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REVIEW

Analysis of the Functional Maturation of Olfactory Neurons in Chicks Before and After Birth

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Abstract

There has been indirect evidence that the olfactory system of mammals could be functional shortly before birth. Taking advantage of the accessibility of bird embryos, we studied the functional maturation of the olfactory mucosa during embryonic development in birds. Using the combination of electrophysiological EOG recordings and immunohistochemical studies, it was possible to directly demonstrate for the first time that the olfactory system is functional during embryogenesis from embryonic day (ED) 13 and that the beginning of olfactory function coincides with the first localization of the calcium dependent calmodulin kinase II (CaMKIIα) in the dendrites of the olfactory receptor neurons. CaMKII and olfactory receptor genes are expressed much earlier in olfactory neurons, both involved in the sensory transduction, but the pattern of expression of CaMKIIα changes during the ontogenesis. The increase of EOG amplitude between ED13 and ED15 also coincides with the increase of the number of neurons presenting the dendritic localization of CaMKIIα. These results suggest that the enzyme CaMKII might play a role in the functional maturation of the olfactory mucosa.

Key words: CaMKII, chick, EOG recordings, functional maturation, olfactory system

Introduction

Two mechanisms are essential in the formation of a functional peripheral olfactory system: the morphogenesis of olfactory epithelium nerve and bulb, involving the correct organization of epithelio-bulbar connections, and sensory neuronal differentiation (for reviews, see Farbman, 1992; Ayer-Le Lièvre *et al.*, 1995).

In the chick, the morphogenesis of the olfactory epithelium starts at embryonic day (ED) 3, with a finger-like invagination at the level of olfactory placode, from which the nasal cavity will develop. At the same stage, a few neurons differentiate in the medial part of the olfactory epithelium primordium and send the first axonal outgrowth to contact the anterior prosencephalon. In addition, some of the newly formed neurons leave the placode and migrate along the forming olfactory nerve. The contact of the first axons with the anterior prosencephalon is known to be necessary for the induction of the olfactory bulb morphogenesis from the anterior prosencephalon (Van Campenhout, 1937). The first sign of olfactory bulb bulging out is visible around ED7, and the beginning of synaptogenesis between sensory axon terminals and the main olfactory bulb neurons, the mitral and the tufted cells, occurs between ED8 and ED10 (Ayer-Le Lièvre *et al.*, 1995).

Besides these morphogenesis events, a large range of molecules are expressed by the differentiating neurons, and these play a fundamental role in the maturation and the sensory function of these olfactory neurons between ED3 and ED10 (Nakamura, 2000). The molecules synthesized after the first sensory fiber outgrowth can be classified into three groups:

1. Transcription factors involved in neuronal differentiation: Mash-1 (mouse achaete scute homologue 1; Guillemot *et al.*, 1993), which activates a cascade of bHLH regulators in olfactory neurons progenitors (Cau *et al.*, 1997), and Cash-1 gene, which is an early chicken marker of neuronal progenitors equivalent to Mash-1 (Jasoni *et al.*, 1994).

2. Molecules necessary to signal transduction pathway, such as G protein, Golf protein and adenylate cyclase.
3. Specific markers of olfactory neurons, such as olfactory receptor (OR), which is involved in the mechanisms of odorant binding; olfactory marker protein (OMP), the function of which is still not completely known; and CaMKII, which is also involved in the regulation of olfactory response (Margolis, 1988; Buck and Axel, 1991; Farbman *et al.*, 1998).

The majority of these molecules have been identified in rodents, though some of them are still unknown in birds. From the comparison of the main stages of avian olfactory system development with rat counterparts, it is possible to hypothesize by extrapolation their stage of expression in birds (Ayer-Le Lièvre *et al.*, 1995). However, OMP has not been found in the olfactory epithelium of birds (Margolis, 1988); in the bird's olfactory system, only two types of differentiation markers have been characterized: chicken olfactory receptor (COR) and Ca^{2+}-dependent calmodulin kinase II (CaMKII).

OR and COR have been mainly demonstrated to play key roles in olfactory axonal guidance to the bulb presumptive area (Leibovici *et al.*, 1996; Mombaerts, 1996; Wang *et al.*, 1998). Nevertheless, their first expression at ED4 and the strong increase of their level of expression after the beginning of synaptogenesis until hatching (ED21) suggest that COR might be involved in the establishment of a functional olfactory system (Leibovici *et al.*, 1996).

