Semaphorin5A expression in the developing chick telencephalon

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Abstract

In the present study, we analyzed the expression of *Semaphorin5A* (*Sema5A*), a gene implicated in axon guidance and many other processes of neuronal development, in the developing chick telencephalon. By using a heterologous mouse probe and in situ hybridization techniques, we showed distinct patterns of *Sema5A* expression within the chick telencephalon. In early development, *Sema5A* was present in pallial regions, mainly in the neuroepithelium and in the deep mantle of ventral and lateral pallia, and in the subpallium. As development proceeds, some ventral pallial derivatives maintained a moderate to strong *Sema5A* expression, whereas other lateral or dorsal pallial derivatives showed low to moderate expression of *Sema5A*. The overall expression of *Sema5A* during development in the chick telencephalon was similar to that reported in mouse. Moreover, the expression of *Sema5A* in mesencephalic, diencephalic, and telencephalic centers related to the tectofugal system suggests an important role of this gene in the development.

Keywords: Gene expression pattern; Olfactory system; Visual system; In situ hybridization; Birds

1. Introduction

Semaphorins constitute a large and growing family of both secreted and transmembrane proteins [8]. These molecules play an important role in creating the complex pattern of neuronal connectivity, serving as nerve growth cone contact and chemotropic guidance signals, allowing axonal growth cones to navigate long distances along specific pathways [10]. Semaphorins usually act as repulsive axon guidance molecules but some have also been shown to be attracting guidance mediators. In general, semaphorins have been involved not only in axon guidance [5] but also in many other processes of neural development, including axonal fasciculation, neural migration, target selection, and dendritic guidance, as well as in remodeling and repair of the adult nervous system [3].

So far, more than 30 members of the semaphorin family have been identified, which are divided into eight classes, all of them sharing a conserved 500-amino acid length "Sema" domain at their amino terminus [5]. Although some studies have reported *Sema5A* in the developing mouse brain [4], little is yet known about its anatomical distribution in other vertebrate brains. In the present study, we report the expression of *Sema5A* in the chick telencephalon during development.

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2. Material and methods

Chicks between embryonic day 6 (E6) and hatchling (P0) were used for the present study. Embryos were first anesthetized by cold or with ethyl ether, and then perfused transcardially with 0.1 M phosphate-buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde in PB. The brains were dissected, postfixed overnight at 4 °C, and embedded in 4% agarose for sectioning; 150-μm-thick transverse sections were obtained with a vibratome. Brain sections were processed for in situ hybridization following a standard procedure, using digoxigenin-labeled riboprobes. The gene analyzed was *Semaphorin 5A* (formerly known as *SemaF*; [8]). We used a heterologous mouse probe, which was obtained from A. Chédotal (INSERM U106, Paris, France).

The probe was screened against a chick EST database (BBSRC Chicken ESTs). BLASTA program analysis showed that primary sequence alignment of our probe exhibited significant identity with sequences identified as *Sema5A* in chick (96–100%).

Digital photographs were taken on a Nikon Eclipse E800 microscope equipped with a Nikon DMX1200 digital camera. Digital images were adjusted for brightness/contrast using Adobe Photoshop software and the figures were mounted and labeled using PageMaker 7.0.

For avian telencephalon, we used the revised nomenclature proposed by Reiner et al. [7].

3. Results

Below, we describe *Sema5A* expression patterns in the chick embryo at three developmental ages. Under early development, we group embryonic day 6 (E6) to E10. At intermediate development, an important change in the expression pattern of *Sema5A* was observed, and we chose E14 as a representative intermediate embryonic day to describe this fact. In later ages (from E16 on), several differences in the expression pattern gradually appeared.

3.1. Early development

The expression of *Sema5A* in the chick brain began early in development. At the earliest embryonic day studied, E6, this expression was mainly observed at all rostrocaudal levels of telencephalic ventricular zone; besides, a moderate expression in the subventricular zone and a slight signal in the mantle layer was found that abruptly stopped at the pallial–subpallial boundary (Fig. 1A). During development the expression increased gradually; thus at E8, the pallial ventricular zone was strongly stained (Fig. 1B). At this embryonic day, a moderate expression in the pallial mantle was also observed, especially in the ventral and lateral pallia. At caudal telencephalic levels the signal was stronger in the lateral pallial mantle than in the ventral one. The mantle layer of the subpallial area was still devoid of signal. Neither the anterior commissure nor the lateral forebrain bundle showed *Sema5A* expression (Fig. 1B; see below how this changed later). At E10, *Sema5A* expression could be identified in the nucleus basorostralis pallii and in the olfactory bulb (Fig. 1C). Also of note, the subpallial ventricular zone was strongly stained whereas the subpallial mantle layer still showed very weak expression at this embryonic day.

