Cell migration from the chick olfactory placode: a light and electron microscopic study

By A. S. MENDOZA,† W. BREIPOHL† AND F. MIRAGALL†

From the Institut für Anatomie, Universitätsklinikum Essen, Bundesrepublik Deutschland

SUMMARY

The differentiation of the olfactory placode in the chick has been studied using light and electron microscopy. Special attention was paid to the appearance of neuronal cells within the placodal ectodermal thickening, the migration of cells out of this tissue and the appearance of the first fila olfactoria in the differentiating olfactory mucosa.

Between the third and fifth day of incubation a large number of cells is observed leaving the base of the invaginating olfactory placode, often in contact with thin axon bundles. These cells are characterized by a well-developed Golgi apparatus, a considerable number of mitochondria and dense-core vesicles. The morphology of these migrating cells resembles that of cells observed near the basement membrane within the developing olfactory epithelium and is clearly different from the mesenchymal cells which are filled with polyribosomes. At the sixth day of incubation thick axon bundles can be observed within the epithelium and the underlying lamina propria. The possible fate of the migrated epitheloid cells is discussed.

INTRODUCTION

Cell migration from the olfactory placode was first described by His (1889) and Kölliker (1890). Since these early investigations, cell migration from the olfactory placode has been described for a variety of species: rat (Lejour, 1967), humans (O'Rahilly, 1967), rabbit (Filogamo & Robecchi, 1969), mole (Milaire, 1974), chick (Robecchi, 1972; Milaire, 1974; Mendoza, 1979; Laugwitz, 1980). Recently we have observed cell migration also from the anlage of the accessory olfactory epithelium in the rat (Breipohl and co-workers, unpublished results). Little is known about the ultrastructure and fate of these migrating cells. It is the aim of the present investigation therefore to report some morphological observations of these cells in the early chick embryo.

MATERIALS AND METHODS

HNL Nick chick eggs were incubated for 3, 3-5, 4, 4-5, 5-5 and 6 days at 38 °C. From the developing chicken embryos the olfactory epithelium was examined both light- and electron microscopically. For each stage of development some 20 animals were used. The specimens were fixed by immersion in

† Authors' address: Institut für Anatomie, Universitätsklinikum Essen, Hufelandstr. 55, 4300 Essen 1, Federal Republic of Germany.
Cell migration from the chick olfactory placode

a mixture of 3.2% glutaraldehyde and 2.6% paraformaldehyde in cacodylate buffer (0.09 M, pH 7.35). The tissue samples were dissected free while immersed in the fixative and kept in the same aldehyde solution another few hours at 4 °C. After fixation the tissue pieces were washed in Hank’s solution, post-fixed in 1% osmium tetroxide (Dalton, 1955) for 2 h, washed and block stained in dark glass bottles with 1% uranyl acetate in sodium acetate buffer (0.2 M, pH 5.0) for 30 min. The samples were then washed, dehydrated in ethanol and embedded in Epon. Semi-thin sections (1 μm thick), as used for light-microscopic study, were stained with 1% Azure II-methylene blue. Thin sections were picked up on Pioloform F-coated 200 mesh copper grids, contrasted according to Reynolds (1963) and investigated in a Philips 200 or 400 electron microscope.

To demonstrate the amount of extracellular material underneath the olfactory placode in 4-day-old chick embryo the ruthenium red reaction was performed according to the technique of Luft (1971a, b) and Mendoza (1980).

RESULTS

Light microscopy

Olfactory placodal thickening of the head ectoderm of chicken embryos was first observed between 60 and 72 h of incubation at 38 °C. The maturing sensory epithelium then showed a sharp and even demarcation from the underlying mesoderm (Fig. 1). From 72 h of incubation onwards a pronounced irregular transition between the ectodermal and mesodermal tissue appeared. This was caused by the appearance of cell groups at the epithelial base that bulged towards the lamina propria. Between 72 h and 96 h of incubation, groups of these cells migrated from the epithelium into the underlying mesenchyme (Fig. 2). Here they could be found till the beginning of the sixth day of incubation, forming epitheloid islands or elongated cellular bands towards the developing forebrain. Around the fifth day of incubation these cell groups often lay in close association with the first outgrowing olfactory axons (Fig. 3).

Fig. 1. Olfactory placode (OP) from a 3.5-day-old chick embryo. Arrows indicate the irregular contour of the epithelial base. × 590.
Fig. 2. Base of the invaginating olfactory placode (OP) from 5-day-old chick embryo. Note groups of migrating cells (MC). Arrows indicate large intercellular spaces communicating with the subjacent mesenchyme. × 590.
Fig. 3. Tangential section through the base of the invaginating olfactory placode (OP) from a 4.5-day-old chick embryo. Note groups of migrating cells (MC) in the subjacent mesenchyme in close association with developing axons (A), bv: blood vessels. × 240.
Fig. 4, 5. Olfactory epithelium from the superior nasal concha of a 6-day-old chick embryo. The epithelium is composed of five to six cell layers. Small asterisks indicate thick axon bundles within the epithelium. Large asterisks indicate axon bundles in the lamina propria. × 480 and ×1200, respectively.
Fig. 6. Base of the olfactory placode on the fourth day of incubation. Arrow heads indicate basal lamina and extracellular fibrils. Arrows show mesenchymal cell processes. $\times 15500$.

