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Postnatal Development of the Rat Vomeronasal Organ

Elke Weiler

Department of Neurobiology & Physiology, Northwestern University, 2205 Tech Drive, Evanston, IL 60208-3520, USA
Present address: Department of Neurophysiology, Ruhr-University, Universitaetsstr. 150, 44801 Bochum, Germany

Correspondence to be sent to: Elke Weiler, e-mail: weiler@neurop.ruhr-uni-bochum.de

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Introduction

The vomeronasal organ (VNO), also know as Jacobson's organ, is a paired chemosensory organ mediating the perception of chemical stimuli related to social and/or reproductive behavior (for review, see Farbman, 1992). In rodents, it is an elongated tube, with a crescent-shaped sensory epithelium (SE) consisting of basal cells, bipolar neurons and supporting cells. Proliferation continues throughout life (Weiler *et al.*, 1999), so the question arose how this influences the VNO structure. We investigated the VNO with respect to size, histological structure and composition, location within the nasal cavity and proliferative parameters, as well as cell death, from birth to senescence, in both sexes of the rat.

Materials and methods

Male and female Sprague–Dawley rats, postnatal day 1 (P1) to P666, were injected with BrdU. Two hours later they were anaesthetized and perfusion-fixed as approved by Northwestern University Animal Care and Use Committee. Heads were decalcified, embedded in Paraplast®, and 10 µm sections processed immunohistochemically against (i) BrdU, to reveal proliferating cells, and (ii) OMP (olfactory marker protein), to reveal mature olfactory sensory neurons (Farbman and Margolis, 1980). Adjacent sections were stained with hematoxylin and eosin for light microscopic evaluation and tabulation of apoptotic cells.

Results

Morphological data

Location

The rat VNO is located throughout life on the vomer, on the ventral end of the nasal septum. Its position in relation to other olfactory organs, however, changes. In newborns the rostral end is posterior to the anterior end of the main olfactory epithelium (OE) and the caudal end extends to the olfactory bulb, whereas in adults, the anterior end is anterior the rostral extent of OE and posteriorly ends before the anterior end of the septal organ and the nasopharyngeal duct.

Structure

In all age groups, the VNO maintains its crescent shape with its trilaminar SE. Capillaries frequently intrude into the epithelium pushing up to the height of the apical row of the spindle shaped supporting cell nuclei.

Twisting of the VNO

Anteriorly, the VNO-SE lies adjacent to the nasal septum, whereas at the posterior end the tube twists so that SE lies ventrally (Figure 1).

Size of the VNO

Length: The rat grows until senescence and so does the length of the VNO, increasing in males from 1.7 mm at birth to 7.3 mm at P450. Females have slightly shorter VNOs due to their smaller body size. *Epithelium thickness:* The SE-thickness changes from anterior to posterior with an interim plateau before reaching a maximum where the tube twists. During development, thickness reachs a maximum around P40, decreasing afterwards to adult levels of ~120 µm. *Area:* The area increases from anterior to a maximum in the posterior half. During development, the area reachs maximal values around sexual maturity (~P66), when the thickness does not increase, so the area increase reflects an increase in the perimeter of the tube. *Volume:* The calculated volume of the VNO-SE increases in males between birth (0.08 mm^3) and P66 (0.70 mm^3), then declines to adult levels (0.65 mm^3).

Proliferation in the VNO

Proliferation was measured by the occurrence of BrdU-labeled cells in the VNO-SE. Proliferating cells were counted in four equal 'quad-

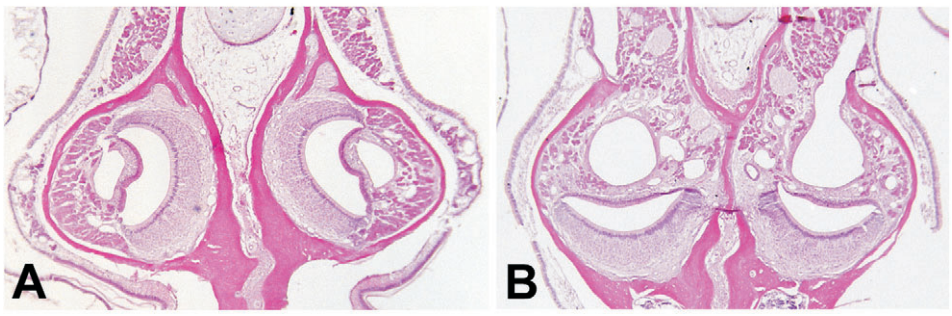

Figure 1 The sensory epithelium of the VNO twists from anterior **(A)** to posterior **(B)** from a medial to a ventral position, allowing the blood vessels and nerves to project dorsally, especially where the nasal wall retracts from the hard palate and the nasopharyngeal duct emerges.