3.2. Embryonic day 14 (E14)

At E14, the avian brain is relatively mature and it is possible to distinguish most telencephalic cell groups. *Sema5A* expression increased during development being much higher at this embryonic day (Fig. 1D).

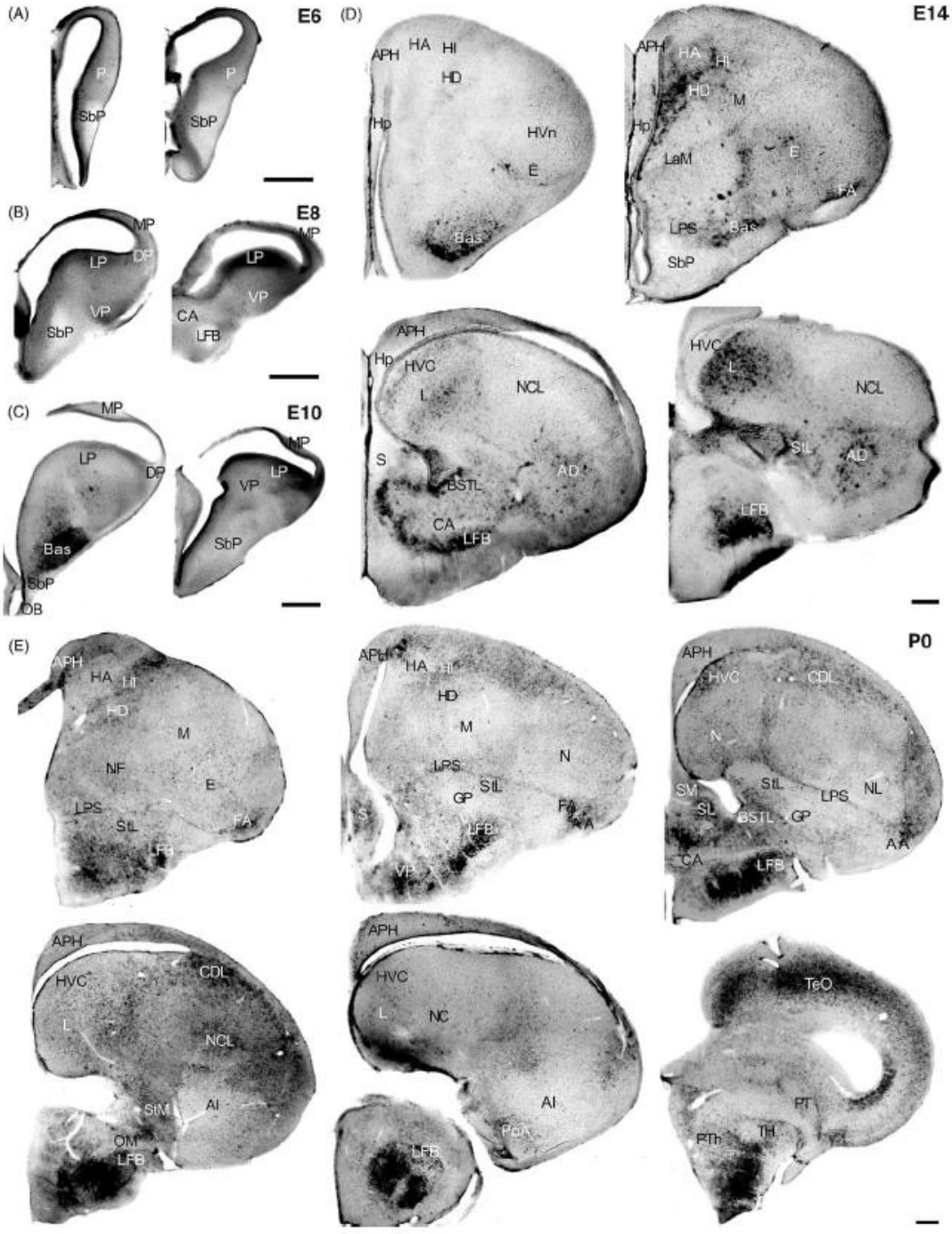
Regarding telencephalic areas, expression of *Sema5A* was found in all four histogenetic divisions of the developing pallium. In the medial pallium, a patch of *Sema5A* signal was located in the rostral part of hippocampus, whereas expression in the area parahippocampalis was absent. In the dorsal pallium, a strong expression was observed in the hyperpallium intercalatum; however the hyperpallium densocellulare showed weaker expression. The expression pattern in the lateral pallium was restricted to the nucleus of mesopallium ventrale at the rostral levels. At caudal telencephalic levels, the arcopallium dorsale, part of the arcopallium intermedium and the caudal part of hyperstriatum ventrale were also stained. In the ventral pallium, the most rostral signal was found in the anterior olfactory nucleus. Both entopallium and nucleus basorostralis pallii also showed a visible expression. At the caudal most levels of this division, the area L pallii displayed a strong *Sema5A* signal, increasing its expression at caudal levels. Besides these nuclei, different structures in the telencephalon expressed *Sema5A*; those were the lamina mesopallialis, the lamina pallio-subpallialis, and the lamina frontalis superior.

