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RESEARCH ARTICLE

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Expression of regulatory genes in the embryonic brain of a lizard and implications for understanding pallial organization and evolution

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Abstract

The comparison of gene expression patterns in the embryonic brain of mouse and chicken is being essential for understanding pallial organization. However, the scarcity of gene expression data in reptiles, crucial for understanding evolution, makes it difficult to identify homologues of pallial divisions in different amniotes. We cloned and analyzed the expression of the genes *Emx1*, *Lhx2*, *Lhx9*, and *Tbr1* in the embryonic telencephalon of the lacertid lizard *Psammodromus algirus*. The comparative expression patterns of these genes, critical for pallial development, are better understood when using a recently proposed six-part model of pallial divisions. The lizard medial pallium, expressing all genes, includes the medial and dorsomedial cortices, and the majority of the dorsal cortex, except the region of the lateral cortical superposition. The latter is rich in *Lhx9* expression, being excluded as a candidate of dorsal or lateral pallia, and may belong to a distinct dorsolateral pallium, which extends from rostral to caudal levels. Thus, the neocortex homolog cannot be found in the classical reptilian dorsal cortex, but perhaps in a small *Emx1*-expressing/*Lhx9*-negative area at the front of the telencephalon, resembling the avian hyperpallium. The ventral pallium, expressing *Lhx9*, but not *Emx1*, gives rise to the dorsal ventricular ridge and appears comparable to the avian nidopallium. We also identified a distinct ventrocaudal pallial sector comparable to the avian arcopallium and to part of the mammalian pallial amygdala. These data open new venues for understanding the organization and evolution of the pallium.

Abbreviations: ac, Anterior commissure; ADVR, Anterior dorsal ventricular ridge; Ac, Nucleus accumbens; AO, Anterior olfactory area; AOB, Accessory olfactory bulb; AOBc, Accessory olfactory bulb, caudal part; AOBr, Accessory olfactory bulb, rostral part; AOd, Anterior olfactory area, dorsal part; AOm, Anterior olfactory area, medial part; aon, Accessory olfactory nerve; aot, Accessory olfactory tract; AOv, Anterior olfactory area, ventral part; Bst, Bed nucleus of the stria terminalis; Bste, Eminential part of Bst; Bstm, Medial part of Bst; Cb, Cerebellum; CB, Calbindin; Ch/SPa, Chiasmatic/Subparaventricular domain; DC, Dorsal cortex; DC1, Dorsal cortex, medial subdivision; DC2, Dorsal cortex, intermediate subdivision; DC2 lat Lateral part of DC2; DC2 med, Medial part of DC2; DC3, Dorsal cortex, lateral subdivision; Di, Diencephalon; DL, Dorsolateral thalamic nucleus; DLA, Dorsolateral amygdaloid nucleus; DLAp, posterior part of DLA; dLCp, Area deep to LCp; DLP, Dorsolateral pallium; DM, Dorsomedial thalamic nucleus; DMC, Dorsomedial cortex; DP, Dorsal pallium; gr, Granular layer; Hb, Habenula; LA, Lateral amygdala; LC, Lateral cortex; LCa, Lateral cortex, anterior part; LCd, Lateral cortex, dorsal part; LCp, Lateral cortex, posterior part; LCv, Lateral cortex, ventral part; lcs, Lateral cortical superposition; LEC, Lateral entorhinal cortex; LP, Lateral pallium; MC, Medial cortex; MeA, Medial amygdala; MEC, Medial entorhinal cortex; Mes, Mesencephalon; ml, Mitral cell layer; MOB, Main olfactory bulb; MP, Medial pallium; Naot, Nucleus of the accessory olfactory tract; Ncp, Nucleus of the pallial commissure; NdB, Nucleus of the diagonal band of Broca; Nlot, Nucleus of the lateral olfactory tract; NS, Nucleus sphericus; oc, Optic chiasma; on, Olfactory nerve; OT, Optic tectum; P, Pallium; pc, Pallial commissure; PM, Posteriomedial thalamic nucleus; PDVR, Posterior dorsal ventricular ridge; PO, Preoptic area; psb, Pallial-subpallial boundary; Pt, Pretectum; PT, Pallial thickening; PTd, Dorsal part of PT; PTv, Ventral part of PT; PTh, Prethalamus; PThE, Prethalamic eminence; Rh, Rhombencephalon; Rot, Nucleus rotundus; SAT, Striato-amygdalar transition area; Se, Septum; SeP, Pallial septum; SeS, Subpallial septum; Sm, Medial septum; Sp, Subpallium; SPO, Septo-preoptic area; SPV, Supraopto-paraventricular domain; Str, Striatum; Tel, Telencephalon; TO, Olfactory Tubercle; Th, Thalamus; v, Lateral ventricle; VAA, Ventral anterior amygdala; VCP, Ventrocaudal pallium; VP, Ventral pallium; VPA, Ventral posterior amygdala.

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KEYWORDS
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1 | INTRODUCTION

A major question for evolutionary neurobiologists is to understand the mechanisms behind conservation, divergence and innovation in brain structure and function. This has been particularly challenging for understanding the origin and evolution of the cerebral cortex (and the pallium, in general), which plays an essential role in cognition and emotion, and in control of behavior and is one of the brain regions with the highest growth and divergence during amniote evolution (Nieuwenhuys, ten Donkelaar, & Nicholson, 1998; Striedter, 2005). After more than 100 years of exhaustive and rigorous studies of the pallium across different vertebrates (reviews by Bruce, 2007; Butler, 1994; Northcutt, 1995; Northcutt and Kaas, 1995; Puelles et al., 2017; Reiner, 1993; Striedter, 1997), the exact number of pallial sectors, their comparison across species, and the mechanisms behind their evolution remain essentially unsolved. However, the emerging field of evolutionary developmental neurobiology (neuro-evo-devo; Medina, 2007a; Medina and Abellán, 2009; O'Connell, 2013) is opening new venues for deciphering the mystery of pallial evolution (Aboitiz, Morales, & Montiel, 2003; Charvet, Striedter, & Finlay, 2011; Charvet & Striedter, 2009, 2011; Medina & Abellán, 2009; Molnar et al., 2014; Nomura, Gotoh, & Ono, 2013a, 2014; Puelles et al., 2000; Puelles, 2001; Wullimann & Mueller, 2004). Basically, the brain develops under the influence of perfectly orchestrated networks of developmental regulatory genes, which determine much of its mature structure and function (reviewed by Medina and Abellán, 2009)[1]. Variations in the expression of these regulatory genes, often determined by permanent modifications at their noncoding regulatory region (including enhancers) and/or the factors/cofactors that bind to it, are behind evolutionary changes (Carroll, 2008; Carroll, Grenier, & Weatherbee, 2001; Davidson, 2006; Sakabe & Nobrega, 2013). Both the coding region and the regulatory region of genes active at early stages of forebrain development show a high level of sequence conservation (thus, there is a high constraint against variation), but the level of constraint appears to decrease in the regulatory regions of genes active from mid-gestation to adults (Capra, Erwin, McKinsey, Rubenstein, & Pollard, 2013; Nord, Pattabiraman, Visel, & Rubenstein, 2015). This explains the results of both neuroanatomical studies (Striedter, 1997; 2005) and transcriptome analysis (Belgard & Montiel, 2013; Belgard et al., 2013) showing important differences between species in adult structures derived from homologous developmental fields. Given the high conservation during early development, the combinatorial expression patterns of developmental regulatory genes at early stages—always referred to the brain topological coordinates (Nieuwenhuys, 1998)—are very powerful for determining the basic brain divisions and their comparison across vertebrates (Medina, 2007a,b; Medina & Abellán, 2009; Nieuwenhuys & Puelles, 2016; Puelles & Medina, 2002; Puelles & Ferrán, 2012). Puelles and colleagues (2000) were among the first to apply this approach for trying to understand pallial evolution, by comparing the expression of several transcription factors in the embryonic telencephalon of mouse and chicken (see also Fernández, Pieau, Repérant, Boncinelli, & Wassef, 1998). Their analysis, based on the combinatorial expression of the transcription factors Pax6, Emx1, Tbr1 and Dlx2, led them to propose the existence of four pallial divisions, comparable as fields between mouse and chicken (Puelles et al., 2000), departing from the three-part scheme of pallial divisions that was prevalent until then (reviewed by Butler, 1994; Johnston, 1915; Northcutt, 1995; Northcutt and Kaas, 1995; Reiner, 1993; Striedter, 1997). All pallial divisions strongly express Pax6, Emx1, and/or Tbr1 in different combinations across layers (ventricular zone, mantle, or marginal zone) and subdomains, but are poor in cells expressing the subpallial marker Dlx2 at early embryonic stages, although later Dlx2 is expressed in the pallium in subsets of interneurons that migrate from the subpallium (for a review on interneurons see Marín and Rubenstein, 2001; Puelles et al., 2000). The four-part pallial scheme implied the division of the classical lateral pallium or piriform lobe into two parts: a redefined lateral pallium, rich in expression of Pax6, Emx1, and Tbr1, giving rise to a dorsal part of the claustroamygdaloid pallial complex in mouse and the mesopallium and part of arcopallium in chicken; and a novel ventral pallium, rich in Pax6 and Tbr1 but poor in Emx1, giving rise to a ventral part of the claustroamygdaloid complex in mouse and the nidopallium and part of the arcopallium in chicken (Medina et al., 2004; Puelles et al., 2000). According to this view, the dorsal pallium is the only division producing the neocortex in mouse and other mammals, while the same division produces the hyperpallium or Wulst in chicken and other birds (Puelles, 2001; Puelles et al., 2000). The four-part pallial scheme is now followed by most comparative embryologists (e.g., Bachy, Berthon, & Rétaux, 2002; Medina, 2007b; Medina & Abellán, 2009; Moreno & González, 2006; Mueller & Wullimann, 2009), and has been reinforced by addition of other conserved transcription factors which expression is restricted to different pallial divisions during development (Medina et al., 2004), such as Lhx9, typically expressed in the ventral pallial division in all tetrapods, but not in the lateral and dorsal pallium (Abellán, Legaz, Vernier, Rétaux, & Medina, 2009, 2014; García-López et al., 2008; Moreno, Bachy, Rétaux, & González, 2004; Moreno & González, 2006). In addition, Lhx9 is expressed in the developing medial pallium in mouse and chicken (Abellán et al., 2009, 2014; García-López et al., 2008), but not in amphibians (at least not in the anuran *Xenopus laevis*; Moreno et al., 2004; Moreno & González, 2006), while data in reptiles

