Changes in Cytochrome Oxidase Activity of the Rabbit Olfactory Cortex Following Unilateral Olfactory Bulbectomy

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Summary The effects of unilateral olfactory bulbectomy on cytochrome oxidase (CO) activity in the olfactory cortex were studied in adult rabbits. In intact rabbits, layer Ia of the olfactory cortex stained symmetrically for CO activity as a continuous brown band. In animals with unilateral lesion of the olfactory bulb (OB), a great decrease in the level of CO activity was found in layer Ia ipsilaterally. The change became prominent about 12 hr after OB lesion. Thereafter no further decrease in CO activity was observed up to 6 months. A decrease in the level of CO activity was observed in only the denervated lamina to which the OB neurons project directly, but not along the synaptic chain in the olfactory cortex.

Key Words: cytochrome oxidase (CO) activity, unilateral bulbectomy, olfactory cortex.

Ablation of the olfactory bulb has been known as a simple manipulation for deafferentiation to the olfactory cortex. To display effects on deafferented areas visually, a histochemical technique, staining for cytochrome oxidase (CO) activity, has been developed. In fact, recent studies using this technique demonstrated a decrease in CO activity at several synaptic stations in the visual (Wong-Riley, 1979) and auditory system (Wong-Riley et al., 1978) consequent to experimental deafferentation. However, there have been no such histochemical studies on the olfactory system. The objective of this work is to examine CO activity in the olfactory cortex of the intact rabbit and effects of unilateral olfactory bulbectomy on staining patterns of CO activity. Some of this work has been reported in abstract form (Onoda and Imamura, 1983).

The right olfactory bulb (OB) in seven adult albino rabbits was removed by suction under Na-pentobarbital anesthesia. Two rabbits were kept intact as
controls. At various post-lesion intervals, from 1 hr to 6 months, animals were deeply anesthetized with Na-pentobarbital, and glutaraldehyde-paraformaldehyde in 0.1 M PO₄ buffer (pH 7.4) was perfused transcardially. Prior to the perfusion, animals underwent tracheal cannulation to prevent stimulation of the olfactory mucosa by perfusion odors. At 1 mm intervals, three series of sequential 100 \( \mu \)m frontal sections from the brain were collected. The histochemical procedure was that of WONG-RILEY (1979). All sections were incubated in a buffered medium of 3,3'-diaminobenzidine and cytochrome c (Sigma, Type III). The first sections in each sequence were counterstained with thionin to observe anatomical outlines. The second ones were filmed under a microscope for densitometric analysis. The third ones were preincubated in buffer with

**Fig. 1.** Cytochrome oxidase (CO) activity of the olfactory cortex in the intact rabbit (A, B) and effect of unilateral olfactory bulbectomy on CO activity (C, D). A, C: Microphotographs of frontal sections through the anterior olfactory nucleus (AON) from the intact rabbit (A) and the rabbit with the post-lesion interval of 24 hr (C). Sections A and C were cut at the plane indicated by the lines in the inset figures. The lesioned OB is shown in black (C). Lines (1-12) vertical to the lateral cortical surface indicate scan runnings. rh.s., rhinal sulcus. B, D: Data obtained from densitometric analysis. Each record, corresponding to the scan number in A and C, shows changes in the relative amplitude of CO activity. Horizontal arrows indicate a scanning direction, which was run from the lateral surface to a deep portion of the AON. Dotted vertical lines show a boundary between layers Ia and Ib. Open triangles indicate the lateral surface of the lateral olfactory tract (LOT). Filled triangles give a boundary between the LOT and layer Ia.
0.1 mM KCN, an inhibitor of CO, for histochemical controls.

The relative levels of CO activity were analyzed by a reflectance densitometer (Joyce Loebl, UK). A filtered light spot (530 nm) through a small aperture (1 x 1 mm) was scanned on enlarged color prints. Thus several linear scans were made through the depth of the cortex.

