The migration of luteinizing hormone-releasing hormone (LHRH) neurons from the medial olfactory placode into the medial basal forebrain

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Summary. Over the years, investigators have noticed, in a wide variety of species of vertebrates, large numbers of cells migrating from the olfactory placode to the forebrain. These cells were considered to be Schwann cells or ganglion cells of the terminalis nerve. Recently, immunocytochemical localization studies have shown that many of these migrating cells contain luteinizing hormone-releasing hormone (LHRH), a brain peptide that regulates reproductive functions by evoking the release of luteinizing hormone and follicle-stimulating hormone from the anterior pituitary gland. The origin of LHRH cells in the epithelium of the medial olfactory placode, their migration across the nasal septum and into the forebrain, with branches of the terminalis nerve, also a derivative of the medial part of the olfactory placode, has led to some interesting speculations, from evolutionary and physiological perspectives, about the origin of these cells and the role of the terminalis nerve in their migration.
Key words. Terminalis nerve; olfactory pit; nasal placode.

Development of the olfactory placodes

In most vertebrates, the olfactory placodes appear about midway in gestation as thickenings of the ectoderm on the ventrolateral sides of the head. Within a day, the thickened epithelia of the olfactory placodes invaginate to form the olfactory pits, and soon after a secondary recess, the anlage of the vomeronasal organ, forms in the medial wall of either olfactory pit. The right and left olfactory pits are separated from each other by the developing nasal septum. The terminalis and vomeronasal nerves arise from the medial part of either olfactory pit and the olfactory nerves arise from the dorsal regions of the pit, just lateral to the anlage of the vomeronasal organ. The central processes of the olfactory nerves grow toward the forebrain and terminate in the main olfactory bulb. The central processes of the vomeronasal nerves also grow toward the forebrain but these axons end in the accessory olfactory bulb, found on the dorsocaudal aspect of either main olfactory bulb. The terminalis nerves, in contrast to the olfactory and vomeronasal nerves, are accompanied by clusters of ganglion cells as they cross the nasal septum and enter the mediobasal forebrain in company with branches of the anterior cerebral artery. The central processes of the terminalis nerves have been traced into the medial septal and the medial preoptic nuclei[22,23,25].

General phenomenon of cell migration from the olfactory placode

The migration of large numbers of cells from the olfactory placode during development has been observed in a wide variety of species of vertebrates including mice[9]; rats[11,24]; frogs[21]; turtles[22]; porpoise[33]; whales[7]; swine[2]; chickens[27,28,55] and humans[3,34,36,37]. At least two types of cells have been described in migration from the epithelium of the olfactory placode[9,11,24,27,54], one of which is considered to be a progenitor of the Schwann cells which accompany fascicles of the olfactory nerves, and the other ganglion cells of the terminalis nerve. Pearson, in his studies of the development of the olfactory[37] and terminalis nerves[36] in human fetuses, traced the migration of cells from the medial part of the olfactory placode along fibers of the terminalis and vomeronasal nerves into the ganglion terminale[25]. This is the major ganglion of the terminalis nerve and it is found medial to the olfactory nerves on the cribriform plate of the ethmoid bone. Pearson noted that the external limiting membrane of the brain had disappeared at the point of contact between the ganglion terminale and the forebrain, and that fibers of the terminalis nerve continued into the forebrain. He interpreted the continuous stream of cells that accompanied these fibers as a 'migration of cells from the brain out into the ganglion', and he suggested that the terminalis nerve develops from sensory components derived from the olfactory placode and autonomic components derived from the forebrain.

Influence of olfactory placode-derived elements on differentiation of the forebrain

The influence of the olfactory nerves on development of the olfactory bulb and forebrain was observed in experimental studies in *Amblystoma*, in which removal of the nasal placode or pit resulted in an absence of the olfactory bulb and a reduction in size of the forebrain[8]. These data were extrapolated to human arhinencephalic fetuses[16], in which the arhinencephaly was postulated to be the result of defective development of the olfactory placode. The strongest evidence in support of this postulate came from the experimental studies of Graziadei and co-workers in mammals[12,13] and in amphibians[26,53]. These investigators showed conclusively that elements of the embryonic olfactory placode affect the organization of the forebrain[6].