[1]Internal and external factors (hormones, nutrients, toxins, temperature, activity, stress, etc.) also have an influence on development, often by way of epigenetic modifications affecting DNA methylation and expression (Jaenisch and Bird, 2003; Feil and Fraga, 2012).

are missing. During development, Lhx9 is also expressed in both the ventral and medial pallia, but not in the pallial sector between them, in cartilaginous fishes (Quintana-Urzainqui, Rodríguez-Moldes, Mazan, & Candal, 2015). Moreover, analysis of new region-specific transcription factors, such as Nr4a2 (previously known as Nurr1), typical of the lateral pallium and parts of the medial pallium (Puelles et al., 2016a; Puelles, 2014), and Lef1, expressed in the medial pallium (Abellán, Desfilis, & Medina, 2014), has led to reconsider these pallial sectors in mouse and chicken. Based on Nr4a2/Nurr1 expression, the lateral pallium is now proposed to include a claustro-insular region in mouse and the mesopallium in chicken, and appears to end more rostrally than previously thought, without including any part of the mouse amygdala and chicken arcopallium (Puelles et al., 2016a; Puelles, 2014). This proposal implies questioning the so-called claustro-amygdaloid pallial complex, which makes nonsense in the current scheme (Puelles, 2014). In addition, the combinatorial expression of Lef1, Lhx2, Lhx9, Lmo3, and Lmo4 throughout development has led to a better definition of the medial pallium in mouse and chicken, and the proposal of new pallial sectors to account for some parts of the mouse and chicken pallium, which do not appear to belong to any of the four divisions considered until now (Abellán et al., 2014). This proposal includes considering the medial entorhinal cortex as a derivative of the medial pallium, and the lateral entorhinal cortex a derivative of a newly defined dorsolateral caudal pallial sector (Abellán et al., 2014), recently renamed as dorsolateral pallium (Medina, Abellán, & Desfilis, 2017b). This proposal requires more investigation and the inclusion of reptiles, which are crucial for trying to understand the evolution of the pallium in amniotes (Aboitiz et al., 2003; Nomura, Kawaguchi, Ono, & Murakami, 2013b). However, data on gene expression patterns during early development in the brain of reptiles are quite scarce, and either lack enough detail (Fernández et al., 1998) or show a narrow focus to identify specific cell types (Suzuki & Hirata, 2014; Wang et al., 2011), without considering the divisions of the whole pallium and their position within the topological framework of the neural tube. Thus, the aim of this study was to clone a battery of developmental regulatory genes in the long-tailed lacertid lizard *Psammodromus algirus*, and to study their expression in the telencephalon during development. The genes cloned and analyzed included several regulatory genes encoding transcription factors known to be relevant for pallial (Lhx2, Lhx9, Emx1, and Tbr1) or subpallial (Dlx2) development in other vertebrates (Abellán et al., 2009; Brox, Ferreiro, Puelles, & Medina, 2003, 2004; Moreno & González, 2006; Puelles et al., 2000). Within reptiles, we chose the lizard *P. algirus* because: (1) it is easy to obtain and incubate their eggs in laboratory; (2) there are abundant publications on the neuroanatomy, neurochemistry and connections of the brain of this species (e.g., Dávila, Guirado, & Puelles, 2000, 2002; Guirado, Dávila, Real, & Medina, 1999, 2000); and (3) according to modern molecular phylogeny, it appears to be well positioned for comparative purposes in order to infer the ancestral condition in sauropsids, since it belongs to a phylogenetic branch (Lepidosauria) leading to the tuatara, lizards and snakes, which is a sister branch of that (Archelosauria) giving rise to birds, crocodiles, and turtles (Crawford et al., 2015; Meyer & Zardoya, 2003; Wang et al., 2013).

2 | MATERIAL AND METHODS

In this study, we used embryos ($N = 25$) of the long-tailed lizard *Psammodromus algirus* (Linnaeus, 1758) (Sauria: Lacertidae) from embryonic stage 34 until hatching. The embryos were extracted from fertilized eggs, which were incubated at 25–27°C until the desired stage. In order to obtain them, pregnant females ($N = 19$) were captured during late spring in the mountains near Madrid (under permission from Dirección General del Medio Ambiente of Madrid, reference numbers: 10/193908.9/11, 10/179954.9/12, and 10/068493.9/16) and in L'Albí (Lleida) (permission from Direcció General de Medi Natural i Biodiversitat of Catalonia, reference number SF/282–284). Lizards were kept in captivity in the Animal Facilities of the University Complutense of Madrid or of the University of Lleida until they laid eggs. Following this, females were kept in captivity during a short recovery period and then released in the same point of capture. During the whole process, the females were treated following the Spanish and European Union regulations for care and handling of animals in research (Spanish Royal Decrees 1021/2005 and 53/2013; Spanish Law 32/2007; European Directive 2010/63/UE). The eggs were placed in humid vermiculite, transported to the Reptile facility of the University of Lleida and put in an incubator at 70% humidity and 26°C until use. The experimental procedure was approved by the Committee of Experimental Research Animals of the University of Lleida and the Direcció General de Medi Natural i Biodiversitat of Catalonia (reference no. 6127).

Upon extraction, the embryos were placed in cold Ringer solution (4°C) containing 100 mg/L of the anesthetic Tricaine methanesulfonate (MS222, Sigma Chemical Co., St. Louis, MO), and they were staged, registered and photographed. The embryos were staged according to Ramos Steffens (1980; summarized in Medina, Puelles, & Smeets, 1994) and Wise, Vickaryous, and Russell, (2009). The embryos were then decapitated and their heads immersed in 0.1M phosphate buffered 4% paraformaldehyde (pH 8). The brains were dissected and postfixed in the same fixative solution for 6 to 12 hr at 4°C. After fixation, the brains were washed in 0.1 M phosphate buffered saline (PBS, pH 7.4) and either embedded in 4% agarose in PBS for vibratome sectioning or cryoprotected and stored at −20°C until use. Brains were sectioned in coronal or sagittal planes using a Leica VT1000S vibratome (80–100 μm thick). We obtained two or three parallel series of sections of each brain that were processed for in situ hybridization or for immunohistochemistry.

2.1 | In situ hybridization

Sections were processed for in situ hybridization using digoxigenin-labeled riboprobes. The riboprobes were synthesized from cDNAs containing fragments of *Dlx2*, *Emx1*, *Lhx2*, *Lhx9*, or *Tbr1* from the lizard *P. algirus*, cloned at the Molecular Marker Service (SCSIE) of the University of Valencia (Spain). The lizard genes were cloned into pCRII Vector (Invitrogen/Thermo Fisher Scientific Inc), after PCR amplification of the transcripts using the following degenerate primers based on the genome of *Anolis carolinensis* (GenBank Assembly ID GCA_000090745.1):

- *pDlx2*, forward: GAGGTCCGSATGGTCAAY; reverse: CTGHG TCTGGGTGAGKCC
- *pEmx1*, forward: GGMGASGTGTCCCAGGAGAG; reverse: GTCTC GGASAGRCTGAGGCTGC
- *pLhx2*, forward: TGAGCTGCAATGAGAACGAT; reverse: GGAGVA CTCTYTTGGTCAGG
- *pLhx9*, forward: AATGAGAATGAGGCAGACCA; reverse: GTTTTC TGGGCAAGCTGCTT
- *pTbr1*, forward: GTCYTRCAGTCSCTSCAC; reverse: GGTGTTC TGGTAGGCGGTKA

We synthesized the antisense digoxigenin-labeled riboprobes for the genes mentioned above using Roche Diagnostics's (Mannheim, Germany) protocols. Before hybridization, the sections were washed in PBS containing 0.1% Tween-20 (PBT 1X), prehybridized in hybridization buffer (HB) for 2 hr at 58°C, and then hybridized in HB containing the riboprobe overnight at 58°C (1–2 μg/ml, depending on the probe and brain size). The hybridization buffer contained 50% of deionized formamide, 1.3X standard saline citrate (SSC; pH 5), 5 mM ethylenediamine-tetraacetic acid (EDTA; pH 8.0; Sigma-Aldrich, Steinheim, Germany), 1 mg/ml of yeast tRNA (Sigma-Aldrich), 0.2% Tween-20, 100 μg/ml of heparin (Sigma-Aldrich), completed with water (free of RNAase and DNAase; Sigma-Aldrich). Following hybridization, the sections were washed with a mix 1:1 of MABT 1X (1.2% maleic acid, 0.8% NaOH, 0.84% NaCl, and 0.1% Tween-20) and HB at 58°C during 20 min and washed abundantly at room temperature with MABT 1X (about 2 hr). Following this, the sections were blocked with a solution containing blocking reagent (Roche), MABT 1X and sheep serum (Sigma) for 4 hr at room temperature, then incubated in an antibody against digoxigenin (alkaline-phosphatase coupled antidigoxigenin; diluted 1:3500; Roche Diagnostics) overnight at 4°C, later washed with MABT 1X and finally revealed with BM purple (Roche Diagnostics). Sections were then mounted with glycerol gelatin (Sigma, Cat. No. GG1).

3 | IMMUNOHISTOCHEMISTRY

Some sections were processed for immunohistochemistry to detect calbindin (CB), using a rabbit polyclonal antibody CB38 against recombinant rat calbindin D-28k (Swant, Cat# CB-38, lot# 9.03, RRID AB_10000340). This antibody crossreacts with calbindin D-28k from many species, including human, monkey, rat, mouse, chicken, and fish, and it has been previously used to detect calbindin in adult *Psammodromus algirus* (Dávila, Andreu, Real, Puelles, & Guirado, 2002; Guirado et al., 1999, 2000). Our staining with this antiserum is identical to that formerly described in adult lizards of this species (Dávila et al., 2002; Guirado et al., 1999, 2000).

The primary antibody was diluted at 1:2000 in PBS containing 0.3% Triton X-100, and the tissue was incubated for 2 days at 4°C, under constant and gentle agitation. Following this incubation and standard washes in PBS-Triton, the sections were incubated in a secondary antiserum for 1 hr at room temperature. The secondary antisera used was biotinylated goat antirabbit (diluted 1:200), purchased from Vector (Burlingame, CA, USA). After washing, the sections were incubated in the avidin-biotin complex (ABC kit; Vector; 0,003% dilution) for 1 hr at room temperature. The immunolabeling was revealed with 0.05% diaminobenzidine (DAB; Sigma-Aldrich, Steinheim, Germany) in 0.05 M Tris (pH 7.6), containing 0.03% H_2O_2. Finally, the sections were rinsed and mounted with glycerol gelatin.