Remarkably, subpallial regions also expressed *Sema5A*. Thus, some nuclei related to the extended amygdala including the lateral and medial parts of the bed nucleus of stria terminalis, and the area subpallialis amygdalae displayed strong expression. Furthermore, the most medial part of septum displayed a strong signal, and a moderate patch was observed throughout the ventral pallidum (results not shown in this age; see Fig. 1E). A patch drawing a curved band in the subpallium showed a strong expression. This curved band formed a continuum connecting the lateral forebrain bundle with the septal telencephalic midline at rostral levels and with the ventral tip of the lateral ventricle more caudally, just in the region of the lateral part of the bed nucleus of the stria terminalis.

The lateral forebrain bundle and the tractus fronto-arcopallialis showed the highest *Sema5A* expression in the telencephalon. However, the tractus occipito-mesencephalicus, the tractus septopallio-mesencephalicus, the olfactory tract and the anterior commissure were not stained.

3.3. Late development

The distribution of *Sema5A* during late development was studied in different embryonic days, including E16, E18 and P0. The expression pattern of *Sema5A* in these ages was similar to that described for E14. Thus, only results on P0 are shown (Fig. 1E). In general, later developmental ages showed a slightly stronger signal and a more widespread expression. Some new areas of expression were observed. A narrow band between area parahippocampalis and hyperpallium apicale, which lacked expression at E14, began to express *Sema5A* gene from E18 on. Besides, the septum was strongly stained, with the expression in the nucleus septalis lateralis being higher than expression in the nucleus septalis medialis. At late development, the superficial areas of the pallium in the caudal


(A)
E6
P
SbP
P
SbP
(B)
E8
MP
LP
DP
VP
SbP
MP
LP
VP
CA
LFB
(C)
E10
MP
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Bas
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OB
MP
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E14
APH
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telencephalon displayed a strong expression, which was increasing from E16 to P0. *Sema5A* was expressed strongly in the diencephalic nucleus rotundus and in tectal and pretectal areas related to mesencephalic ascending visual pathways, as it was observed from E10 (results not shown).

4. Discussion

In this report, we present an account of developmental changes in the expression of the *Sema5A* in the avian telencephalon. To our knowledge, this is the first study dealing with the anatomical distribution of *Sema5A* in the developing brain in a non-mammalian vertebrate. We observed variations in the patterns of *Sema5A* expression within the chick telencephalon in the different embryonic days. So, in early development, *Sema5A* was present both in pallial regions, mainly in the ventricular zone and in the deep mantle of the ventral and lateral pallium, and in the subpallium. As development proceeds, some ventral pallial derivatives, entopallium, and area L pallii maintained a moderate to strong *Sema5A* expression, but other lateral or dorsal pallial derivatives also expressed low to moderate signals of *Sema5A*.

Previous studies in mouse suggested that the differential expression of *Sema5A* (together with other developmental regulatory genes) could be relevant for the analysis of the main divisions of the pallium, in particular the lateral and ventral divisions [4]. In this sense, a strong to moderate expression of *Sema5A* characterizes the subventricular zone and mantle of the ventral pallium, from early to later developmental ages, whereas the lateral pallial derivatives show only weak *Sema5A* expression in the mantle [4]. While this differential pattern of *Sema5A* expression is useful to distinguish the derivatives of the lateral pallium from those of the ventral pallium, other pallial or subpallial regions also expressed *Sema5A*. Thus, it is described that the expression of *Sema5A* in the early development in mouse is strong in the ventricular zone and deep mantle of the ventrolateral pallium, and decreases gradually along the dorsal pallium and the subpallium [4]. Therefore, the overall expression of *Sema5A* during early ages in the developing chick brain is similar to that reported for the mouse brain.

