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Authors: Tarozzo, Glauco, Peretto, Paolo, and Fasolo, Aldo

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REVIEW

Cell Migration from the Olfactory Placode and the Ontogeny of the Neuroendocrine Compartments

Glauco Tarozzo, Paolo Peretto and Aldo Fasolo*

Dipartmento Biologia Animale University of Torino, via Accademia Albertina, 17 10123 Torino, Italy

ABSTRACT—The olfactory placode and its derivative, the olfactory pit, give rise to several different populations of migrating cells, which contribute to drive the organization of the prosencephalon, but also to form a part of the central neuroendocrine compartments. Some cell types are seemingly transient and can play a role in the establishment of the final connections. The understanding of the mechanisms involved in the migration and differentiation of these cell populations can give an insight on the interplay between peripheral structures and central nervous system and on the mechanisms of commitment, phenotype selection and control for neuroendocrine cells able to selectively "colonize" the brain.

INTRODUCTION

The olfactory system represents a beautiful model in order to study neuronal plasticity and differentiation and recently it was argued that the analysis of its development and of mechanisms subserving learning and behavior can be very profitable in order to test neural darwinism and selectionist theories in brain function [46]. Among others, a challenging arguments is the heterogeneity of developmental products issued from the olfactory placode. The olfactory placode gives rise in fact to several different cell populations, including neuronal, glial, epithelial and glandular cells (Table 1). The main point concerning neurons is that during early development, both in mammals and birds, several cell types, with different functions, fate and differentiation program are generated, i.e.:

1) Olfactory receptor neurons (ORNs) within the olfactory neuroepithelium, which are able to proliferate and differentiate *in situ* during the embryonic and juvenile life, but even in adulthood show active neurogenesis [42, 54], since they preserve a stem cell compartment.

2) Neurons expressing the mammalian form of the decapeptide gonadotropin releasing hormone (GnRH, sometimes referred also as LHRH), which are early committed and migrate from the nasal region into the brain [122, 152].

3) Other cell types, sometimes labelled by olfactory markers, which are seemingly migrating together with GnRH positive neurons or along the olfactory pathways during fetal life. The fate and the final phenotype of such cells remain still obscure.

In addition, several lines of evidence suggest that during development and later on under experimental conditions, a complex interplay of inductive actions between the olfactory placode and its target, the olfactory bulb (OB), takes place [40, 52, 69]. Accordingly, it may be relevant to understand the fine relations between these key developmental events and address several questions on cell differentiation and on the relations between peripheral structures and central neuroendocrine systems. In the present review data about

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* To whom correspondence should be addressed.

FOOTNOTES

A-N-CAM, neural cell adhesion molecule, adult form
AMOG, adhesion molecule on glia
B-50/GAP 43, B-50 phosphoprotein, growth associated protein
CARN, carnosine
CN-Ch, cyclic nucleotide gated channel
CNS, central nervous system
E-N-CAM, neural cell adhesion molecule, embryonic form
E 10.5, E12, ...embryonic day 10.5, 12,...
GAP, GnRH associated peptide
GFAP, glial fibrillary acidic protein
GnRH, gonadotropin-releasing hormone
LH, luteinizing hormone
LHRH, luteinizing hormone-releasing hormone
MAM, methylazoxymethanol acetate
N-CAM, neural cell adhesion molecule
Ng-CAM, neural-glial cell adhesion molecule
NO, nitric oxide
NOS, nitric oxide synthase
NSE, neuron-specific enolase
OB, olfactory bulb
OMP, olfactory marker protein
ORNs, olfactory receptor neurons
P1, P2,...postnatal day 1, 2,...
RA, retinoic acid
SST, somatostatin
UEA, *Ulex europaeus* lectin-I
VNO, vomeronasal organ

Table 1. Different cell populations derived from the olfactory placode (Adapted and modified from [43])

Cells derived from the olfactory placode
Neuronal
Basal cells of olfactory epithelium, progenitor of neurons
Olfactory sensory cells, main nasal cavity
Olfactory sensory cells, vomeronasal organ
Olfactory sensory cells, septal organ
GnRH (LHRH) secreting neurons, septal-preoptic area
Ganglion cells, terminal nerve
Migratory neuronal populations
Non-neuronal
Olfactory supporting cells, main nasal cavity
Olfactory supporting cells, vomeronasal organ
Ciliated cells of respiratory epithelium, main nasal cavity, nonsensory region of vomeronasal organ, paranasal sinuses
Glandular cells of respiratory epithelium in main nasal cavity, vomeronasal organ, paranasal sinuses
Bowman's gland epithelium, main nasal cavity
Ensheathing cells of olfactory and vomeronasal nerves and nerve layer of olfactory bulb
Submucosal glands, nonsensory region of main nasal cavity
Brush cells and other microvillous cells in both olfactory and respiratory epithlium