3.1 | Digital photographs and figures

Digital photographs from hybridized and immunostained sections were taken on a Leica microscope (DMR HC) equipped with a Zeiss Axiovision digital camera. Selected digital images were adjusted for brightness/contrast using Corel PHOTO-PAINT 12 and figures were mounted and labeled using CorelDRAW 12.

4 | RESULTS

We studied the combinatorial expression patterns of *pEmx1*, *pLhx2*, *pLhx9*, *pTbr1* and *pDlx2* in the telencephalon of the long-tailed lizard *P. algirus* throughout development. At early stages, all these genes showed expression patterns in the lizard forebrain identical to those of the orthologous genes in mouse and chicken (Abellán et al., 2009; Puelles et al., 2000). In the telencephalon, these expression patterns allowed the delineation of the two major divisions, pallium and subpallium. In addition, the combinatorial expression patterns, when followed throughout development with careful consideration of the topological position, allowed the distinction of six major pallial subdivisions and analysis of their main derivatives.

To better show the temporal as well as spatial variations in the expression patterns, the figures are presented following a sequence from early (S35-S36: Figures 1–8) to intermediate and late stages of embryonic development (S37: Figure 9; S38: Figures 10 and 11), and each figure shows series of sections following a rostrocaudal order from top to bottom, which sometimes continues in the following figure (e.g., the series of frontal sections of S35 shown in Figure 1 continue with those in Figure 2 and then in Figure 3). Moreover, to better understand the expression patterns, in some cases we show adjacent sections of the same brain hybridized for two different genes (e.g., *pDlx2* versus *pTbr1* in Figures 1–3; *pEmx1* versus *pLhx9* in Figures 1, 7, 8; *pLhx2* versus *pLhx9* in Figures 4, 5; or *pTbr1* versus *pLhx9* in Figure 6). In frontal section, where the two hemispheres were shown, we labeled the right side using our interpretation of six pallial divisions (as explained below; see Tables 1 and 2), while the left side was labeled using the classical nomenclature of brain nuclei and areas. At the end, we include three additional figures to help clarify the results and our interpretation: two of them show schemes of the pallial divisions in different views or section planes using a color code (Figures 12 and 13), and the last one (Figure 14) shows a series of Nissl-stained frontal sections combining the labeling of six pallial divisions (on the right side) with the classical nomenclature (left side).

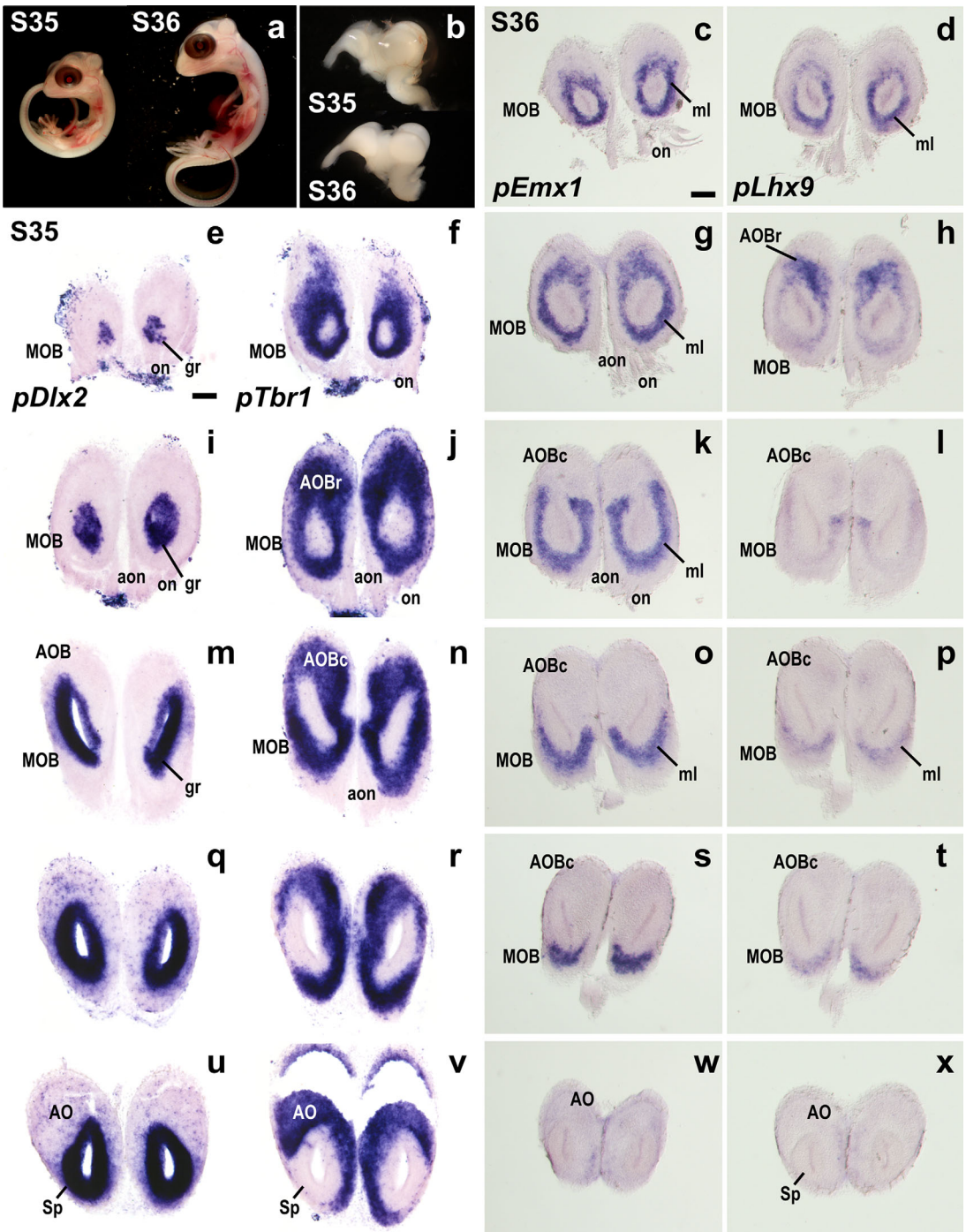

FIGURE 1 (a) Lateral views of embryos of the lizard *P. algirus*, at developmental stages 35 (S35) and 36 (S36). (b) Lateral views of the brain of the lizard *P. algirus*, at S35 and S36. (c–x) Serial frontal sections through the embryonic olfactory bulbs of *P. algirus*, at S35 or S36, from rostral (top) to caudal (bottom) levels, hybridized for *pEmx1*, *pLhx9*, *pDlx2*, or *pTbr1*. For abbreviations see list. Scale bar (in c, applies to all): 100 μm

4.1 | Pallium versus subpallium

The combinatorial expression patterns of *pEmx1*, *pLhx2*, *pLhx9*, *pTbr1*, and *pDlx2* allowed the distinction of the pallial and subpallial telencephalic divisions from early stages (S34/S35 and S36) (Figures 1–8). The subpallium shows strong expression of *pDlx2* and light or moderate expression of *pLhx2* in the progenitor cell layer (ventricular zone; vz), and is mostly or completely devoid of *pEmx1*, *pLhx9*, and *pTbr1*, with a few exceptions mentioned below (Figures 1–7). In contrast, the pallium shows intense or moderate expression of *pLhx2*, *pLhx9*, *pEmx1*, and *pTbr1* in the vz and/or in the mantle zone, with differences between subdivisions that will be explained in next section (Figures 1–7). The pallium also includes subpopulations of cells showing expression of *pDlx2*, which are very large in

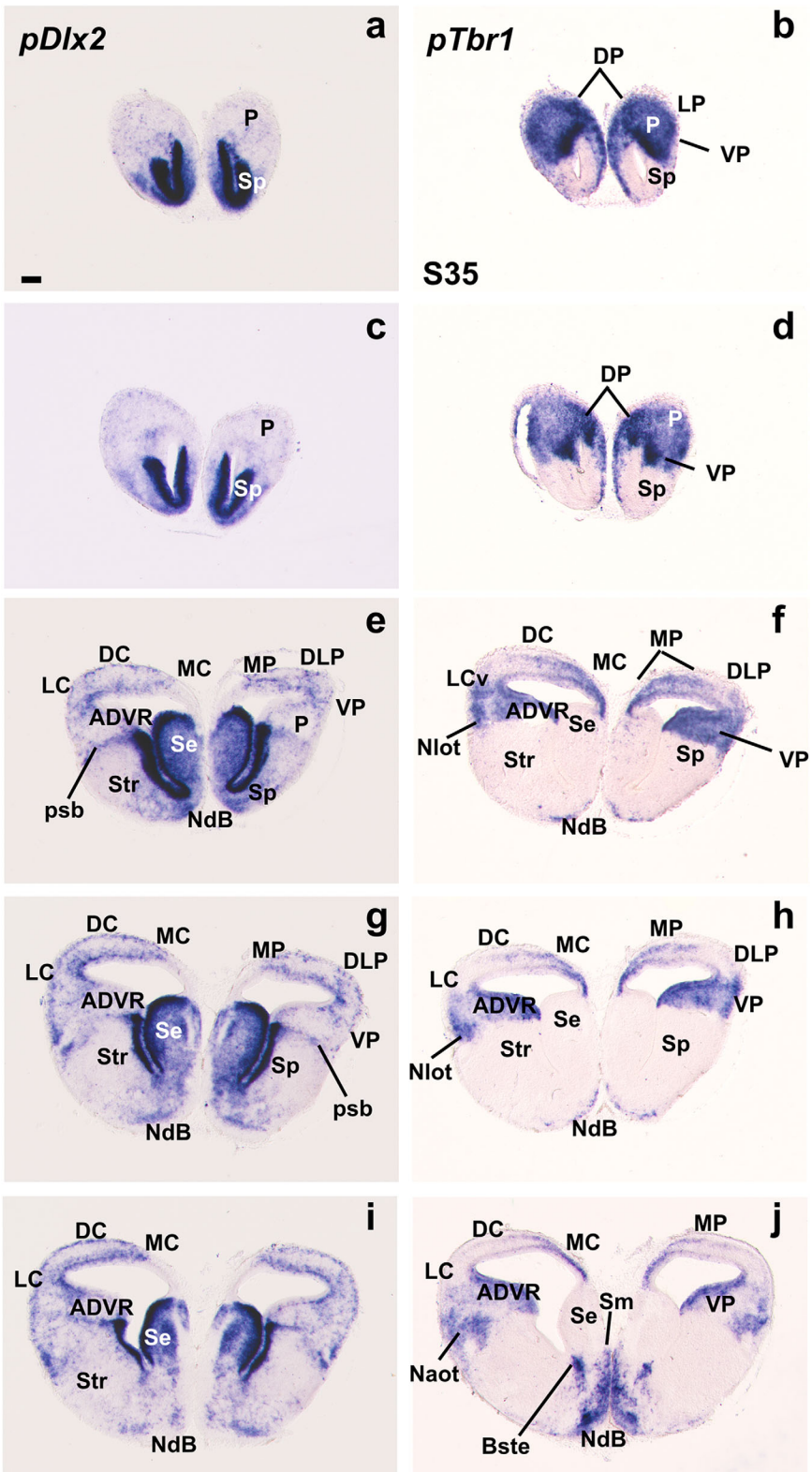

FIGURE 2 Series of adjacent frontal sections through the embryonic telencephalon of *P. algirus*, at S35, from rostral (top) to intermediate (bottom) levels, hybridized for *pDlx2* or *pTbr1*. For abbreviations see list. Scale bar (in a, applies to all): 100 μm

