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Recurrent Excitation of Secondary Olfactory Neurons: A Possible Mechanism for Signal Amplification

Abstract. *Secondary neurons of the olfactory bulb can be excited monosynaptically after activation of neighboring secondary neurons by antidromic and orthodromic volleys. Recurrent collaterals of secondary neurons are proposed to synapse with other secondary neurons, thus forming a direct recurrent excitatory pathway. Such a positive feedback system could strengthen the original input signal.*

One of the most striking features of the olfactory system is its high degree of acuity. It has been estimated that as little as a few molecules of an odorant can be detected by some species (*1*). Thus one might expect the olfactory system to contain neuronal mechanisms for signal amplification. Although the concept of signal amplification in sensory systems has been proposed by a number of investigators (*2–4*), there is little physiological evidence for the presence of such a process. The results presented below suggest one possible mechanism in olfaction which might also function in other sensory systems.

The olfactory bulb is well suited for the study of sensory processing because the afferent and efferent pathways are easily accessible for stimulation and the neuronal elements are organized into discrete layers. Olfactory nerves arise from bipolar receptor cells in the nasal mucosa and terminate in the glomeruli of the olfactory bulb (Fig. 1). Secondary neurons of the olfactory bulb, mitral and tufted cells, receive synaptic excitation from olfactory nerves and send axons to the lateral olfactory tract (LOT) (*5–7*). Cajal showed anatomically that both mitral and tufted cells have axon collaterals that terminate in the external plexiform layer, and he proposed that such pathways might spread excitation to "an increasing number of conductors (avalanche conduction)" (*2*). I report here evidence for the monosynaptic recurrent excitation of secondary neurons; this would provide a positive feedback system that could strengthen the input signal.

Experiments were performed on rabbits with transected olfactory peduncles. Such a lesion would remove the centrif-