Origin and migration of LHRH cells from the olfactory placode

Interest in the olfactory placode, in this laboratory, stemmed from the discovery, in mice, that luteinizing hormone-releasing hormone (LHRH) neurons originate in the olfactory placode and migrate into the forebrain along branches of the terminalis and vomeronasal nerves[48,49] (fig. 1). Similar observations in another strain of mice[59,60], in rhesus monkeys[41] and in gray opossums[1] are at present being extended in our laboratory to other species of vertebrates, including rats, chickens and humans.

Luteinizing hormone-releasing hormone, (also known as gonadotropin-releasing hormone or GnRH), is a decapeptide found in the brain of all species of vertebrates, including humans, and it is essential for reproductive functions. It regulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the gonadotropic cells of the anterior pituitary gland[14,42] and it facilitates reproductive behavior[29,38]. In adult animals, LHRH has been localized, by immunocytochemical procedures, in neurons and fibers in specific areas of the brain, including the septum, the preoptic area, the organum vasculosum of the lamina terminalis, the hypothalamus and the median eminence[52]. In addition, it has been found in a population of ganglion cells and fibers of the terminalis nerve in guinea pigs[43,44], rats[19,45,58]; mice[47]; hamsters[18,56]; opossums[46]; rhesus monkeys[41,57]; platyfish[15]; dolphins[39] and amphibians[31,32].

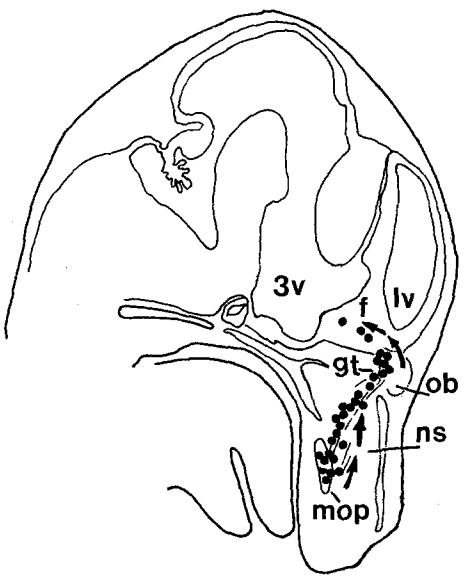

Figure 1. Microprojection drawing of an 8-µm sagittal section through the whole head of a 14-day-old fetal mouse. The migration pathway of LHRH neurons (represented by black dots) is shown from the epithelium of the medial olfactory placode (mop), across the nasal septum (ns), through the ganglion terminale (gt), seen caudal to the olfactory bulb (ob), and into the forebrain (f). Fibers of the terminalis and vomeronasal nerves (fine black lines) accompany the LHRH cells along the migration route. Third ventricle: 3v; lateral ventricle: lv.

In immunocytochemical localization studies of the development of LHRH neurons in guinea pigs, rats and opossums[44–46]; teleost fishes[30]; and platyfish[15] LHRH-immunoreactivity was first detected in the nose, in ganglion cells of the terminalis nerve before it was seen in any other area of the brain. Muske and Moore[31,32] observed that all LHRH immunoreactive cells, in the amphibian *T. granulosa* were found along or close to the terminalis nerve, a finding which prompted them to speculate that LHRH cells are 'embryologically derived from the nervus terminalis, which originates from the olfactory placode....' Similarly, Munz and Class[30], in teleost fishes, observed '...all GnRH neurons innervating the hypothalamus and pituitary region seem to belong to the terminalis nerve.' In other species, specifically guinea pigs[44], rats[19,45], and opossums[46], also Abraham and co-workers (personal communication), LHRH-immunoreactive neurons were seen only occasionally in the epithelium of the olfactory placode, and were found for the most part in streams of migrating cells just outside of the placode, on the nasal septum. These data lead us to speculate that while LHRH neurons may originate in the olfactory placode, in these species, the majority of the LHRH cells begin synthesis of the decapeptide (recognized by antibodies to LHRH) after they have migrated out of the placode. On the other hand, fetal rhesus monkeys are similar to mice, in that LHRH-immunoreactive neurons are seen first in the nasal epithelium and then with nerve fibers on the nasal septum[41].

