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[REVIEW]

Neuronal Development, Differentiation, and Plasticity in the Mammalian Vomeronasal System

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ABSTRACT—The vomeronasal system is one of the chemosensory systems. It is believed to play an important role in the processing of pheromonal signals and the expression of reproductive function. The sensory mechanism in the vomeronasal system has not been studied as well as those in the other chemosensory systems such as the main olfactory system and the gustatory system. Recently information on the neuronal development and plasticity in the vomeronasal system has gradually increased. Neuronal development in the rat vomeronasal system continues until about the 4th week after birth. The vomeronasal system exhibits a high degree of plasticity. In this article we shall review neuronal development and plasticity in the mammalian vomeronasal system.

INTRODUCTION

Olfaction is a very important sensory function in mammals. The olfactory system has two completely separate neural pathways in mammals: main olfactory system and vomeronasal (accessory olfactory) system (Wysocki, 1979; Halpern, 1987; Halaz, 1990; Farbman, 1992). The main olfactory system consists of the olfactory organ, the main olfactory bulb, and other central nervous system that receive input fibers from the main olfactory bulb (anterior olfactory nucleus, olfactory tubercle, piriform cortex, entorhinal cortex, etc.) (Fig. 1). The main olfactory system plays an important role in the various behaviors associated with food intake and the detection of enemies' odors, required for survival. The vomeronasal system consists of the vomeronasal organ (VNO), the accessory olfactory bulb (AOB), and other CNS regions that receive input fibers from the AOB (Fig. 1). Male hamsters show a significant preference for nonvolatile high-molecular-weight vaginal secretions and it is postulated that the preference is exhibited only when the male snout has been in contact with the secretion (Singer *et al.*, 1984). Removal of the vomeronasal organ in hamsters results in a loss of responsiveness to the aphrodisiac stimulus of the high-molecular-weight fraction of the vaginal discharge (Clancy *et al.*, 1984). The expression of both c-fos and egr-1 increases in the granule cells of the AOB during mating behavior (Brennan *et al.*, 1992). These reports strongly suggest that the vomeronasal system is involved in mediating the effects of nonvolatile secretions from non-self animals (pheromones). Pheromonal stimuli may alter the course of puberty, modulate estrus cycles of females, elicit courtship, and modulate reproductive and aggressive behaviors (see review by Vandenberg, 1983). The vomeronasal system plays a major role in the perception of stimuli related to social and/or reproductive behavior in many species of mammals. This behavior is very important for conservation of species.

Recently, Dulac and Axel (1995) identified a gene family encoding putative pheromone receptors in mammals. Berghand and Buck (1996) reported that G-proteins and adenylcyclase play major roles in pheromone signaling pathways. The processing mechanism of sensory information in the vomeronasal system may be elucidated in several years. However, the vomeronasal system is thought to be a useful for studying basic neural events (development and plasticity), since the functions of this system have been well characterized. Thus, we studied the morphological characteristics of neuronal development and the plasticity in the vomeronasal systems of rat and hamster. The aim of this article is to review recent studies in this field.

MORPHOLOGY OF VOMERONASAL SYSTEM

The morphological characteristics of the vomeronasal system are described briefly. The VNO is located in the base of the nasal septum (Fig. 1). The organ is composed of paired, elongated, tube-shaped structures, each of which is enclosed in a cartilaginous capsule. Posteriorly the tube ends blindly while anteriorly it opens via a narrow duct into the nasal or oral cavity. The wall of the vomeronasal organ consists of two types of epithelia; the sensory epithelium forms a thick medial

wall and the non-sensory epithelium forms a thin lateral wall (Fig. 2A). The sensory epithelium consists of sensory and supporting cells (Fig. 2B). Sensory and supporting cells extend from the basal surface of the epithelium to the apical surface. Just below the luminal surface of epithelium, the cell bodies of the supporting cells are arranged in a single row. Multiple rows of sensory cell bodies are located deep to the row of supporting cell bodies. Sensory cells have many microtubules and mitochondria at the distal end, whereas supporting cells are characterized by a large amount of free ribosomes and electron-dense cytoplasm. The sensory and supporting cells have a large number of long microvilli on the luminal surface (see Fig. 4C). The diameter of the microvilli is shorter in the sensory cells than in the supporting cells in the rat (Taniguchi and Mochizuki, 1983; Yoshida *et al.*, 1995). Epithelial cells in non-sensory epithelium were characterized by the presence of microvilli, which were distributed densely on the surface of the cells.

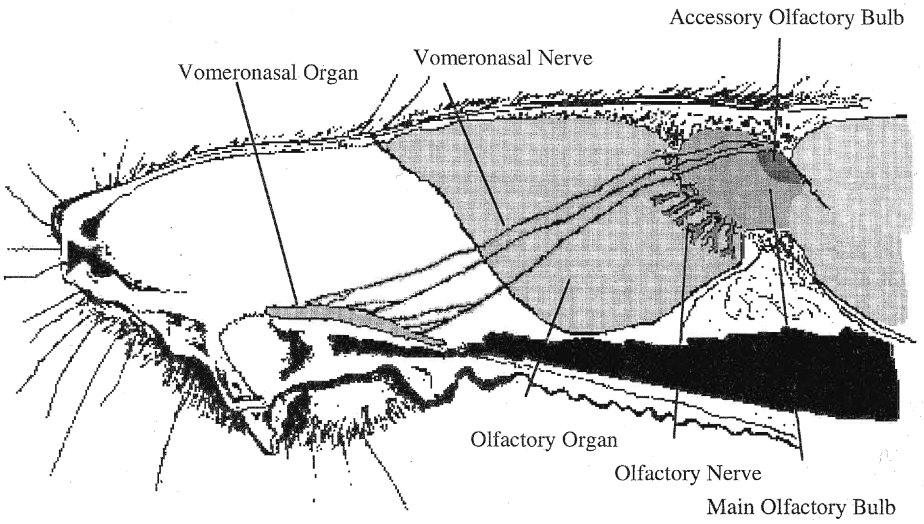

Fig. 1. Diagram of a sagittal representation of two olfactory systems: main olfactory system (olfactory organ, olfactory nerve, and main olfactory bulb) and vomeronasal system (vomeronasal organ, vomeronasal nerve, and accessory olfactory bulb).