CaMKII is well known to regulate the formation and function of several sensory systems, as well as the synaptic plasticity of the mechanisms of long-term potentiation in the neocortex (Wu *et al.*, 1998; Hardingham *et al.*, 2003). In adult rodents, evidence has been provided that CaMKII participates in complex mechanisms of odor adaptation by inhibiting olfactory adenylyl cyclase via Ca^{2+}-induced phosphorylation (Wei *et al.*, 1996, 1998; Leinders-Zufall and Zufall, 1999). Although it has been noted that CaMKII is abundantly expressed in olfactory cilia, the cellular localization and its exact function in the olfactory system is still only incompletely known (Zou *et al.*, 2002). In late embryos and postnatal chicks, the distribution changes of CaMKIIα immunoreactivity in the cell body and fibers of mature neurons permit its use as a marker of differentiation of mature neurons (Leibovici *et al.*, 1996). However, very little is known of its expression and function before birth, except that it is expressed between ED16 and hatching (ED21) in bird embryos, and its drastic decrease during the 48 h following experimental olfactory axotomy corresponds with the peak of olfactory neuron death (Leibovici *et al.*, 1996; Mathonnet *et al.*, 2002).

Regarding the functional aspect of the olfactory system in birds, behavioral studies have indicated that odor learning can take place in embryos (Tolhurst and Vince, 1976; Sneddon *et al.*, 1998; Burne and Rogers, 1999), suggesting that a functional olfactory system is available at this stage. For instance, olfactory presentations beginning by day E15–E20 were followed by recognition of the presented odors after hatching (Burne and Rogers, 1999). However, it was not known when the chemical sensitivity of the chick olfactory mucosa started. In fact, there are very few studies concerning the developing sensitivity of the olfactory mucosa of vertebrate embryos. In the rat, while anatomical olfactory differentiation (cilia genesis) in the olfactory area is completed on day E15 of embryonic life (Menco and Farbman, 1985), the first electroolfactograms in response to chemical stimuli were recorded by day E14 (Gesteland *et al.*, 1982).

Furthermore, it appears that the main molecules involved in changes of sensitivity of the olfactory mucosa in the chick are still unknown. In order to specify the involvement of CaMKII in the olfactory system, we have analyzed the spatio-temporal pattern of expression of CaMKII in the olfactory epithelium during the embryonic development of the chick olfactory system. The expression of this molecule was compared with the functional maturation of the olfactory epithelium analyzed by taking an electroolfactogram (EOG) recording of the developing olfactory epithelium at different stages of development. Early electrophysiological responses from the olfactory mucosa in fetuses or newborns have been recorded only rarely (Gesteland *et al.*, 1982; Hudson and Distel, 1998).

Materials and Methods

Animals and Embryos

Fertilized chicken eggs (*Gallus gallus* Linné) from commercial sources (commercial label chicken heterozygous for naked neck gene, Couvoir du Faget, Lot, France) were incubated at 38°C in a humidified atmosphere. Embryos were staged according to the developmental timetable of Hamburger and Hamilton (1951) (H&H). All animals were maintained in accordance with NIH guidelines for care and use of laboratory animals.

For immunocytochemistry, the embryos were sacrificed at different stages between ED10 (stage 36 H&H) and ED15 (stage 41 H&H), their heads were frozen to be sectioned on a cryostat.

For electrophysiological recordings, day 8, 10, 11, 12.5, 13, 14, 17, 18 and 20 chick embryos and 2-day-old neonates were used in this study. Embryos were obtained following the opening of the eggshell. The animals were anaesthetized by i.p. injections of equithesine (0.1–0.2 ml/ animal), a mixture of pentobarbital and chloral hydrate. Then the head of the animal was isolated and fastened on rhodorsil (Rhone Poulenc, France) in a Petri dish. The bony top of the nasal cavity was carefully excised exposing the olfactory conchae (Figure 1).

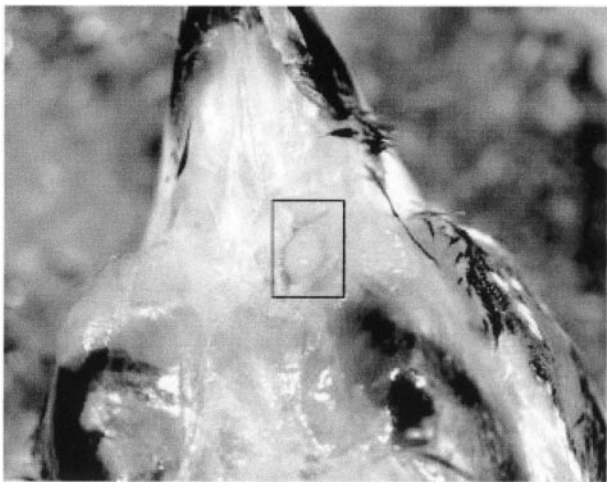

Figure 1 The olfactory conchae in a ED14 chick embryo. The skin and the bony roof of the right nasal cavity were dissected, making the superior aspect of the olfactory conchae accessible to EOG recording and odorant stimulation. The rectangle delineates the limits of Figure 4.