the case of the olfactory bulbs (Figure 1) but less numerous in other parts of the pallium (Figures 2 and 3). These *pDlx2* expressing cells of the pallium seem comparable to the interneurons shown to migrate from the subpallium to the pallium in other vertebrates, following the rostral migratory stream or other pathways (Marín & Rubenstein, 2001; Moreno, Gonzalez, & Retaux, 2008; Quintana-Urzainqui et al., 2015).

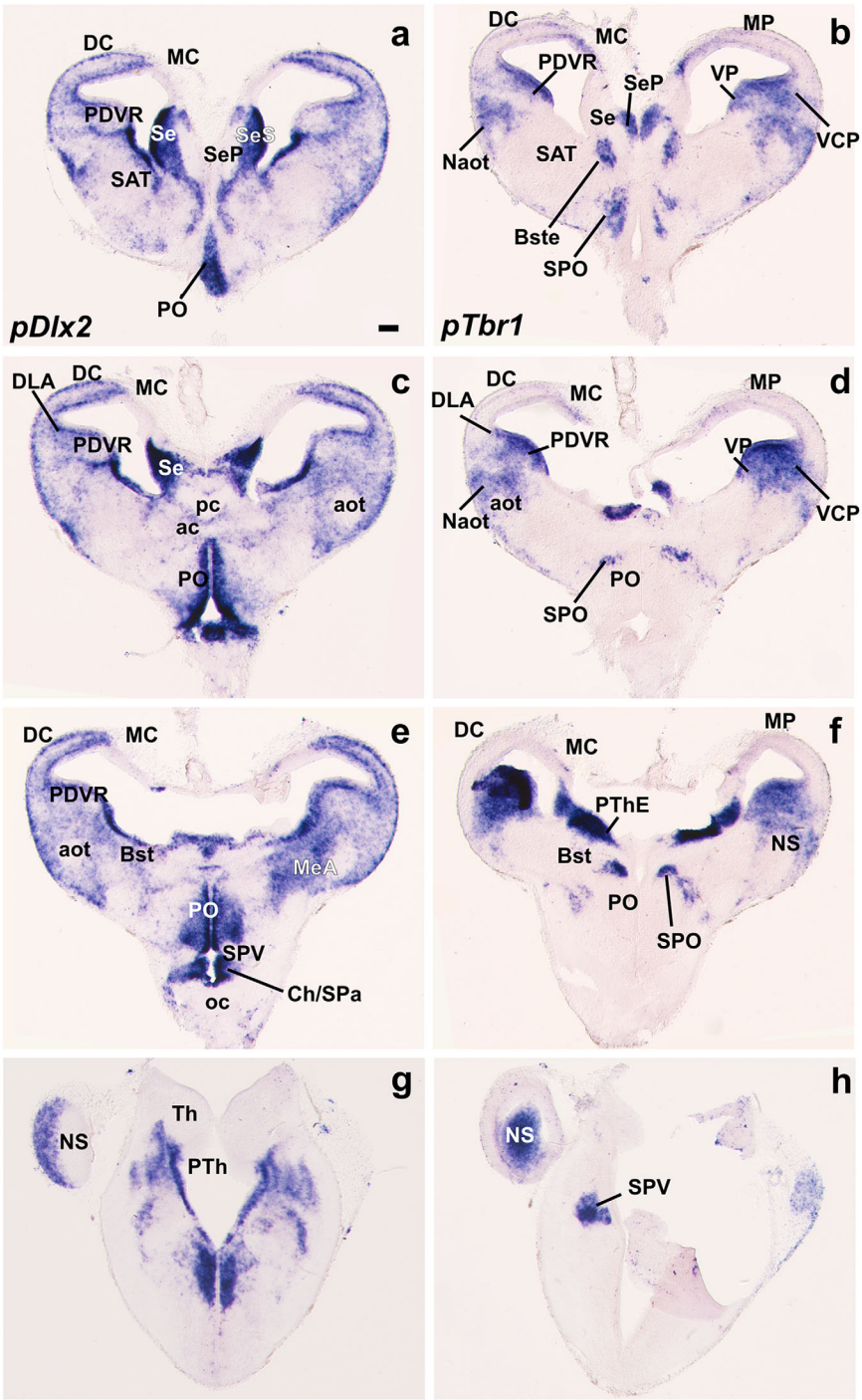

FIGURE 3 Series of adjacent frontal sections through the embryonic telencephalon of *P. algirus*, at S35, from intermediate (top) to caudal (bottom) levels, hybridized for *pDlx2* or *pTbr1*. This series of sections is the caudal continuation of that shown in Figure 2. For abbreviations see list. Scale bar (in a, applies to all): 100 μm

Based on these patterns, the subpallium includes the primordia of the basal ganglia and centromedial extended amygdala (laterally), most of the septum (medially) and the preoptic area (in the nonevaginated telencephalon; Figures 2 and 3). On the contrary, the pallium includes the primordia of the medial, dorsomedial, dorsal and lateral cortices (MC, DMC, DC, and LC, respectively), and the dorsal ventricular ridge (DVR), including the primordia of nuclei considered part of the pallial amygdala such as the dorsolateral amygdala (DLA) and nucleus sphericus (NS) (Figures 2–5). In addition, the pallium includes a small part of the septum, observed at intermediate levels of the telencephalon. This relates to a small dorsal septal ventricular sector expressing *pLhx9* (SeP, Figure 4l,n) but devoid of *pDlx2* (Figure 2d,e). More caudally, a compact cell group expressing *pLhx9* and *pTbr1*, but not *pDlx2*, is observed in the septal pallial mantle, occupying the dorsomedial region (SeP,

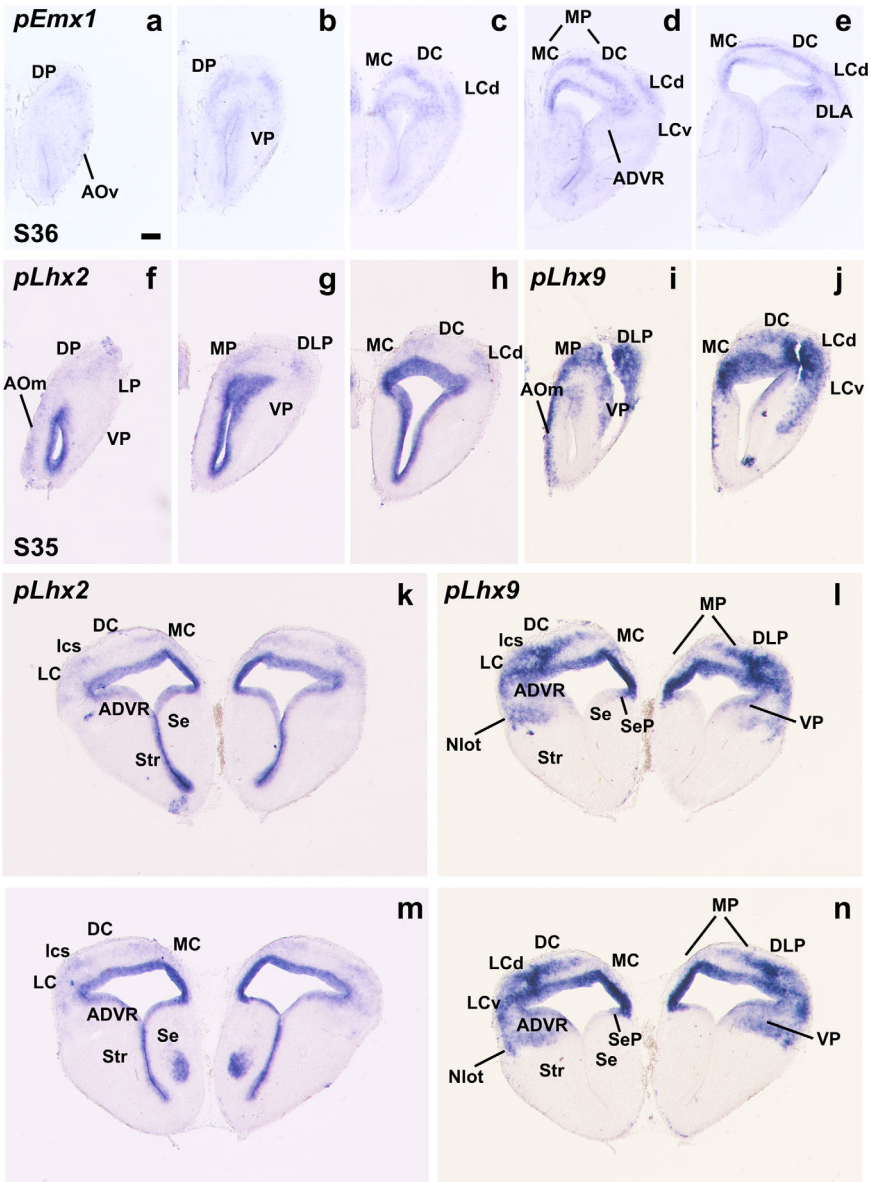

FIGURE 4 (a–e) Serial frontal sections through the embryonic telencephalon of *P. algirus*, at S36, from rostral (a) to intermediate (e) levels, hybridized for *pEmx1*. (f–n) Series of adjacent frontal sections through the embryonic telencephalon of *P. algirus*, at S35 (late), from rostral (f,i) to intermediate (m,n) levels, hybridized for *pLhx2* (f–h,k,m) or *pLhx9* (i,j,l,n). The section plane in these series is a little oblique when compared to the series of frontal sections shown in other figures, so that the top part of the sections shown in this figure is more caudal than the bottom part (note the lateral horn of the ventricle, already visible in c, g and j). For abbreviations see list. Scale bar (in a, applies to all): 100 μm

Figures 3a,b and 5d). This dorsomedial part of the septum seems contiguous to the prethalamic eminence (Figure 6a–c; see last section of Results). From this compact dorsomedial septal group, a stream of cells expressing *pLhx9* and *pTbr1* appears to extend ventromedially, to reach the medial septum (Sm) and nucleus of the diagonal band (NdB) (Figures 2f,h,j and 5b,d), where they occupy a superficial stratum adjacent to another one (deeper) with *pDlx2* expressing cells of subpallial origin (compare panels g and h in Figure 2). Analysis at later stages suggests that part of the cells from the dorsomedial septal group will form the nucleus of the pallial commissure (Ncp, Figure 8d,f).