rants' of a cross section [the two margins, the boundaries to the non-sensory epithelium (NE) and the two central regions], as well as in the different height within the epithelium, representing compartments of basal cells, neurons and supporting cells.

Proliferation density

Proliferation density dramatically decreases postnatally from a maximum of 115 cells/section in newborns to 47 cells/section by P11, 27 cells/section by P21 and 12 cells/section by P40, until adult levels are reached around sexual maturity (10 cells/section). No difference in proliferation density between males and females were detected, nor between the two sides of the paired VNO.

Distribution of proliferating cells

Proliferating cells are not distributed evenly. Whereas in newborns all quadrants show approximately the same number of BrdU-labeled cells, in adults most proliferation in the VNO-SE occurs in the basal cell population at the margins adjacent to the NE and at the caudal end, thereby increasing size of the organ (growth). Only a few proliferating cells were found on the basement membrane in the mid-region of the VNO, even adjacent to capillaries intruding into the epithelium. These mid-region proliferating cells are likely the pool for replacing dying neurons.

Height of proliferating cells

The proliferating cells might produce either neurons or supporting cells. We tabulated the proliferating cells according to their height within SE. Apically located labeled cells usually showed a more elongated oval-shaped nucleus, very likely representing supporting cells. The intermediate height of the epithelium where the neurons are located and the basal regions, in contrast, showed more round labeled nuclei, representing the neuronal precursors. Whereas in newborns a high percentage of labeled cells appeared in the apical region, this percentage decreased to just 10% in adults. Accordingly, the percentage of cells in the basal region increased.

Cell death in the VNO

Apoptotic cells, a sign for cell death, occurred predominantly in the marginal regions of the crescent-shaped SE, at the boundaries with NE in adult rat VNO, thus exactly in those regions of high proliferation. This suggests that when growth slowed down, newly generated cells are quickly eliminated, keeping the effective rate of neuronal turnover low and not replacing established neurons.

Discussion

The rat has a very well developed VNO in both sexes. As the rat grows, the VNO increases in size, the result of cell division. In newborns a large proportion of proliferating cells belong to the supporting cells as in OE (Weiler and Farbman, 1998) whereas with age this proportion decreases as the percentage dedicated to the generation of neurons increases. Further, in adults, proliferating cells are found predominantly in the margins of SE. Additionally, most cell death also occurs in this regions of highest proliferation.

Comparison with main olfactory epithelium

In contrast to OE (Weiler and Farbman, 1997), where the area continues to increase in adults, the VNO area shows a maximum around sexual maturity. Thus, the development of the VNO follows a pattern different from the OE, indicating an independent regulation.

Structurally, the VNO is divergent from OE neuronally (Menco, 1997), additionally, blood capillaries intrude into VNO-SE. Along those capillaries, pushing the basement membrane into the epithelium, BrdU-labeled basal cells are found, able to replace dying neurons right at the spot where new neurons are needed.

VNO for both sexes

The rat VNO length increases with increasing body size, independently of the sex. Shorter VNOs in females are related to the smaller body size compared to males, not to the sex itself. The lack of a sex difference applies also to the proliferation density between males and females of the same age.

Two populations of proliferating cells—growth and replacement

Proliferating cells are already concentrated in the margins as early as P21, when the VNO has reached only ~50% of its adult size. Growth thus occurs predominantly at these margins. This is consistent with observations in mice (Barber and Raisman, 1978), in which [^{3}H]thymidine-labeled cells were first seen at these margins, but with increasing time after injection closer to the center, suggesting cells were added by accretion at the margins. This is consistent with our observation of increasing perimeter with age. Thus proliferating cells at the margins represent a 'pool for growth'.

On the other hand, we found BrdU-labeled cells on the basement membrane also of intruding capillaries in the central regions. These cells are situated right at the location where they are needed to replace dying established neurons. They represent a 'pool for replacement'.

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