bryos were comparable to normal embryos cultured on serum from untreated subjects with respect to body curvature, forelimb buds, pharyngeal development, and nasal placodes (Fig. 1g). Somite pairs ranged in number from 17 to 24. Protein and DNA values for embryos cultured in four of the six serum samples did not differ significantly from those obtained for embryos cultured with serum from untreated subjects (Table 1).

Thus we demonstrated that growth of rat embryos in human serum can be appreciably enhanced by glucose supplementation and that growth and development varies with the individual source of the culture serum. Indeed, we have observed death and abnormalities in rat embryos cultured with serum from humans suffering from colds and with serum from rats suspected of having pneumonia.

Culture medium prepared from serum from persons who were receiving drugs that are teratogenic to animals (*9*) or implicated as teratogenic to humans (*10*) was lethal or teratogenic to rat embryos. Although this demonstrates that cultured rat embryos can be used to detect teratogenic substances, no attempt was made to characterize the deleterious substances in the serum samples. Such an analysis is required to establish a relation between the effects of human serum on rat embryos in vitro and the occurrence of congenital defects in man.

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5 November 1979

## Dendrodendritic Inhibition: Demonstration with Intracellular Recording

Abstract. *The isolated turtle olfactory bulb was used to characterize synaptic interactions between mitral and granule cell dendrites. First, blockade of antidromic invasion of mitral cell dendrites caused a large decrease in the size of the inhibitory postsynaptic potential (IPSP) recorded in mitral cells, indicating that the IPSP results in large part from activity in the dendrites of mitral cells. Second, direct depolarization of mitral cells was followed by an IPSP. In the presence of tetrodotoxin, depolarization of mitral cells evoked calcium spikes (as would be expected for presynaptic membranes) followed by IPSP's. These findings demonstrate that regenerative sodium spikes and axonal pathways are not required for this inhibitory pathway and that the inhibition is indeed a result of local dendritic interaction. Such a pathway provides an obligatory synaptic inhibition of mitral cells following their activation and emphasizes the tight coupling that exists with reciprocal dendrodendritic synapses.*

Local circuits of the central nervous system (CNS) are crucial components in the integration of sensory input (*1*). A particularly important example of local neuronal action is provided by dendrodendritic synaptic inhibition, which is likely to occur at numerous sites in the CNS. The specific properties of this synaptic interaction are still poorly understood, however, and, except for the olfactory bulb, the evidence is limited almost entirely to anatomical findings. In the olfactory bulb, the physiological evidence is based largely on the analysis of extracellular field potentials with some supporting intracellular data (*2*). In the bulb (Fig. 1A) it is envisaged (*2*) that depolarization of mitral cells and their dendrites activates excitatory synapses which depolarize granule cell dendrites. This depolarization in turn activates inhibitory synapses made by granule cells that release $\gamma$-aminobutyric acid (GABA) back onto the same, and neighboring, mitral cells. This pathway has also been proposed to be activated by graded electrotonic depolarization. A number of predictions can be made: (i) calcium channels should exist in mitral cell soma-dendrites since these cells are presynaptic; (ii) depolarization of mitral cells should result in a local self-inhibition that is independent of axonal pathways; (iii) the inhibitory pathway should remain intact in the absence of action potentials; (iv) the inhibition of a mitral cell should be greater when an antidromic impulse invades the soma-dendritic membrane than when the impulse is limited to the axon and initial segment of the mitral cell. We have used the isolated turtle olfactory bulb (*3*) to test these predictions.

Turtles (*Pseudemis scripta elegans*) were decapitated, and the olfactory bulb was removed and placed in Ringer solution (*4*). The bulb was hemisected to facilitate the placement of electrodes. We used a bipolar concentric electrode to stimulate the olfactory nerves and a bipolar needle electrode to stimulate the mitral cell axons antidromically. Microelectrodes (100 to 200 megohms) filled with 2*M* potassium methyl sulfate or 3*M* potassium chloride were used for intracellular recording. A bridge circuit was used for passing current through the recording electrode. Switching between


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Ringer solutions containing known concentrations of drugs was accomplished by means of a solenoid switching valve (General Valve). Since many neurons generate calcium-activated potassium responses (*5, 6*), which could be confused with true inhibitory postsynaptic potentials (IPSP's), we usually used KCl-filled electrodes, which inverted the IPSP's into depolarizing potentials. Mitral cells were identified by antidromic activation of their axons and by their location in the mitral cell layer, which was defined as the region in which the orthodromic field potential reversed polarity (*7*). The results were obtained from 53 neurons with a mean resting potential of 57.4 mV (range, 50 to 75).

