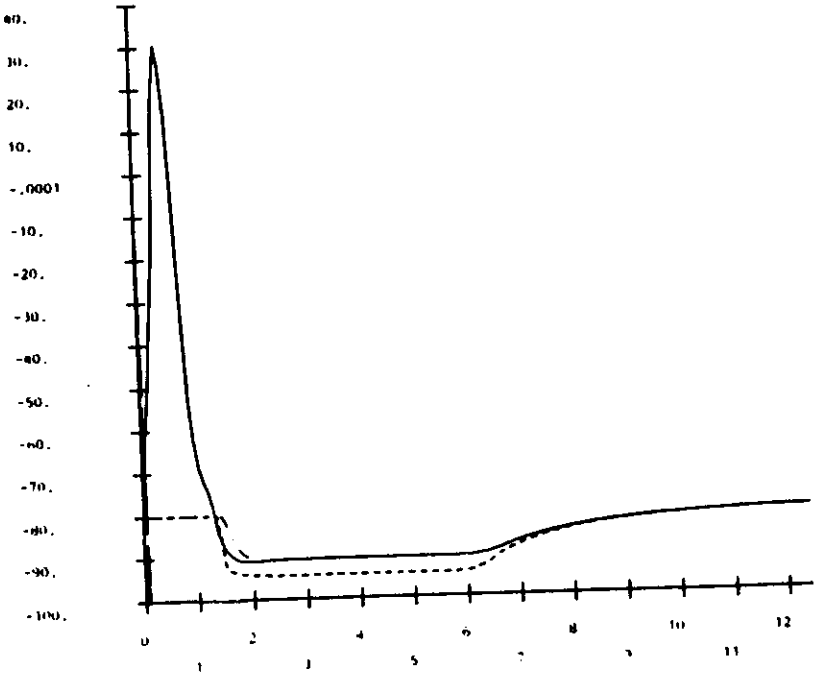
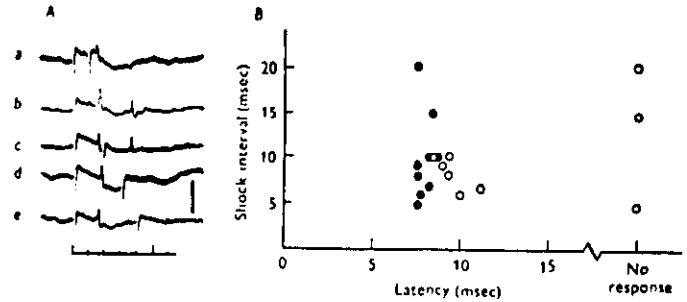


FIGURE 6 Computed activity in the dendrodendritic circuit of Figure 5, elicited by an impulse generated in compartment 1 of M_1. The impulse recorded from M_1 is shown as a solid line; it spreads electrotonically through compartments 2–5, activating the reciprocal synapse connecting mitral compartment 3 to granule spine head 1, which feeds back self-inhibition onto mitral compartment 3 (note the hyperpolarizing IPSP in M_1 dendrite in the aftermath of the impulse). The depolarization set up in granule-cell spine head 1 spreads into the granule dendrite and into the second spine 3 (see Figure 5), activating the inhibitory part of the reciprocal synapse onto compartment 3 of the M_2 dendrite. This mediates lateral inhibition onto the M_2 dendrite, as seen in the hyperpolarizing IPSP in compartment 3 (dashed line) and compartment 1 (dashed-dotted line). Note the similarity of these traces to the experimental recordings in Figure 2B.

the glomeruli. Following this excitatory period, there is characteristically a period during which the response to a second volley is suppressed or shortened in duration. This period may last for several hundred milliseconds. A similar period occurs in mitral cells, where, of course, the IPSP due mainly to granule-cell inhibition occurs (see Figure 2). The suppression of PG cells at longer testing intervals can be shown to be independent of the olfactory-nerve recovery cycle, leaving us to assess the effects of PG-cell impulse activity.

