

Unilateral Naris Occlusion and the Rat Accessory Olfactory Bulb

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Abstract

Blocking airflow through half of the nasal cavity during early life results in a 25% reduction in the size of the ipsilateral main olfactory bulb. The present study indicates that the size of the accessory bulb is relatively unaffected by the procedure.

Introduction

A large effort has been made to understand the role afferent activity plays in the development of sensory systems. One common approach to studying the issue has been to reduce the amount of sensory stimulation available during early life and then to assess the development of various central processing stations. In the olfactory system, the procedure most often employed has been to reduce odorant access to the sensory epithelia by blocking airflow through one half of the nasal cavity (Philpot *et al.*, 1997). The results of the manipulation are striking, and include macroscopic reductions in the size of the ipsilateral olfactory bulb. Considerable effort has been expended in examining the process through which these changes emerge (e.g. Brunjes, 1994). One reason why experimental bulbs are smaller than contralateral controls is that regions of the main olfactory bulb (MOB; e.g. the glomerular, external plexiform and granule cell layers) are reduced in size by >25% due at least in part to selective cell loss.

The olfactory bulb houses not only the MOB but also the accessory olfactory bulb (AOB). In rodents, the AOB gets input from the tube-like vomeronasal organ (VNO) found ventromedially in the anterior nasal cavity. Much has been written describing the anatomical and functional differences between the main and accessory pathways (e.g. Wysocki, 1979; Halpern, 1987; Meredith, 1991; Segovia and Guillamón, 1993). One common theme is that the VNO/AOB system serves to recognize higher molecular weight compounds that are unlikely to be airborne, and/or that it is involved in chemoreception subserving species typical tasks such as reproduction. The present study was designed to determine if the AOB, like the MOB, is affected by unilateral naris occlusion. The vomeronasal organ does receive stimulation from chemicals entering through the external naris (e.g. Coppola and Millar, 1994), and so blockade of this route may affect its development.

Materials and methods

Tissue gathered by Brunjes and Borror (1983) was used. The four rats underwent unilateral (right) naris occlusion on postnatal day 1 (the day after the day of birth) and were killed 30 days later. This age group was chosen as it has repeatedly been demonstrated (including through the use of this tissue) that during this time period the MOB is extremely susceptible to the effects of unilateral naris occlusion (Brunjes, 1994; Cummings *et al.*, 1997). The rats were perfused with aldehydes, the brains cryoprotected and complete, serial sets of 30- μ m-thick frozen sections were produced and stained with thionin. The procedure yielded ~40 sections/AOB/bulb. The volumes of three laminae within the AOB were determined: the glomerular (GLM: defined as the region containing spherical shaped regions of neuropil), the combined external plexiform/'mitral' cell layer (E/M: the area extending from the deep border of the glomeruli to the much lighter internal plexiform zone) and the granule cell (GCL: a cell-packed zone containing strands of profiles oriented roughly parallel to the internal plexiform layer; Figure 1) layers. The vomeronasal nerve layer (a region populated almost solely by small glial profiles) was not measured due to difficulties in defining its medial boundary, nor was the internal plexiform layer as it contains axons from MOB mitral cell (Macrides *et al.*, 1985), and thus its measure would not reflect changes peculiar to the AOB.

The methodology employed (serial section planimetry) has been extensively described elsewhere (e.g. Brunjes and Borror, 1983; Cummings *et al.*, 1997). Briefly, sections were viewed at low power through a microscope and the area of each lamina measured using an image analysis program (MCID4, Imaging Research, St Catharines, Ontario). Each projected lamina was measured twice and mean areas were calculated to increase the accuracy of the measurement. To determine the proper sampling ratio, every section through one AOB was measured, the laminar areas were multiplied