4.2 | Interneurons of subpallial origin in the pallium

From S34, the pallium of *P. algirus* contains Dlx2 expressing cells, which presumably may originate in the subpallium, as described in other vertebrates (Abellán & Medina, 2009; Anderson, Eisenstat, Shi, & Rubenstein, 1997; Brox et al., 2003; Cobos, Puelles, & Martínez, 2001a; Marín & Rubenstein, 2001; Moreno et al., 2008; Quintana-Urzainqui et al., 2015). These cells do not distribute homogeneously, but show a trend to aggregate along major migration streams that follow the pallio-subpallial boundary, the pallial subventricular zone and the pallial marginal zone (Figures 1–3). One of these putative *pDlx2*-expressing cell migration streams of

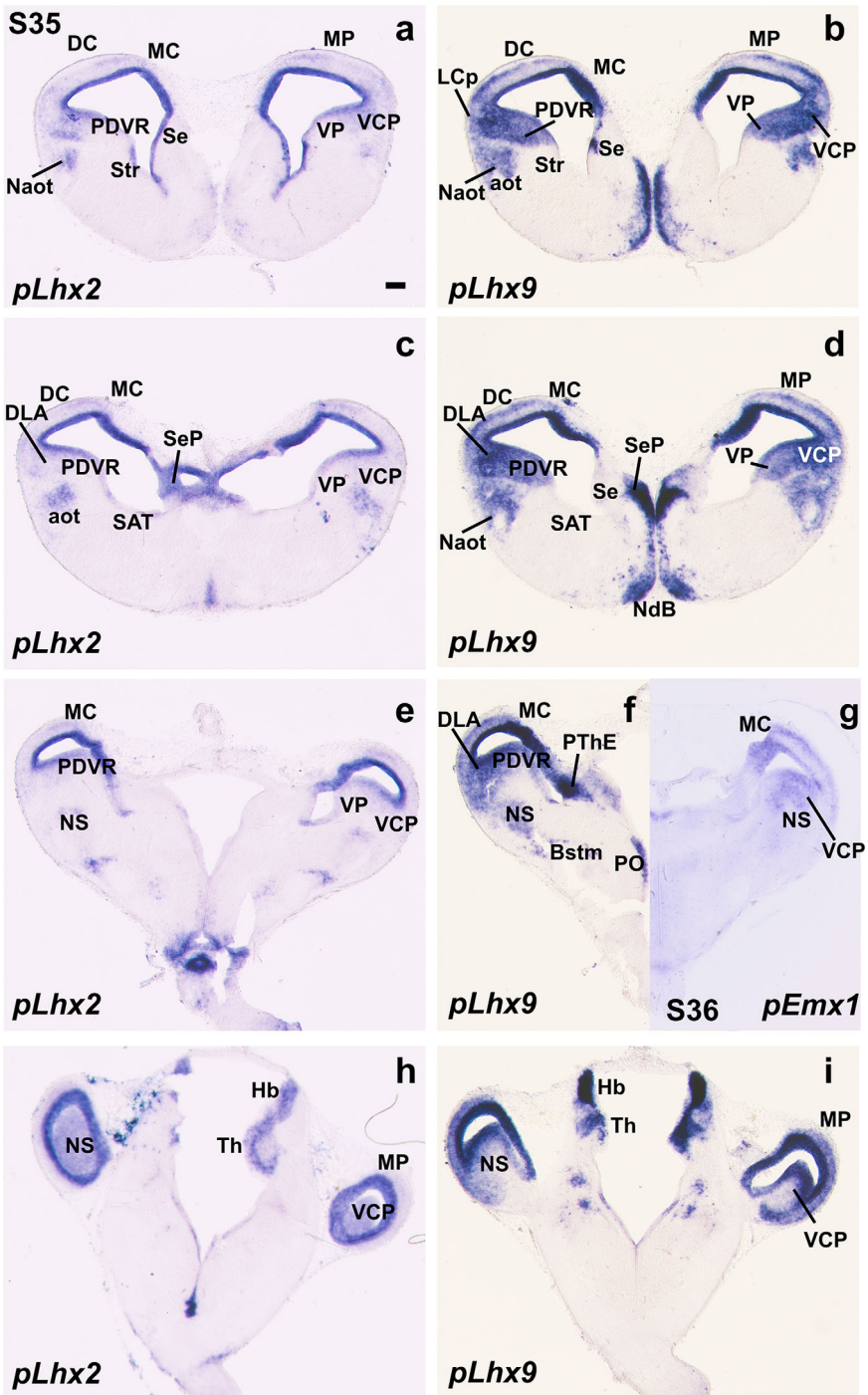

FIGURE 5 (a–i) Series of adjacent frontal sections through the embryonic telencephalon of *P. algirus*, at S35 (late), from intermediate (a,b) to caudal (h,i) levels, hybridized for *pLhx2* (a,c,e,h) or *pLhx9* (b,d,f, i). (g) Image of a frontal section similar to that shown in f-left, hybridized for *pEmx1* (S36 brain). For abbreviations see list. Scale bar (in a, applies to all): 100 μm

the lizard that is particularly massive is the one seen in the periventricular area of the olfactory bulbs, where the granular cell layer is forming (Figure 1). This appears to correspond to the so-called "rostral migratory stream," which in other vertebrates was shown to follow the subventricular zone to reach olfactory bulbs, giving rise to subpopulations of interneurons as the granular and the periglomerular cells (Alvarez-Buylla & Garcia-Verdugo, 2002). The "rostral migratory stream" has also been described in lizards and appears to retain cells with mitotic activity throughout life, being described as a neurogenic niche in the adult brain (Font, Desfilis, Pérez-Cañellas, & García-Verdugo, 2001).

4.3 | Pallial subdivisions

The combinatorial expression of *pEmx1*, *pLhx2*, *pLhx9*, and *pTbr1*, together with the topological position, allowed the distinction of the following six subdivisions within the pallium of the lizard *P. algirus*:

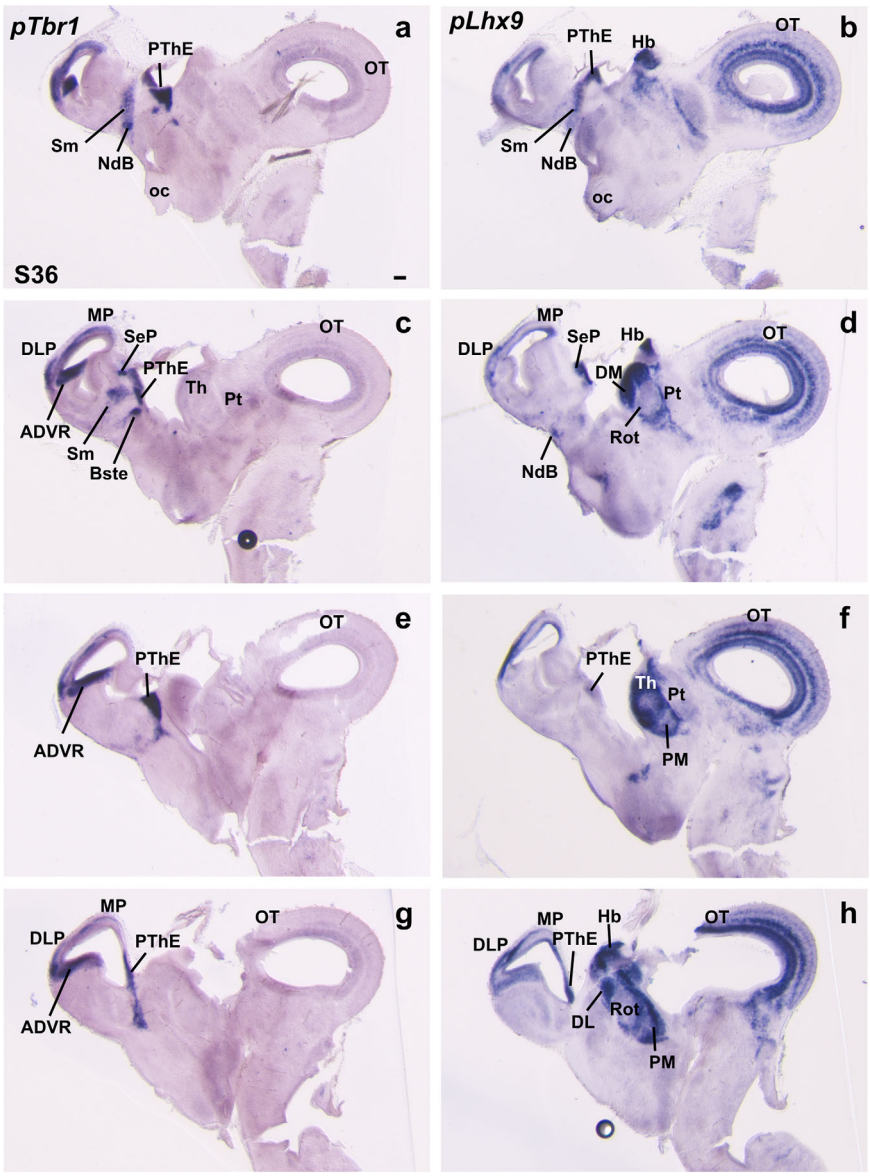

FIGURE 6 Series of adjacent sagittal sections through the embryonic telencephalon of *P. algirus*, at S36, from lateral (a,b) to medial (g,h) levels, hybridized for *pTbr1* (a,c,e,g) or *pLhx9* (b,d,f, h). For abbreviations see list. Scale bar (in a, applies to all): 100 μm

1. Medial Pallium (MP). As in mouse and chicken (Abellán et al., 2014), the MP of the lizard *P. algirus* was characterized by moderate or strong expression of *pEmx1*, *pLhx2*, and *pLhx9* in the vz and mantle (Figures 4–8) and *pTbr1* expression in the mantle (Figures 2, 3 and 6) at early developmental stages (S34/S35 and S36). Based on this, the medial pallium of *P. algirus* includes most of the medial and dorsal aspects of the future cortical plate, giving rise to MC, DMC, and most DC, except the lateral cortical superposition, which shows a different expression pattern (details explained below) and appears to belong to a different dorsolateral pallial sector.