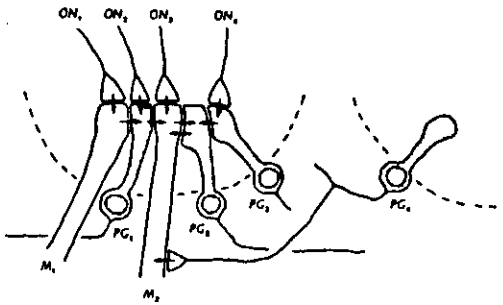
To do this we studied PG-cell units at threshold for single-spike initiation by weak ON volleys. In some cells the test response was facilitated in terms of threshold and/or latency; this response characteristically began early and could last up to 30 msec. In some cases the test response was suppressed. In Figure 7, for example, there is early blockage due to absolute refractoriness, a relative refractory recovery phase, and, finally, blockage at 15 msec intervals and beyond. Although electrophysiologists often use strong volleys to elicit clear-cut response patterns, the use of very weak ones, as in these studies, may be more productive of evidence about synaptic connections and the properties of small intrinsic neurons.

Mitral cells undergo similar periods of facilitation and suppression following an ON volley (Getchell and Shepherd, 1975a); this raises the interesting question of the relation between the PG-cell and mitral-cell responses. The Renshaw-cell model (Eccles, Fatt, and Koketsu, 1955) requires the IPSP mediated by an interneuron to be coincident with an impul



IGURE 7 Extracellular unit responses in glomerular layer paired ON volleys. (A) Responses shown at increasing ock intervals; note suppression of test response at short- it (a) and longest (d, e) intervals. Time in 5 msec divisions. (B) Plots of data; filled circles indicate spike responses to conditioning volleys, open circles to testing volleys. (From Getchell and Shepherd, 1975b.)

ischarge in the interneuron; but how can the PG ell be an inhibitory interneuron for the mitral cell hen it appears to be inhibited itself? The answer es in a consideration of the dendrodendritic arangements in the glomeruli. As shown in Figure 8, eginning on the left, the simplest types of synaptic athways identified in EM studies are the monosynptic olfactory-nerve connections onto mitral-cell nd PG-cell dendrites and the disynaptic pathways hrough dendrodendritic synapses. PG cells also have eciprocal synapses onto mitral cells. A circuit thus xists whereby the PG-cell dendrite can inhibit the nitral-cell dendrite and, by so doing, block further xcitatory input to itself; that is, it presynaptically nhibits itself from responding to further olfactory- erve input. This mechanism provides a tentative xplanation for the physiological results described bove and indicates that dendrodendritic pathways an provide for a relationship between impulse firing



IGURE 8 Schematic diagram summarizing evidence from M and electrophysiological studies for synaptic circuits in he glomerular layer of the olfactory bulb. (From Getchell nd Shepherd, 1975b.)

and the inhibitory action of a short-axon cell that is different from that of the Renshaw-cell model.

This example also underscores the caution one must exercise in basing interpretations of synaptic interactions on extracellular spike data. Studies of spike discharge patterns in other parts of the nervous system may warrant reinvestigation on this account, particularly in regions where dendrodendritic synapses are known to exist or are suspected. In many such regions, "impulse flow" may give only a limited indication of the actual information flow through the synaptic circuits. This consideration appears to be important in developing strategies for studying the functional properties of local neuronal circuits.

Natural stimulation

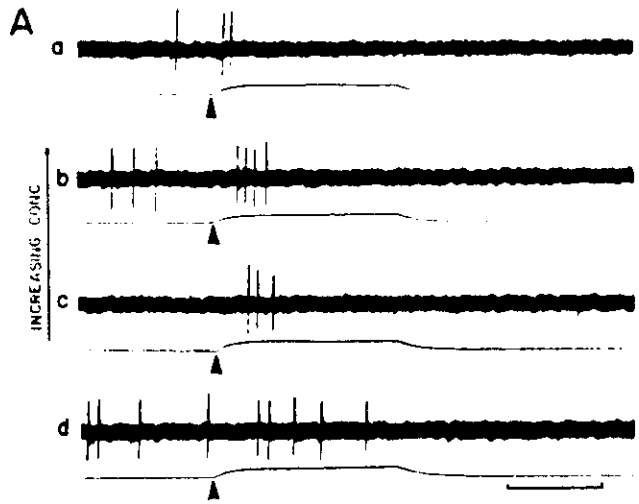
Thus far we have considered methods of analysis that involve brief, artificial activation of local circuits from volleys produced by electrical shocks. The next step is to use natural stimulation. For this purpose we need synchronous activation by a stimulus defined in time course and in spatial locus. The ability to control stimuli in this way has been fundamental to the analysis of neuronal properties and mechanisms in the visual (Kuffler, 1953), auditory (Galambos and Davis, 1943), and somatosensory (Mountcastle, 1957) systems, to name only the best-known examples. In these systems it has been relatively easy to generate well-defined step pulses, ramps, and sinusoidal stimuli. In the olfactory system, however, we are dealing with stimuli—volatile gases—that are very difficult to work with. In fact, when we began our experiments several years ago there were no methods for delivering and monitoring the stimuli equivalent to those used for other sensory systems.