Immunocytochemistry with anti-CaMKIIα antibodies

Serial 12 μm cryosections from the heads of chick embryos were fixed in 4% paraformaldehyde and treated overnight at 4°C with anti-CaMKIIα antibodies, diluted 1/100 (Roche Molecular Biochemistry, Germany), which are specific for the α sub-unit. After rinsing in 1× phosphate-buffered saline, the second antibody, an anti-mouse immunoglobulin conjugated with horseradish peroxidase (anti-mouse-POD; Sigma, St Quentin Fallavier, France), was applied. The immunostaining was revealed using diaminobenzidine (Roche Molecular Biochemistry, France) as the substrate. Microscopic observations were made using a Leica stereomicroscope (Leica System, Toulouse, France), with Nomarsky equipment. The pictures were taken using a digital camera and stocked using the Leica Lida Lite base (Leica system).

Odorant delivery system

In a preliminary study, we used 1,8-cineole, carvone, oct-3-en-1-ol, 5α-androst-16-en-3-one, galaxolide, cedryl acetate (a gift from Givaudan), β-ionone, phenylethyl alcohol, trimethyl amine, isovaleric acid, anisole, acetophenone, cadaverine, purified Civette extracts (a gift from Firminich), 2-heptanone, limonene (–), cyclohexanone, vanilline, β-hexenol, iso-amyl acetate. In this case, odorized puffs were delivered using syringes.

During the main experiment, four chemical stimuli were delivered using a gas dilution olfactometer. The gas used as vector or dilutor was charcoal-filtered air, water saturated and delivered at room temperature (25°C). The odorized gas was obtained by rinsing a glass flask filled with polymeric pellets adsorbing pure liquid odorant chemicals. The final stimulating gas was prepared diluting the airflow saturated by the odorant vapors by a flow of pure air before delivery. The stimulus was delivered to the olfactometer nozzle switching pneumatically the pure air by the odorized air. The operator started the sequence, i.e. the stimulus preparation (30 s), stimulus delivery (2 s) and the delay (1 min) between the stimuli. The ratio between the odorized air and the vector air was settled at 1/5: the concentration of the delivered stimulus was 1/5 of the saturated vapor of the tested chemical. The stimulating flow was fixed to 75 ml/min and the delivery nozzle, inner diameter 2 mm, was placed at 1 cm in front of the olfactory conchae.

Four pure compounds (*purum* or >99%) were chosen to test the sensitivity of the olfactory mucosa of the chick embryos and neonates: isoamyl acetate, 1,8-cineole, oct-3-en-1-ol (Aldrich) and lilial or 2-methyl-3-(4-tert-butylphenyl) propanal (Givaudan).

EOG recordings

EOG were recorded using polyethylene electrodes filled with a saline gel (agar–agar, 9 g/l NaCl), tip diameter 0.5 mm, inserted on an Ag/AgCl wire. A reference electrode (Ag/AgCl, 1 cm^2) was wrapped in a wet gaze and inserted under the head of the preparation. The electrical signal was amplified by a conventional DC amplifier and digitized (Daqcard-516, 16 bits, National Instruments) before visualization and storage using a homemade program (sample rate 100 Hz, LabView). For each recorded sequence, a 1 mV calibration top which was used for the normalization preceded the chemical stimulation. The maximum amplitudes of the EOG responses were determined off-line by a peak/valley determination procedure (Windaq, DataQ Instruments) and normalization.

Results

Spatio-temporal expression of CaMKII between ED10 and ED15

The spatio-temporal expression of CaMKII between stages ED10 and ED15 is shown in Figure 2.

Stage ED10

At ED10 (Figure 2A), the immunoreaction with anti-CaMKIIα antibodies showed that the expression of CaMKIIα was limited to the inferior third of the olfactory epithelium corresponding to a majority of basal cells, but also including at this stage a high percentage of early differentiating olfactory neuroblasts. It is likely that the immunostained cells could be a globose cell subpopulation that constitutes the main neuronal precursors. A few positive patches in the submucosa between the ectodermal epithelium and the cartilage of the nasal conchae corresponded to cross-sections of olfactory nerve fibers (arrows).