Stimulation of olfactory nerve or mitral cell axons resulted in an action potential followed by a prolonged hyperpolarization of mitral cells. With electrodes filled with potassium methylsulfate, the response remained hyperpolarizing for the duration of the impalement. With KCl-filled electrodes, the responses were typically slightly hyperpolarizing immediately after impalement (Fig. 1B, first trace) but gradually became depolarizing (Fig. 1B, second trace). The potential was associated with a marked fall in membrane resistance as shown by the reduction in the size of the responses to hyperpolarizing constant current pulses. Hyperpolarizing the mitral cell membrane resulted in a large increase in the depolarizing response (Fig. 1B, third trace). These properties indicate that there is a prolonged IPSP in mitral cells following orthodromic and antidromic stimulation. The IPSP's could be generated by orthodromic and antidromic stimuli that were below threshold for action potentials in the impaled mitral cells, but a large increase in the size of the IPSP generally occurred when the cell responded with an action potential. This result suggests that the IPSP is linked, in part, to the firing of the cell under observation. Indeed, single spikes occurring either spontaneously or in response to depolarizing current pulses were capable of eliciting IPSP's in more than 95 percent of the cells (Fig. 1D). Large depolarizing current pulses increased the size of the IPSP (Fig. 1C). As would be expected for synaptic potentials, the IPSP's were blocked by cobalt. These findings indicate that mitral cells are self-inhibited and suggest that recurrent axon collateral pathways are not necessary for the inhibition, since in neurons that receive only collateral inhibition, IPSP's do not follow spikes evoked by intracellularly applied depolarizing pulses (*6, 8*).

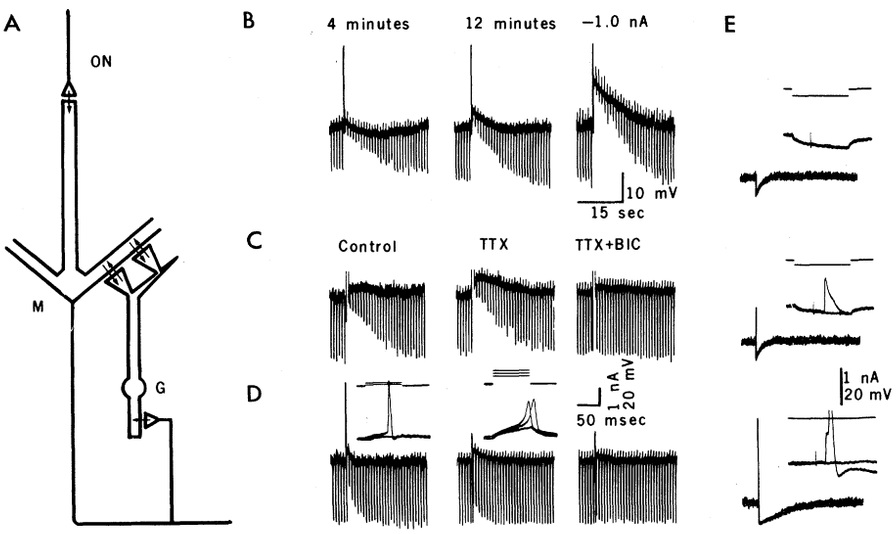


Fig. 1 (A) Schematic diagram of neuronal circuit; *ON*, olfactory nerve; *M*, mitral cell; *G*, granule cell. Arrows indicate synaptic polarity. (B to D) IPSP's recorded in turtle olfactory bulb mitral cells. (B) Chart records of responses to orthodromic stimulation 4 and 12 minutes after impalement and during passage of 1-nA d-c hyperpolarizing current through the KCl-filled recording electrode. Downward deflections are hyperpolarizing responses to constant current pulses through the recording electrode. The increase of resting membrane resistance between the 4-minute record and the following records is due to improved sealing of the membrane around the electrode. (C) Responses to large depolarizing current pulses through the recording electrode (6 nA for 800 msec) in control Ringer solution, in $10^{-6}M$ TTX for 15 minutes, and in $10^{-6}M$ TTX with $10^{-4}M$ bicuculline methiodide added for 23 minutes (*TTX + BIC*). (D) Responses to threshold depolarizing current pulses in the same conditions as in (C). The first inset shows the directly produced action potential and current trace in control Ringer. The second inset shows the calcium spikes to direct stimulation in TTX. Note the great increase in threshold for the calcium responses. The records in (B), (C), and (D) are from the same cell. (E) Antidromic responses recorded in another mitral cell with an electrode filled with potassium methylsulfate. In the top records, a strong hyperpolarizing current pulse injected during antidromic stimulation entirely blocked invasion. In the middle records, invasion was limited to the initial segment, whereas in the bottom records, invasion of the soma-dendrites occurred. The resting potential of both cells was 55 mV. The calibration in (B) applies to all chart records. The sweep speed for the insets in (E) was twice that in (D).