The instrumentation we have developed (Kauer and Shepherd, 1975a,b) consists of a system of concentric nozzles that deliver step pulses of odor to the exposed olfactory receptor sheet of a salamander. The step pulses have a relatively abrupt onset, a steady plateau phase, and an abrupt termination. The duration and concentration of the stimulus can be independently varied. For monitoring the stimulus a low percent CO_2 is introduced into the odor carrier stream; this is measured by a CO_2 analyzer with a small inlet port positioned over the olfactory receptor sheet at the site of stimulation.

Typical results obtained with these methods are shown in Figure 9. These are recordings of extracellular spike activity in a mitral cell. The stimulus is the odor of amyl acetate, delivered in step pulses lasting 4 sec, as shown by the lower traces. At the lowest concentration (d), just over threshold for this unit, there is a prolonged impulse discharge. At a higher concentration (c), the discharge has a higher frequency, shorter onset latency, and briefer duration, and it is followed by a period of suppression. These changes become more pronounced at higher concentrations (b, a). Note that at the highest concentration (a), the response consists of only two spikes separated by a brief interval and followed by complete suppression for the remainder of the pulse.

We found that there are three main types of activity correlated with pulse stimuli: initially excitatory, initially suppressive, and no change (unaffected). These categories were originally described by Kauer (1974), and our results confirm them and show the precise relation between the timing of the response and the stimulus. As in the earlier study, cells are not specific for particular odors; among the battery of odors used, a given cell may show all three response categories.

With regard to synaptic circuits, the results have some interesting implications. The prolonged discharge at threshold suggests a prolonged impulse input through the receptor axons and correspondingly prolonged excitatory synaptic drive in the glomeruli, but with minimal inhibitory synaptic actions. As odor concentration increases, it appears that the receptor impulse activity increases pari passu, causing earlier and more intense excitatory synaptic drive to the mitral cells, but it also activates circuits that provide for suppression, which cuts off the impulse response; suppression then continues for the duration of stimulation. It is tempting to correlate this suppression with synaptic inhibition through the dendrodendritic pathways as described above. Our present work is directed to obtaining further evidence for the properties of this inhibition and attempting to identify the contributions at the external plexiform and glomerular levels.



IGURE 9 Extracellular unit responses in salamander olfactory bulb to odor pulse stimuli delivered to the olfactory mucosa. Lower traces are pulse monitors, with onsets indicated by arrows. Successive trials at increasing concentration as shown; test odor was amyl acetate. Time, 4 msec. (From Kauer and Shepherd, 1977.)

These results demonstrate that odor stimuli can be controlled and monitored in a manner equivalent to that in other sensory systems. This control greatly facilitates the analysis of response properties of neurons and synaptic circuits under conditions of natural stimulation; and in some cases findings can be directly compared with results from electrical stimulation. For example, the prolonged discharge at threshold, with little evidence of inhibition, correlates well with the finding that the mitral-cell response to a test volley in the olfactory nerves shows little evidence of suppression when the shocks are very weak. With odor stimulation at higher concentrations, the excitatory-suppressive sequence in some mitral cells is very clear, which correlates with the excitatory-inhibitory sequence in the mitral-cell response to strong olfactory-nerve volleys. We need close correlations of this type, using both electrophysiological and natural modes of stimulation, in the analysis of local circuits. Our results indicate that they are possible in the olfactory bulb; they show further that the functional properties of local circuits are not rigidly set but may change dramatically in relation to submodality of stimulus and levels of activation.

