Response Patterns of Olfactory Bulb Neurons to Stimulation of Distilled Water and Odorous Solutions

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Summary Responses of 50 olfactory bulb neurons of the bullfrog to odorous solutions of ethyl *n*-butyrate were composed mainly of facilitation, inhibition and no response, whereas those of the same neurons to water were mainly of facilitation and delayed facilitation. The responses to water tended to continue longer than those to the odorous solutions.

In a preceding paper (ARITO *et al.*, 1978), the water response of the frog olfactory epithelium was studied by recording discharges of the olfactory bulb neurons, and its underlying mechanism was discussed with reference to the water response of the gustatory organs. The present study was undertaken to clarify probable differences between the responses to distilled water and those to odorous solutions in the olfactory bulb neurons.

Bullfrogs (*Rana catesbiana*) were used. The surgical procedure and technique for recording the spike potentials of olfactory bulb neurons were the same as previously described (ARITO *et al.*, 1978). Three stimulants were applied: distilled water prepared by distillation of deionized water (100 μ l); and 1.5 mM ethyl *n*-butyrate (ENB) and 15 mM ENB, both of the reagent grade, dissolved in isotonic NaCl solutions (50 μ l each). When recording of stable neuronal activity was successful in the olfactory bulb, the three stimulants were dripped one after another on the olfactory epithelium of the recording side. One minute before the application of a stimulant, 100 μ l of an isotonic (116 mM) NaCl solution was applied as an adapting solution (ARITO *et al.*, 1978). This adapting solution was also effective in removing any possible remaining stimulant applied previously. It has been shown already that dripping itself does not elicit any response at all (TAKAGI *et al.*, 1978). These experimental procedures were carried out every 2 or 3 min: and the response types of 50 olfactory bulb neurons were examined. The numbers of the spontaneously

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Fig. 1. Response types of 50 olfactory bulb neurons elicited by 1.5 mM and 15 mM ENB solutions and distilled water (D.W.). The response types are indicated by six differentially-shaded blocks below the matrix. The horizontal column indicates the number of the olfactory bulb neurons examined.

occurring spikes were counted every 5 sec bin for 20 sec before stimulation, and they then were averaged. Next, this mean number was compared with the spike numbers counted during the poststimulation period of every 5 sec. By these countings the responses were classified into the following six categories: (1) facilitatory response (an increase in the number of spikes), (2) inhibitory response (a decrease in the number of spikes), (3) no response (no significant change in the number of spikes), (4) facilitation followed by inhibition, (5) inhibition followed by facilitation, and (6) delayed facilitation (an increase in the number of spikes which occurred later than 5 sec after the onset of stimulation).

All the responses are summarized in Fig. 1. When 1.5 mM ENB solution was applied, the response types of the 50 neurons were composed of facilitation in 36%, inhibition in 34%, no response in 26%, facilitation followed by inhibition in 2% and inhibition followed by facilitation in 2%. When 15 mM ENB solution was applied, however, the response types of the same neurons were composed of facilitation in 40%, inhibition in 40%, no response in 16%, facilitation followed by inhibition in 2% and inhibition followed by facilitation in 2%. When distilled water was applied, the response types of the same neurons were composed of facilitation in 42%, delayed facilitation in 34%, inhibition followed by facilitation in 12%, inhibition in 6%, facilitation followed by inhibition in 4% and no response in 2%. Some characteristic differences were found between the water and odorous responses: Firstly, the delayed facilitatory response was observed only in the water response. Secondly, the inhibitory

Fig. 2. Histograms for response durations of 50 olfactory bulb neurons to 1.5 mM ENB (A) and 15 mM ENB solutions (B) and distilled water (C). The response types are shown by the same six kinds of blocks as in Fig. 1. The ordinate of each histogram indicates the number of olfactory bulb neurons.

or no response type was observed more frequently in odorous stimulation than in water stimulation. Our results concerning the occurrence rates of each response type to the odorous solutions were not significantly different from the results obtained by other investigators who applied odorous gases of different kinds (DÖVING, 1966; HIGASHINO *et al.*, 1969; MATHEWS, 1972; KAUER, 1974). Therefore, as IINO and TAKAGI (1978) suggested, there may be no essential difference between the responses to odors in a gaseous phase and in an aqueous phase.

In the present experiment, stimulation with water caused the facilitatory and delayed facilitatory responses in 76% of the 50 neurons and no response in 2%, whereas in the previous experiment (ARITO *et al.*, 1978) 53% of the 45 olfactory bulb neurons showed facilitation, including delayed facilitation,

and no response in 33%. This discrepancy between the two studies can be explained from our hypothesis on the generation of the water response (ARITO *et al.*, 1978): The volume of distilled water applied was 50 μ l in the previous study and 100 μ l in the present study. This increased volume of water is considered to be more effective in removing the adsorbed cations from the surface of the olfactory epithelium and thus results in an increase of the number of olfactory bulb neurons responding in the facilitatory way and a decrease of the number of neurons showing no response.

Durations of the responses of olfactory bulb neurons to distilled water, 1.5 mM and 15 mM ENB solutions were examined in the same 50 neurons. The histograms A, B and C shown in Fig. 2 indicate relations between the response durations and the response types. The neurons which showed a facilitatory response of less than 5 sec to the ENB solutions were most numerous, and the neurons which showed longer facilitatory responses generally decreased with increasing durations. The inhibitory responses elicited by the ENB solutions showed a tendency to continue longer than the facilitatory ones. In contrast, distilled water elicited responses with durations longer than 60 sec most frequently. With respect to the sustained nature, the water response in the olfactory epithelium is similar to the one in the frog tongue (KOKETSU, 1951; AKAIKE and SATO, 1976). The water response was depressed immediately by the application of an isotonic NaCl solution on the olfactory epithelium (ARITO *et al.*, 1978), while it was reported that rich sodium ions are secreted from Bowman's glands (Ref. (7) of MOULTON, 1976). Consequently, it is considered that the water response continues until sodium ions secreted from Bowman's glands increase to a certain concentration on the surface of the olfactory epithelium. Thus, it may be said that the sustained nature of the water response of the olfactory bulb neurons reflects a long-lasting activity of the olfactory cell. However, KAUER and SHEPHERD (1977) correlated temporal patterns of the responses to odors of the salamander olfactory bulb neurons with some basic properties of synaptic circuits in the bulb. Consequently, there remains a possibility that the long duration of the water response and the short duration of the odorous response may be due to the activity of the synaptic circuits in the bulb. In order to clarify this problem, it is necessary to record the responses to distilled water and to odorous solutions directly from the olfactory cell.

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