replaced. The solution flowing from the distal cannula was collected in graduated tubes for determining volume outflow per unit time. The rate of water absorption was determined by the difference between inflow and outflow rates of the solutions. Aliquots of the incoming and outgoing fluids were analyzed for calcium, sodium, and potassium by flame photometry; the radionuclide was estimated by standard radioisotopic procedures with a thin-window Geiger-Muller counter. At termination, the rats were bled by cardiac puncture. The plasma was collected and analyzed for total calcium, total protein by the biuret method, and radiocalcium.

Efflux is defined as the unidirectional movement of ion from lumen to plasma, and influx as the unidirectional movement from plasma to lumen. Net calcium flux is the difference between efflux and influx rates. The various flux rates, in micromoles per centimeter per hour, were calculated from the following equations:

$$\text{Ca efflux} = \frac{(Ca^{45}{}_i)(W_i) - (Ca^{45}{}_o)(W_o)}{[(SA_i + SA_o)/2]\,(L)} \quad (1)$$

$$\text{Net Ca flux} = \frac{(Ca^{40}{}_i)(W_i) - (Ca^{40}{}_o)(W_o)}{L} \quad (2)$$

$$\text{Ca influx} = (\text{Ca efflux}) - (\text{net Ca flux}) \quad (3)$$

The symbols are defined as follows: Ca^{45} = radiocalcium content of fluids in counts per minute per milliliter; Ca^{40} = total calcium of fluids in micromoles per milliliter; SA = specific activity of calcium in fluids in counts per minute per micromole; W = rate of water flow in milliliters per hour; and L = length of duodenal segment in centimeters. The subscripts i and o indicate inflowing and outflowing solutions, respectively. The assumptions in these calculations are that the backflow of Ca^{45} from plasma to lumen is small, and that the mean specific activity of calcium in the intestine is approximated by the average of the specific activities in the inflowing and outflowing fluids. Since the incoming and outflowing specific activities differed by only 20 percent, it was estimated that the error in using the arithmetic mean instead of the geometric mean would be small.

The essential data on the unidirectional fluxes and net transfer of calcium across the rat duodenum are presented in Table 1. First, it may be noted that the efflux and net flux of calcium increased with increasing concentration of calcium ion in the lumen; however, the influx rates were not significantly altered although there may have been a depression at the highest level of calcium (1.97 mmole). Especial note should be given to the observation that there was a net efflux of calcium at even the lowest calcium concentration. The net flux of water also was unaffected by calcium concentration or the length of time that the animal was under study. Although the data are not included here, it was further observed that the flux rates of calcium were not correlated with the net movement of sodium from lumen to plasma or with the net movement of potassium from plasma to lumen.

Total plasma calcium was about 2.6 ± 0.5 mmole (mean ± standard error of the mean) and within the normal range for the rat. The ionic calcium in plasma, as estimated from the nomograph of McLean and Urist (*7*) relating total plasma calcium, total plasma protein, and ionic plasma calcium, was found to be about 1.3 mmole, or 50 percent of the total. The potential gradient from lumen to plasma averaged about −8.1 mv; with these values and the concentration of ionic calcium in the lumen, the theoretical flux ratio was calculated from the diffusion equation of Ussing (*8*), as given in the footnote of Table 1. If the transfer of calcium from lumen to plasma and from plasma to lumen was due only to passive physicochemical forces, the observed ratio would be approximately the same as the theoretical ratio. From Table 1, it may be seen, however, that the observed flux ratio exceeded the theoretical ratio at all levels of calcium concentration in the inflowing solution. According to the usual definitions, this would constitute evidence for the active transport of calcium in the intact animal and, therefore, substantiates observations made in vitro. The contribution of this process to the total absorption of calcium by the intestine, and its importance in calcium homeostasis, is unknown at present and remains to be assessed.

R. H. Wasserman
F. A. Kallfelz
C. L. Comar

Department of Physical Biology, New York State Veterinary College, Cornell University, Ithaca

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Olfactory Bulb Response of Rabbit

Abstract. An approach to understanding the properties of dendrites is to record the response of the olfactory bulb where the dendrites of mitral cells form the glomeruli. After the stimulations of the bulb and nasal mucosa, the responses appear different, but they are fundamentally composed of three successive potentials, suggesting that the last one is the action potential of glomerular dendrites.