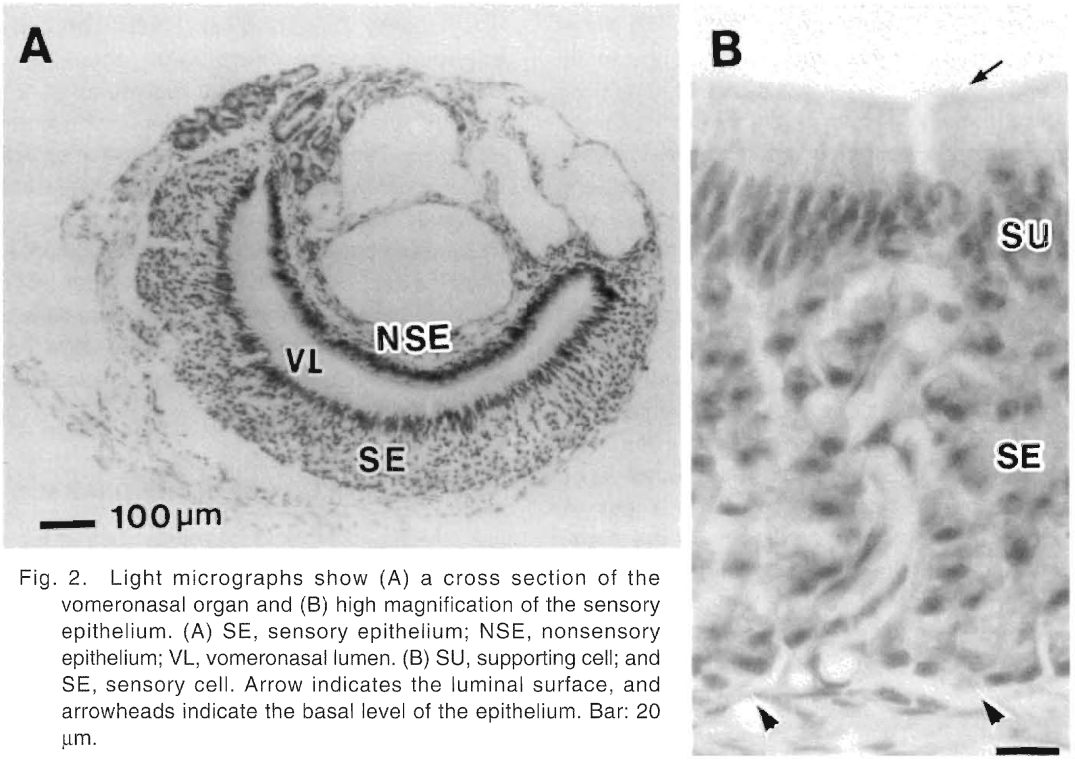

Fig. 2. Light micrographs show (A) a cross section of the vomeronasal organ and (B) high magnification of the sensory epithelium. (A) SE, sensory epithelium; NSE, nonsensory epithelium; VL, vomeronasal lumen. (B) SU, supporting cell; and SE, sensory cell. Arrow indicates the luminal surface, and arrowheads indicate the basal level of the epithelium. Bar: 20 µm.

Sensory neurons in the vomeronasal epithelium are bipolar in structure, detect chemical signals (pheromones) from the external environment and send this information to the AOB. The vomeronasal sensory cells send their axons (vomeronasal nerve) to the AOB (Fig. 3). The rodent AOB consists of five layers: vomeronasal nerve layer, glomerular layer, mitral/tufted (MT) cell layer, olfactory tract layer and granule cell layer. The synaptic circuitry of the AOB is comparatively simple and is similar to that of the main olfactory bulb. The vomeronasal nerves reach the dorsal surface of the AOB to form the vomeronasal nerve layer, and penetrate into a deeper layer in which they form the glomeruli. In this layer, designated as the glomerular layer, the vomeronasal nerves establish synaptic contacts with the dendrites of MT cells whose somata are located in the MT cell layer. The rodent MT cells are quite different from the mitral and tufted cells of the main olfactory bulb in terms of cell architecture (Takami and Graziadei, 1991). At the second level of organization, the main type of microcircuit is the reciprocal dendrodendritic synapses between MT cells and granule cells. Somata of granule cells are located in the granule cell layer. The granule cells receive the collateral axons of MT cells and the centrifugal fibers from other areas of the central nervous system. The MT cells project to the medial region of the amygdala (the bed nucleus of the AOB, the medial amygdaloid nucleus and the posterior corticomedial amygdaloid nucleus). The main projection fibers from these nuclei pass through the stria terminalis and terminate in the bed nucleus of stria terminalis, the preoptic area and the ventromedial nucleus of hypothalamus. Thus, the information perceived by the vomeronasal system regulates the reproductive and aggressive behaviors.

DEVELOPMENT AND DIFFERENTIATION

1) Development of the VNO

The development of the rat VNO has been morphologically studied (Garrosa and Coca, 1991; Garrosa *et al.*, 1986, 1992; Yoshida *et al.*, 1993). The primordium of the VNO is observed first on 11 days of gestation (E11) as a thickening of the ectodermal layer on the mediorostral wall of the olfactory pit. The ectodermal thickening occurs in the area distant from the olfactory epithelium. In the rat fetus, the vomeronasal placode appears one day later than the olfactory placode. The vomeronasal nerves are observed on E13 and immature vomeronasal cartilage cells accumulate around the VNO on E14. On E15, the VNO is clearly distinguished on both sides of the base of the nasal septum under a binocular microscope. Using microscopy, the two different epithelial tissues occupying the lateral and medial walls, can already observed in the organ. Sensory epithelium forms a thick medial wall, and the non-sensory epithelium forms a thin lateral wall. Electron microscopic observation shows that both the sensory and supporting cells are identified in the sensory epithelium. The sensory cells have a few microvilli on the luminal surface and