In the late development, we also found some similarities between chick and mouse. Thus, the expression of *Sema5A* in the chick telencephalon was still present in the four histogenetic divisions of the pallium, whereas in mammals, the signal of this gene is mainly observed in the ventral and lateral pallial regions, although parts of the dorsal and medial mantle in the mouse telencephalon also expressed *Sema5A* [4]. Therefore, the general pattern of *Sema5A* expression is quite similar between birds and mammals from early to late ages of development.

Several lines of evidence suggest that secreted semaphorins could play a role in the development of olfactory projections in mammals. Thus, neuropilin-1 is highly expressed on the axons of embryonic mitral cells, neuropilin-2 mRNA is present in all components of the olfactory circuitry [2], Sema3A/collapsin-1 mRNAs are found in the olfactory bulb and in the nucleus of the lateral olfactory tract [1], and Sema3F and *Sema5A* expression is observed in the olfactory epithelium [1,6]. In this context, it is interesting to note that we observed expression of *Sema5A* in the neuroepithelium of the olfactory bulb, in the anterior olfactory nucleus, and in the most medial part of the septum. Some authors have suggested that the septum produces a diffusible repellent factor, which would prevent the axons from the nucleus of the lateral olfactory tract crossing the midline [1]. This factor has not yet been characterized, but on the basis of the high expression in the septum we observed in this study, *Sema5A* might be a potential candidate to play this role.

Additionally, it has been suggested that *Sema5A* is essential for the development of the visual system in mammals [6]. Thus, both *Sema5A* mRNA and protein were specifically expressed in neuroepithelial cells surrounding retinal axons at the optic disc and along the optic nerve. The retinal axons could continually respond to *Sema5A* inhibition as they progressed through the developing retinal pathway [6]. Moreover, *Sema5A* is expressed in the lateral geniculate nucleus in mammals [9].

We found that *Sema5A* was expressed in most components of the chick visual tectofugal pathway. Thus, *Sema5A* was expressed in the optic tectum from early embryonic days, and in the thalamic nucleus rotundus, an important relay center, which receives tectal information and projects to the telencephalon. Also the entopallium, the main pallial target for the rotundal projections, and the lateral forebrain bundle, which conveys ascending thalamo-telencephalic axons, displayed a high expression of *Sema5A*.

It is to note that, in contrast to mammals, the retinofugal axons and some of the retinorecipient areas, such as the dorsal geniculate nucleus and the superficial tectal layers did not

Fig. 1. Expression pattern of *Sema5A* in frontal sections through the telencephalon of chick embryos at E6 (A), E8 (B), E10 (C), E14 (D) and P0 (E). Sections are rostro-caudally arranged within each panel (rostral is to the upper left corner of the panel). Abbreviations: AA, arcopallium anterius; AD, arcopallium dorsale; AI, arcopallium intermedium; PoA, nucleus posterior amygdalopallialis; APH, area parahippocampalis; Bas, nucleus basorostralis palii; BSTL, bed nucleus of the stria terminalis, lateral part; CA, anterior comissure; CDL, area corticoididea dorsolateralis; DP, pallium dorsalis; E, entopallium; FA, tractus fronto-arcopallialis; GP, globus pallidus; HA, hyperpallium accesorium; HD, hyperpallium densocellulare; HIS, hyperpallium intercalatum; Hp, hippocampus; M, mesopallium; HVC, hyperstriatum ventrale, pars caudalis; HVn, nucleus of the ventral hyperstriatum; L, area L pallii; LFB, lateral forebrain bundle; LaM, lamina mesopallialis; LP, pallium lateralis; LPS, lamina pallio-subpallialis; MP, pallium medialis; N, nidopallium; NC, nidopallium caudalis; NCL, nidopallium caudalis, pars lateralis; NF, nidopallium frontalis; NL, nidopallium lateralis; OB, olfactory bulb; OM, tractus occipito-mesencephalicus; P, pallium; PT, pretectum; PTh, prethalamus; S, septum; SbP, subpallium; SL, nucleus septalis lateralis; SM, nucleus septalis medialis; STI, striatum, lateralis pars; STm, striatum, pars medialis; TeO, optic tectum; TH, thalamus; VP, pallium ventrale. Scale bars = 500 μm.

show expression of *Sema5A* in the developing chick brain. These and other differences may be attributed to the use in our study of a heterologous mouse probe, which may not always hybridize with the mRNA of this gene, resulting in a false negative (also, this probe could hybridize with other related semaphorins, resulting in a false positive). However, we found that the sequence of the mouse probe exhibited a significant identity, between 96% and 100%, with sequences identified as *Sema5A* in the chick, so we believe that our results are quite reliable. Therefore, it is possible that only part of the avian developing visual system (the tecto-thalamo-telencephalic visual pathway) expresses *Sema5A*.

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