At S35, there was evidence for both lateromedial and rostrocaudal gradients of development in the medial pallium. The MC sector was the most immature, showing a very thick vz and a very thin mantle, with the cortical plate being only barely visible (Figures 4 and 5). At S36, the cortical plate was better defined and was more clearly visible in all sectors of the medial pallium: MC, DMC and DC (Figures 6–8). However, the vz was thinner throughout the medial pallium at S36, and showed lighter expression of *pLhx9* than at S35. Nevertheless, the expression patterns were similar at S35 and S36, with *pLhx2*, *pEmx1* and *pLhx9* showing mediolateral decreasing gradients in the vz: these genes were expressed moderately (*pLhx2*, *pEmx1*) or strongly (*pLhx9*) in the MC vz but gradually decreased laterally (Figures 4, 5, 7 and 8). For *pLhx9*, this decreasing gradient abruptly stopped when reaching the vz sector related to the lateral cortical superposition (as noted above, not part of the medial pallium), where *pLhx9* was very strong again (Figure 4l,n).

In contrast to the vz, the expression of *pLhx2* and *pLhx9* in the cortical plate of the medial pallium showed opposite gradients at S35 (e.g., compare Figure 4k with Figure 4l): *pLhx2* was moderate in the

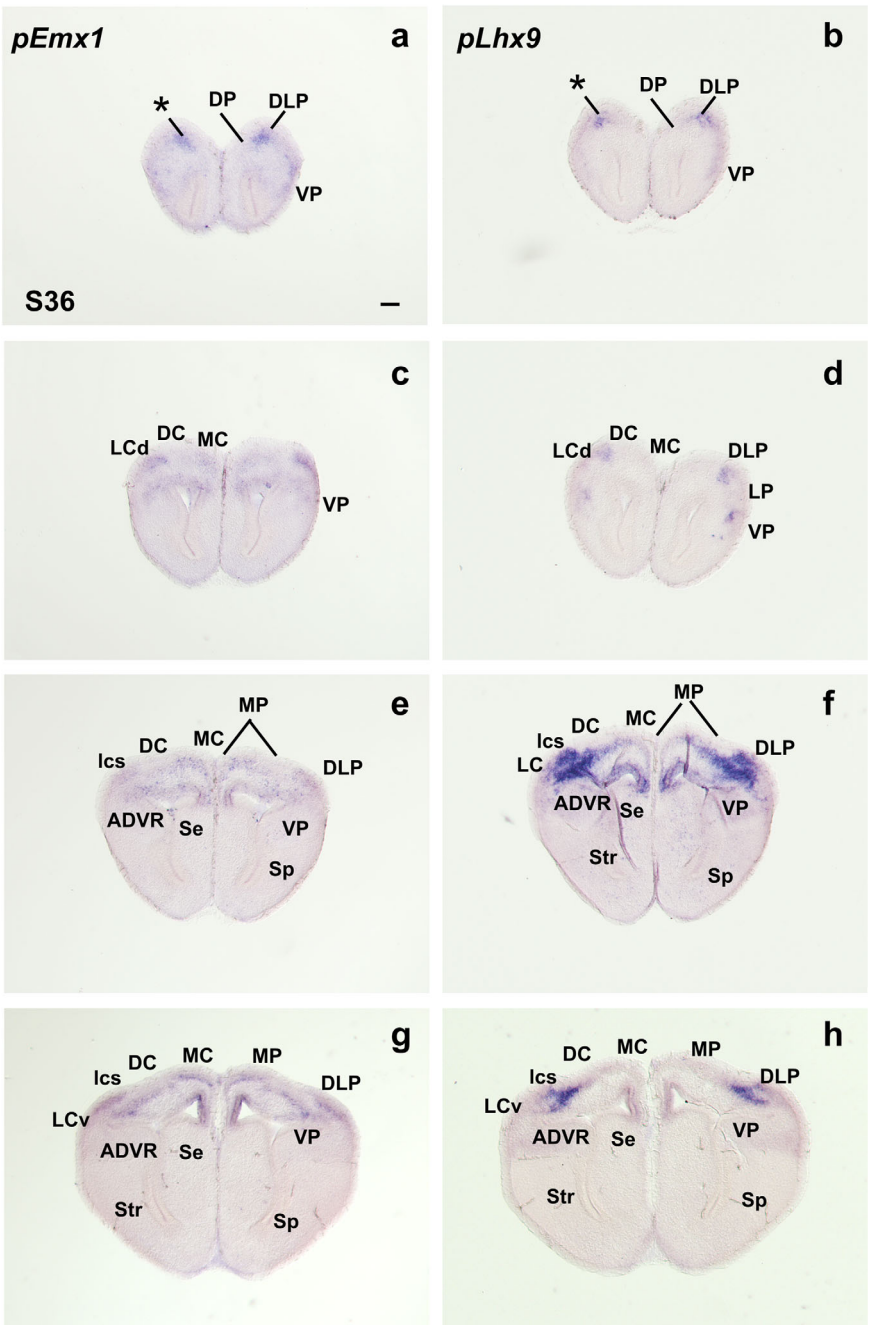

FIGURE 7 Series of adjacent frontal sections through the embryonic telencephalon of *P. algirus*, at S36, from rostral (a,b) to intermediate (g,h) levels, hybridized for *pEmx1* (a,c,e,g) or *pLhx9* (b,d,f, h). This series of sections is the caudal continuation of that shown in Figure 2. For abbreviations see list. Scale bar (in a, applies to all): 100 μm

cortical plates of MC and DMC, but was extremely light in DC (except the area of the lateral superposition); however, *pLhx9* expression was strong in the cortical plate of DC (particularly in the intermediate sector or DC2), moderate in DMC and light in MC (Figures 4l,n and 5b,d). The patterns were similar at S36, although the expression of *pLhx9* became very light in the DMC cortical plate, and almost absent in the MC cortical plate (Figures 7 and 8). At these early stages, the cortical plates of MC, DMC, and DC showed moderate expression of *pEmx1* (Figures 4c–e, 7e,g, and 8a,c,e,g), and expression of *pTbr1*, ranging from strong at rostral levels (Figure 2f), to moderate at intermediate levels (Figures 2j and 3b), to negligible caudally (Figure 3d,f), with minor differences between areas.

At intermediate (S37, S38) and late embryonic stages (S39), the expression pattern of *pLhx9* remained similar in the medial pallium, although the expression in the vz was much lighter (Figures 9–11). In the cortical plate, each sector shows a specific pattern: MC plate was devoid of *pLhx9* expression, DMC plate and the medial sector of DC plate (DC1) showed very light expression, while the intermediate sector of DC (DC2) plate show moderate expression medially and strong expression laterally (Figures 9f–j and 10d–f). Based on its distinct