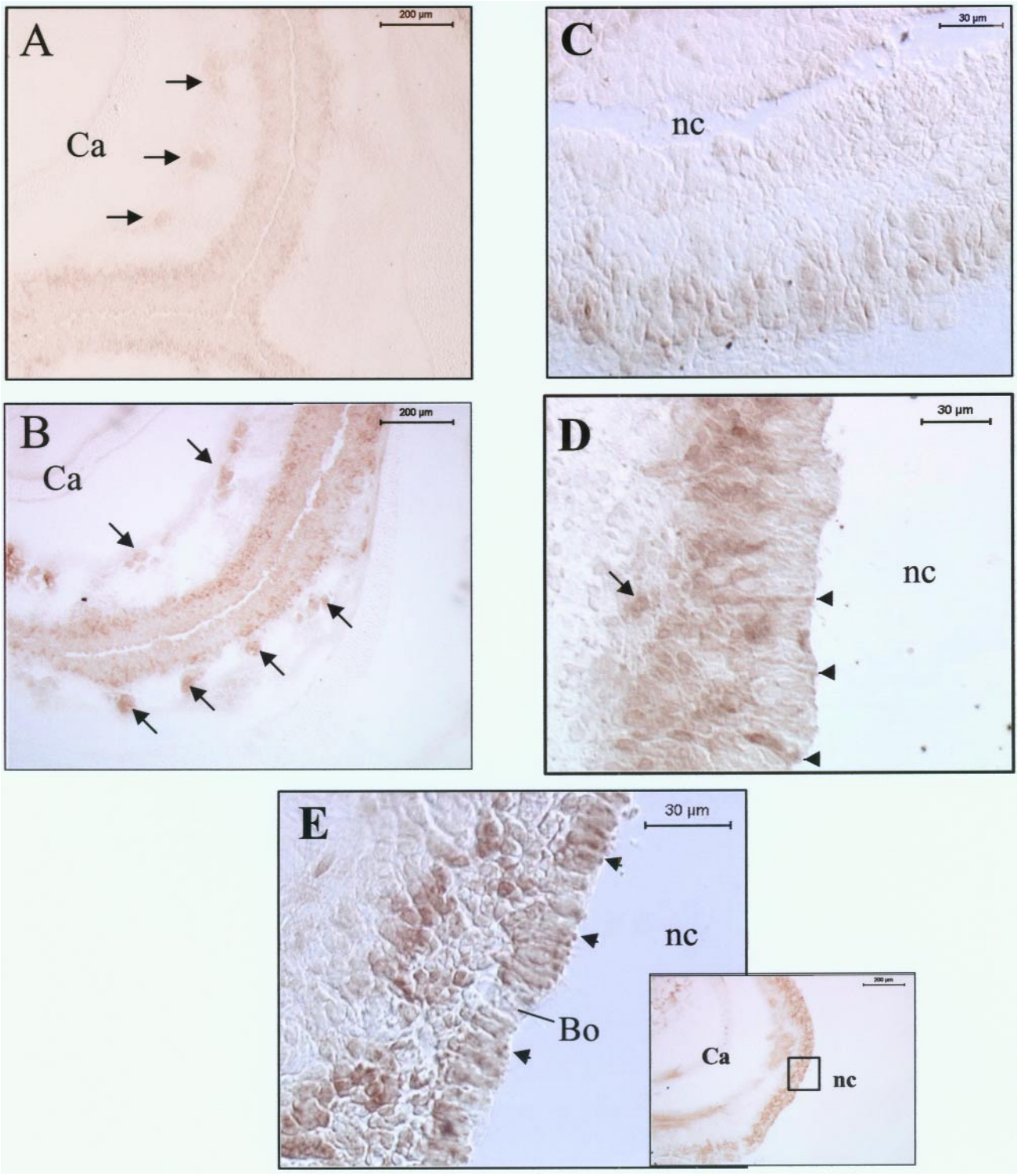

Figure 2 Changes in the expression pattern of CaMKIIα in chicken embryos between ED10 and ED15 (coronal sections). During the first stages of CaMKIIα expression in the peripheral olfactory system the expression pattern is similar at ED10 **(A)**, ED11 **(B)** and ED12 **(C)**. The labeling is first restricted to mainly basal cells close to the basal lamina, in the olfactory epithelium, and then appears in the cell bodies of a few immature neurons. The axonal bundles can be visualized outside the basis of the basal lamina (arrows) (nc, nasal cavity). From ED13 **(D)**, the number of CaMKIIα positive neuronal cell bodies in the median part of the epithelium increases. A few of them extend dendritic positive fibers, ending as positive dendritic knobs (arrowheads) to the apical epithelial surface. At ED15 **(E)** (higher magnification), the density of CaMKIIα immuno-positive dendritic knobs and cilia of the sensory epithelium has already drastically increased. Some cell bodies of immature neurons and basal calls are still also labeled The lower magnification allows to check that the observed region is situated in the superior conchae of the sensory olfactory epithelium. ca, cartilage of the superior conchae; Bo, opening of a Bowman's gland primordium.

Stages ED11 and ED12

There were only limited changes in the expression pattern of CaMKIIα between ED10 to ED12. However, the intensity of labeling and the density of positive cells were increased. The immunostained cells were still mainly localized to the basal third of the olfactory epithelium, but an increasing number of positive cells reached the median part of the olfactory epithelium and a few of them presented a bipolar neuron-like spindle shape, in agreement with the fact that this region includes mainly cell bodies from mature and immature neurons, the later being the more numerous at these stages [ED11 (Figure 2B), ED12 Figure 2C)]. The number of positive fibers in the submucosa also increased.

ED13

The brown staining corresponding to the CaMKIIα immunoreactive cells was not restricted only to basal cells and cell bodies of immature neurons, in contrast with the patterns observed at previous stages (ED10–ED12). At ED13 (Figure 2D), CaMKIIα expression in mature neurons localized in the medial third part of the olfactory epithelium was not limited exclusively to the cell bodies, but was also detectable in a few dendritic fibers up to their dendritic knobs at the apical limit of the epithelium with the nasal cavity (arrowheads).