This past year, we had the rare opportunity to examine the brain and nasal regions of a human fetus that had Kallmann syndrome (hypogonadotropic hypogonadism with anosmia[20]), and to compare the distribution of LHRH-immunoreactive cells and fibers in this fetus with those of three normal fetuses of the same age and sex[50]. In the three normal fetuses we found LHRH-immunoreactivity present in cells and fibers in the brain and a few LHRH cells in ganglia of the terminalis nerve on the nasal septum. The pattern of distribution of LHRH-immunoreactive neurons and fibers was in agreement with descriptions of the LHRH systems in human fetal brains, provided by other investigators[1,35]. In the Kallmann fetus, we did *not* detect any LHRH-immunoreactivity in the brain. However, in the nose of this fetus, on either side just lateral to midline, we found thick fascicles of LHRH-immunoreactive fibers and clumps of LHRH-immunoreactive cells, ending in the meninges on the cribriform plate, below the forebrain. The distribution of LHRH-immunoreactivity in the Kallmann fetus was restricted to the nasal regions, in a pattern consonant with that of the terminalis nerve[4], an important component of the migration path of LHRH neurons early in development. In the Kallmann fetus, the olfactory bulbs were not present, and neither the olfactory, vomeronasal nor terminalis nerves were in contact with the brain. We surmise that the absence of LHRH-immunoreactivity in the brain of this fetus is due to a failure of the LHRH neurons to

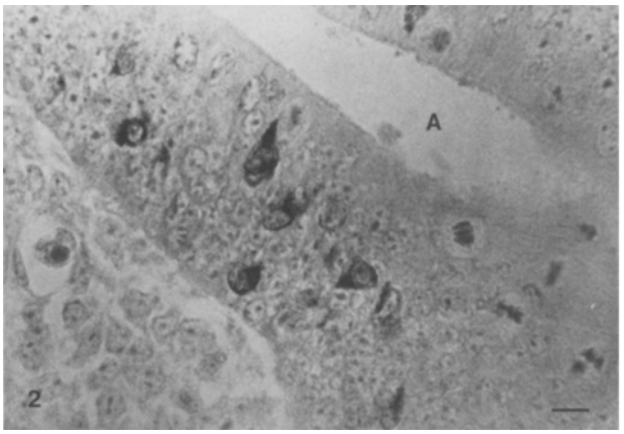

Figure 2. The medial olfactory placode (anlage of the vomeronasal organ) of an 11-day-old fetal mouse. LHRH-immunoreactive neurons, oriented perpendicular to the apical (luminal) surface (A) of the developing vomeronasal organ, are seen among non-immunoreactive cells in the apical, intermediate and basal layers of the epithelium. 8-µm sagittal section, lightly counterstained with cresyl violet. 1 cm bar = 10 µm.

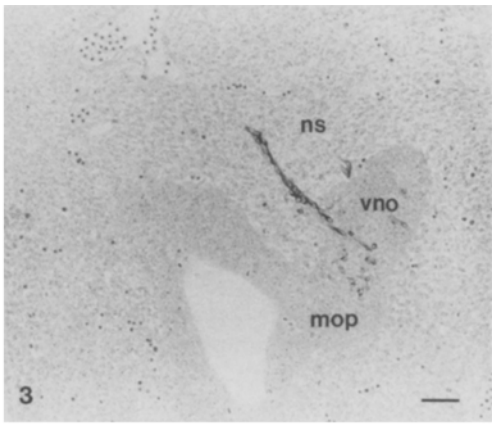

Figure 3. At 11.5 days of gestation, LHRH-immunoreactive neurons are seen leaving the epithelium of the developing vomeronasal organ (vno) and migrating onto the nasal septum (ns), towards the forebrain. These LHRH neurons appear to accompany axons of the terminalis and vomeronasal nerves, which are also derived from the medial part of the olfactory placode (mop). 8-µm sagittal section, lightly counterstained with cresyl violet. 1 cm bar = 100 µm.