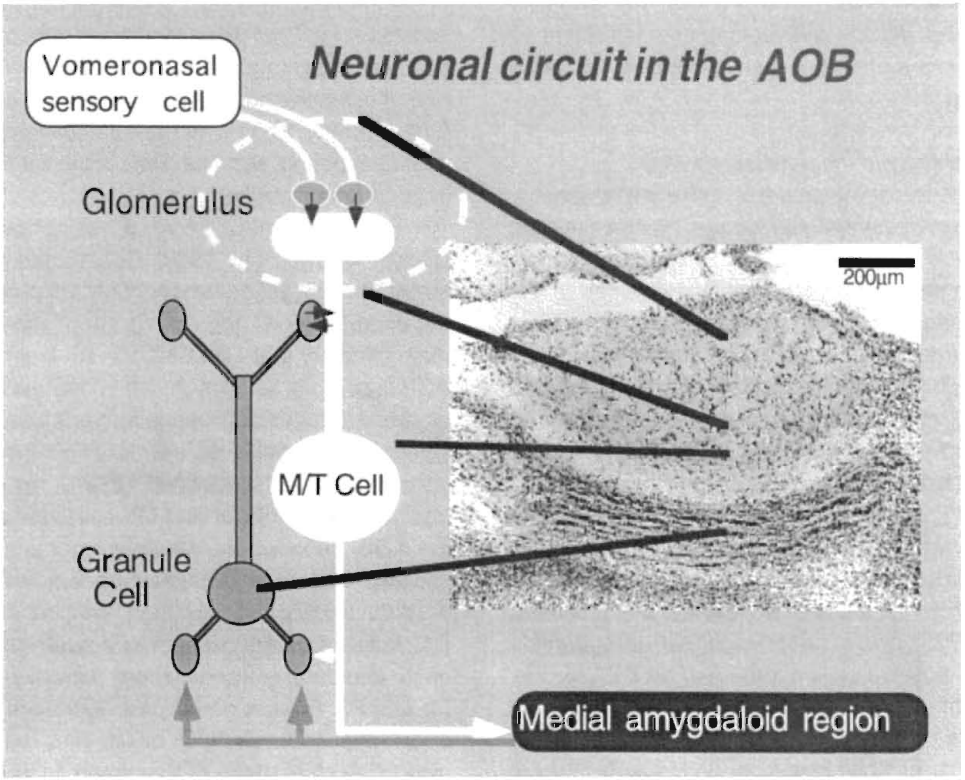

Fig. 3. Diagram of the neural circuit in the AOB. Arrows indicate the synapses and its direction. Photograph shows a sagittal section of AOB. M/T Cell, mitral/tufted cell. (Courtesy of Dr. M. Matsuoka).

contain microtubules and centrioles in the distal processes while the supporting cells have processes which contain many free polyribosomes and mitochondria and a few microvilli on the luminal surface (Fig. 4A). In the VNO of postnatal day (P)3, sensory and non-sensory epithelia are clearly identified, forming thick medial and thin lateral walls, respectively. In the sensory epithelium, two types of cells, sensory cells and supporting cells, are distinguishable. Sensory cells are characterized by microtubules and a variable number of centrioles in the distal end of dendritic processes (Fig. 4B). On the luminal surface of the sensory cells, a few microvilli are visible. The microvilli on P3 are longer than those on E15. Supporting cells have a large amount of free polyribosomes in the distal processes. On the luminal surface, a large number of microvilli are observed. On P7, the size of the VNO increases. Sensory and supporting cells can be distinguished using electron microscopy. The morphological characteristics of each cell type are similar to those of P3 rats, but the length and number of microvilli of the sensory cells are greater on P7 than on P3. During the third week microvilli are more frequently observed and appear to be longer and more branched. The sensory cells show morphological signs of maturity. By the end of the third week the vomeronasal epithelium morphologically matured. In adult rats, the sensory and supporting cells have a large number of long microvilli on the luminal surface. Sensory cells have many microtubules and mitochondria at the distal end, whereas supporting cells are characterized by a large amount of free polyribosomes and electron-dense cytoplasm (Fig. 4C).

Taniguchi *et al.* (1982) reported that the sensory epithelium in hamster VNO is delayed and not matured at 10 days after birth. Development of rat VNO shows the same delayed maturation.

2) Immunocytochemistry in the developing VNO

In order to study the development of vomeronasal sensory cells, the expression of several substances (nestin; N-CAM, neural cell adhesion molecule; and OMP, olfactory marker protein) has been immunocytochemically examined in the developing VNO (Osada *et al.*, 1995). Nestin is a useful marker for neuronal precursor cells (Frederiksen and Mckay, 1988; Osada *et al.*, 1995). N-CAM is a marker for developing and matured neuronal cells (Edelman, 1985; Rutihauser and Jessel, 1988), and OMP is a marker for mature olfactory neurons (Farbman and Margolis, 1980; Monti-Graziadei *et al.*, 1980). On E15, E17, and E19, most cells in the vomeronasal epithelium are stained with an antibody to nestin. From P1 to P22, the distribution of nestin-positive cells becomes progressively restricted to the area adjacent to the basement membrane. ON P29, a few nestin-positive cells can be observed. N-CAM-positive cells appear first on E17, and as the development of vomeronasal organ progresses to E19, the N-CAM-positive cells increase in number. Postnatally the N-CAM-positive cells increase in number and form multilayers in the epithelium. On E17 and E19, OMP-positive cells are occasionally observed although the number of these cells is relatively small. The number of OMP-positive cells increases postanatally.

Monoclonal antibodies have been raised and selected for reactivity with the luminal surface of the rat vomeronasal organ. Schwarting *et al.* (1991) examined the developing VNO using an antibody (CC1) which reacts with N-acetyl galactosamine containing glycolipid. Immunological staining of the VNO using CC1 is first on E15. At this stage the staining is faint and punctate. On E20 the intensity increases, and during the first postnatal week, CC1 reactivity is strong in the central region and becomes progressively weeker in the marginal area in the sensory epithelium of VNO. On P34, although a central/marginal gradient still exists, the difference between positive/negative regions of CC1 reactivity is less distinctive. This pattern continues in the adult rats. Osada *et al.* (1994) demonstrated that of the monoclonal antibodies generated, one, VOM2, reacts specifically with antigens on the luminal surface of the rat vomeronasal sensory epithelium. The VOM2 antigen is present low on the luminal surface on P14. After P21, VOM2 immunoreactivity is as strong as that in the adult sensory vomeronasal epithelium (Osada *et al.*, 1994; Yoshida *et al.*, 1994).

Results of these immunocytochemical studies suggest that development of the VNO continues in the third-fifth postnatal week.

3) Development of the AOB

Neurogenesis in the rat AOB was examined with ^{3}H-thymidine-radioautography (Bayer, 1983). Some neurogenesis of mitral/tufted (MT) cells occurs as early as E12. E13 is the peak day of MT cell generation when 60% of the cell population in the rat AOB originates. Most of the MT cells originate by E15. A extensive neurogenesis of granule cells occurs on E17-E19 and only 10-13% of granule cells are generated in the postnatal period. Most neurons in the rat AOB are generated in the prenatal period.

Developmemt of the AOB has not been studied extensively (Ichikawa *et al.*, 1994). On E16, the laminar structure of the AOB is not yet developed; only small fibrous bundles and the underlying cell aggregates are observed posterior to the main olfactory bulb. On E18, the laminar organization of the AOB is partially developed; the vomeronasal nerve layer and glomerular layer are recognizable, although the remaining layers, the MT cell layer, olfactory tract layer and granule cell layer, cannot be distinguished. On E20, the vomeronasal nerve layer, glomerular layer and MT cell layer can be identified in the AOB, although the olfactory tract layer and the granule cell layer cannot be distinguished. Between E20 and P1, the AOB develops drastically; the five layers are recognizable on P1. Antero-posterior length and depth of the vomeronasal nerve layer and glomerular layer increase markedly between P1 and P3. Further developed AOB is observed on P7 with each layer increasing in depth and length. On P14, the cytoarchitecture of the AOB appears to be very similar to that of adult rats.