To verify the presynaptic role of mitral cell dendrites. In the first, we added tetrodotoxin (TTX) to the Ringer solution to block impulse propagation out the mitral cell axon and thereby localize the effects of depolarizing current injection to the soma-dendritic membrane. The TTX ($10^{-6}M$) blocked propagated action potentials in the olfactory nerves and mitral cell axons and, thus, both orthodromic transmission and antidromic invasion. The fast action potentials elicited by the intracellular stimulation of mitral cells were also blocked. However, increasing the strength of the depolarizing current pulses evoked smaller and broader action potentials (insets in Fig. 1D), which were entirely blocked by the calcium antagonist cobalt (5 m$M$). Thus, the presence of these calcium spikes indicates that mitral cells do have well-developed calcium channels. In addition, the IPSP's evoked by calcium spikes demonstrate that the reciprocal synapses were activated (Fig. 1D, second trace). The existence of IPSP's insensitive to TTX indicates that granule cell dendrites also release transmitter in the absence of propagating sodium spikes. Graded depolarization below threshold for calcium spike initiation failed to activate the reciprocal synapse. The fact that the IPSP is produced in TTX demonstrates that the inhibitory pathway can be activated by the mitral cell body and dendrites and does not require axonal pathways, since mitral cell axons are inexcitable in TTX. Finally, the IPSP is blocked by the GABA antagonist bicuculline (Fig. 1, C and D) as would be expected from the release of GABA from granule cells (*9, 10*).

In a second approach to verifying the presynaptic role of mitral cell dendrites, we compared the size of the IPSP generated in the absence and presence of impulses in the soma-dendritic membrane. By applying hyperpolarizing current through the recording electrode, which is presumably located in the soma, the antidromic action potential can be fractionated into two distinct steps (Fig. 1E). By

analogy with other neurons (*11*), the first step is the action potential generated in the initial segment, and the second is that generated in the soma-dendritic membrane. The records in Fig. 1E demonstrate that the presence or absence of an impulse in the initial segment has little effect on the size of the IPSP. However, when the impulse is permitted to invade the soma-dendritic membrane, there is a large increase in the size of the IPSP. It can be concluded, then, that the observed increment in the IPSP is attributable to a synaptic pathway involving the soma-dendritic membrane.

Our results directly demonstrate the presynaptic action of dendrites, for both mitral and granule cells of the olfactory bulb, and show that the reciprocal pathway remains intact when all sodium-dependent propagated action potentials are blocked by TTX. Such a pathway provides for an all-or-none self-inhibition of mitral cells (*12*). These cells are also subjected to lateral inhibition, as is apparent from observations that orthodromic and antidromic stimuli below threshold for generating action potentials in the impaled cell nevertheless evoke small IPSP's.

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20 August 1979; revised 19 December 1979

## Role of Nitrogen Dioxide in the Biosynthesis of Nitrosamines in Mice

Abstract. *Groups of three to four mice were gavaged with aqueous solutions of 2 milligrams of morpholine, after which they were exposed to nitrogen dioxide in inhalation chambers at concentrations of 0.2 to 50 parts per million for up to 4 hours. At sequential intervals during the exposure, mice were frozen and pulverized in liquid nitrogen, and the mice powder was extracted with ice-cold 35 percent aqueous methanol and dichloromethane; organic-phase concentrates were analyzed for* N-*nitrosomorpholine with a thermal energy analyzer interfaced to a gas chromatograph. The* N-*nitrosomorpholine yields, ranging up to about 2.3 micrograms per mouse, were time-dependent relative to the duration of exposure to nitrogen dioxide and dose-dependent relative to the concentrations of nitrogen dioxide; control levels (in mice that were gavaged with morpholine or distilled water and then exposed to air instead of nitrogen dioxide) were less than 5 nanograms per mouse. These preliminary studies demonstrate the in vivo nitrosating potential of nitrogen oxides.*