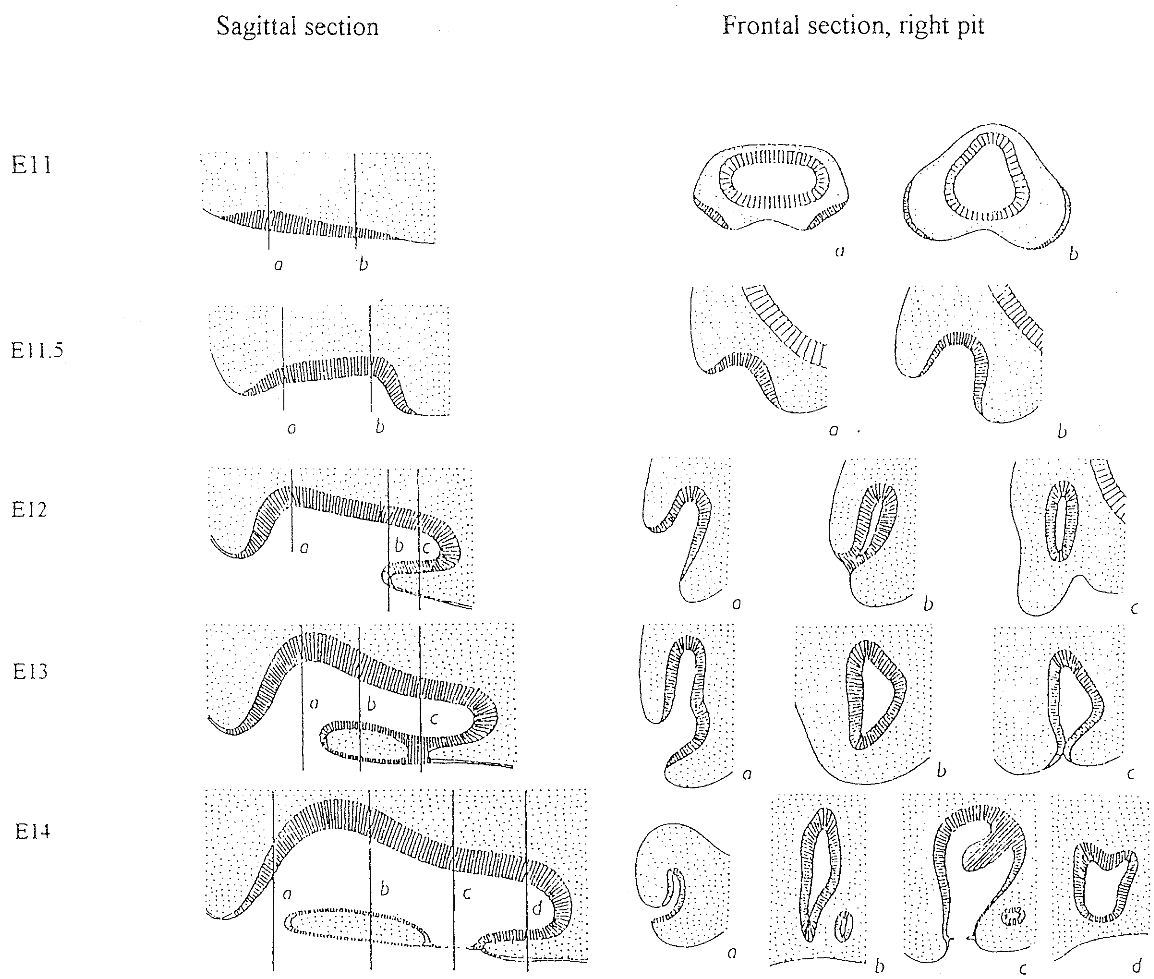


Fig. 1. Development of the olfactory pit in the rat [70].

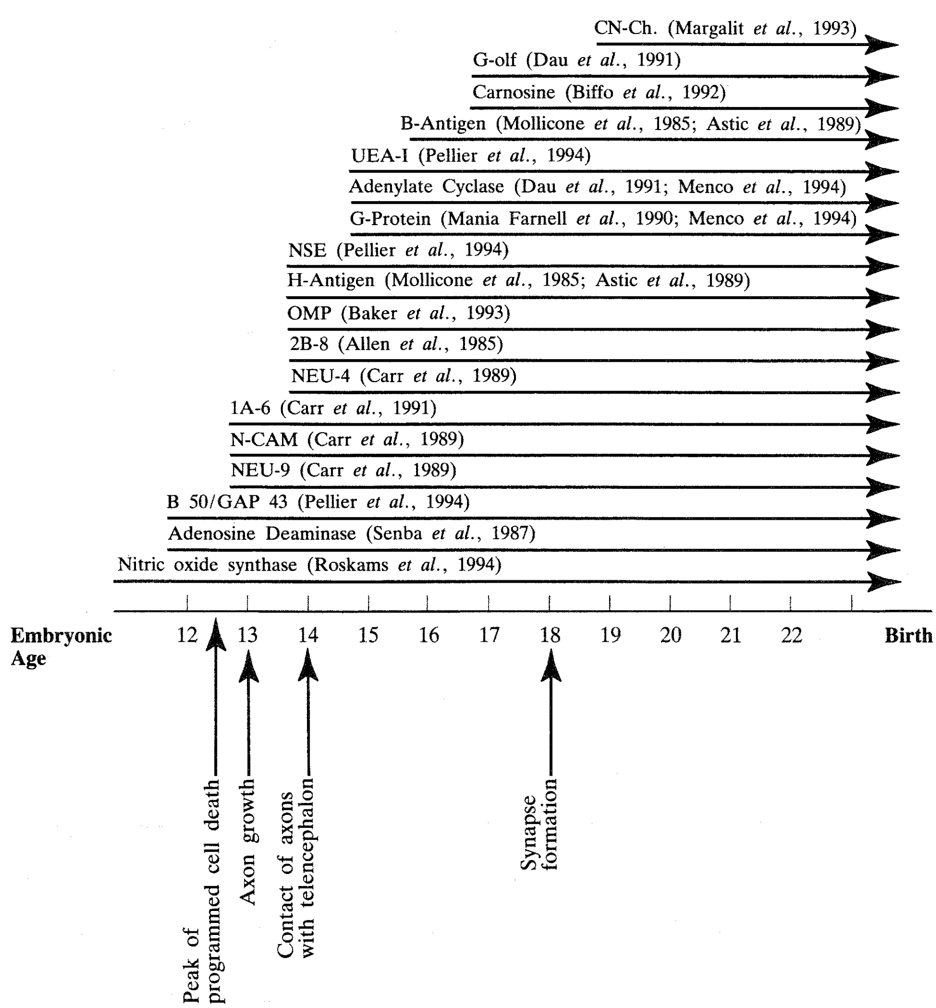
mammals and birds will be essentially discussed, since information about other taxonomic groups is even more fragmentary.

THE OLFACTORY PLACODE

The olfactory epithelium develops bilaterally on the ventrolateral aspects of the head from thickened patches of ectoderm, called olfactory placodes (Fig. 1) [70, 71]. The placodes, in turn, are derived from the primitive placodal thickening, which arises very early, concurrently with the formation of the neural plate [20, 112]. The subsequent folding of the neural plate into the neural groove results in a thinning of the ectoderm along the lateral border of the plate. In this way, the primitive placode is separated from the developing central nervous system (CNS) and from the neural crest. The placodal band then undergoes segmentation into a number of regional placodes, including the anterior "sense plate". This latter structure arises from the anterior of the head and differentiates into the paired olfactory placodes and the hypophysial placode. In the mouse, the olfactory placodes form around embryonic day E9 and at that day they have already a pseudostratified appearence. With continued growth the placode begins to invaginate, forming the early nasal pit, then the nasal pit becomes confluent with the oral region when the thin nasobuccal membrane ruptures, resulting in the formation of the nasal cavity. The central region of the pit gives rise to the olfactory epithelium proper, while a portion of the medial wall develops into the vomeronasal organ. Initially, cell division is largely restricted to the apical zone of the olfactory epithelium, but after E12, proliferating cells become prevalent in the basal layer. Comparison of the features of the olfactory epithelium, with those shown by other neuroectodermal derivatives, as epidermis and CNS, suggests that the olfactory epithelium has unique intermediate phenotype [75]. Several authors have also

TABLE 2. Appearance of different molecules in the olfacotry epithelium during embryonic development in rats (Adapted and modified from [43])

Time-line of expression of molecules in rat olfactory epithelium during embryonic development



stressed that in several vertebrates a close anatomical relation exists between the paired olfactory placodes and the hypophysial placode [53, 61, 133].

NEURONAL AND GLIAL POPULATIONS FROM THE OLFACTORY PLACODE

Olfactory receptor neurons (ORNs)