It has been suggested that brain waves are due to the synchronization of dendritic potentials lasting 10 to 20 msec (*1*). Therefore, we studied the direct cortical response of the olfactory bulb because of its relatively simple cortical lamination. In a preliminary report (*2*) we showed that the bulb response induced by direct stimulation is composed of two successive potentials, referred to as the spike and slow potential. It seems probable that the spike potential is assigned to an action potential in olfactory nerves, the slow potential to the activity of glomerular dendrites.

Recently, Ottoson (*3*) reported that the olfactory bulb response, lasting about 150 msec, is induced by electrical stimulation of the nasal mucosa of frog and is composed of two waves, P_1 and P_2. It is thought that P_1 is a synaptic potential arising in the glomeruli, while P_2 is due to the propagated activity in secondary neurons. The present report is confined to the analysis of the potential form that can be induced by direct stimulation of the olfactory bulb and nasal mucosa.

Rabbits were anesthetized with urethan (1 g/kg), and the olfactory bulb was exposed by removal of the overlying bone and dura. Stimulation (with a 0.03- to 0.1-msec pulse) was applied bipolarly to the bulb or nasal mucosa through the hole made in the ethmoid bone. Surface recording was made from a monopolar silver-silver chloride electrode with a tip 0.1 mm in diameter. The temperature around the subject was kept at 28° to 30°C with a heating device. All recordings were made with a resistance-capacitance coupled amplifier of time constant 0.03 second. Figure 1 (*A* and *B*) demonstrates wave forms of the response recorded 1 to 2 mm from the site of stimulation, and the graded nature with increasing stimulus strength.

When it is fully developed, the response is composed of two potentials, a spike and a succeeding slow potential. The amplitude of the spike potential increases almost linearly without any detectable steps when the stimulus strength is increased. However, the slow

potential is linearly graded in the lower range of stimulus strength and reaches a plateau at about 30 volts. It can be said that these potentials behave independently of the stimulus strength. The spike reaches a summit in about 3 msec and lasts approximately 3 to 5 msec; maximum amplitude ranges from 3 to 5 mv. The slow potential shows a slow time course which lasts about 15 msec.

Sometimes the response shows different patterns owing to the ratio of amplitude between spike and slow potential. Figure 1*B* illustrates such a potential variation in which the slow potential is depressed, leaving the spike fully developed. Although the rising phase of the spike is usually rapid, in this case an inflection on the rising phase of the spike can be recorded by a fast sweep. From the experiment of twin shocks it is shown to give a summating effect, and possibly this is a sign of postsynaptic potential.

Figure 1*C* shows the record under the deteriorated state of the superficial cortex. The spike in isolation does not develop at maximal stimulation; the entire response appears as a monophasic potential. The pattern in this experiment is interpreted as due to damage and bleeding of the superficial plexuses of the olfactory nerve. Thus, it is supposed that the slow potential can be ascribed to the directly induced action potential in the secondary neuron, presumably dendrites of glomeruli, not to the transmitted activity.

As the stimulating electrode is placed on the nasal mucosa, the response induced on the bulb appears after a definite latency and has a considerably different pattern (Fig. 1*D*). The configuration and duration of the second negativity are practically the same as those of the slow potential observed at direct stimulation of the bulb. However, the initial part of the response (initial negativity) characteristically differs from the spike at bulb stimulation; its duration is about 10 to 20 msec, longer than that of the spike. In most cases the spike appears as a rising phase of the initial negativity. Therefore, it is very difficult to record the spike clearly, even by increasing the stimulus strength. This is apparently due to the asynchronous volley entering the bulb with a longer traveling distance.

It is important to determine whether the nature of such a potential is comparable to the earliest inflection and the spike observed above. To test this possibility, twin shocks at maximal strength were applied to the nasal mucosa. An interesting phenomenon observed was that the initial negativity summated when the interval was 6 to 25 msec. The main fraction of initial negativity seems to correspond with the inflection observed in Fig. 1*B*. On the contrary, the absolute refractory period measured 5 msec in the second negativity and was followed by a gradual recovery. However, in this recording the refractoriness of spike was not obvious, for the absolute refractory period is only half that of the slow potential.