ED15

Two main changes in the immunostaining pattern were observed at ED15 (Figure 2E). First, this stage was characterized by the shifting of most positive cell bodies from the basal third to the median third of the epithelium. Secondly, the staining was more intense in dentritic terminals crossing the apical third of the olfactory epithelium and in the corresponding dendritic knobs bulging into the lumen of the nasal cavity.

A few Bowman's gland primordia were also detected at this stage, but their epithelial cells were not immunoreactive to the CaMKIIα antibodies, the immunostaining being restricted to the neuronal subpopulation of the epithelium.

Distribution of anti-CaMKIIα immunoreactive cells in the different regions of the olfactory epithelium

At the beginning of CaMKIIα expression, at ED10 the positive immunoreaction was limited to the olfactory epithelium. The distribution of positive cells was identical along the developing olfactory epithelium lining both the cartilage of the upper nasal conchae and the corresponding part of the nasal septum.

In the following stages, positive cells and fibers were detected on both side of the deep part of the nasal cavity, but they were more dense in the lateral epithelium covering the conchae than in the septal part of the epithelium or in the epithelium surrounding the very deepest aspect of the cavity. This contrast between the two sides (medial and lateral) increased particularly from ED13 to ED15.

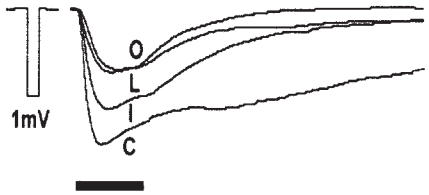

Figure 3 Electrophysiological responses (EOG) of the olfactory mucosa of a chick neonate to the different odorant chemicals. The responses were recorded successively at the same point and presented different shapes specific of the chemical stimuli. C, 1,8-cineole; I, isoamyl acetate; L, lilial; O, oct-3-en-1-ol. Horizontal bar, time = 1 s.

Electrical responses to chemical stimuli

During a preliminary experiment, we observed that all 20 odorant stimuli delivered were able to elicit typical electrical responses from the olfactory mucosa of 2-day-old neonates. Air delivered in the same conditions did not elicit any response. In the main study, the four chemical stimuli elicited EOG responses in all the 2-day-old preparations tested. EOG responses are odorant specific in amplitude and shape, as illustrated in Figure 3. Due to the duration of the stimulation and the dynamic of the transduction processes, the presence of a short plateau can be observed. The maximum of the response corresponds consistently with the first curve shoulder occurring after the beginning of the stimulation. The response shapes are similar to those recorded in other animal species. For instance, cineole usually elicits a long-lasting depolarization.

The amplitude of the responses to a given stimulus depends on the recording site. Thus, in order to compare electrophysiological sensitivities on preparations of different ages, we decided to record the response from a unique point, in the middle of the antero-posterior axis and on a medial location of the exposed aspect of the conchae (point 1 in Figure 4), which was found most consistently sensitive to the chemical stimulation.

No electrophysiological responses could be recorded in 8- to 10-, 11- or 12-day-old embryos ($n = 11$). Small but clear responses to the chemical could be observed on day 13 (eight embryos), and from this stage the mean of the response amplitudes gradually increased with time (Figure 4). Data were distributed into four classes of ages for statistical analyses ($n_{min} = 3$, $n_{max} = 13$) and were analyzed using one-way analyses of variance (Figure 5). Differences between EOG amplitude means were considered significant at $P < 0.05$. A first ANOVA was achieved on the total data matrix (4 odorant chemicals × 4 periods). The analysis confirms a temporal effect ($F = 35.344$, d.f. = (3,3), $P < 0.001$) but did not reveal a clear odor effect ($F = 2.445$, d.f. = 3,3, $P = 0.067$).

The analyses by odorant show that the EOG amplitudes increase during ontogenesis ($F_{cin} = 12.184$, d.f. = 3, $P = 0.000$;