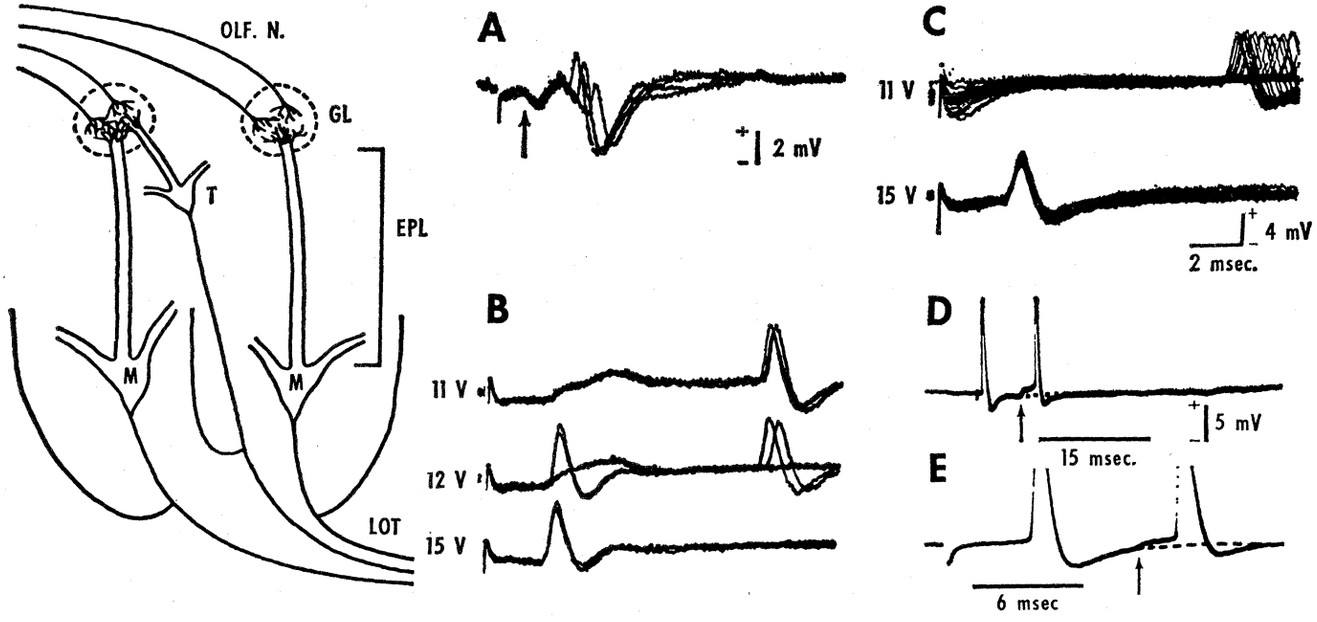

Fig. 1. Diagram at left illustrates basic anatomical features of the olfactory bulb. Olfactory nerves (*Olf. N.*) run along the surface of the bulb to form excitatory synapses with dendrites of mitral (*M*) and tufted (*T*) cells in glomeruli (*GL*). Axons of both cell types travel in the lateral olfactory tract (*LOT*) and send recurrent collaterals to the external plexiform layer (*EPL*). (A) Extracellular records of a presumed tufted cell showing variable latency to threshold stimuli to the LOT (five superimposed traces). Arrow shows onset of negative field that indicates antidromic invasion of the mitral cell. The time scale is the same as in C. (B) Extracellular recording from a mitral cell. The LOT was stimulated with 11 volts, 12 volts, and 15 volts as indicated (three to five superimposed sweeps). (C) Upper record: the LOT was stimulated with 11 volts at a frequency of 110 cycle/sec. Lower trace: the LOT was stimulated with 15 volts at a frequency of 110 cycle/sec. (D) Intracellular recording from a mitral cell responding to LOT volley (stimulus artifact just precedes first spike). Arrow indicates beginning of EPSP. (E) Intracellular record from another mitral cell at faster sweep responding to olfactory nerve volley (stimulus artifact at beginning of trace). Lack of inhibitory postsynaptic potential is presumably due to Cl^- leakage from the electrode.

ugal fibers which might produce effects similar to responses attributed to recurrent collaterals. The electrophysiological recording and stimulating procedures used were standard and recently described (*8*).

Tufted cells (*9*) often responded to antidromic stimulation of the LOT with features suggesting synaptic excitation. The response obtained from a presumed tufted cell as a result of stimulation of the LOT at threshold intensity has a variable latency (Fig. 1A). In general, most of these cells responding with variable latencies to stimulation of the LOT failed to follow stimulus frequencies greater than 30 to 40 cycle/sec, which is consistent with synaptic excitation by LOT volleys. As the intensity of the stimulus to the LOT was increased, there was usually a decrease in the latency of the spike to 0.5 to 1.3 msec after the onset of the field potential indicating antidromic invasion of mitral cells (arrow in Fig. 1A); this latency is in the order of one synaptic delay from mitral cell axons. In agreement with other investigators (*10*), I conclude that tufted cells can be excited monosynaptically by mitral cell axon collaterals. Such a monosynaptic connection from mitral cell axon collaterals to tufted cells is illustrated schematically in Fig. 2A.

Extracellular recording from mitral cells also suggested that mitral cells can be excited synaptically after antidromic volleys. Figure 1, B and C, shows the responses from a mitral cell. At low stimulus intensities the cell responded at a long and variable latency (Fig. 1B) and failed to follow a stimulus frequency of 110 cycle/sec (Fig. 1C). With higher stimulus strengths (Fig. 1B), the latency shortened to 1.8 msec without variability in the latency and faithfully followed stimulus frequencies of 110 cycle/sec (Fig. 1C). These results with extracellular recording suggest that low stimulus strengths excited the cell synaptically, whereas at higher intensities the cell was invaded antidromically (*11*). More direct evidence for synaptic excitation by orthodromic and antidromic volleys was obtained with intracellular recordings. Figure 1D illustrates an intracellular response of a mitral cell which discharged twice after a single stimulus to the LOT—an antidromic response and a later action potential arising from an excitatory postsynaptic potential (EPSP). The broken line in this record demonstrates the response obtained with stimuli below threshold for the EPSP. Such double responses were also produced by stimulation of the olfactory nerves as shown in Fig. 1E (*12*). The minimal latency after antidromic invasion for mitral cell synaptic excitation was 0.5 msec which indicates a monosynaptic pathway. Thus mitral cell collaterals appear to synapse directly with neighboring mitral cells (Fig. 2A). Presumably the synaptic excitation of mitral cells with longer latency (Fig. 1, B and C) occurs by way of axon collaterals of the slower-conducting tufted cell axons (Fig. 2A) (*13*).