Fig. 7. Base of the olfactory placode on the fourth day of incubation. Note strong positive ruthenium red reaction in the region of the basal lamina. $\times 18500$. 
Fig. 8. Base of the olfactory placode from a 4-day-old chick embryo. Arrows indicate cytoplasmic processes of the placodal cells, CP presumably processes of the mesenchymal cells (Mes). × 12000.

Fig. 9. Base of the olfactory placode from a 3-5-day-old chick embryo. Note group of migrating cells (MC). Arrow indicates a widened intercellular epithelial space communicating with that in the underlying mesenchyme. × 4750.
Fig. 10. Group of migrating cells (MC) from a 4-day-old chick embryo. Note the well developed Golgi apparatus (G) and the presence of dense-core vesicles (arrow heads). A: axons. $\times 15350$. 
Fig. 11. Migrated cells (MC). 4-5-day-old chick embryo. Arrow heads indicate dense-core vesicles in the perikarya and in the cytoplasmic processes (P) filled with polyribosomes. Arrows indicate dense-core vesicles in the axon-like processes containing microtubules. G: Golgi apparatus. ×14700.

Fig. 12. Mesenchymal cell (Mes) and migrated cells (MC) in a 4-5-day-old chick embryo. Note the large number of polyribosomes in the cytoplasm. Arrow heads indicate dense-core vesicles of the migrating cells (MC). ×14000.
Fig. 13. Base of the developing olfactory epithelium from a 5.5-day-old chick embryo. Note the intraepithelial axon bundles (AB) which also contain abundant dense-core vesicles (arrows). N: nucleus of epithelial cells. ×11350.
Cell migration from the chick olfactory placode

At the end of the sixth day of incubation, the outgrowing axons were more numerous and arranged in thick bundles within the epithelial base and in the lamina propria (Fig. 4). In the latter, they were ensheathed by thin, elongated cells (Fig. 5). Typical epitheloid cell groups were no longer seen at this developmental stage.

Electron microscopy

Electron microscopical investigation of the developing olfactory pit revealed, up to the third day of incubation, a sharp demarcation from the underlying lamina propria and a normal width of the intercellular space between the basal feet of the epithelial cells. After this stage of development a more irregular demarcation between both tissues became obvious. From 72 h of incubation onwards growing numbers of fine processes and cell protrusions were observed that bulged against the underlying mesodermal tissue (Fig. 8). In addition to these, fine processes of mesenchymal cells were also observed in close contact with the basal lamina of the developing olfactory epithelium (Figs. 6–8). Both mesenchymal cell processes and the base of the developing olfactory epithelium gave a positive ruthenium red reaction (Fig. 7). The reaction was much stronger beneath the epithelial cells showing cytoplasmic processes and extensive bulging. During further invagination of the olfactory placode the intercellular cleft at the epithelial base was often enlarged and continued with wide intercellular spaces in the subjacent mesenchyme (Figs. 8, 9). Groups of epitheloid cells ‘leaving’ the epithelial base were characterized by a well-developed Golgi apparatus, considerable numbers of mitochondria and some cisterns of rough endoplasmic reticulum (Figs. 9–11). The main characteristic of the perikaryon of these cells was the presence of dense-core vesicles with diameters between 100 and 200 nm (Fig. 11).

In the migrating cells two different cytoplasmic processes could be observed. The first one was filled with polyribosomes and the second one lacked these but contained many microtubules. In both these cell processes, dense-core vesicles could be found (Fig. 11). The migrated epitheloid cells were similar to some cells observed within the developing olfactory epithelium and clearly different from the mesenchymal cells which were filled mainly with polyribosomes (Fig. 12). The axon bundles, which could be seen with the light microscope by the sixth day of incubation, were characterized by the presence of microtubules and mitochondria, by regularly occurring dense-core vesicles and by the absence of ribosomes (Fig. 13).

DISCUSSION

The present investigation documents the differentiation of the olfactory pit and groove in chick embryos between the third and sixth day of incubation. Special attention has been paid to the fine structure of the epitheloid cells.
leaving the differentiating olfactory sensory epithelium, and to the appearance of the first bundles of the fila olfactoria.