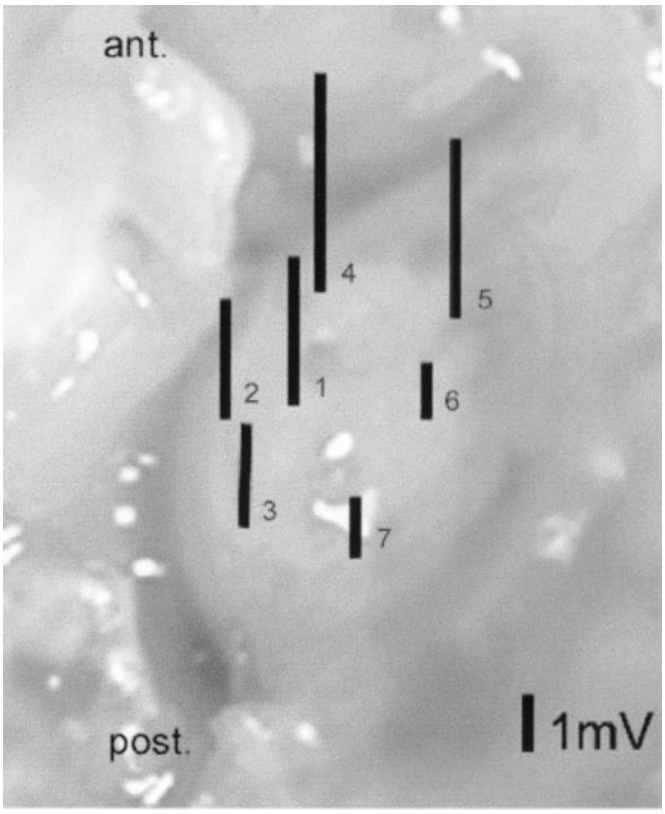

Figure 4 Spatial variations of the sensitivity of the olfactory mucosa to isoamyl acetate stimulations. As measured on the dorsal aspect of the conchae in a chick neonate, the amplitude of the electrophysiological response to a given stimulus (isoamyl acetate) depends on the site of the recording electrode (locations are labeled 1–7).

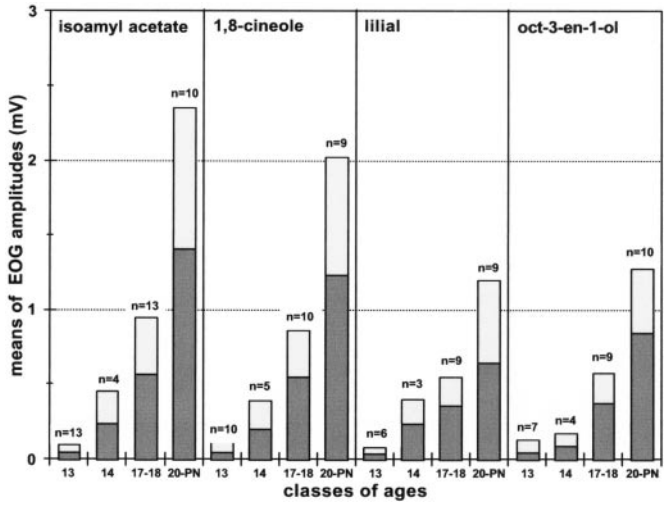

Figure 5 Means of the maximum amplitudes and standard errors of the EOGs recorded in chicken at different stages of the ontogenesis. Data were distributed into four classes of ages, corresponding respectively to embryos of 13 days; 14 days; 17 and 18 days; finally embryos of 20 days and neonates of 2 days. An increase of the sensitivity to the chemical stimuli with age can be observed. The effect is not odorant specific. At the 2 day neonate stage, while the stimuli were delivered in identical dilutions, isoamyl acetate and 1,8-cineole appeared more efficient than oct-3-en-1-ol or lilial.

F_{iso} = 13.211, d.f. = 3, P = 0.000; F_{lil} = 3.941, d.f. = 3, P = 0.020; F_{oct} = 14.007, d.f. = 3, P = 0.000). The obtained electrophysiological response amplitudes for the ED20 to post-hatching period are significantly greater than those of the previous periods. For cineole and isoamyl acetate only, the EOG amplitudes obtained during the third period (17–18 days) are significantly greater than those of the previous periods.

Discussion

Chemical sensitivity of the olfactory mucosa in the chick embryo

Specificity of the responses

For the first time, we observed electrical responses of putative olfactory areas to chemical stimulation in pre- and post-hatching chicks.

As the air stimuli were unable to elicit responses, the observed potential variations following odorant stimuli can be interpreted as true chemical responses, EOG. In several experiments, we have also observed that some small displacements (<1 mm) of the recording electrode on the mucosal surface from a responsive site could extinguish the response. In addition, despite the physical consistency of the stimuli, the variety of the response shapes can be considered as an indication of the specificity of the sensitivity.

Increasing responses with age

We further observed that as the age of the preparation increases, the maximum amplitudes of the responses increased. Taking into account that no significant difference could be shown between the four tested odorants, the EOG variation seems to be independent of the stimulus quality.

An increase in the sensitivity of the olfactory mucosa to the chemicals can result from different processes: (i) each of the cells participating to the response could be more sensitive. This could be the result of biochemical improvement of elements of the receptor/transduction cascade. As, in chick embryo, a receptor expression can be expected from ED4 (Leibovici *et al.*, 1996), the implication of other regulatory elements of the transduction in the olfactory maturation must be envisaged. (ii) The density of responsive cells in the olfactory mucosa could increase as the density of mature olfactory neurons. This is also in agreement with the localization of the zone of the olfactory mucosa where the high sensitivities were observed. At ED16 this zone exhibited the highest density of olfactory neurons expressing olfactory receptors belonging to the three identified COR subfamilies (COR2, 3, 4) (Leibovici *et al.*, 1996), thus corresponding to the highest density of functional mature olfactory neurons.