migrate into the brain. We believe these data provide further evidence in support of the origin of LHRH neurons from the olfactory placode and extend the generality of the phenomenon of LHRH cell migration to humans. In mice, LHRH-immunoreactivity was first detected in the cytoplasm of a number of neurons within the epithelium of the medial part of the olfactory pit (the anlage of the vomeronasal organ) at 11 days of gestation (the first day of pregnancy was counted as day 1 of gestation). These cells were oval or fusiform in shape, oriented perpendicular to the apical (luminal) surface, and seen in the apical, intermediate and basal layers of the epithelium (fig. 2). No LHRH-immunoreactivity was seen in the medial olfactory pit until formation of the anlage of the vomeronasal organ. By day 11.5 LHRH-immunoreactive neurons were seen emerging from the epithelium, accompanied by axons of the developing terminalis and vomeronasal nerves (fig. 3). At 12 and 13 days of gestation, cords of LHRH-immunoreactive cells were seen on the nasal septum (fig. 4) and by day 14 LHRH cells were observed entering the forebrain medial and caudal to the developing olfactory bulbs, traversing the ganglion terminale and the central roots of the nervus terminalis. It was interesting to note that no LHRH-immunoreactive cells were seen in forebrain, if these structures which form a bridge between the nasal septum and the forebrain, were not present. In the forebrain, the LHRH-immunoreactive cells formed a continuum, arching from the ganglion terminale through the medial forebrain into the septum, the preoptic area and the hypothalamus. The migration of LHRH-immunoreactive cells, from the nose into the forebrain, covers a distance of approximately 1320 µm (just lateral to midline, between the olfactory bulbs) at 14 days of gestation (fig. 1). By 16 days of gestation, the migration is essentially over, although a few LHRH cells may still be seen in the epithelium of the

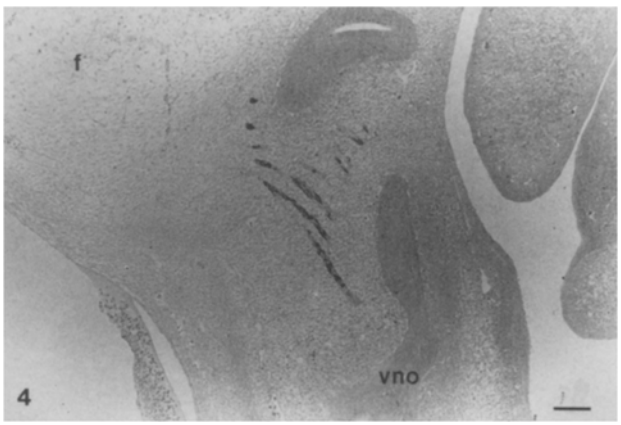

Figure 4. Cords of LHRH-immunoreactive cells are seen on the nasal septum, coursing toward the forebrain (f) with branches of the terminalis and vomeronasal nerves, from the anlage of the vomeronasal organ (vno). 8-µm sagittal section of the nasal septum of a 13-day-old fetal mouse, lightly counterstained with cresyl violet. 1 cm bar = 214 µm.

medial olfactory pit and along the migration route into postnatal life. These LHRH-immunoreactive cells are found in ganglia or along branches of the terminalis nerve[44], which makes up a part of the migration route. Days 14, 16, 18 and 20 showed an increase in the number of LHRH cells found in the septum, the preoptic area and the hypothalamus, while a concomitant decrease in the numbers of these cells was observed in the epithelium of the olfactory pit, on the nasal septum, and in the traverse (ganglion terminale and central roots of the terminalis nerve, see fig. 5). The increase in LHRH cells in the brain appears to be due to the rostral to caudal migration of cells from the nose rather than cell division. Our studies, using tritiated thymidine autoradiography in combination with LHRH immunocytochemistry[48,49], showed the uptake of the radioactive precur-