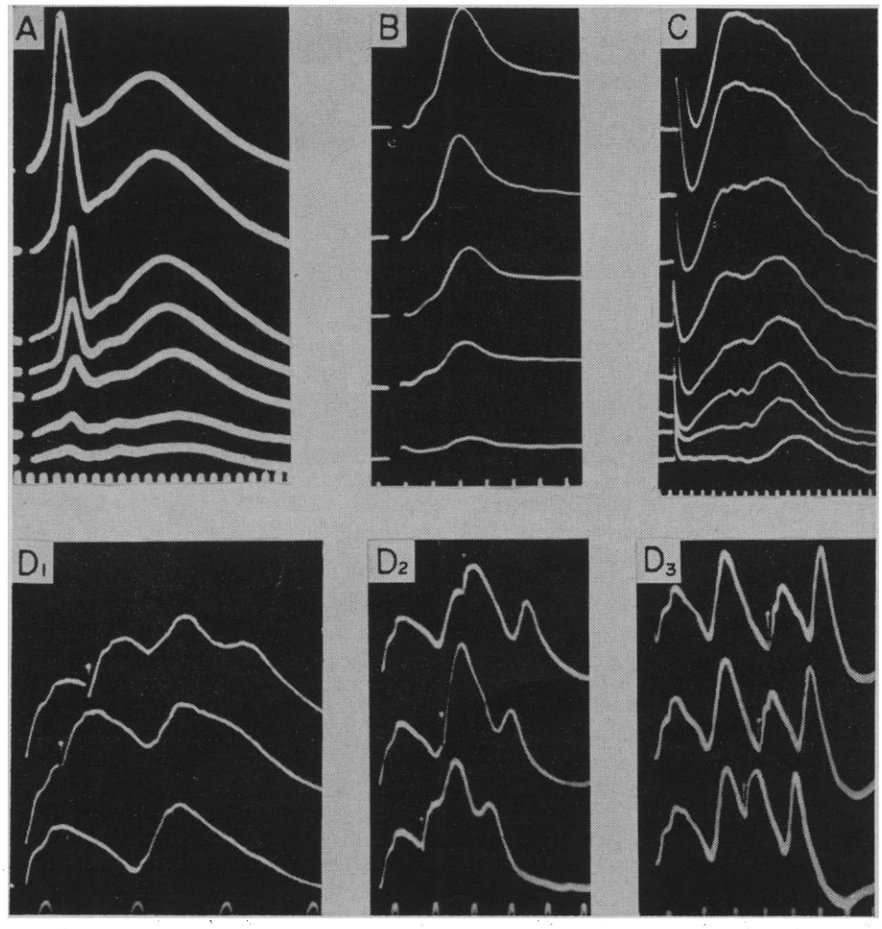

Fig. 1. Olfactory bulb response induced by direct stimulation of olfactory bulb (*A, B, C*) and nasal mucosa (*D*). In all series successive records are read from below upward. Potential was recorded monopolarly with negativity upward. *A* and *B*, Records in normal cortex; *C*, record in deteriorated cortex. Effect of increasing stimulus strength from 8 to 40 volts. Time in milliseconds. D_1, D_2, and D_3, Continuous records of twin shocks demonstrating the summation and subsequent gradual recovery. Lowest record in D_1: single response. Time interval of twin shocks: 6 to 11 msec in D_1, 16 to 32 msec in D_2, and 40 to 50 msec in D_3. Time mark, 60 cy/sec.

The summating potential and the slow one obtained in the present work are in striking accordance with the P_1 and P_2 in the frog reported by Ottoson. If we consider it from the anatomical point of view, it will be supposed that the spike originates from the superficial plexuses of the olfactory nerve, whereas the summating and slow potentials are due to the activity of the secondary neuron. Usually the summating potential appears in a depressed or masked form in the stimulation of the olfactory bulb. On the other hand, in the stimulation of the nasal mucosa, it is very difficult to record the spike in isolation because of the asynchronous arrival of nervous impulses to the bulb. It is concluded that the response of the olfactory bulb is built up of three potentials, that is, summating, spike, and slow. Most likely, these potentials change in amplitude and form according to the stimulating sites, the stimulus strength, and the cortical excitability, but may be distinguished from one another with twin shocks.

YOSHIHIKO IWASE
MASANOBU URUHA
Department of Physiology, Kyoto Prefectural University of Medicine, Kyoto, Japan

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