The observed changing subcellular localization of the CaMKII in the depth of the olfactory mucosa (in fact, in

neuronal compartments) can be correlated with functional changes, while the increases of CaMKIIα-labeled cells suggest that the number of mature neurons does increase.

An increase of EOG responses during the olfactory ontogenesis has been mentioned only in the rat (Gesteland *et al.*, 1982). Another model has been tentatively proposed to study the developmental properties of the olfactory mucosa: increasing EOG responses during the regeneration following olfactory mucosa lesions have been observed (Simmons and Getchell, 1981; Lidow *et al.*, 1987; Delaleu and Sicard, 1995). In these conditions, the authors could correlate the functional recovery with the increasing density of the mature neurons. As in the present study, in such models, the recovery as estimated by EOG recordings seemed to be chemically unspecific (Lidow *et al.*, 1987).

The beginning of the olfactory function

At ED13, in a few cases, we observed small responses to the odorant stimulation (see Figure 6). However, the absence of any detected EOG response before ED13 does not necessarily mean that no mature neurons are present in the olfactory mucosa (Lidow *et al.*, 1987). According to several observations, the appearance of cilia which are essential to the olfactory sensitivity (Simmons and Getchell, 1981; Mair *et al.*, 1982; Adamek *et al.*, 1984) is a good criterion for determining the functional maturity of the olfactory neurons

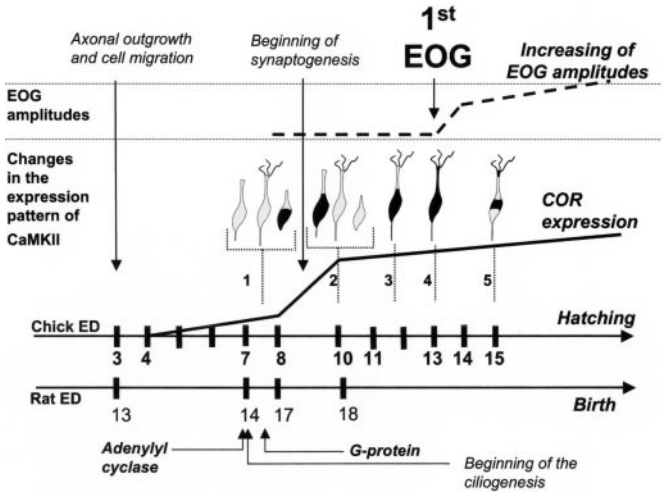

Figure 6 Comparison of the time lines, showing the stages at which the molecules involved in signal transduction pathway are expressed in rat olfactory epithelium with changes of expression pattern of CaMKII in the olfactory sensory neurons from chick embryos. (1) The basal cells express CaMKIIα. (2) CaMKIIα is present in immature neurons. (3) A majority of mature neurons express CaMKIIα. (4) The first EOG recorded correspond to the beginning of expression of CaMKIIα in dendritic extensions and in few dendritic knobs. (5) Changes of cell localization of CaMKIIα in dendritic knobs and cilia coincide with the increasing of the amplitude of EOG recordings (dotted line). A curve represents the evolution of the level of expression of COR (dark line) and some important developmental events are mentioned in italics at the top and at the bottom of the scheme.

(Menco and Farbman, 1985). While a mucosa presenting few ciliated olfactory neurons failed to show any EOG response, a relationship between the density of ciliated olfactory neurons and the EOG amplitudes has consistently been put forward (Lidow *et al.*, 1987). In the chick embryo, immature neurons with star-like emerging profiles have been observed at ED7, while more mature neurons bearing long cilia have been observed at ED11 (Breipohl and Fernandez, 1977). The expression of an EOG could thus be a matter of the number of responsive neurons, and as a consequence, the first time that a working odor representation appears at the periphery of the olfactory system cannot be determined precisely on the basis of EOG recordings only. The question is even more complicated considering that the selectivity of the rat olfactory neurons could increase during the ontogenic process (Gesteland *et al.*, 1982). So, the detection of a response on ED13 suggests that the neurons are functional at least by ED13.

The correlation at ED13 between the first EOG recorded after stimulating the olfactory mucous membrane with different chemical stimuli and the changes of the cellular distribution of CaMKIIα suggest that this enzyme might play a role in the functional maturation of the olfactory mucosa.

The possible candidates in the functional maturation

Several molecules, such as those of the signal transduction pathway or COR, have been considered as potential candidates in the final maturation of the olfactory system. However, these two elements are expressed in the olfactory cilia before the embryonic stage ED13. So, the equipment in COR and in the main molecules involved in the transduction pathway is complete by the time CaMKIIα starts to be expressed in the dendritic terminals at ED13. These data suggest that all the elements necessary to a functional system are expressed and present in dendritic knobs and cilia before the cell localization of CaMKIIα is modified at ED13, but not sufficient for the elaboration of an electrical response. Therefore, CaMKIIα in dendritic terminals and knobs appears to constitute a critical element in the functional maturation.