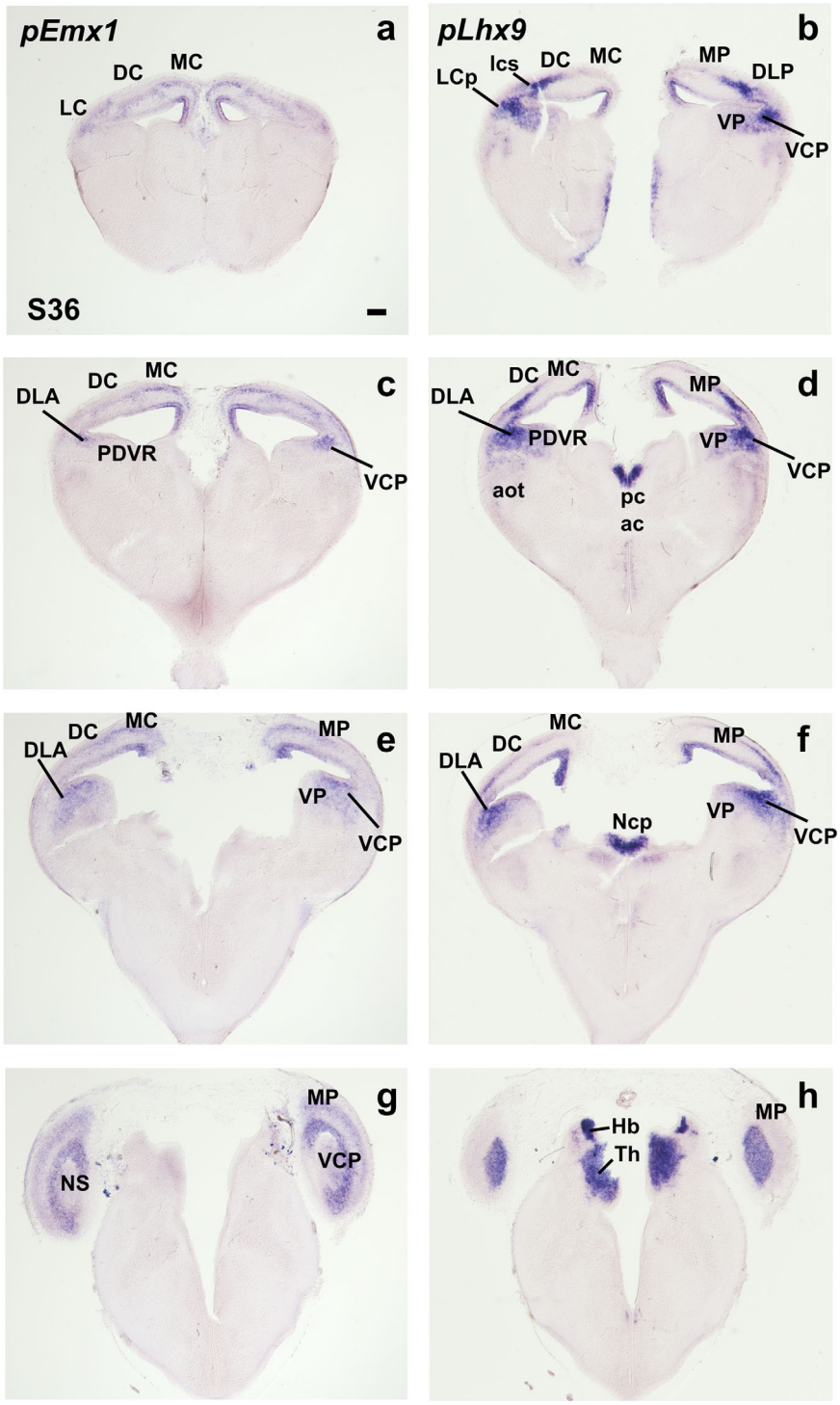

FIGURE 8 Series of adjacent frontal sections through the embryonic telencephalon of *P. algirus*, at S36, from intermediate (a,b) to caudal (g,h) levels, hybridized for *pEmx1* (a,c,e,g) or *pLhx9* (b,d,f, h). This series of sections is the caudal continuation of that shown in Figure 7. For abbreviations see list. Scale bar (in a, applies to all): 100 μm

pLhx9 expression, DC2 appeared to extend laterally at caudal levels, as the lateral cortical superposition disappears (Figures 9l,m and 10g). At very caudal levels, DC2 is no longer present and MC and DMC occupy all the cortical plate (Figure 10h).

2. Dorsal Pallium (DP). Since the lizard DC appears to be part of the medial pallium, an important question is where the dorsal pallium of lizards is. In mouse and chicken, the dorsal pallium expresses *Lhx2* and *Emx1* in the vz and mantle, *Tbr1* in the mantle, but is generally free of *Lhx9* except for a transitory light expression restricted to the preplate at very early stages; however, DP vz is always devoid of Lhx9 expression (Abellán et al., 2009; Rétaux, Rogard, Bach, Failli, & Besson, 1999). Using these combinatorial expression patterns, and considering

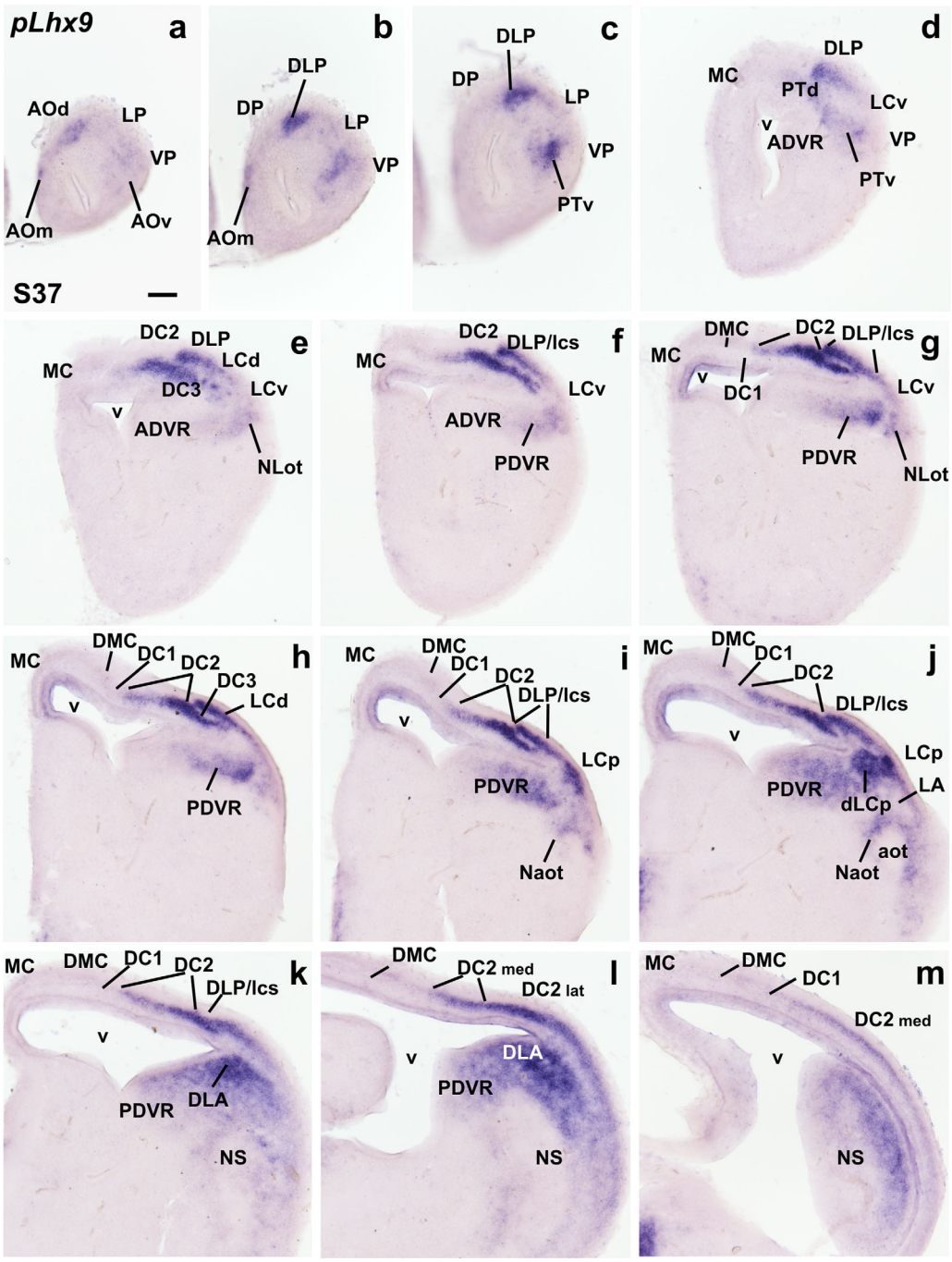

FIGURE 9 Series of frontal sections through the embryonic telencephalon of *P. algirus*, at S37, from rostral (a) to caudal (M) levels, hybridized for *pLhx9*. For abbreviations see list. The caudal part of the dorsolateral pallium (DLP) includes the lateral cortical superposition (lcs). Scale bar (in a, applies to all): 100 μm

the rostral and dorsomedial location of the dorsal pallium in birds (giving rise to the hyperpallium; Medina, 2007b; Puelles, 2001), we found a small rostral and dorsomedial pallial area in the lizard *P. algirus* that may represent the dorsal pallium (DP in Figures 2b,d, 4a,b, 7a, and 9b, c). According to our interpretation, this rostral and dorsomedial pallial sector is distinct from DC and from any other part of the medial pallium, since it expresses *pEmx1* and *pLhx2* in the vz and mantle, and *pTbr1* in the mantle, but lacks *pLhx9* vz expression (*pEmx1*: Figure 4a,b; *pLhx2*: Figure 4f; *pLhx9*: Figures 7b and 9b,c; *pTbr1*: Figure 2b,d). The putative DP was seen as a column of *pTbr1* and *pEmx1* expressing cells that extended dorsally from the ventricular zone (Figures 2b,d and 4b), highly resembling the avian apical hyperpallium. This same area was previously considered to be the most rostral pole of the so-called general pallium (including DC) by Johnston (1915; see his Figures 26 and 27). In our Nissl material of adult lizard, we also identified this area as a column of stained cells resembling the avian apical hyperpallium (Figure 14a,b). As in mouse and chicken, this pallial sector of the lizard *P. algirus* appears to show transient *pLhx9* in the mantle at very early stages

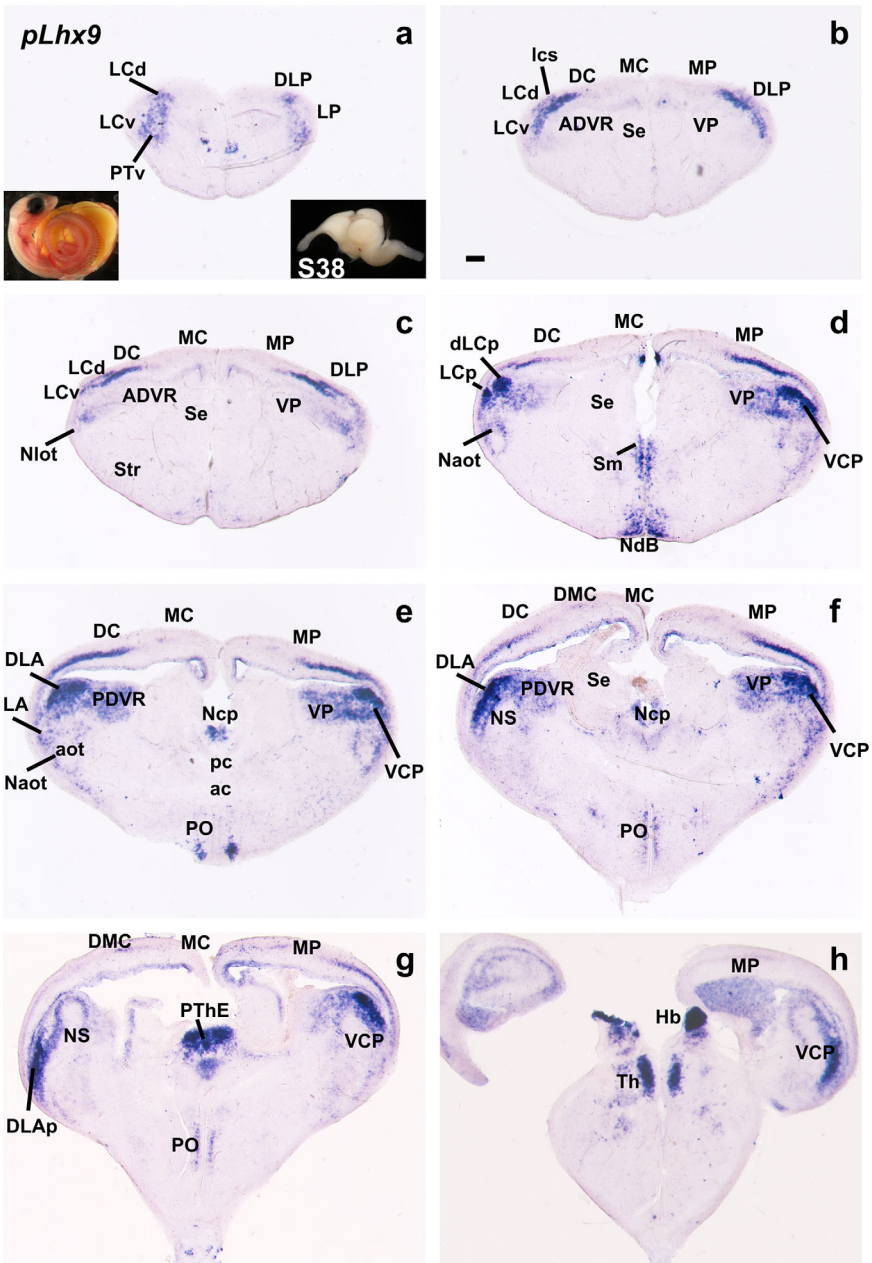

FIGURE 10 Series of frontal sections through the embryonic telencephalon of *P. algirus*, at S38, from rostral (a) to caudal (h) levels, hybridized for *pLhx9*. The inserts in a show an embryo of *P. algirus* and its brain at S38. For abbreviations see list. Scale bar (in b, applies to all): 100 μm