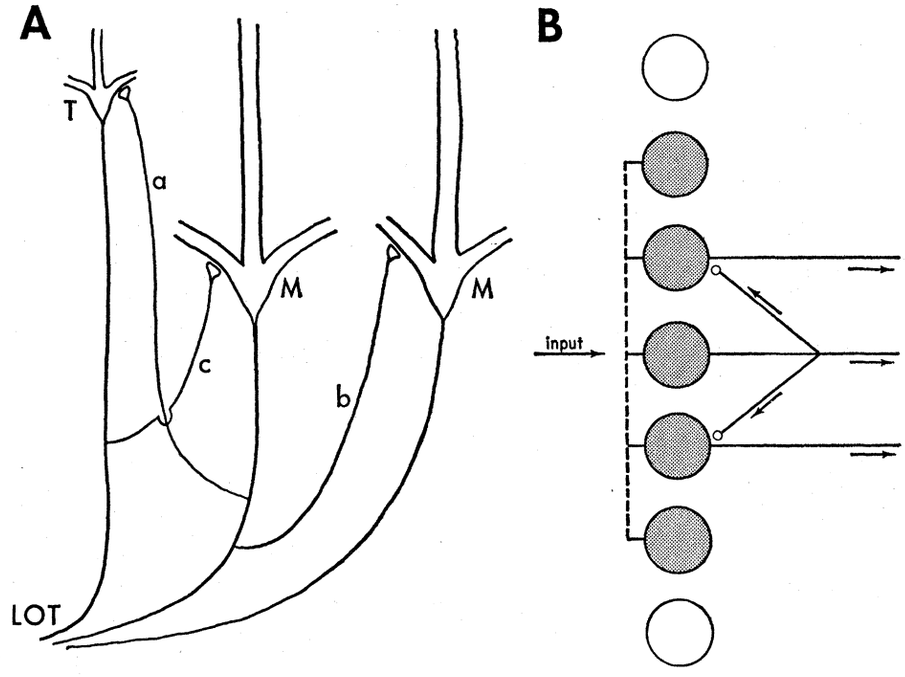

Fig. 2. (A) Diagram of proposed recurrent pathways. Mitral cell recurrent collaterals are proposed to make direct synaptic contact with tufted cells (*a*) and other mitral cells (*b*). In addition, the recurrent collaterals of tufted cells axons are proposed to make direct synaptic contact with mitral cells (*c*). (B) Schematization of possible interaction of dendrodendritic inhibition and recurrent excitation of secondary neurons [adapted from von Békésy (*4*)]. Broken line indicates lateral spread of inhibition (shaded neurons are inhibited).

From psychophysiological studies on numerous sensory systems, von Békésy (*4*) has developed the concept of "funneling." He proposes that funneling involves two processes: (i) inhibition of small laterally spreading stimulus effects which would improve the signal-to-noise ratio of the system and (ii) an increase in the magnitude of the sensation. There is neurophysiological evidence supporting the existence of lateral inhibition in many sensory systems (*4*, *14*). In the olfactory bulb, dendrodendritic inhibition has been proposed to serve this function (*15*). However, there is little neurophysiological evidence for the second concept. The present findings of monosynaptic recurrent excitation of secondary sensory neurons could serve to increase the magnitude of the sensation. Figure 2B shows a schematization of the above hypothesis. The broken line represents the lateral spread of the disynaptic dendrodendritic inhibition to a series of secondary neurons. Within this area secondary neurons can be excited monosynaptically by recurrent collaterals, thus increasing the magnitude of the original signal. It is assumed that the area of inhibition is larger than the area of feedback excitation (*4*). It should be noted that the spatial characteristics of these two systems must be elucidated and quantitative data on the interaction of these two systems must be obtained to further test this hypothesis.

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9. These are cells recorded in the external plexiform layer that discharge one or two spikes to orthodromic activation. Some of these cells can be invaded antidromically at a long latency with LOT volleys (*7*). They are distinguished from periglomerular cells in that periglomerular cells fire a long burst of spikes upon stimulation of the olfactory nerve.
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11. The delayed synaptic activation presumably does not appear with the stronger stimuli because of the powerful postsynaptic inhibition following antidromic invasion (*8*, *15*).
12. The first spike arises from the base line because the excitation of the olfactory nerve occurs approximately 500 μm from the soma whereas the EPSP giving rise to the second spike is evidently generated closer to the soma.
13. Although an interneuron cannot be entirely ruled out for the excitations of longer latency, the major interneuron of the olfactory bulb is the granule cell and this has been shown to be inhibitory (*15*).
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Illness-Induced Aversions in Rat and Quail: Relative Salience of Visual and Gustatory Cues

Abstract. *Bobwhite quail, like the rat, learn in one trial to avoid flavored water when illness is induced by a drug ½ hour after drinking. In contrast to the rat, quail also learn to avoid water that is merely darkened by vegetable dye. The visual cue is even more salient than the taste cue in quail.*