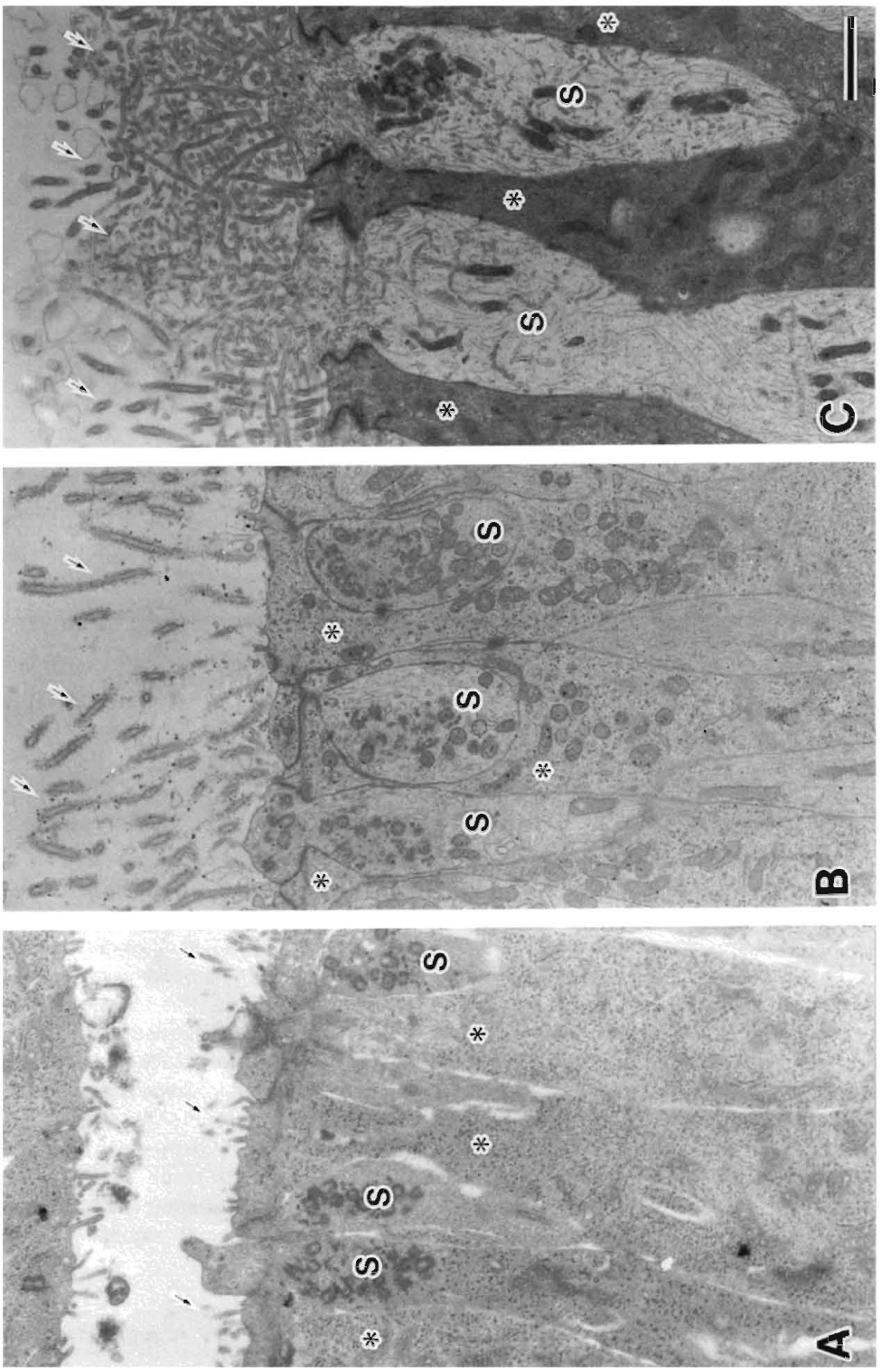

Fig. 4. Electron micrographs show the sensory epithelia of E15 (A), P3 (B), and mature (C) rat. Sensory (S) and supporting (asterisks) cells bear microvilli (arrows) on the luminal surface. Bar: 2 μm.

4) Innervation of the vomeronasal axons and synaptogenesis in AOB

Synaptic contacts of vomeronasal axons on the dendrites of MT cells in the AOB are present at birth though the density is low. Figure 5 shows the time course of synaptic density in the glomerulus of the developing rat AOB. The density of synapses increases with postnatal age. In the third week, it reaches a peak , then decreases, and remain at this level in the adult rat. The pattern is not different from that of other areas in the central nervous system. Glomerular arbors of MT cells that have characteristic morphological features are, however, not observed at birth (Fig. 6). The glomerular arbors of the MT cells develop gradually in rats after 1 week; 2-week-old rats have the adult-type glomerular arbors of the MT cells,

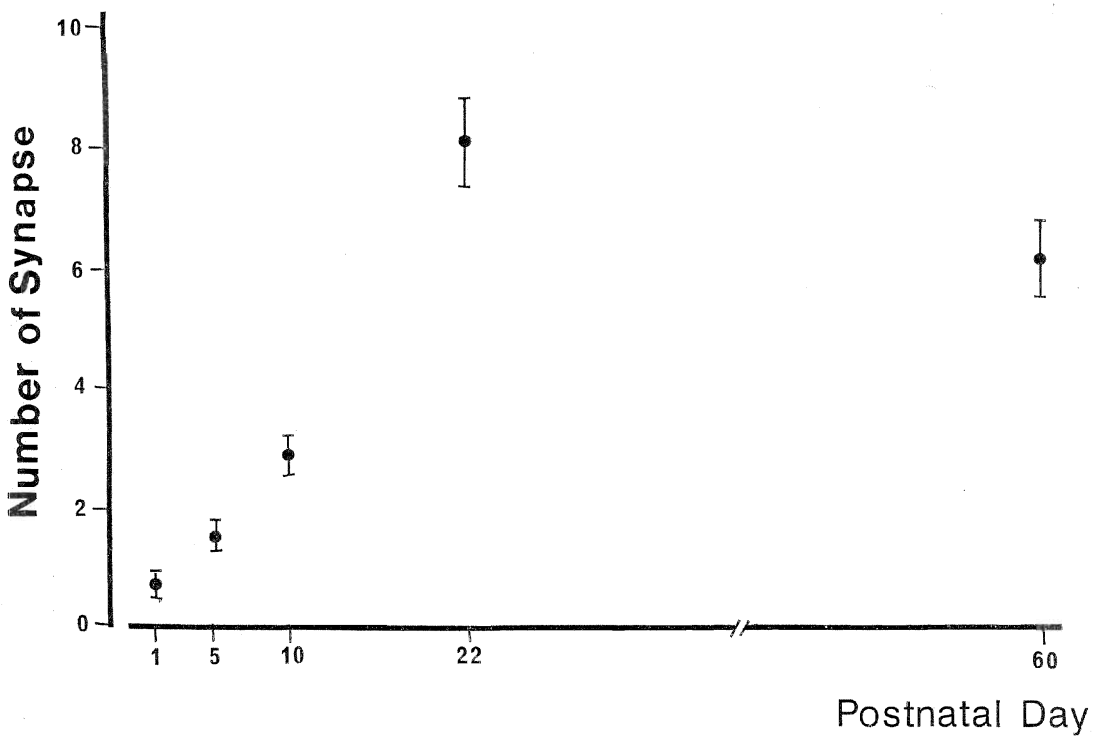

Fig. 5. Time-course of numerical density of synapses in the glomerular layer of the developing rat AOB. Ordinate indicates the number of synapses per 48 μm^2.

and the number of the arbors is increased by the 4th week (Fig. 6). These observations indicate that the time course of glomerular arbor development is very similar to that of synaptogenesis as determined by electron microscopic study.