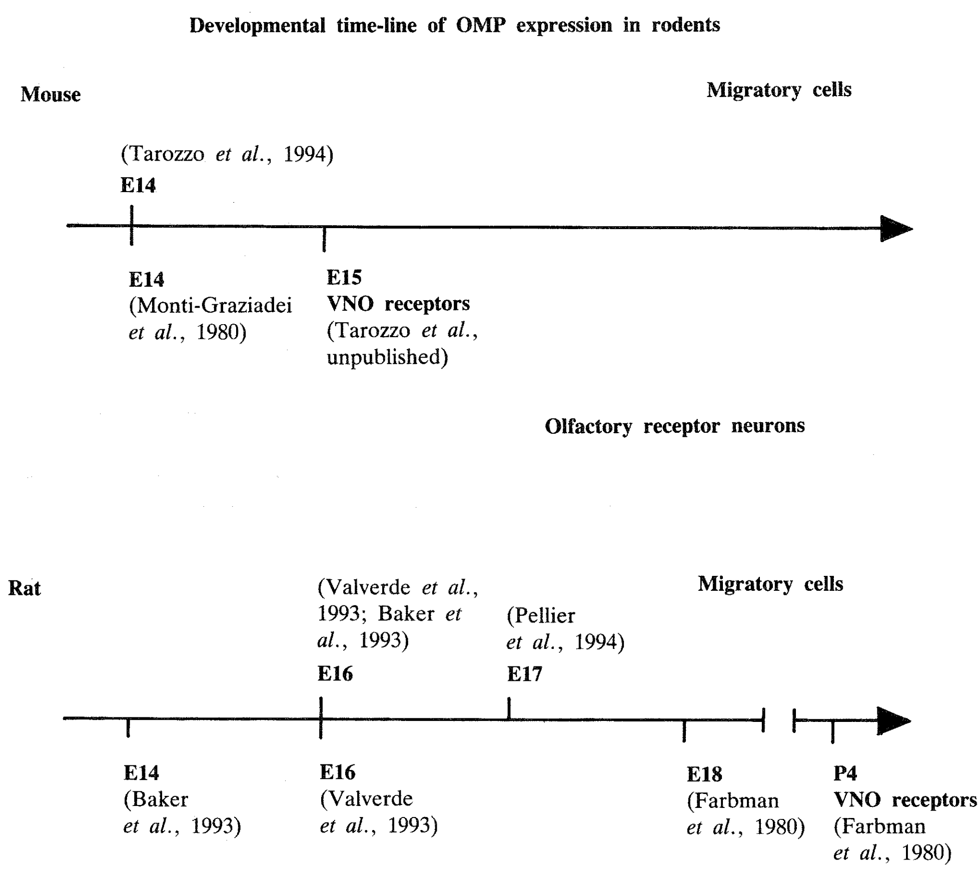
ORN development was carefully described in several species, but detailed information is available for rodents, in particular for the rat (Table 2). Since its relevance for the present review, special attention is paid to two specific olfactory substances, i.e.: olfactory marker protein (OMP) and carnosine. A specific marker for primary olfactory neurons is OMP, a 19 kD acidic protein first observed in the olfactory system of rodents [78], where it is synthesized by mature receptor neurons of the olfactory neuroepithelium [62]. Some discrepancies occurr concerning its ontogeny in the main and accessory olfactory system, since in the rat it was detected by immunocytochemistry at E18 in the main mucosa ORNs and at P4 in the vomeronasal organ [44], whereas recently, using more sensitive methods, a reliable staining has been detected early on (at E14 in the ORNs of the main epithelium) [8]. An overview of the data about the OMP onset in rodents in the ORNs and in the peripheral olfactory system is drawn in Table 3. OMP is phylogenetically conserved and it is present in the olfactory system of virtually all vertebrates [21, 110, 111, 132]. Immunocytochemical analysis has also revealed that OMP is expressed by some populations of neurons in the preoptic and hypothalamic regions of rats, mice and hamsters [9]. Although the genetic sequence coding for OMP has been determined [32] and the regulation of its expression have been studied [67], nothing is yet known about its function.

Another reliable marker is carnosine, a dipeptide (β-alanyl-L-histidine) which labels olfactory neurons in mammals, birds and reptiles [5, 12, 79]. In the mammalian CNS, its expression is associated with glial cells [12] and in amphibians with neurons [5, 6]. The role of carnosine in the olfactory system is still unknown but circumstantial evidence suggests it could be an excitatory neurotransmitter of primary olfactory neurons [80] or perhaps a modulator of glutamatergic olfactory transmission [11, 117].

GnRH neurons

The ontogeny and differentiation of GnRH neurons was authoritatively reviewed by Schwanzel-Fukuda in several instances [120, 123]. In general a very close association with

TABLE 3. Expression of OMP in primary receptor cells of the olfactory epithelium and of the vomeronasal organ, compared to the developmental pattern of OMP in migratory cell



the *nervus terminalis* and the medial nasal region (especially related to the presumptive vomeronasal organ) was shown. GnRH cells are early committed to form the gonadotropic compartement of the hypothalamus [122, 152, 154] and migrate in clusters along the nasal septum, penetrate the cribriform bone, reach the telencephalon and through a pathway, defined by a scaffold of embryonic N-CAM expressing cells [118] and possibly on a subset of peripherin positive/N-CAM-negative olfactory axons both *in vivo* and in embryonic olfactory explant cultures [48, 153]. For instance in mouse GnRH neurons undergo their final division between E10 and E11 when are still located in the nasal system. However they do not express the GnRH molecule until E11. In postnatal mice about 800 cells (90% of the all neurons containing the mammalian form of GnRH of the adult mouse brain) have initiated GnRH systhesis as assessed with *in situ* hybridization [152] (Table 4). The short time-span, during which GnRH neurons are generated, suggests that the mitotic division is highly synchronized and that they are all derived from one distinct group of progenitor cells. Once GnRH cells express the decapeptide they begin to migrate from the nasal system into the forebrain using as major guiding system the *nervus terminalis* (Fig. 2A, B), where some ganglion cells are altogether GnRH positive [60]. They then reach their final site in the septum-diagonal band complex, in the preoptic and hypothalamic areas, and there they take their mature connections and phenotypes. A similar pattern of differentiation and migration was described for other mammals even if the exact birthdate was not clearly shown and in some instances, as in the case of the rat, GnRH neurons appear simultaneusly in the nasal system and in the forebrain [30]. Extensive studies in our laboratory were unable to disrupt GnRH cells in the rat, after *in utero* treatment using the DNA alkylating agent MAM at different embryonic days (from E11.5 to E15). These results (Fasolo *et al.*, unpublished data) might be due to a failure of the drug treatment, which impairs mitoses for about 12 hr after the injection, but probably reflect a longer and rather plastic neurogenesis of GnRH neurons in the rat.

The reality of the migration of GnRH cells was demonstrated experimentally in several vertebrates, using lesion experiments, fluorescent dye-labelling, transplants and organotypic cultures [1, 31, 55, 95].

A failure in the migration of the GnRH cells from the olfactory system into the brain was shown as the cause of the so-called Kallmann syndrome [119]. The human Kallmann syndrome involves hypogonadotropic hypogonadism, coupled with anosmia. They hypothesis that this syndrome is

TABLE 4. Stage of appearance and numbers of GnRH neurons during development in rodents

Number of GnRH-expressing migratory cells in rodents during embryonic development

Mouse

Embryonic Age	11	12	13	14	15	16	17	18	19	20	BIRTH	Reference
	75	190	510	450				480				Schwanzel-Fukuda *et al.* 1989
		90	880	500	700	760					850	Wray *et al.* 1989
	19	293	814	442		195					108	Zheng *et al. 1992*

Rat

Embryonic Age	14	15	16	17	18	19	20	21	22	BIRTH	P6	P10	Reference
			42	155	319	502	529	642	872	815	815	881	Sétáló *et al.*, 1992
		6	176	395	339								Daikoku-Ishido *et al.*, 1990
		28		375		520							Schwanzel-Fukuda *et al.*, 1985
			522	656	1158								Tobet *et al.*, 1992

due to a failure of GnRH neurons to migrate into the brain is indirectly supported by the demonstration that the Kallmann syndrome-related gene, cloned in man and in chicken, codes for a substrate adhesion putative molecule [47]. Until now a Kallmann syndrome-related gene has not been shown in mice [74, 115]. Moreover genetic deletion of N-CAMs does not seem to affect the gonadotropic compartment and the reproduction of transgenic mice [27, 145]. Anatomical studies as well as double immunostainings showed that in rodents GnRH neurons are accompanied by other cell types seen on the same migratory route (*see* the following paragraph). These latter are seemingly immunonegative for some neurohormones and neuropeptides. According to Zheng *et al.*, [155] in Bouin's-fixed, paraffin embedded tissue sections through the head of embryonic mice, cells associated to GnRH neurons were negative for thyrotropin-releasing hormone, corticotropin-releasing hormone, oxytocin, vasopressin, neuropeptide-Y and somatostatin. Recently, even in marsupials it was shown the occurrence of other cells on the same route or accompanying the olfactory fibres [140, and manuscript in preparation] suggesting that this phenomenon is widespread among mammals (Fig. 2).