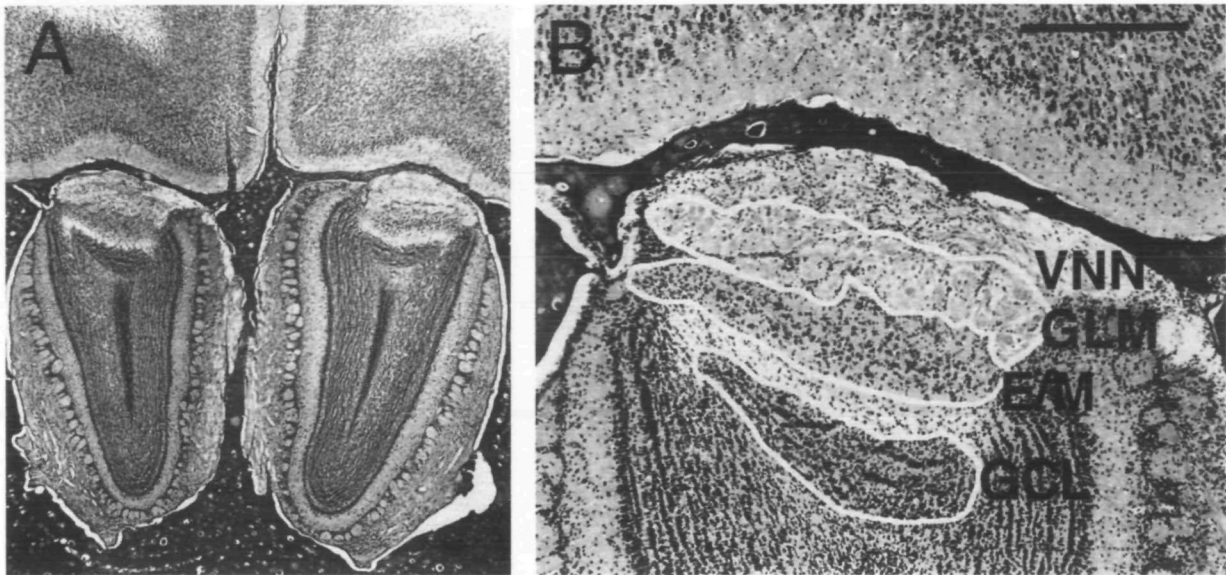


Figure 1 (A) Low power photomicrograph of a frozen horizontal section through a 30-day-old rat forebrain embedded in egg yolk. The bulb on the left side of the figure was ipsilateral to the occluded naris. Note that, while the overall size of the left bulb is smaller, the AOBs are roughly comparable on the two sides. (B) Higher power view of the AOB from the left bulb showing laminar boundaries used in quantification. VNN, vomeronasal nerve; GLM, glomerular layer; E/M, combined external plexiform/‘mitral’ cell layer; GCL, granule cell zone. The scale bar, located in frontal neocortex, represents 0.5 mm.

by the section thickness to convert them to volumes and the values were added to determine the ‘true’ volume of the AOB. The data set was then analyzed to determine the consequences of only measuring every second, third, fourth, etc. section on the calculated total volume. It was determined that every fifth section could be measured and the results would still stay within 5% of the ‘true’ volume; thus this ratio was employed in the remainder of the study. In order to calculate laminar volumes, the average volume for adjacent pairs of measured sections (sections 1 and 6, 6 and 11, 11 and 16, etc.) was calculated. These values were multiplied by the number of intervening sections, and the total volume determined by summing the averages. The percent difference in laminar volume was computed using the formula $[(L - R)/L \times 100]$, where L and R are the volumes of the left and right bulbs respectively.

Results

Only slight and variable differences were found between left and right AOBs in occluded animals (Table 1). Expressed as percentages, measures of both the GLM and GCL were <3% different between sides, while the E/M was ~16% larger on the experimental side when compared with the contralateral control. However, Mann–Whitney tests revealed no significant differences between the left and right sides in any of the three measured layers.

Discussion

The results are consistent with Najbauer and Leon’s (1985) observation that there were no differences in AOB GCL size in animals occluded on P1 and examined of P15, and also

	Mean volumes ($\mu\text{m}^3, \times 10^7$) of AOB laminae (with SEMs)	
	Experimental	Control
Glomerular layer	14.3 (1.45)	14.7 (2.36)
External plexiform/mitral	31.9 (2.65)	27.5 (3.10)
Granule cell	25.0 (1.05)	24.6 (1.76)

with observations that, although the system is functional at birth (e.g. Pedersen *et al.*, 1985), the vomeronasal organ is very immature (Coppola *et al.*, 1993). Nevertheless, the AOB is highly responsive to changes in environmental input. For example, much work has examined learning-like changes in the AOB necessary for the pregnancy block phenomenon (e.g. Brennan and Keverne, 1997). As a second example, 40-day-old male rats reared in either social or isolated conditions develop differences in the synaptic organization of the granule cell layer (Ichikawa *et al.*, 1993). How can our findings of a lack of change be reconciled with these demonstrations of considerable malleability? One difference is that the recorded plasticity seems to be mostly in animals near sexual maturity, rather than the pups observed in the present study. Perhaps, as the reviews cited above suggest, the VNO–AOB system is most adept at processing signals dealing with reproduction, and since these odors are less important during the pre-weanling period, the system has not yet become capable of large-scale synaptic plasticity. Second, since the VNO can receive odor information from several routes, including from the oral cavity via the nasoincisor duct, perhaps naris closure has

relatively less effect on it than the olfactory epithelium, which is more reliant on airborne stimulation. However, as Devor and Murphy (1973) demonstrated that hamster mating behavior can be eliminated by blocking the external naris, it is likely that blocking airflow through this route does affect vomeronasal function. Finally, the present study examined only extremely gross morphological changes; it is possible that naris closure has a more subtle effect on the AOB. Nevertheless, the present study demonstrates that, in tissue where the size of the MOB is reduced by nearly 25%, the AOB is relatively unaffected.

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