Involvement of CaMKIIα in the control of maturation in other systems

Changes of CaMKIIα localization have been reported several times in other systems. Thus, in rodent forebrain embryogenesis, changes in the subcellular distribution of CaMKIIα has already been shown (Kelly and Vernon, 1985; Kelly *et al.*, 1987), and those authors made the assumption that CaMKIIα accumulation in a specific area could represent an important process in the molecular and enzymatic maturation of CNS postsynaptic structures, although the precise mechanisms of action of CaMKIIα remain unknown.

The specific role of CaMKIIα in the retinotectal system has been identified more recently. Its activity appears to be necessary to limit dendritic growth and stabilize dendrite morphogenesis. Therefore, it might act as an activity-dependent mediator of neuronal maturation and promote the maturation of the system (Wu and Cline, 1998).

In comparison with the first analysis of CaMKIIα in various systems, the traffic of CaMKIIα in the olfactory sensory neurons at a specific stage and the first EOG recordings at the same stage point to the role of that molecule in the functional maturation of the olfactory system.

Other roles of CaMKIIα in the olfactory system

The importance of CaMKIIα in regulating the formation and function of several sensory systems is well known. However, most findings concerning the role of CaMKIIα in the olfactory system have been from postnatal animals (rodents). In the olfactory system, the only developmental studies emphasizing the expression pattern of CaMKIIα have been performed in the mouse olfactory bulb, in which CaMKIIα activity could regulate the genesis and the maintenance of granule cell spines, and ultimately the formation and function of the reciprocal dendro-dendritic synapses between the granule and the mitral/tufted cells (Zou *et al.*, 2002). In the peripheral olfactory system, the experiments concerned mainly the critical role of the enzyme in the regulation of the sensory system. Previously, it has been demonstrated that CaMKIIα is expressed abundantly in olfactory cilia and inhibits olfactory adenylyl cyclase via Ca^{2+}/calmodulin-induced phosphorylation (Wei *et al.*, 1996, 1998). In order to test whether CaMKIIα could be implicated in the desensitization process, experiments consisted in disrupting this molecular step. The blockade of CaMKIIα by a specific inhibitor AIP (autocadmide-2-related inhibitory peptide) provokes impaired odor adaptation. This result supports the hypothesis that CaMKIIα-mediated attenuation of adenylyl cyclase activity plays a key role in the phases of odor adaptation (Leinders-Zufall and Zufall, 1999; Zufall and Leinders-Zufall, 2000). Consequently, our developmental study is the first evidence of changes in the expression pattern of CaMKIIα and its relation with the functional maturation of the peripheral olfactory system. The correlation which is established between the modification of CaMKIIα subcellular distribution and the first EOG recording permits us to hypothesize that the beginning of the electrophysiological response of the olfactory neurons to chemical stimuli demonstrating the chemical sensibility of the olfactory mucous membrane might be closely linked to the cell localization of CaMKIIα in the dendritic terminals.

At the critical stages of ED13–ED15, the analysis of the EOG recordings demonstrated that the range of the EOG recorded increased when the majority of CaMKIIα enters into the dendritic knobs close to the cilia. The presence of the enzyme in the dendritic extension or in few dendritic knobs at ED13 and the delocalization of its expression pattern in the dendritic knobs during the following stages support the hypothesis that the cell localization of CaMKIIα could play a key role in the functional maturation of the olfactory system. CaMKIIα could also participate in the functional maturation processes by stabilizing the dendritic tree, represented here by dendritic cilia bearing the COR and emerging in the nasal cavity. As has been proved in other systems, this function could be a determinant for the maturation of olfactory neurons (Wu and Cline, 1998). Thus, CaMKIIα may play a dual role in the olfactory neurons in favouring the maturation process and in participating in the desensitization mechanisms. These changes also contribute to the adaption of the sensory olfactory system to its definitive function.

During a bird's embryonic life, the nasal cavity is bathed by liquid and the nostrils are closed until the beak penetrates the inner shell membrane (our own visual observations suggest that the olfactory mucus changes during this period). Consequently, the olfactory mucosa can access a gaseous environment only during the last 2 days before hatching. Taking the present results into account, the olfactory epithelium seems to be mature earlier, as are other parts of the olfactory system, and can discriminate stimuli dissolved in the amniotic fluid. Nevertheless, the present study shows that the olfactory mucosa precociously responds to molecules that are good airborne stimuli, and thus is ready to face a new (and future) environment. The functional significance of the precocity of this chemical sense can be hypothetically related to the maturation of sensorimotor ability, this sense serving as a template for sensory and cognitive operations, preparing the animal for access to a new world.

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