Several issues remain however still controversial, i.e.:

1) Even if there is a large agreement on the origin of the GnRH cells from the nasal system, some data are suggesting that at least in the chicken they can derive from the ectoderm of nasal cavity presumptive territory [41]. After the unilateral removal of the olfactory placode anlage, the distribution pattern of GnRH cells was not disturbed in the operated as well in the control side, although ipsilateral olfactory structures were greatly reduced. In contrast, when the presumptive ectoderm of the nasal cavity was unilaterally removed, GnRH neurons were detected only in the control side, where this territory was left intact.

2) The GnRH migrating neurons are closely associated to the vomeronasal organ and intermingled with GnRH-positive cells of the *nervus terminalis*. The fine ontogenetic and anatomical relations with the *nervus terminalis* ganglion cells need however further analysis [101].

3) Some electron microscope observations suggest that the decapeptide GnRH is stocked, but not secreted by migrating cells [73, 155]. The GnRH immunopositive material is in fact accumulated outside the nuclear envelop and in the lumen of the rough endoplasmic reticulum and when the cells start to migrate and assume a fusiform appearance, the immunoreactive product extends through the cytoplasm, but is not detectable in the Golgi apparatus or secretory granules. Evidence obtained by Daikoku-Ishido *et al.*, using intraventricular transplant of the nasal placode in the rat, suggests that GnRH neurons acquire secretory activity in the presence of the medial basal hypothalamus [31]. It can be of interest to mention that the onset of GnRH neurons precedes in most species the first production of pituitary gonadotropins (discussion in [60]). Immunohistochemical studies have shown that in the rat, LH can be detected on E16 or E17, while GnRH immunopositivity is present in the *nervus terminalis* at E15 [60, 121]. Since a similar sequence on initiation of LH and GnRH synthesis was observed also in rhesus monkeys [113], it was hypothesized that GnRH released from terminal ganglion cells into the subarachnoid space could have an inductive role on the pituitary differentiation [60].

4) The cues controlling the migration and the final site remain elusive. A common view is that the migrating GnRH cells might follow a chemically labelled path. According to Schwanzel-Fukuda *et al.* [118, 125], GnRH neurons migrate along a scaffold of N-CAM positive cells and fibres, whereas Murakami *et al.* [96] described in the chicken positivity for polisialylated N-CAM on the GnRH cells themselves. Other markers of GnRH cells, possibly involved in cell migration, are some unique carbohydrates as CC2 [142]. It is puzzling to note that peripherin is expressed in axons accompanying GnRH cells during mouse development [153], whereas in the adult rat vimentin and peripherin are markers of GnRH cells themselves [59].

Another related problem is to understand what are the cues inducing the co-expression of other neuropeptides as galanin; in the preoptic region of the male rat brain 15–20% of GnRH-like galanin immunoreactive cells coexpress GnRH [84]. The differentiation of GnRH neurons in subgroups, related to different functional activities represent another puzzling question. In guinea pig, for instance, a subgroup of GnRH neurons expresses progestin receptors and is centrally positioned within the total population of GnRH neurons [65]. These cells are possibly foci of activity, capable of activating a larger component of GnRH cells in certain neuroendcrine conditions, such as prior to the LH surge.

5) Finally it remains unclear if the olfactory region is the sole source of GnRH. Norgren and Gao [99] showed in fact that GnRH neuronal subtypes have multiple origins in chickens: GnRH neurons in the thalamus are not continuous with the olfactory nerve and ablation of the olfactory placode eliminates GnRH neurons in the telencephalon, originated in the placode [98], but does not eliminate the ones in the thalamus. The problem is complicated by the fact that recently it has been shown that in vertebrates different molecular forms of GnRH exist and can be present simultaneously in the same species, but expressed in different neuronal systems [97]. In birds and even in mammals, using specific antibodies against the GnRH forms, different pattern of disributions have been reported. In birds, chicken GnRH-I (cGnRH-I) is present in the telencephalic and diencephalic regions, whereas cGnRH-II has a widespread distribution and occurs also in the brain stem [136]. In mammals, the mammalian GnRH (mGnRH), which varies from the cGnRH-I for one amino acid at position 8, was found in the prosencephalon of the musk shrew [37], while cGnRH-II, which has three amino acid substitutions in respect to mGnRH, is present in the midbrain [63, 64]. A recent report indicates, for instance, that GnRH neurons in the posterior tubercle in urodeles do not originate in the olfactory placode [100].