Metabolic mapping

One traditionally thinks of functional analysis of the nervous system in terms of electrophysiological methods; but other approaches are possible for detecting changes in activity of cells. One of the most promising is the use of 2-deoxyglucose (2DG), as introduced by Sokoloff and his colleagues (Kennedy et al., 1975; Plum, Gjedde, and Sampson, 1976). This glucose analog is taken up by nervous tissue and phosphorylated by the same mechanisms as glucose, but it cannot be further metabolized. Thus, when radioactively labeled with ^{14}C and introduced in tracer amounts, 2DG can be used to give autoradiograms revealing regions in which nerve cells have changed their activity and hence their glucose requirements. Early results have shown local regions of activity-related 2DG uptake in relation to sciatic-nerve stimulation, ocular ablations, and focally induced epilepsy (Kennedy et al., 1975; Collins et al., 1976). These results have been consistent with evidence from previous studies using electrophysiological methods, but they have gone further in providing maps of the simultaneous activity changes throughout the nervous system.

Our interest in this method was based on the rationale that it was particularly sensitive to activity changes in neuropil, that is, in synaptic terminals and local synaptic circuits. It was therefore well suited for determining whether or not spatial patterns of activity are present in olfactory bulb neuropil during odor stimulation. The possibility of such patterns had been suggested (Mozell, 1971; Moulton, 1976), but the electrophysiological evidence was fragmentary and unsatisfactory.

Our experiments involve injecting rats with a pulse of ^{14}C-2DG and placing them in a closed glass chamber with a controlled olfactory environment for 45 minutes (Sharp, Kauer, and Shepherd, 1975, 1977). The brains are then rapidly removed and frozen, the bulbs are sectioned, and X-ray autoradiograms are prepared according to the Sokoloff method.

In control rats breathing room air, there is a broad band of relatively high activity in the autoradiograms of the olfactory bulb, extending from the glomerular layer to the granule layer. Individual layers can be discerned in favorable sections, and the glomerular and mitral-cell layers typically have high levels of resting activity. A characteristic finding is the presence of scattered very small dense foci, and careful correlation with the histology shows that these are located over groups of glomeruli. It appears that these foci are in some way related to olfactory input under conditions of minimal stimulation of olfactory receptors by odors in the ambient room air.

Under conditions of odor stimulation, larger areas of increased activity are characteristically found in the olfactory bulb. Figure 10 shows a typical result for the case of stimulation with the odor of amyl acetate. A broad band of increased activity is found in the anterolateral aspect of the bulb, and another similar band is found in the posteromedial aspect. Histological correlations show that these bands have peak density in the glomerular layer and spread into adjacent olfactory-nerve and external plexiform layers. This general pattern of activity is found in different animals subjected to this odor, suggesting that the pattern may represent a spatial distribution of activity important for transmission of this olfactory input. Furthermore, preliminary evidence indicates that patterns may be different for different odors and may thus reveal an essential aspect of the mechanisms of discrimination between odors. The results in the rat olfactory bulb have been confirmed and extended in studies of the olfactory bulb of the tree shrew (Skeen, 1977).

Synaptic transmitters

I shall conclude this review of experimental methods by considering briefly the identification of transmitter