The ectodermal thickening occurring in olfactory placode formation is caused by a pronounced local cellular proliferation. This local acceleration of mitosis is accompanied by two morphological features: first, special apical cell contacts and secondly, special morphological differentiation at the placodal base (Mendoza, Miragall & Breipohl, 1980; Mendoza, 1980). The appearance of gap junctions may play an important role in embryonic differentiation and cell division (cf. Bennett, 1973; Loewenstein, 1973). In our opinion such a hypothesis is also supported by the investigations of Decker & Friend (1974), who found a temporary formation of gap junctions in the course of amphibian neurulation. Moreover a temporary appearance of these specialized cell contacts could be seen during olfactory placodal differentiation (Mendoza et al. 1980), and in the early stages of retinal development (Dixon & Cronly-Dillon, 1972). According to Fujisawa, Moriioka, Watanabe & Nakamura (1976) ‘the fact that the number of cells with gap junctions attains a maximum at the stage slightly before the cessation of cell proliferation may suggest that the gap junction allows the passage of some factor(s) controlling the halt of cell proliferation, which is followed by cell differentiation’. Another stimulus for local ectodermal thickening may also result from developmental changes in the underlying basal lamina.

Acid mucopolysaccharides and other acidic groups that are present in the cell coat and in the basal lamina show a positive ruthenium red reaction (Luft, 1971a, b; Blanquet, 1976a, b). As shown above the ruthenium red reaction is less strong around the mesenchymal cell processes and scarcely distributed fibrils deeper in the lamina propria than it is at the epithelial base. We believe that this is due to the assembly of extracellular material forming the underlying basal lamina (cf. Mendoza, 1980). The accumulation of extracellular ruthenium-red-positive material underneath the differentiating olfactory placode is accompanied not only by enhanced epithelial mitosis but also by a migration of cells out of the overlying epithelium. Investigating the process of neurulation in chick embryo, Ebendal (1977) has assumed that the orientation of the extracellular fibrils in the underlying mesenchyme apparently influences the direction of migrating neural crest cells. Migration of epithelial cells is influenced also by hyaluronate-containing extracellular matrix (Toole, 1973; Pratt, Larsen & Johnston, 1975). A remarkably high content of hyaluronate was shown by Ebendal (1977) in the mesenchyme surrounding the neural tube of the chick embryo. With respect to the investigations of these authors we argue therefore that the strong ruthenium red reaction that we obtained in the region of the basal lamina of the developing olfactory placode is at least partly due to an enhanced content of hyaluronate.

Migrating cells from the olfactory placode have been reported by different authors (cf. Mendoza, 1979; Laugwitz, 1980). The nature and fate of these
remains to be discovered and more detailed fine structural investigation has been missing so far. Filogamo & Robecchi (1969) have observed neuroblasts in the olfactory pit of rabbits with a Koelle-positive reaction for acetylcholinesterase. Moreover they described Koelle-positive cells among the developing fila olfactoria and they suggested that such cells were 'migratory olfactory neuroblasts'. On the other hand Robecchi (1972) stated that at the basal surface of the chick olfactory placode and in the underlying mesenchyme along the outgrowing fila olfactoria, a nodular formation of cells appears that are 'not of a nervous nature'. From the present description of the ultrastructural characteristics of these migrating cells, that shows them to be similar to some cells observed within the developing olfactory epithelium, we believe that they are migrating neurons. Whether all the observed axons within such groups of migrating cells are fila olfactoria or have to be interpreted at least partially as axons of the nervus terminalis (Brookover, 1914) requires further investigation.

The thick bundles of olfactory fila seen in the base of the developing olfactory epithelium and in the subjacent lamina propria from the sixth day onwards were not seen in later developmental stages within the epithelium (Mendoza, 1979). This fact may suggest that these bundles are formed primarily only by the grouping of developing axons from the sensory cells. Later on they will be separated in the lamina propria by Schwann cells, probably migrated from the neural crest, and within the epithelium by supporting cells. Due to the observed close association of migrated epitheloid cells with the outgrowing fila olfactoria it cannot be excluded, however, that Schwann cells of the olfactory nerve may also originate from the olfactory placode. Such an interpretation appears to agree with the investigations of O'Rahilly (1967) on the developing olfactory nerve of man.

The dense-core vesicles found in the axon bundles as well as in placodal and migrated cells have the typical appearance and diameter of norepinephric granules described by Bloom & Fawcett (1975) in the adrenal medulla and other paraneurons (Fujita, 1980). Cornwell-Jones & Marasco (1980) have found a steroid-dependent content of norepinephrine in the olfactory bulb of rats. Whether norepinephric granules do appear in neurons of the olfactory bulb of the adult chicken as well and whether such granules may eventually show an analogous behaviour as in the rat remains obscure, however.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 114) and (Br. 358/5-1).

REFERENCES


*Received 20 July 1981, revised 14 December 1981*