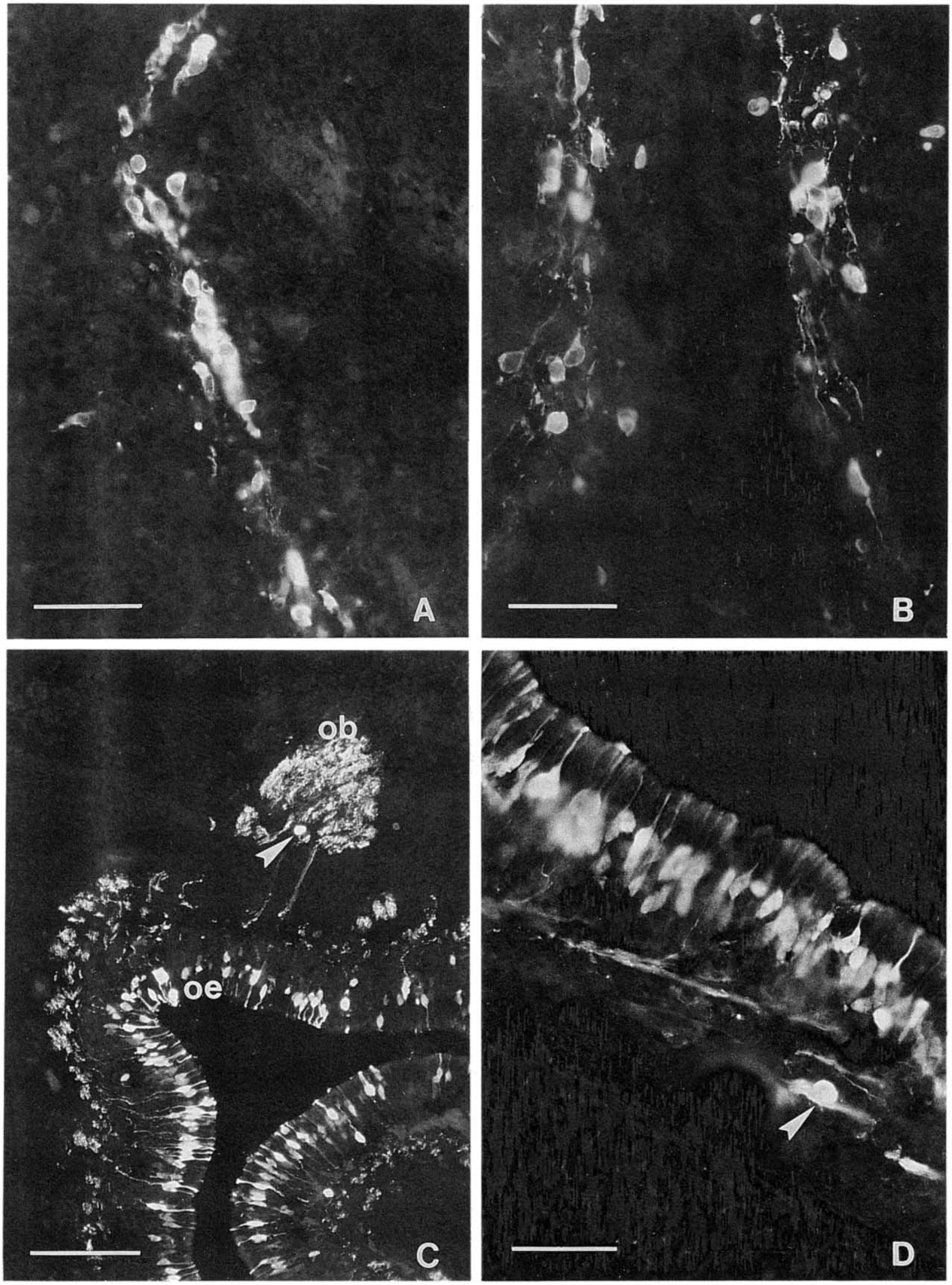


FIG. 2. Different migratory cells from the olfactory pit during development. Migratory cells can express several markers such as GnRH, carnosine, OMP. (A) Migratory cells in the olfactory mesenchima of an E14 mouse expressing GnRH (scale bar, 55 μm); (B) GnRH expressing-migratory neurons in a P1 opossum *Monodelphis domestica* in the medial rostral telencephalon (scale bar, 55 μm); (C) carnosine expressing migratory cell (arrowhead) in the rostral tip of the presumptive olfactory bulb of a E15 mouse (ob, olfactory bulb; oe, olfactory epithelium; scale bar, 140 μm); (D) OMP expressing cell in the submucosa of an E18 mouse, migrating along branches of the olfactory nerve (scale bar, 55 μm).

Other cell types

Several different cell populations have been identified along the migratory route of GnRH neurons or in close relation with the olfactory nerves [83] (Table 5). Some problems are still pending. In particular, no final proof exists that these cells are actually migrating, since the published papers report their occurrence along the olfactory fibres and in a well defined time gap, but data on their exact birthdate or exprerimental manipulation of their putative migration are not available. Another puzzling point is how to cope with the different reports in order to identify unequivocally the different populations and their specific markers.

At a very first approximation we can try to discriminate

TABLE 5. Markers for migratory cells in the olfactory system of birds and mammals during development

Markers for Migratory Cells

Antigens	Species	Stages	Remarks	References
OMP	Rat	E16-P2	colocalized with GnRH-neurons	Valverde *et al.* 1993
		E15-E22		Baker *et al.* 1993
		E18-P3		Pellier *et al.* 1994
CARN	Mouse	E14-E19	colocalized with OMP	Tarozzo *et al.* 1994
	Opossum	P1-P7	colocalized with OMP	Tarozzo *et al.* 1994
B-50/GAP-43	Rat	E13-after birth	colocalized with NSE	Pellier *et al.* 1994
	Mouse	E12.5-E16.5	colocalized with a subset of GnRH cells	Livne *et al.* 1993
	Rodents	neonatal and adult		Monti-Graziadei *et al.* 1993
NSE	Human	12 to 36 weeks		Boehm *et al.* 1994
	Rat	E14-P9	colocalized with a subset of UEA I cells	Pellier *et al.* 1994
	Rodents	neonatal and adult		Monti-Graziadei *et al.* 1993
UEA I	Rat	E15-P9		Pellier *et al.* 1994
CC2	Rat	E14-E19	colocalized on a subset of GnRH-ir cells	Tobet *et al.* 1992
H and B	Rat	E14-P3	determinant of ABH blood antigens	Pellier *et al.* 1994
B		embryo		Astic *et al.* 1989
GFAP	Rat	from E16	ehsheathing cells	Chuah and Au 1991 (cited in Pellier *et al.* 1994)
CONNEXIN-43	Mouse	post natal and adult	ensheathing cells	Miragall *et al.* 1992
NPY-like immunoreactivity	Rat	newborn and adult	ensheathing cells colocalized with S-100	Ubink *et al.* 1994
NEU 5	Rodents		recognized N-CAM epitopes	Carr *et al.* 1989 (cite in Pellier *et al.* 1994)
S-100	Opossum	P5-10-20	colocalized with migrating GnRH nuerons	Cummings *et al.* 1994
	Human	12-13 weeks		Boehm *et al.* 1994
KERATIN	Mouse	postnatal		Suzuki *et al.* 1994
SST	Chick	E3-E11		Murakami *et al.* 1994
FMRFamide	Bird			Northcutt *et al.* 1994
N-CAM-H	Chick	E7………	colocalized with GnRH neurons	Murakami *et al.* 1991
N-CAM	Mouse	E10.5-newborn		Schwanzel-Fukuda *et al.* 1992
		embryo	in the plasma membranes of ensheathing cells	Doucette 1990
	Rodents	neonatal and adult		Monti-Graziadei *et al.* 1993
A-N-CAM	Mouse	E13.5-adult	ensheathing cells	Miragall *et al.* 1992
E-N-CAM		E13.5-P7	ensheathing cells	Miragall *et al.* 1992
N-CAM 180		E13.5-adult	ensheathing cells	Miragall *et al.* 1992
L1/Ng-CAM		embryo	in the plasma membranes of ensheathing cells	Doucette 1990
		E13-adult	ensheathing cells	Miragall *et al.* 1992
AMOG			ehsheathing cells	Miragall *et al.* 1992
	Rat	E13/14-E19	9-O-acetylated GD3 and GQ1c	Mendez-Otero *et al.* 1994
		E12-P2	ehsheathing cells	Valverde *et al.* 1992
"Migratory mass"			ganglion cells of the terminalis nerve	
			precursor of the glial capsule of the olfactory glomeruli	
			precursor of periglomerular cells	

different cell types on the basis of their phenotypes, their location and the time dependent-evolution. Accordingly, it seems possible to identify an early group of cells, which form a blastema and are N-CAM positive [118]. In the mouse early in embryogenesis (at about E10), these cells form an aggregate, on either side of the midline, in the mesenchyme between the olfactory pit and the forebrain, in a vertromedial location. The axons of the olfactory, terminal and vomeronasal nerves, which are also N-CAM positive, grow into this cell aggregate. As development proceeds, the N-CAM immunoreactive aggregate becomes adherent to, or continuous with the rostral tip of the forebrain, and together with the central processes of the olfactory, terminal and vomeronasal nerves, form a scaffold, linking the olfactory pit to the rostral forebrain. This aggregate is not disrupted by treatment at E10 with antibody against N-CAM, whereas is significantly reduced the number of GnRH positive cells outside the placode [126].