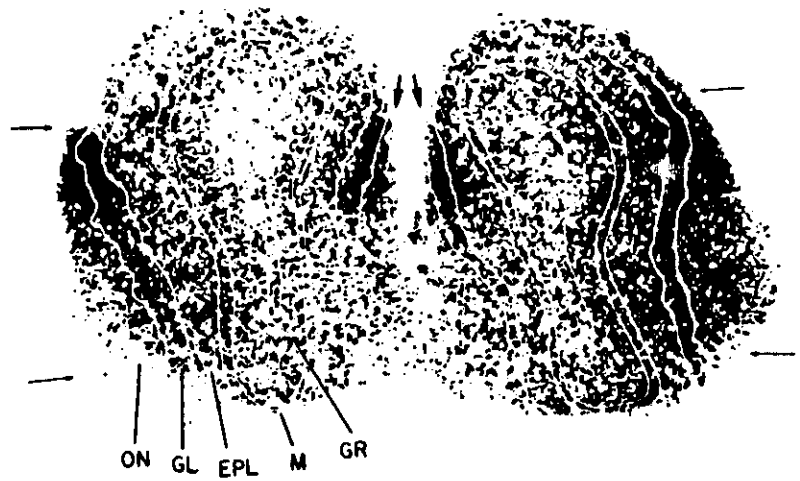


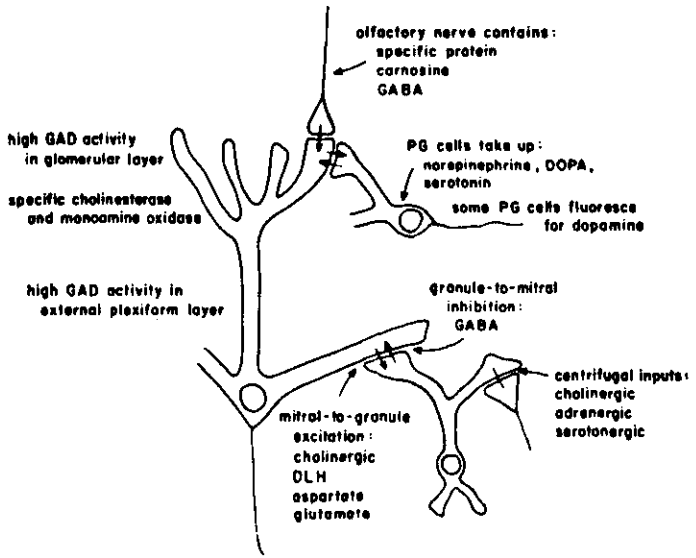
FIGURE 10 Autoradiograph of frontal sections of olfactory bulbs of a rat exposed to strong odor of amyl acetate. The outlines of the histological layers of the olfactory bulb, as determined from the subsequently stained sections, are shown superimposed on the autoradiographs. Small arrows indicate extent of lateral active regions; large arrows indicate medial active regions. Scale bar is 500 μm. (From Sharp, Kauer, and Shepherd, 1977.)

substances. Only in very recent years have adequate techniques become available so that one can begin to approach this problem with confidence in the central nervous system. A number of later chapters in this volume testify to the range of methods now available.

In the olfactory bulb the best case for a specific transmitter substance can be made for the granule-to-mitral dendrodendritic synapse. Microiontophoretic studies, combined with single unit recordings, have provided evidence that GABA is the transmitter substance at this synapse (McLennan, 1971; Nicoll, 1971). Iontophoresis of GABA produces suppression of mitral-cell activity, mimicking the suppression following LOT volleys. Iontophoresis of bicuculline, a known blocker of GABA, blocks the LOT-induced inhibition of mitral cells. More recently it has been shown that radioactively labeled GABA is taken up by granule cells. (Halász, Ljungdahl, and Hökfelt, 1978). Moreover, an immunocytochemical study has provided evidence for the localization of the GABA-synthesizing enzyme GAD in granule cells (Ribak et al., 1977). High GAD activity has been reported in the external plexiform layer and presumably reflects GABA synthesis in granule-cell dendrites and spines in that layer (Graham, 1973).

With regard to the other half of the reciprocal synapse in the external plexiform layer, microiontophoretic studies have shown that mitral cells are also suppressed by amino acids such as aspartate and D-L homocysteate (McLennan, 1971; Nicoll, 1971). At first this seemed paradoxical, since these substances have excitatory actions at many other central synapses. The explanation appears to be that they may exert their excitatory action on the granule cells thereby mimicking the excitatory dendrodendritic synaptic actions of the mitral-to-granule half of the reciprocal synapse. The excited granule cells then inhibit the mitral cells reciprocally, so that the observed effect is mitral-cell inhibition. This interpretation awaits further testing, but it serves as an important caution for the interpretation of microiontophoretic studies in other parts of the nervous system. It is clear that one cannot assume that the substance iontophoresed acts directly on the cell recorded from. Any synaptic terminal or local circuit element that carries receptors, specific or nonspecific, for that substance may be activated by it and transmit the ultimate effect on the cell recorded from by its own local action or that of the circuits of which it is a part.