A frontal section processed by histochemical procedures (2nd series) in the intact animal is shown in Fig. 1A. It can be seen that layer Ia of the anterior olfactory nucleus was intensely stained. The densitometric measurement (Fig. 1B) indicates that relative reflecting amplitudes of CO activity have the highest peak in layer Ia of every scanning run, and that the lateral olfactory tract (LOT) is less reactive. The degree of staining for CO activity in layer Ia was bilaterally symmetric: 0.91±0.05 (S.D.) (n=6) on the left and 0.93±0.06 (S.D.) (n=6) on the right. In caudal sections, the intensely stained band was also in the most superficial layer (Ia) of the pyriform cortex (PC) and the olfactory tubercle. Sections (3rd series), preincubated with KCN, were nerve stained for CO activity. In contrast to the intact side of experimental animals, layer Ia of the lesioned side was lightly stained for CO activity (Fig. 1C). From densitometric analysis (Fig. 1D), relative reflecting amplitudes of CO activity in layer Ia were 0.91±0.04 (S.D.) (n=6) in the intact side and 0.67±0.07 (S.D.) (n=6) in the lesioned side. The difference was statistically significant (t-test, p<0.0001). A similar decrease in the level of CO activity was observed in layer Ia throughout the rhinencephalon. On the other hand, the deep portion in the intact side was 0.43±0.12 (S.D.) (n=6)

![Graph](image-url)

Fig. 2. Changes in LOT-evoked field potentials in the rabbit olfactory cortex after unilateral olfactory bulbectomy. Amplitudes of initial-positive components in the intact side (open circles) and in the lesioned side (filled circles) are plotted against post-lesion intervals. Zero day means immediately after OB-lesion, and 1 day 24 hr after OB-lesion.
and that in the affected side was 0.39±0.05 (S.D.) (n=6). Thus, it was concluded that no changes occurred in subsequent synaptic stations (e.g. layer III).

Until 4 hr after OB lesion the difference of CO reactivity between lesioned and intact sides was not clear, while a slight decrease in the level of CO activity was discernible in the lesioned side 6 hr after OB lesion. After 12 hr the level of CO activity reached its minimum. As long as we checked animals with 6-month post-lesion intervals, neither further decrease in CO activity in layer Ia, recovery of the decrease, nor the complementary increase in CO activity in deep layers was found in adult rabbits.

In two cases with unilateral OB-lesion, evoked potentials of the PC in response to hourly electrical stimulation of the LOT were chronically recorded in both sides (Fig. 2). A marked decrease in an early positive component in LOT-evoked potentials in the affected side began 36 hr after the OB lesion. The potentials went on decreasing and disappeared after 80 hr. Thus changes in oxidative enzyme activity preceded electrical changes in the olfactory cortex, suggesting that a capacity of synaptic transmission is maintained longer than CO activity.

The superficial layer of the olfactory cortex has been distinguished by two major fiber systems which have different laminar patterns of termination (HEIMER, 1968; WESTRUM, 1969; PRICE, 1973; SCALIA and WINANS, 1975; HABERLY and PRICE, 1978; HABERLY and BEHAN, 1983). The OB fibers end in layer Ia, whereas layer Ib receives association fibers (which arise in the other parts of the olfactory cortex on the ipsilateral side) or commissural fibers (which arise in the contralateral olfactory structures). The present histochemical study reveals that in intact animals the degree of CO activity in layer Ia is the highest in the olfactory cortex (Fig. 1B). Electron microscopic study has demonstrated that CO reaction products are in mitochondria (WONG-RILEY, 1976). It is suggested, therefore, that layer Ia is mitochondria-rich or oxidative metabolism in layer Ia is comparatively high. Next, our results also show that following OB lesion a decrease in CO activity is restricted to the ipsilateral layer Ia (Fig. 1D). Thus, in adult animals the laminar pattern of termination of sensory afferents could not be complemented by that of the layer Ib system.

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REFERENCES


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