The origin of glial ensheathing cells (corresponding to Schwann cells of the olfactory axons), which are altogether N-CAM positive, represents a further difficulty. According to Doucette [38, 39] these cells migrate early in development in the mouse (at the Theiler stage 19, corresponding to E10.5) and take a dorso-caudal route. Since the observations by Doucette are relying mainly on electron microscopic data, it remains unclear if these cells correspond, in part at least, to the N-CAM positive blastema aforementioned. A similar problem can be envisaged for the rat embryogenesis: according to Valverde *et al.* [148], from E12, developing olfactory axons from the olfactory placode are accompanied by migratory cells, also derived from the placode, that reach the prospective olfactory bulb by E13. The mass of migratory cells accumulates superficially on the telencephalic vesicle. Within the mass, the cells increase in number by mitotic divisions. Seemingly the majority of these cells represent precursor elements that will later develop into ensheathing cells of the olfactory nerves and olfactory nerve layer of the bulb. This migratory mass is also positive for 9-O-acetylated GD3 and GQ1c [82]. Subsequent experimental studies suggest that around E12 and the following days, some migrating cells enter the telencephalic vesicles or are around its dorsal and lateral surfaces. Later on there is an increase in the number of migrating elements enter the dorsal surface of the telencephalon and might contribute to the preplate [34].

Other cell types are encountered during embryogenesis. Extensive work by Pellier *et al.* [104, 106], showed in the rat, from E13 to birth, a pool of putative neuronal elements, which are neuron specific enolase (NSE)-positive, labelled by *Ulex europaeus* lectin (UEA) and B-50/GAP-43 antibody. These cells, that migrate toward the ventromedial aspect of the presumptive olfactory bulb are arising from both the medial and the lateral olfactory pits. It has to be stressed that GnRH neurons are encountered only together with the nerve fascicles from the medial pit. One prominent population of these cells along the olfactory nerves is expressing olfactory markers (such as OMP and carnosine) (Fig. 2C, D), approximately at the same time when these molecules are first seen also in the ORNs (Table 3). They are in fact immunolabelled at E16 in the rat [8, 147] and E14 in the mouse [139]. Carnosine and OMP-positive cells outside the epithelium were also seen in neonatal pups of the marsupial, *Monodelphys domestica* ([140] and personal unpublished data) (Fig. 3).

The distribution of these cells at the different develop-

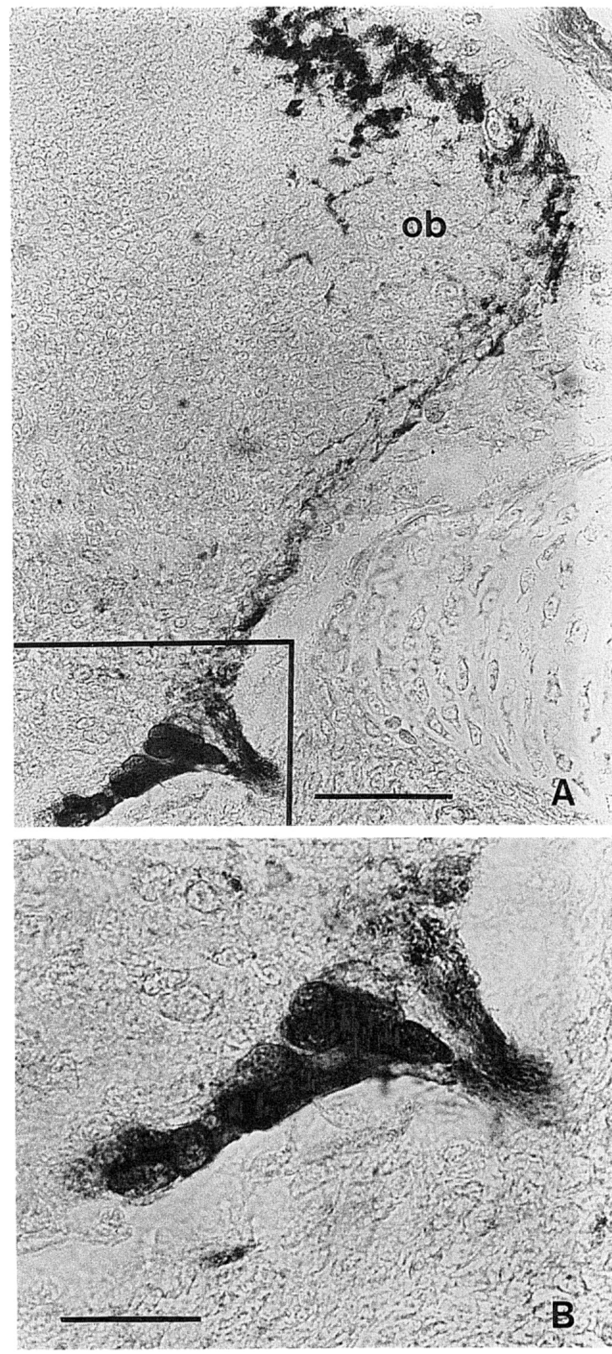


FIG. 3. Features of OMP positive cells in opossum. Cluster of OMP immunopositive cells entering in the forebrain along branches of OMP positive olfactory fibres in a P1 opossum *Monodelphis domestica* (A, scale bar, 55 μm; ob, olfactory bulb). A detail of the cluster is shown in B (scale bar, 27 μm).

mental stages strongly suggests a migratory event. While OMP and carnosine are colocalized in the same extramucosal cells (Fig. 4A, B), comparing the distribution of carnosine/OMP cells with GnRH migrating neurons in the opossum as well as in mice, we never saw colocalization even though the two populations were present in close contact. This observation, which is consistent with the results reported by Baker and Farbman [8] in rats, could account for the existence of two sets of migrating neurons, one expressing GnRH, the other expressing typical olfactory markers. Similarly, after transplantation of olfactory epithelia into adult rat brain OMP-positive cells migrate into the host tissue [10, 92]. In recent experiments made on newborn opossum CNS *in vitro* we have observed a similar migration from explants of the olfactory region to the brain (Tarozzo *et al.* in preparation) (Fig. 4C). The fate of cells expressing carnosine and OMP