In the glomerular layer there has long been evidence for catecholamine uptake and synthesis (Lichtensteiger, 1965). Recently Halász et al. (1977) have shown with immunocytochemical methods that periglomerular-cell bodies are positive for dopamine (DA)-synthesizing enzymes. The reaction exter



. 11 Summary of evidence for neurotransmitter nces in the olfactory bulb. For details see text. (Modified from Shepherd, 1977.)

he dendrites, suggesting that DA may function r transmitter at dendrodendritic as well as axoritic synapses of the PG cells. However, only a of the population of cells around the glomeruli in these enzymes. This fact provides a most ining hypothesis—that the periglomerular-cell lation may consist of metabolically distinct sublations that use different transmitter substances therefore, could have different synaptic actions. hypothesis suggests a biochemical fractionation anatomically defined and otherwise homoges cell type. A new complexity is thus introduced the functional significance of cell types in the ous system—a complexity that is obviously rele- to the organization of the local circuits within a region. Similar observations have been made in macrine-cell population in the retina (see Miller, volume). In the olfactory bulb further work is ed to determine the subtypes of PG cells that rve the inhibitory actions attributed to PG cells e electrophysiological studies. The PG cell ap- s to be the first short-axon cell type in the CNS hich DA has been identified as a possible neuansmitter. Some tufted cells also appear to syn- ze DA (Halász et al., 1977). Recently a second opulation of PG cells, which synthesize GABA, een identified (Ribak et al., 1977).

The biochemical constituents of the olfactory nerves have been studied intensively in recent years. A protein has been described that is unique to these nerves and the receptor cells from which they arise (Margolis, 1974). A dipeptide, carnosine, has also been found in these cells and axons; it is present in higher concentration there than in any other region of the nervous system (Margolis, 1974; Neidle and Kandera, 1974). Its function and possible relation to chemical transmission by the nerve terminals in the glomeruli is intriguing but as yet unknown.

The other main extrinsic fiber systems to the olfactory bulb are the efferent pathways from the CNS. The bulb is one of the regions that receives terminals from the adrenergic and serotoninergic fibers arising in the brain stem (Halász, Ljungdahl, and Hökfelt, 1978). These terminals are found on granule-cell spines, where they are strategically located for producing inhibition of the mitral cells or biasing the mitral- and granule-cell interactions. Some connections to the periglomerular regions are also known.

From this brief review it can be seen that there is evidence regarding possible neurotransmitters for most of the main neuronal types and extrinsic fiber systems in the olfactory bulb. In this respect progress is equivalent to that in regions such as retina or cerebellum. A special significance of the olfactory bulb for these studies may rest in the evidence for neurotransmitters at dendrodendritic synapses. The optic tectum is another central region where considerable progress has been made, including evidence for possible transmitters at dendrodendritic synapses (see Cuenod, this volume). Other regions, such as the basal ganglia, have also been studied intensively, and there is tentative evidence for important synaptic interaction between dendrites in some of these regions. Progress in neurochemical studies will be closely dependent on knowledge of the organization of the local circuits within the regions under study.

Discussion

The experiments that have been reviewed illustrate the proposition set out in the introduction to this section—that a correlation of results from different methodologies is essential in the effort to understand the nature of nervous organization. In addition, general comment may be made on two further aspects, one practical and the other theoretical.