Two types of lectins (*Bandeiraea simplicifolia* lectin-I (BSL-I) which specifically recognizes terminal α-D-galactose and α-N-acetylgalactosamine sugar residues, and *Vicia villosa* agglutinin (VVA) which specifically recognizes terminal α- and β-N-acetylgalactosamine sugar residues) are used as markers for vomeronasal axons because these two lectins bind specifically to the vomeronasal axons in the AOB, but not to the olfactory axons of adult rats (Ichikawa *et al.*, 1992). Differential binding patterns of these two lectins in the AOB are also observed (Takami *et al.*, 1992). Intense binding of BSL-I is observed in the whole vomeronasal nerve layer and glomerular layer. In contrast, VVA shows a position-specific binding pattern; intense binding of VVA is observed in the posterior two-thirds, whereas only weak binding for this probe is observed in the anterior one-third of the vomeronasal nerve layer and glomerular layer.

Innervation of vomeronasal axons was studied in the AOB using the two lectins as markers (Ichikawa *et al.*, 1994). Developing vomeronasal axons are labeled with VVA earlier than with BSL-I; binding of VVA to vomeronasal axons in the AOB is first observed on E18. The position-specific binding pattern of VVA has already been established at birth. In contrast, the binding of BSL-I is observed in the posterior half of the vomeronasal nerve layer and the glomerular layer at birth. The binding area expands to the anterior area and the binding intensity increases as the development proceeds. On P28, binding of BSL-I is observed in the entire vomeronasal nerve layer and glomerular layer which is identical to the situation in adult rats. This indicates that vomeronasal axons consist of two populations possessing different glyco-conjugates and develop differentially and that by the end of the 4th week the innervation is established.

These developmental studies suggest that the vomeronasal system of the rat is morphologically mature by the end of the 4th week. It has been suggested that morphological maturation is relatd to the expression of reproductive function (Taniguchi *et al.*, 1982; Garrosa and Coca, 1991).

PLASTICITY

1) Replacement of vomeronasal sensory cells

The main olfactory system undergoes continuous replacement of its sensory neurons (Graziadei and Monti-Graziadei, 1978; Monti-Graziadei and Graziadei, 1979). Sensory neurons in the vomeronasal organ as well as those in the olfactory epithelium undergo continuous neurogenesis throughout the life span of the animal (Barber and Raisman, 1978a,b). Wilson and Raisman (1980) suggested, based on the results of quantitative study, that the entire population of

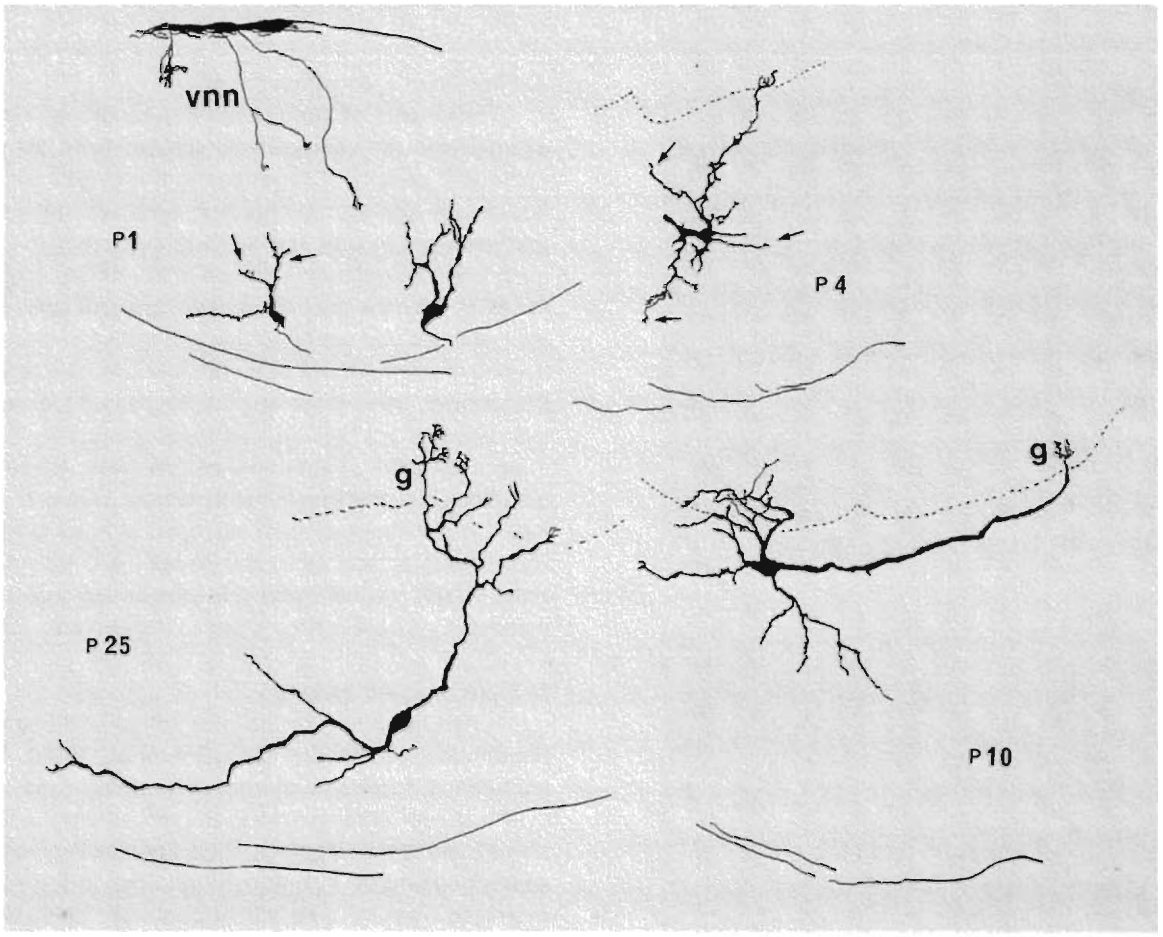

Fig. 6. Camera lucida drawings of Golgi staining of MT cells in the developing AOB of the rat. g, branching pattern in glomerulus; vnn, vomeronasal nerve.