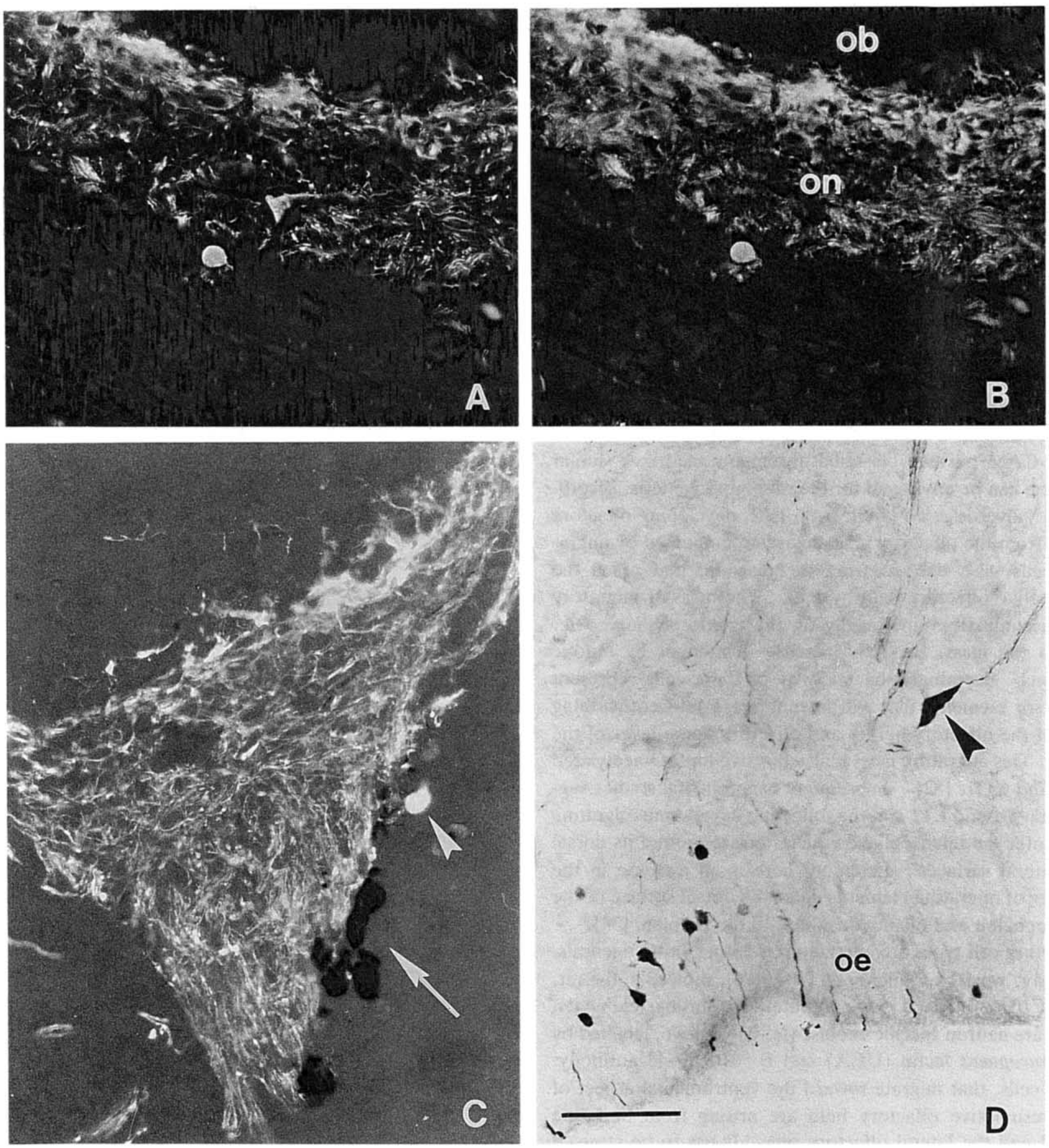


Fig. 4. Relations between different cell populations. Carnosine (A) and OMP (B) are coexpressed by the same migrating neurons (mouse embryo, E18), while no colocalization is seen between OMP (arrowhead) and GnRH (arrow), even in explants of the olfactory area of opossum pups (C); cell migration from the olfactory pit can also be followed in a transgenic line harboring a truncated portion of OMP promoter driving the *E. coli* β-galactosidase gene as a reporter. In (D) a cluster of β-galactosidase expressing cells are seen migrating along fibres of olfactory receptor neurons also stained by the anti-β-galactosidase antibody, in a transgenic mouse embryo E16 (scale bar, 55 μm).

in early life remains unknown yet. In the adult *Monodelphis domestica*, carnosine immunoreactivity was seen in cells with glial-like morphology while OMP staining was present in few subsets of fibers in the telencephalon, outside of the mediobasal areas.

In order ot assess the occurrence of olfactory marker-positive cells outside the neuroepithelium, recently an approach using transgenic mice was developed. Transgenic mice harboring 0.3 kb of upstream 5' promoter region of the OMP gene fused to the *E. coli* β-galactosidease (lacZ) gene expressed lacZ in subsets of olfactory receptor neurons [149] and in clusters of migratory cells as revealed by X-gal histochemistry performed on whole-mount preparations. Cells migrating along branches of the olfactory nerve towards the presumptive olfactory bulbs were seen from the 14th day of embryonic development (E14) until the 1st day of postnatal life (P1), while no such elements could be detected in adult animals. Further immunocytochemical characterization with a panel of different antibodies (anti-OMP, anti-*E. coli* β-galactosidase, anti-carnosine) has been carried out on sections of olfactory mucosae of transgenic mice between E14 and P1 (Fig. 4D). Double immunofluorescence labelings demonstrate that transgene-expressing migratory cells belong to the neuronal population of migratory cells described above, since some of the transgene-expressing cells are also labelled by OMP antibody.

FATE OF MIGRATING CELLS

On the whole the occurrence of several cell populations migrating into the olfactory bulb and contributing to telencephalic development seems well established. As argued in the previous section, these cells give rise to the ensheathing cells of the olfactory nerve and to the glial elements (ensheathing cells and possibly astrocytes) of the fibrous and glomerular layers of the olfactory bulb [148] and to the whole GnRH system of the prosencephalon. Some cells seemingly remain outside the brain, scaffolding the migrating elements. The fate of other cell populations is elusive. A new fascinating hypothesis can however be explored. Some cells could contribute to other neuroendocrine compartments or also may induce the development of the telencephalic vescicle. de Carlos *et al.* [34] suggest in particular that the fate of dorsally migrating cells may be the preplate. It is necessary however to identify reliable markers (natural or after cell manipulation) or phenotypic traits enabling to follow their route and fate.

As described by Murakami and co-workers in the chicken, some migratory elements express transiently somatostatin [93]. Even in rodents, transient expression could be considered for the neuronal elements migrating medially toward the telencephalon. In this case cells might change their phenotype, switching to the expression of other neuropeptide/neurotransmitter or alternatively they could be selectively eliminated. Interestingly enough, Pellier *et al.* [103] have shown, combining Hoechst 433342 DNA labelling in order to recognize pycnotic nuclei and UEA I lectin staining for migrating neurons, that some of these cells in the olfactory nerve layer may die for apoptosis. In agreement with this assumption, the peak of cell death process within the olfactory nerve layer is around E16, coincident with the stage where a marked decrease in the number of migrating neurons has been reported. Since some UEA-negative cells are showing also nuclear picnosis, Pellier and her co-workers discussed if this UEA negativity could be due to the final degenerative stage of cells initially UEA-positive, or else involved different cell populations (ensheathing cells, even their number is increasing between E16 and E19, other neuronal elements). It is tempting however an analogy to what happens in the subplate, which represents a transient neocortical structure, playing a key role in the development of connections between thalamus and neocortex [4].

INDUCTION OF THE OLFACTORY BULB BY THE OLFACTORY PLACODE

The inductive role exerted by the olfactory placode on the development of the olfactory bulb is well established in the classical model for experimental embryology, i.e. the *Xenopus* [40].

In mammals the data are scanty, but nervertheless they point for an inductive role of the olfactory placode on the development of the OB. A classic work by Giroud [50], studying experimentally induced cyclocephalic mouse embryos, could demonstrate that correlatively to the absence of olfactory nerves or the lack of connections between these fibres and the encephalon, the OB did not develop. This was shown to be true also for humans, both in terathological cases and in Kallmann syndrome. In this syndrome the OB is lost or reduced [74, 115].