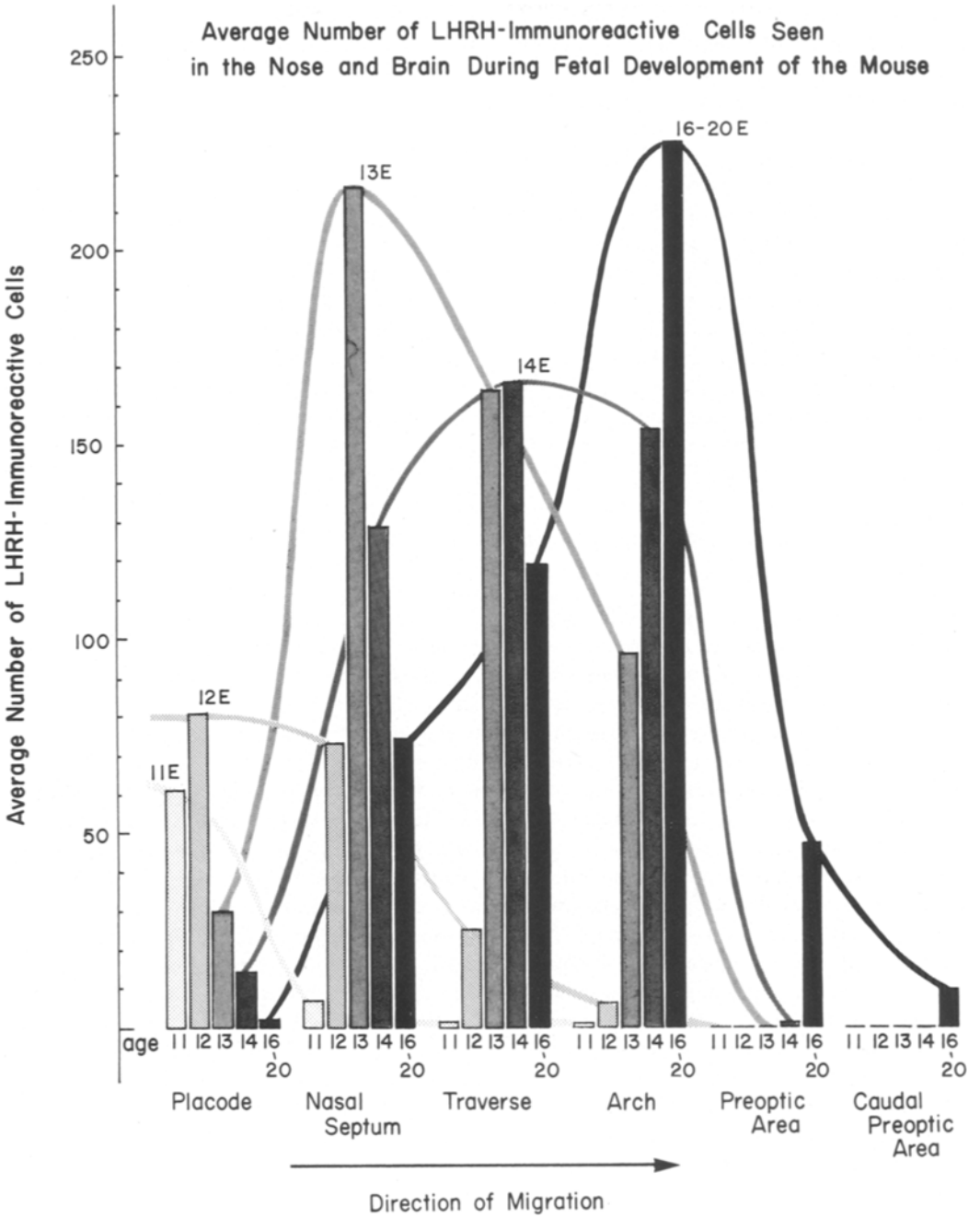

Figure 5. The distribution of the average numbers of LHRH-immunoreactive cells counted on embryonic days 11 (n = 6), 12 (n = 3), 13 (n = 4), 14 (n = 5) and 16–20 (n = 7) is plotted to show the proposed migratory route. Placode: the epithelia of the medial olfactory placode; Nasal septum: area on the nasal septum below the cribriform plate of the ethmoid bone; Traverse: the area above the cribriform plate and ventromedial to the olfactory bulbs, including the ganglion terminale and the intracranial roots of the nervus terminalis; Arch: the central roots of the terminalis nerve, the anterior olfactory nucleus, the nucleus and tract of the diagonal band, the medial septal nucleus, the anterior hypothalamic area, the medial and lateral preoptic areas, the organum vasculosum of the lamina terminalis, and the areas dorsal and caudal to the optic chiasm. Each gestational age shows a characteristic pattern of distribution of LHRH-immunoreactive cells, and any sample of the distribution of LHRH cells in the fetal mouse fitted into one of these curves with less than one day's difference. The migration appears to be over by day 16 of gestation. The vertical bars show the number of cells found at a particular fetal age. The lines joining the bars illustrate the 'envelope' characteristic of a particular age.

sor in the nuclei of some LHRH cells on the nasal septum. However, no evidence of LHRH cell division was seen in the brain. The pattern of distribution of the migrating LHRH-immunoreactive neurons is so distinctive, that the age of a fetal mouse could be determined ($\pm$ 1 day) by noting the whereabouts of the LHRH neurons. Examination of the olfactory placode of fetal mice with radioactive molecular probes, specific for LHRH mRNA, has confirmed LHRH gene expression by these cells from the olfactory placode[60, 61]. In an ultrastructural study of LHRH-immunoreactive cells in the olfactory placode and migrating on the nasal septum, LHRH-immunoreactivity was found to be associated with the cytoplasmic side of the nuclear envelope, and no neurosecretory granules were seen, suggesting that these LHRH neurons may not have a secretory function until they have attained their target organs[61].