sensory neurons would be replaced in every 2-3 months. Barber (1981) demonstrated, in a study of the retrograde axonal transport of HRP combined with ^{3}H-thymidine labeling of dividing cells, that the axons of the newly formed sensory cells in the VNO reach their designated target, the AOB. Sensory cells in the vomeronasal epithelium are also replaced following experimentally induced degeneration (Barber and Raisman, 1978a,b; Ichikawa and Costanzo, 1995). Unilateral section of vomeronasal nerves in adult hamsters results in the degeneration of sensory cells followed by replacement of the receptor cell population (Fig. 7). We have performed a quantitative analysis to determine the time course and degree of cell replacement in the vomeronasal epithelium following unilateral section of vomeronasal nerves in adult hamsters. There is gradual degeneration of sensory cells, the number decreasing to 50% by day 2 after section and 16% by day 6. During days 7-15 maximum cell replacement is observed. Cell number increases significantly and reaches a level of 118% of that on the control side by day 15. At long recovery time of 40-60 days cell number is restored at 90% of control. The pattern of replacement of sensory cells is similar to that for olfactory epithelium (Costanzo and Graziadei, 1983; Costanzo, 1985). In the main olfactory system, restoration of olfactory-mediated behavior after olfactory bulb deafferentation has been reported (Yee and Costanzo, 1995). Assessment of the functional capacity of the replaced cells requires further study in the vomeronasal system.

2) Environmentally induced synaptic plasticity

It has been suggested that the complexity of the environment to which animals are exposed has an effect on the structure of synapses, and that the effect varies among different regions of the central nervous system and different animal species (see reviews by Greenough and Chang, 1988; Petit, 1988). For example, the synaptic contacts in the occipital cortex are larger in rats reared in an enriched or complex environment than those reared in an impoverished or isolated one (Sirevaag and Greenough, 1985). The ratio of synapses per neuron varies, with the highest ratio observed in animals reared in an enriched environment and the lowest in those reared in an impoverished one (Turner and Greenough, 1985). The complexity of the environment also affects the synaptic structure in the cat visual cortex significantly, but affects that in the motor cortex of the same animal to a much lesser degree (Beaulieu and Colonnier, 1987, 1989). We have studied pheromonally induced synaptic plasticity in the vomeronasal

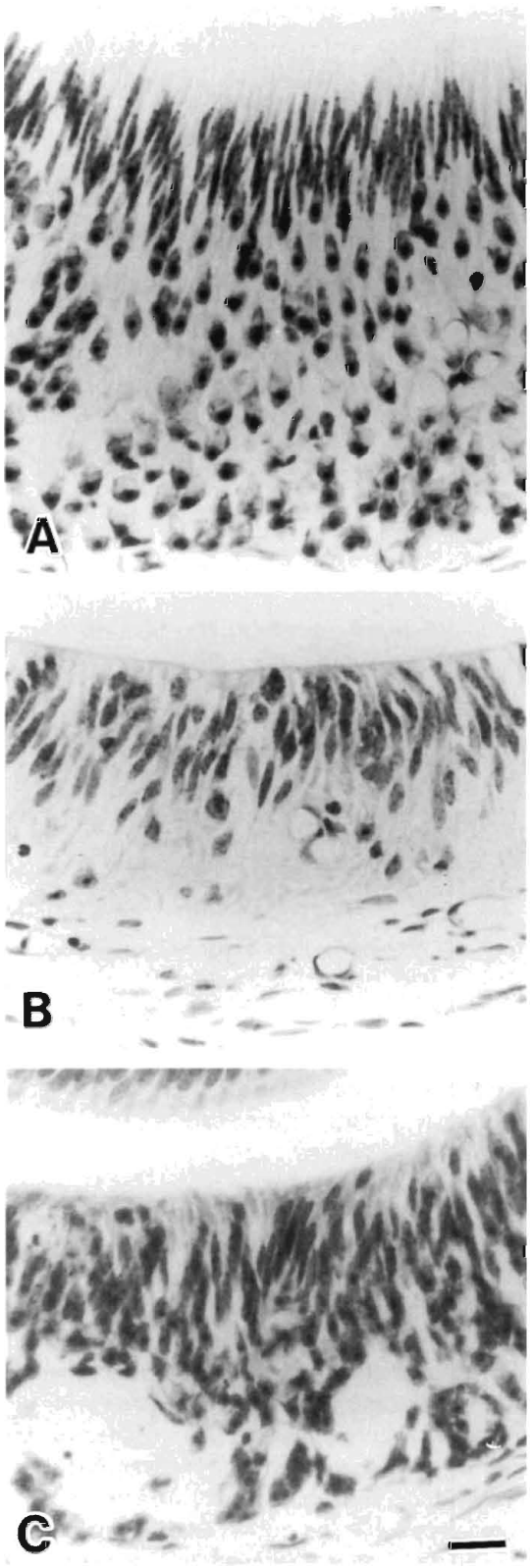

Fig. 7. Light micrographs show the neuroepithelium of the vomeronasal organ after transection of the vomeronasal nerve. (A) 3 hr after transection, degenerative changes are not observed, (B) 6 days after transection, there is a marked reduction of sensory cell, (C) 50 days after transection, newly formed dark cells are observed. Bar: 20 μm.

system.

a) Effect of mix rearing on the AOB

The effects of various rearing conditions have been examined in the vomeronasal system. Both the size and numerical density of synapses in the medial amygdaloid nucleus are significantly greater in adult rats that are housed together in a complex social environment than in those that are housed individually (Ichikawa *et al.*, 1993a). Moreover, in the AOB, the mean size of synapses is significantly greater in adult animals that are housed together than in those that are housed alone (Ichikawa *et al.*,1993b; Matsuoka *et al.*, 1994). Presumably, when these animals are reared together, either in a unisexual or a heterosexual environment, they experience a more complex pheromonal environment, which induces morphological changes in the synapses. However, because the AOB also receives other afferents not associated with the vomeronasal system (Price and Powell, 1970; Raisman, 1972; Halaz, 1990), it is not possible to unequivocally conclude that these changes are related solely to vomeronasal influences.

b) Effect of soiled bedding

It has been known that the soiled bedding contains pheromonal substances (Schellink *et al.*, 1993). The AOB presumably receives a more purely vomeronasal influence by the soiled bedding than in the mix rearing in which the animals are reared together. Thus, the effects of exposure to soiled bedding on synaptic morphology in the AOB were examined in adult male rats (Ichikawa *et al.*, 1995). One group was exposed to bedding soiled by male and female rats (male and female group). Another group was exposed to bedding soiled by only males (male group). A third group was exposed to clean bedding (clean group). After 2 months, the size and the numerical density of synapses were measured in the glomerulus. The mean size of the synapses was significantly greater in the male and female group than in the clean group, whereas that in the male group appeared to be intermediate between those in the male and female and the clean groups, but was not significantly different. There was no statistically significant difference in the density of synapses among the three groups. These results suggest that exposure to a more complex soiled bedding environment, i.e., to bedding soiled by both male and female rats, can induce greater structural changes of the synapses in the AOB of male adult rats.