The olfactory placode possibly contributed to the development of telencephalon through cell migration, but also by some inductive actions. Several points are deemed of high interest:

1) A key role for the retinoic acid (RA) in the morphogenesis of both the olfactory mucosa and the bulb was shown by La Mantia *et al.* [69]. Using an *in vitro* assay to identify sources of RA and transgenic mice to identify target domains in the developing forebrain, they were able to show that RA participates in a sequence of events that leads to the establishment of the olfactory pathway. First, the lateral cranial mesoderm activates a RA-inducible transgene in neuroepithelial cells in the olfactory placode and the ventrolateral forebrain. Then neurons and neurites begin to differentiate in these two regions and finally, olfactory axons grow specifically into the ventrolateral forebrain and remain restricted to the OB. These observations imply that RA induction and RA-receptors coordination can help to define a forebrain subdivision.

2) The site of origin of glial cells remains elusive. Recently using probes to DM-20 mRNA (which is a product of proteolipid (PLP) gene, together with PLP protein, and

represents a good marker of olygodendrocytes). Timsit *et al.* [141] were able to show that oligodendrocytes originate in a restricted zone of the embryonic ventral neural tube. In addition DN-20 expressing cells were observed in the peripheral olfactory system, to label possibly ensheathing cells. The problem is even more challenging after the observations by Liu *et al.* [72] that soluble factors from the olfactory bulb attract olfactory Schwann cells. In response to these attractant molecules, these authors hypothesize that groups of cells (in particular ensheathing cells) migrate out of the epithelium with the olfactory axons, resembling a moving carpet on which the olfactory neurites grow.

3) The incoming olfactory axons into the OB have seemingly a double action. First of all, they are not restricted to the presumptive fibrous and glomerular layers of the OB, but form exuberant projections [116]. These exuberant projections, which are subsequently pruned during the first postnatal week, could regulate the formation of the OB itself. In mouse, according to Gong and Shipley [52], some pioneer olfactory axons penetrate into the ventricular zone of the highly restricted region of the telencephalon at E13 and E14. At E15, this same telencephalic region evaginates to form the OB. Experimental observations, using bromodeoxyuridine labelling, demonstrate that the areas reached by pioneer axons show significant changes in the cell cycle kinetics and higher proliferation rate.

4) The olfactory fibres are able to induce a glomerular pattern even on ectopic targets and in adult stages. As reviewed by Dryer and Graziadei [40], after transplant experiments of the cerebellum instead of the OB or after bulbectomy, when the regenerating olfactory fibres reach abnormal telencephalic targets more caudally, glomeruli are altogether induced. This kind of data and many experimental manipulations performed on amphibians [55, 95] suggest an instructive action by the olfactory fibres themselve, but the possible role of the migrating cells which are observed also in these experiments along the olfactory nerves should be carefully explored.

AN AGENDA FOR FUTURE RESEARCH

Future research should elucidate several interesting questions.

Event cascades leading to the differentiation of olfactory-related systems

Some questions are related to the molecular genetic mechanisms controlling the fate and the regional organization of the olfactory placode and the rostral prosencephalon. Several recent reports suggest that expression patterns of homeobox and other putative regulatory genes a neuromeric organization in the embryonic mouse forebrain [109] and in the more cranial parts, including the olfactory placode, several genes should be expressed as members of the *Distal-less* related *Dlx* family [15, 102], of the *Pax* family [135], of the *Emx* and *Otx* family [16, 129, 130, 131]. These genes probably contribute to the regionalization and patterning of the rostral telencephalon. Other genes could be involved in the control of the cell proliferation and differentiation, as MASH-1 gene (the mammalian homologous to *Achaete-scute*) or the mammalian counterpart of the *Fork-head* gene [108, 138]. Genetic deletion of MASH-1 induces several abnormalities in mutant mice, involving defects in development of neuronal progenitor cells in distinct neural lineages [56]. In particular the olfactory epithelium is severely affected, since neural progenitors die at an early stage, whereas the non-neuronal supporting cells are retained. The mouse homolog (*mtll*) of the orphan nuclear receptor *tailess* is also expressed in the developing forebrain [88] and in the olfactory epithelium and may act in a cascade with MASH-1 and other developmental genes. Few other putative regulatory genes have been localized in the primary olfactory system. *Siah-2*, a gene recently identified in the mouse within a family of genes with extensive sequence similarity to the *seven in absentia* gene of *Drosophila*, is highly expressed in the olfactory epithelium, with a close time correspondence to the maturation of the ORNs [36]. FORSE-1, a positionally regulated epitope (probably a surface proteoglycan) in the developing rat CNS, specifically labels the olfactory epithelium [143, 144] and possibly represents a further way for mediating regional specification from the earliest stages of CNS development. Finally some specific transcription factors, controlling the ORN phenotype have been identified and in particular Olf-1 transcription factor. Olf-1 binds on an olfactory specific genomic motif [67] and is able to coordinate the expression of several effector genes giving the mature phenotype to the ORNs [150, 151]. In particular Olf-1 is controlling the expression of OMP gene [67]. It can be a challenging problem to see if some of these genes might be expressed also in migrating cells and play a role in the determination of their phenotype and/or cell guidance. Recently it was produced a polyclonal antiserum to the protein product (DLX-2) of the *Dlx*-2 gene, belonging to the *Dlx* family of homeobox genes, which are candidates for regulating patterning and differentiation of the forebrain [108]. These authors showed that some DLX-2 expressing cells are present in the olfactory placode, the OB and the hypothalamus, suggesting that it might be able to label migrating elements.

Cellular interactions and trophic control

Another puzzling problem is to understand what could be the growth factors (GF), the GF receptors or the activity-dependent stimulations able to warrant survival and terminal differentiation of the migrating cells or else their developmental death. Primary olfactory neurons in culture are dependent for their survival and differentiation on both extracellular matrix [22, 23], some growth factors (especially epidermal growth factor, EGF, and members of the transforming growth factor, TGF) [28] and probably co-cultured glial cells [107]. *In situ* studies localized insulin-like growth factor (IGF) [17], brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) [57] and ciliary neurotrophic factor (CNTF) [134] in the olfactory bulb, i.e. the target of primary olfactory axons. The data on neurotrophin receptor localization appear quite unclear. Expression of mRNA encoding mainly tunctated *trk*B receptors is present on primary olfactory neurons and in the olfactory bulb of cat and rat, whereas *trk*C hybridization was seen in all layers of the olfactory bulb, most dense in the mitral cell layer [35, 85]. The role and the localization of NGF low-affinity receptors in primary olfactory system are more controversial: according to some observations they are expressed during development and in regeneration of the olfactory nerve [51], whereas others were unable to correlate NGF receptors with olfactory fibres [13].

Recent data on the possible developmental functions of gaseous agents, such as nitric oxide (NO) suggest a role for this "unorthodox messenger molecule" [49] in activity-dependent establishment of connections in the early development of olfactory neurons. In fact NO synthase (NOS), is expressed in primary olfactory neurons exclusively during development or after bulbectomy [18, 114] but not in adulthood [66, 68], arguing against a role of NO in signal transduction mechanisms, as suggested by previous studies [19].

Contribution of olfactory system to the central neuroendocrine compartment

The possible contribution of migrating cells to the building of the central neuroendocrine compartments different from the GnRH system is suggested by the data since now discussed, but need further demonstration. The first step should be the identification of their final phenotypes. Another important cue could arise from the comparative data available, as recently discussed by Aubrey Gorbman [53], in order to reinvestigate the relations between the olfactory and the hypothalamo-hypophysial systems both in ontogeny and phylogeny and address the enigma of the olfactory origins and evolution of the brain-pituitary endocrine system.

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