On the practical side, it is worth remembering that physiological study of the olfactory bulb was initially based on traditional methods of electrophysiological analysis suitable for motoneurons and other large impulse-generating projection neurons in the nervous system. Electron-microscopic study of synaptic connections was also begun at a time when only the types of synapses made by projection neurons with long axons had been recognized. These approaches by themselves have been successful in the olfactory bulb up to a point; but one of the lessons emerging most clearly from this work is that our methods and the tactics of applying them must be reevaluated and modified for the task of analyzing local synaptic circuits and local synaptic properties. Evidence of the presence of output synapses on dendrites and their activation by local graded potentials is the most urgent need. I pointed out earlier that "impulse flow" as monitored by extracellular unit recordings can no longer be relied upon as an accurate reflection of "synaptic flow"—that is, of the activity mediated by local synaptic circuits. Even with intracellular recordings, the compartmentalization of dendritic trees severely restricts the utility of a soma recording site in monitoring or testing the input-output relations mediated by circuits into and out of distant dendritic compartments. The development of improved recording techniques and isolated preparations are important steps toward obtaining stable recordings from intrinsic neurons and dendrites; even so, the effects of electrode impalement on local properties and the electrotonic constraints on the sampling of activity will have to be rigorously assessed. With regard to neurochemical aspects, it is likely that the simple picture of brief synaptic potentials mediated by punctate synaptic junctions will be much embellished as more is learned about the variety of morphophysiological relations at different junctions and the variety of longer-term effects a terminal may exert through substances acting on neighboring terminals as well as on itself. Considerations such as these indicate that the practical problems facing experimental analysis of local circuits are considerable; but they also leave little doubt that it is here that the basic mechanisms of neuronal organization are to be found.

On the theoretical side, a key problem is to develop a conceptual framework for the new types of organization that have come into view through the work on local circuits. In the olfactory bulb, some clues have been provided by the organization of synaptic circuits in relation to the dendrites of the principal and intrinsic neuronal populations. I have discussed this elsewhere on several occasions (Shepherd, 1972b, 1974, 1975, 1977, 1978). Here it will suffice to say that, beginning with the single synapse as the basic unit, several levels of organization of increasing extent and complexity can be identified in terms of synaptic clusters, dendritic branching compartments, whole dendritic trees, the whole neuron, and multineuronal chains and loops. This view of the olfactory bulb emerges rather naturally from simple structural considerations. Present analysis of functional operations is being carried out within this context, as illustrated by the model of the reciprocal synapse and the dendritic branch compartments of the mitral and granule cells, as in Figure 5.

There is evidence that similar types of synaptic circuits and functional properties are found in a number of other regions. We thus face two key questions. First, can logical hierarchies of levels of organization be identified in all regions? Second, can the levels in any given region be systematically correlated in a meaningful way with those in other regions? Against these propositions it has been argued that every neuron is unique and that the information processed in each is different in kind from that of any other region. However, the evidence seems to be increasing that, behind the welter of detail, there may indeed be some relatively limited and common principles of organization along the lines indicated above, that apply, to a greater or lesser extent, to all types of nervous organization. Both points of view were expressed in the formal and informal discussions at

this Study Program and represent at least a preliminary attack on this important issue.

Although these matters are speculative, they touch on a requirement of any field for a consensus on the basic units of organization. We have traditionally thought of the basic structural unit as the neuron and its basic functional property as the impulse. But at the level of local circuits, these units lose their force and generality: input-output operations may be carried out through restricted parts of whole neurons, and local synaptic transmission and integration may take place in the absence of impulse activity. Impulse activity of course underlies the excitability that has always been regarded as the hallmark of nervous tissue; yet not only do some nerve cells function without this activity, there are a number of nonnervous types of cells, in both animals and plants, that also exhibit this property (see Mueller, this volume).

It thus appears that the study of local synaptic circuits in the olfactory bulb, retina, and other regions requires a reassessment of our traditional beliefs about the essential nature of nervous organization. The case for retaining traditional tenets in the face of the new evidence has been argued eloquently by Peters, Palay, and Webster (1976). The problem, in fact, is not to discard these tenets, but rather to incorporate them into a new framework. I have suggested elsewhere (Shepherd, 1972b, 1977) that this can be developed around the functional operations carried out at the different levels of organization. In this view, neuronal units are replaced by functional units, and the traditional neuron doctrine is incorporated into a set of functional principles that begins with the single synapse as the simplest unit and builds in complexity through the successive levels of organization. At each level the synaptic circuits comprise the basic units for information processing, the processing being carried out by the ensemble of units at that level of complexity. Since the functional operations are largely defined by synaptic properties and circuits, it is possible that the organizing principles we seek will be eventually subsumed under the heading of a synaptic doctrine. If this can serve as a means to orient the search for common principles that account for the diversity of nervous-system structures and functions, it will have some heuristic value.

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