Concern about the public health hazards of carcinogenic nitrosamines in air, water, food, and consumer products is growing (*1*). This is paralleled by concern over the wide environmental distribution of nitrosamine precursors, nitrite and amines (*2*), from which nitrosamines can be readily synthesized both in vitro and in vivo (*3*, *4*). The role of nitrite as a nitrosating agent is well documented; there is more limited evidence on nitrosation by nitrogen oxides ($NO_x$) of secondary amines in the liquid or solid phase (*5*), in the gas phase (*6*), and in vitro in plasma (*5*) or lung homogenates (*7*). To our knowledge, there are, however, no available data on in vivo nitrosation by $NO_x$. Nitrogen oxides are common atmospheric pollutants, found in a wide range of anthropogenic sources, largely resulting from the combustion of fossil fuels, including auto exhaust, emissions from stationary sources such as utility plants, and a wide range of high-temperature combustion processes such as welding and foundry work (*8*). Addition-

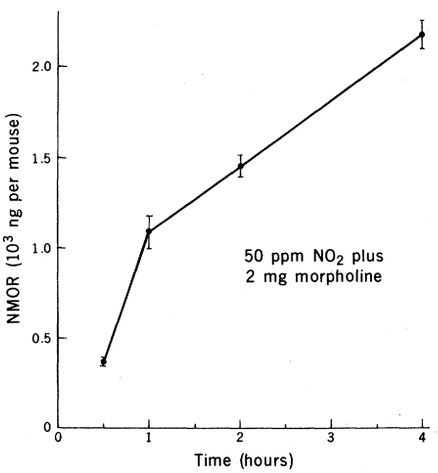


Fig. 1. Time course of NMOR biosynthesis in mice. Groups of three to four male ICR mice were gavaged with freshly prepared solutions of 2 mg of MOR (Aldrich Chemical) in 0.2 ml of distilled water and immediately placed in exposure chambers (Nalge desiccators, modified for gas inflow from the bottom and exhaust from the top). Mice were then exposed to 50 ppm $NO_2$ (three to four mice per chamber, 5 cubic feet per hour, 20 volume changes per hour) at intervals of from 0.5 to 4 hours. The required concentrations of $NO_2$ were produced by mixing stock $NO_2$ (custom grade, Union Carbide) with air at an appropriate flow rate, prior to introduction into the chambers; we checked the accuracy of the exposure mixtures by periodically monitoring and analyzing the $NO_2$ in the exhaust from the chambers, using the Griess-Saltzman reaction (*19*). Concurrent controls consisted of two mice exposed in separate chambers to $NO_2$ alone for 4 hours, additional controls were gavaged with 2 mg of MOR or 0.2 ml of distilled water and exposed to air for identical periods in separate chambers. After exposure to $NO_2$, the mice were killed by freezing in liquid nitrogen and blended to a fine powder (*20*). Two or three aliquots (approximately 8 g each) were taken from each mouse powder and blended with 75 ml of ice-cold 35 percent aqueous methanol in a Waring Blendor (5 minutes, medium speed); a known amount of a nitrosamine standard [152 ng of di-*n*-propylnitrosamine (DPN), Aldrich] was then added, and blending continued for 1 to 2 minutes. Homogenates were divided in half and centrifuged (5000*g*, 25 minutes, 5°C; swinging bucket), supernatant was removed, and the pellets were extracted again with cold 35 percent methanol. The pooled supernates were extracted (twice) with an equal volume (total, 150 ml) of dichloromethane [(DCM), Burdick and Jackson] (*21*), and the organic layer was dried by passage through a cotton gauze (Ex-tube, Analytichem International) and concentrated to 2 ml in a Kuderna Danish concentrator (Kontes, 250 ml) kept in a 65°C bath. Aliquots (20 $\mu$l) of the concentrates from each of two or three powder samples were injected into the thermal energy analyzer–gas chromatograph (Thermo Electron modified model TEA-502) (*22*) for NMOR analysis. Peaks were identified and quantitated by comparison with the retention time and response of reference nitrosamines (*23*). The plotted values are corrected for any background control NMOR levels and for the DPN standard recoveries and represent means of three to four mice ± the standard deviation.


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