Earlier work on illness-induced aversions to eating and drinking shows rather clearly that the rat, at least, must have either a gustatory or an olfactory cue in order to learn to avoid ingesting a substance if the illness that follows ingestion is delayed by ½ hour or more. Visual, auditory, and tactual cues, even though conspicuously present at the time of ingestion, do not become danger signals for the rat in such circumstances (*1*, *2*). On the other hand, blue jays (*Cyanocitta cristata bromia* Oberholser, Corvidae) easily learn to reject toxic monarch butterflies (*Danaus plexippus* L., subfamily Danainae) on sight, although the model suggested for this learning gives emetic reinstatement of taste during illness a prominent, mediating role (*3*).

Impetus for our experiments came from the general view that the behavior of an organism, including what it can and cannot readily learn, is largely a product of its evolutionary history. In view of the rat's highly developed chemical senses, nocturnal feeding habits, and relatively poor vision, its ability to learn to avoid toxic substances on the basis of their taste or smell, rather than their appearance, is not surprising. But how general is this phenomenon across species? Might we not expect a diurnal bird, with its superior visual equipment and greater reliance upon vision in foraging for food and drink, to show a different pattern? Perhaps such birds, even in situations involving long delay between the time of ingestion of some food and the onset of illness, can learn to avoid ingesting substances that are distinctive in appearance only.

We report here two experiments which show that bobwhite quail (*Colinus virginianus*) can associate a purely visual cue with a long-delayed, illness consequence. In the first experiment we investigated the relative salience of a visual cue and a gustatory cue in both rats and quail. In the second experiment, in which we used quail only, we controlled for two variables which, unless accounted for, would not have allowed clear-cut interpretation of the first experiment.

Forty 90-day-old male Sprague-Dawley rats and 40 adult male bobwhite quail were subjects (*4*) in the first experiment. All were caged individually and had free access to food throughout the experiment. At the start, both species were trained over a period of several days to drink all of their daily water from 30-ml glass Richter tubes. Water was presented at the same time each day, and the time allowed for drinking was gradually reduced to a 10-minute period. Baseline drinking was then measured for 1 week, after which experimental treatments were imposed.

On treatment day, subgroups of each species received an initial 10-minute exposure to water that was either dark blue ($N = 8$), sour ($N = 8$), or both blue and sour ($N = 24$). Water was made blue by the addition of three drops of vegetable food coloring to 100 ml of water. Sour water consisted of a weak hydrochloric acid solution (0.5 ml per liter). One-half hour after removal of the distinctive fluid all subjects were injected intraperitoneally with the illness-inducing drug, cyclophosphamide. The dosage for the rats was 66 mg/kg, a dosage known to be effective for establishing one-trial aversions to distinctive tastes in the rat. We used a larger dose (132 mg/kg) for the quail, however, because exploratory use of the drug with the birds showed that the larger dose was necessary in order to produce the primary symptom of illness that rats exhibit, namely, extensive diarrhea.

For 2 days after treatment all subjects drank plain water at the regular 10-minute daily drinking period. This allowed them time to recover from the illness, as evidenced by remission of diarrhea and a return to baseline amounts of water consumption. Extinction tests were then begun to determine whether aversive conditioning had been established to the cues present in the water on treatment day. Five 10-minute tests were conducted, one every third day, with 2 days intervening between tests during which subjects were allowed to drink plain water to reestablish the baseline.

Animals that drank sour water on treatment day were tested with sour water (S : S); those that drank blue water on treatment day received blue water in the extinction tests (B : B). However, the 24 animals of each species that had drunk blue-sour water on treatment day were divided into three subgroups for testing. One group of each species was tested on blue-sour water (BS : BS), another on sour water (BS : S), and the third on blue water (BS : B).

Figure 1 shows a comparison of the amount of water drunk by rats and quail over five extinction trials for each of the five treatment : test conditions. Differences between mean drinking scores on treatment day and the first extinction trial (E_1) were assessed for statistical significance by the *t*-test. Results in the S : S condition show that the sour taste by itself was an effective cue for avoidance in both rat ($P < .02$) and quail ($P < .05$). Only the quail, however, showed reduced drinking ($P < .01$) of water that was colored blue on treatment and test days (B : B). In the BS : BS condition, both species again showed significantly reduced drinking in the tests ($P < .001$).

Perhaps the most striking results