These results support the notion that exposure to a more complex pheromonal environment induces greater morphological changes in the synapses in the glomerulus and the granule cell layer of the AOB. However, in the present study, the nature of the pheromonal stimuli has not been defined. The amount of urine and other secretions as well as numerous other unspecified constituents (bacterial content, presence of food pellets, etc.) varies between treatment groups.

c) Effect of urine exposure

At present we are studying the effect of urinary

pheromones on the synapses of male AOBs, because many reports have suggested that urine contains pheromonal substances (Matsuoka *et al.*, 1995). Urine is collected every day from intact female hamsters, ovariectomized female hamsters and male hamsters. Male hamsters are housed in isolation and exposed to one of four conditions: intact female urine (female group), urine of ovariectomized females (OVX group), male hamster urine (male group), and distilled water (water group). Cotton swabs soaked with urine or water are placed in the cage every day. The male hamsters often sniff, lick, bite or chew the cotton swabs, especially when the swabs contain urine. After being reared for 15 days, the length of synaptic active zone (SAZ) in the glomerulus was measured (Fig. 8). The synaptic active zone in the female group is significantly longer than that in the water and the male groups, and that in the OVX group is intermediate in length between those in the female and the water groups (Fig. 9). That in the male group is not significantly different in length from that in

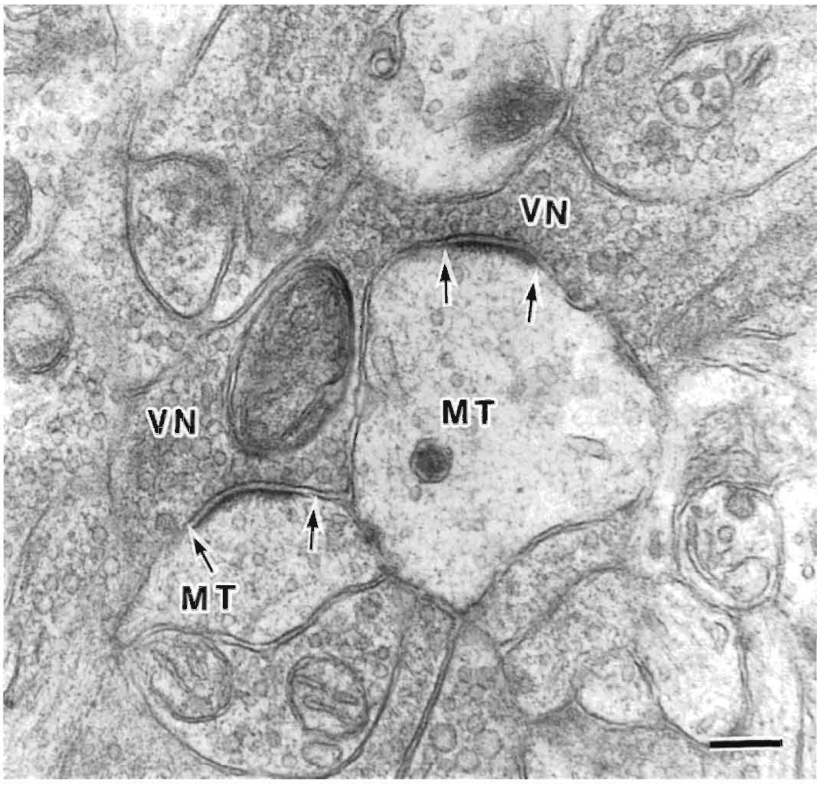

Fig. 8. Electron micrograph shows the synapses in the glomerulus of AOB. Arrows indicate the edges of the synaptic active zone. MT, MT cell dendrite; VN, axon terminal of vomeronasal sensory cell. Bar: 200 nm.

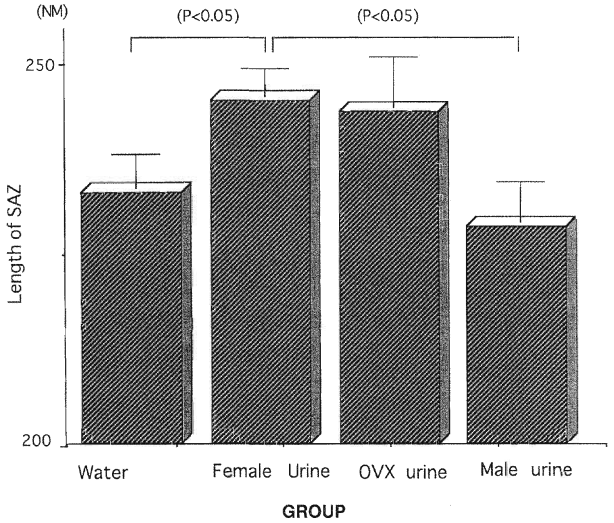

Fig. 9. Graph shows the length of the synaptic active zone in the glomerulus of differentially reared hamsters. Male hamsters are exposed to one of four conditions (water, female urine, ovariectomized urine, and male urine). The synaptic active zone in the glomerulus in the female urine group is significantly longer than that in the water and the male urine groups ($P<0.05$).

the water group. This indicates that exposure to female pheromones induces morphological changes in the synaptic active zone of the glomerulus in the male AOB. It has been known that the SAZ consists of receptor and ion channel proteins (Fig. 10). It is likely that the changes of length of the SAZ induce the efficacy of synaptic transmission.

Recently, synaptic mechanisms of olfactory memory in the vomeronasal system have been studied (see review by Kaba and Nakanishi, 1995). The olfactory block to pregnancy (Bruce effect) occurs most effectively when the strange male is of a different strain from that of the male with which the female has mated (stud male). This mechanism is dependent on the memory formation of the pheromone from the stud male. This memory trace response is localized in the MT-to-granule dendrodendritic synapse in the AOB. It is necessary to examine the relationship between the memory trace and the plasticity of synaptic structures in the dendrodendritic synapses in the AOB.

3) Lesion-induced synaptic plasticity in the medial amygdaloid nucleus (MAN)

Reorganization of neuronal connections after lesion-induced deafferentation in several areas of the central nervous system has been reported (Cotman, 1985; Flohr, 1988). It has been hypothesized that the lesion-induced reorganization of neuronal connections is one of the mechanisms mediating recovery of function after injury of the adult central nervous sytem (Marshall, 1985). However, a correlation between lesion-induced reorganization and functional recovery has not been clearly demonstrated yet. One of the reasons for this is that lesion-induced reorganization of neuronal connections has been primarily observed in neuronal pathways of unknown function. Thus, it is necessary to study lesion-induced reorganization in a neuronal pathway whose function has been well characterized, and to study functional recovery following the injury in the same pathway. Thus, the reorganization of neuronal connections in the MAN and the recovery of olfactory behavior following AOB lesion, in the adult rat, were studied.