Speculations about the origin of LHRH cells from the olfactory pit and mechanisms of migration

What biological sense can be made from an olfactory pit origin for LHRH neurons? First, an evolutionary perspective: Since the hypophyseal placode develops near the olfactory placodes, there may have been (even before Agnatha: hagfish, as well as lampreys) opportunities for LHRH neurons to control gonadotropes through paracrine mechanisms. Then, as Rathke's pouch evolved to form the anterior pituitary gland, LHRH cells had to migrate and establish axonal connections in order to retain their influence over gonadotropes in the anterior pituitary gland. There is evidence from comparative studies of the neuroendocrine system that the LHRH molecule has a central role in reproductive functions throughout phylogeny. Similarities have been found between the primary structures of LHRH molecules across mammalian and submammalian species, including alpha yeast mating factor. It is remarkable that certain characteristics of the LHRH molecule, specifically the sequence of amino acids essential for the release of gonadotropins, have been essentially conserved throughout 500 million years of vertebrate evolution[40, 51].

Secondly, some physiological perspectives: Not only pheromonal but also other environmental controls over reproduction follow nicely from olfactory/nasal relations of LHRH neurons. Even beyond plant and animal chemosensory signals of reproductive importance, water temperature, salinity and hydrostatic pressure can be important for aquatic species and could be signalled through physical changes in the nasal epithelium. With consequent depolarization of LHRH cells, the decapeptide would be released through stimulus-secretion coupling.

Finally, mechanisms contributing to migration: Chemical and mechanical factors with positive and negative influences are under investigation in our laboratory. Cell adhesion molecules are of obvious potential importance (Schwanzel-Fukuda and co-workers, in progress) and are being studied intensively in this laboratory but need not exclude other contributing factors.

What is the role of the terminalis nerve in the migration of LHRH neurons?