The time course of the loss and reappearance of synapses was examined in the molecular layer of the MAN to which the AOB axons project (Ichikawa, 1987a). The number of synapses decreases to less than half of the control density at 4 and 8 days following AOB lesion and thereafter increases gradually, until it reaches about 80% of the density in intact animals 2 months after the lesion. This recovery in the number of synapses following lesion suggests the possibility of synaptic reorganization by the remaining afferent fibers.

The axons from the bed nucleus of the stria terminalis (BNST) terminate in the MAN. Thus, the rearrangement of terminals from the BNST was examined in the MAN 2 months following lesion of the AOB in an electron microscopy and degeneration study (Ichikawa, 1987b). The number of synapses which were formed by the axons of the BNST had increased significantly. This indicates that the axons from the BNST formed new synapses in the molecular layer following denervation of axons from the AOB. Axons from the posteromediocortical amygdaloid nucleus (PMCAN) terminate in the cellular layer of the MAN. The rearrangement of terminals of axons from PMCAN was examined in the MAN following denervation of AOB axons, using immunocytochemistry of anterogradely transported *Phaseolus vulgaris* leucoagglutinin (PHA-L) (Ichikawa, 1988). The AOB was first removed unilaterally. Two months later, PHA-L was injected bilaterally into the PMCAN. On the control side, labeled axons were found in the cellar layer of the MAN, but they were not found in the molecular layer. On the side of AOB removal, labeled axons were found not only in the cellular layer but also in the molecular layer. Electron microscopic observation showed that the labeled axons formed synaptic contacts in the molecular layer. These results indicate that the axons from the PMCAN expand from the cellular layer to the molecular layer in the MAN and form synapses in this layer, following the denervation of AOB axons (Fig. 11).

It has been shown that male rats prefer the odors of female rats (Vandenberg, 1983). This preference is a function of the vomeronasal system. Accordingly, changes in the preference of male rats for the odors of female rats were examined following AOB removal (Ichikawa, 1989). This preference

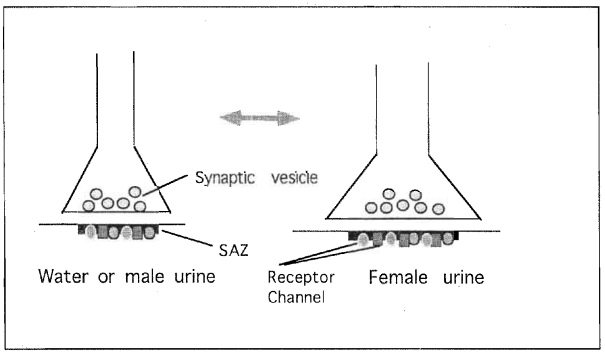

Fig. 10. Schematic representation of pheromonally induced synaptic plasticity. The exposure to female urines induces morphological changes in the SAZ of synapses. The changes of length of the SAZ induce the efficacy of synaptic transmission.

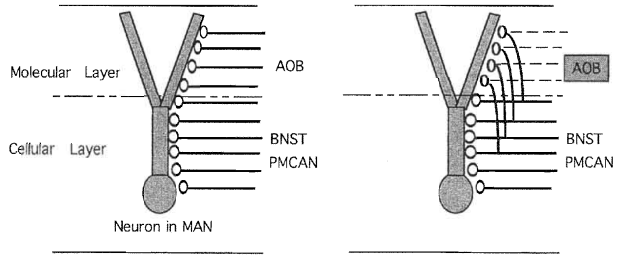

Fig. 11. Schematic representation of the synaptic connections in the MAN. Left, normal connection. The axon terminals from the AOB form the synaptic contacts with the neuron in the molecular layer of MAN. Those from the PMCAN and BNST form the synaptic contacts in the cellular layer. Right, Representation of the reorganization of the neuronal connection in the MAN after denervation of axons from AOB. Broken lines indicate the degeneration axons.

decreases to about 30% of that seen in the intact rat during the first 10 days following AOB removal. Thereafter, it increases gradually and reaches approximately 70% of the level of intact rats 1 month after AOB removal. The time course of recovery of this preference behavior is similar to that of the recovery of number of synapses following denervation of AOB axons. It is not possible to conclude that a causal relationship exists between the synaptic reorganization and the recovery of behavior only on the similarity of their time courses, because many unknown events other than synaptic reorganization may take place during the behavioral recovery after injury. However, the similarities in the time courses supports the hypothesis that lesion-induced reorganization is one of the important events underlying the behavioral recovery after injury (Ichikawa, 1989).

4) Sexual dimorphism in the vomeronasal system

Recently it has been reported that the vomeronasal system shows sexual dimorphism (see review by Segovia and Guillamon, 1993). This suggests that the neurons in the vomeronasal system exhibit a high degree of plasticity in response to sex hormones.

In the VNO, values for several morphometric parameters are higher for males than for females in three aspects: structural volume of VNO, sensoryepithelial volume and the number of sensory neurons. Orchidectomy of males and androgenization of females performed on the day of birth abolishes and inverts this sexual dimorphism, as seen when the subjects are studied in adulthood (Segovia and Guillamon, 1982). Females show significantly greater nuclear size than males. Gonadectomy of males induces a 5% reduction in nuclear size, while ovariectomy of females results in a 20% reduction. Segovia *et al.* (1984a, b) have postulated that during the developmental stage, the VNO undergoes sexual differentiation which depends on the appropriate sex hormone and that the morphological characteristics of the VNO depends on the level of sexual steroids in adulthood.

The AOB also shows sexual differentiation in several structural aspects (Roos *et al.*, 1988; Caminero *et al.*, 1991). Male rats have a larger AOB, more MT cells and granule cells, larger somata and more dendritic branches in MT cells than female rats. The sexual dimorphism depends on the action of sex steroids present in the subjects shortly after their birth. The manifestation of sexual dimorphism found in all of these morphological features is suppressed and/or reversed by orchidectomy in males and androgenization of females.

The vomeronasal system shows a high degree of plasticity by which the animals adapt to their environment.

CONCLUSION

On the basis of morphological studies, the vomeronasal system shows the following characteristics. Development of the rat vomeronasal system continues until about the 4th week after birth. This late maturation is related to the expression of the reproductive function, e.g. the beginning of puberty. It is also suggested that the maturation of the vomeronasal system is closely correlated with the expression of the reproductive function. The vomeronasal system exhibit a high degree of plasticity. This plasticity is necessary for animals to adapt to the changes of their environment. Assessment of the functional background of these characteristics requires further study.

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