The terminalis and vomeronasal nerves originate in the same part of the olfactory placode that gives origin to most of the LHRH neurons. It may be of some significance that LHRH-immunoreactivity is not detected until this medial part of the olfactory placode has formed, which is usually midway in gestation in most vertebrates, and early with respect to differentiation of the brain and pituitary gland and the gonads. It is generally believed that parts of the nervous system that appear early in ontogenesis appeared early in phylogeny[17]. Just as the LHRH molecule has been found to be conserved throughout vertebrate evolution[40, 51], the terminalis nerve has been found to be remarkably constant across diverse species of vertebrates in 1) its content of LHRH-immunoreactivity and 2) its anatomical position in regions of the brain that have undergone considerable modification throughout phylogeny. The presence of the terminalis nerve in adults as well as fetuses, in all species examined[22, 23], including humans[4, 36, 50], with the possible exception of insectivorous bats[5], indicates that it is not a transient embryological structure. Whether the terminalis is the vestige of an ancient nerve or, as proposed by some investigators, mediates certain aspects of reproductive function[10, 56] is the subject of speculation, nearly as much as it was a hundred years ago. We believe that an important role of the terminalis nerve, in development, is to provide a route along which the LHRH neurons migrate into the brain. Since the olfactory nerves, originating in the lateral, dorsal olfactory placode, have been shown to influence the development of the forebrain, including the olfactory bulbs[12, 13, 53], it may be possible that LHRH neurons and the terminalis nerve, originating in the medial olfactory placode, influence the development of parts of the forebrain on and near the midline.

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Reviews

Effects of acid precipitation on reproduction in birds*

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Summary. Acidification in aquatic habitats reduces the reproductive success of both piscivorous and non piscivorous birds, mainly by reducing the food supply. Piscivorous birds find some compensation in an increased transparency of the water, non piscivorous birds in less competition for invertebrate prey by fish. Acidification in forests often has large impacts on insect populations but how this affects forest birds is unknown. Some woodpeckers and nuthatches temporarily benefit from an increase in standing dead timber. In advanced stages of forest dieback the breeding density of forest birds is very much reduced, but species of open woodland increase. Calcium deficiency reduces the reproductive output of some passerine species, but the extent of this phenomenon is unknown. Increased exposure to toxic metals has reduced the reproductive success of some lake dwelling species. It is difficult to assess the effect of acid precipitation on birds since acidification affects ecosystems in many ways, the evidence is largely correlative and reliable estimates of the population size are often lacking. Future studies should concentrate on carefully selected indicator species suitable for detailed data collection.
Key words. Acid deposition; reproduction; birds; insects; calcium.

1 Introduction

The term 'acid rain' was first used in 1852 to describe the effects of industrial emissions on precipitation in Britain[90]. However, only in the 1950's did it become evident that acid precipitation was a worldwide phenomenon, and it took another 20 years before widespread public concern forced governments to initiate studies on the effects of acidification on ecosystems. Many studies have been carried out since the 1970's and an enormous amount of information has become available on the effects of acid precipitation on the water chemistry and the invertebrate life in aquatic ecosystems[23, 79, 90], on soils[14, 34, 52, 68, 106] and on trees[21, 63, 94, 96]. However, little is known about effects on higher trophic levels, in particular on mammals and birds.

Studies of acidification and birds mostly concern wetland birds, especially in North America[62, 66, 67, 93]. One of the first noticed effects of acidification was the decline of fish populations[2, 6, 7, 42] and this focused the attention on wetlands. Population declines of important game species like the Black Duck *(Anas rubripes)* and public concern for the popular Great Northern Diver *(Gavia immer)* also played a significant role. In Europe, data on the effects of acidification on insects and forest birds have only recently become available[24, 59, 77].

This review aims to bring this information together, to identify the mechanisms by which acidification could affect birds, to identify the problems involved in interpreting the data and to make suggestions for future research. Emphasis will be on the effects of acidification on the reproduction of birds, in particular on the reproductive success of birds initiating a clutch. Other important aspects, such as the proportion of females that initiate a clutch, or the survival of adult birds, are much harder to study and there are virtually no data on the subject.

In sections 2.1 and 3.1 changes in the biotic and abiotic environment due to acidification are reviewed. Sections 2.2 and 3.2 deal with effects of changes in availability of food and vegetation characteristics on various groups of birds. Effects of changes in food quality are treated in sections 4.1 and 4.2.