(S35), but later the *pLhx9* expression disappears (S36, Figure 7b; S37, Figure 9b,c). At S36 and S37, the *pLhx9*-free area (putative DP) loses expression of *pEmx1* (Figures 7a and 9b,c), but it is flanked laterally by a pallial sector that expresses both *pEmx1* and *pLhx9* in the mantle, with at least partial if not complete overlapping patterns, which does not appear to belong to DP, but perhaps represents a rostral extension of the dorsolateral pallium (asterisk and DLP in Figure 7a,b; DLP in Figure 9b,c).

3. Dorsolateral Pallium (DLP). We recently identified the DLP in chicken and mouse as a distinct pallial sector topologically located between the DP (rostromedially), MP (caudomedially), LP (rostroventrally), and VP/VCP (ventrally and caudoventrally, respectively; Abellán et al., 2014). During development, it expresses Emx1, Lhx2, and Lhx9 (Abellán et al., 2009, 2014; Puelles et al., 2000). In the lizard *P. algirus*, the comparable pallial division appears to encompass the lateral cortical superposition, which includes a double cortical plate in the mantle formed by the lateralmost part of DC (DC3) and the dorsalmost part of LC (LCd), and the ventricular sector related to it. This pallial division of *P. algirus* shows moderate expression of *pLhx2* and *pEmx1* at early stages, and a very strong and distinctive expression of *pLhx9* that remains high from early (Figures 4–8) through late embryonic stages (Figures 9–11). Based on this

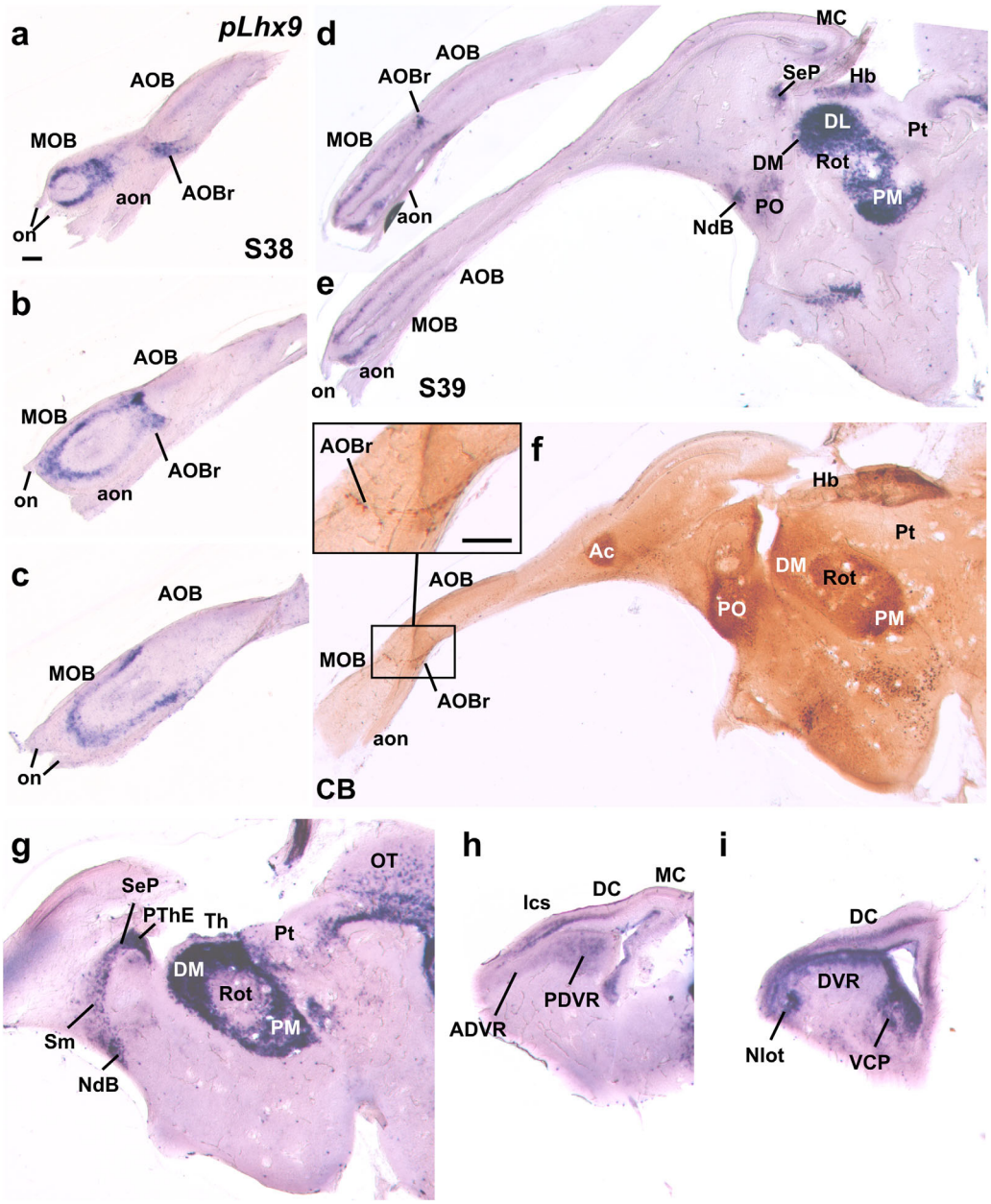

FIGURE 11 (a–d) Series of adjacent sagittal sections through the olfactory bulb of *P. algirus*, at S39, hybridized for *pLhx9*. (e, g–i) Series of adjacent sagittal sections through the telencephalon of *P. algirus*, at S39, at medial (e, g) or lateral (h, i) levels, hybridized for *pLhx9*. (f) Sagittal section adjacent to that shown in e, immunostained for calbindin. The insert in f shows a detail of the rostral part of the accessory olfactory bulb (AOBr), which contains many calbindin-positive cells. This same part expresses *pLhx9* (a,b,d), but the caudal part of the AOB is free of *pLhx9* expression. For abbreviations see list. Scale bar (in a, applies to all): 100 μm

expression profile and its topological position, our results suggest that the DLP includes a caudal (main) part encompassing the lateral cortical superposition, and a rostral part (rostral continuation of DLP (DLP and asterisk in Figures 7a,b and 9b,c) that extends to the vicinity of the dorsal anterior olfactory area (AOd, Figure 9a). As seen in Nissl staining, the rostral DLP includes a dorsal part of the pallial thickening (PTd), which seems to be caudally continuous with DC3 (Figure 9d). Moreover, it relates to a defined cortical plate (Figure 14a), which has sometimes been included as part of the rostral LC (fig. 13A in Martínez-Marcos, Lanuza, & Halpern, 1999). However, the rostral LC defined by Martínez-Marcos et al. (1999) also includes a lateral part that appears to belong to LP.

Overall, the DLP can be distinguished from adjacent pallial sectors regarding the combination of genes expressed (rostrally) and/or the spatiotemporal pattern of expression (caudally). At rostral levels, the *pLhx9*-expressing DLP can be distinguished because it is flanked by two pallial divisions negative for *pLhx9*, DP and LP (Figures 7b,d and 9b,c). Moreover, *pEmx1* shows a stronger expression in DLP as compared to that in DP and LP (Figure 7a). At caudal levels (i.e., the main DLP related to the lcs), it is distinguished from the adjacent medial

TABLE 1 Comparison between different divisional models of the pallium

Three parts model (classical)	Four parts model (Puelles-2000)	Four parts model revisited (Puelles-2014)	Six parts model—current proposal
Medial pallium Hippocampal formation	**Medial pallium** Hippocampal formation	**Medial pallium** Hippocampal complex	**Medial pallium** Hippocampal formation and medial entorhinal cortex
Dorsal pallium Neocortex	**Dorsal pallium** Neocortex	**Dorsal pallium** Neocortex, except insular cortex	**Dorsal pallium** Neocortex, except insular cortex and orbitofrontal cortex
			Dorsolateral pallium **Rostral**: orbitofrontal cortex and dorsal peduncular cortex? **Intermediate**: Insular cortex and dorsal or insular claustrum. **Caudal**: Perirhinal cortex and lateral entorhinal cortex
Lateral pallium Piriform Lobe, including olfactory or piriform cortex, claustrum, endopiriform nuclei, and pallial amygdala	**Lateral pallium** Dorsal or insular claustrum, dorsal part of piriform cortex, and part of the pallial amygdala	**Lateral pallium** Dorsal or insular claustrum and insular cortex; it also gives rise to the dorsal endopiriform nucleus by tangential migration	**Lateral pallium** Dorsal endopiriform nucleus and dorsal part of piriform cortex
	Ventral pallium Olfactory bulbs, ventral part of the piriform cortex, ventral claustrum, endopiriform nuclei, and part of the pallial amygdala	**Ventral pallium** Ventral endopiriform nucleus, piriform cortex, and pallial amygdala	**Ventral pallium** Ventral endopiriform nucleus, ventral piriform cortex, and anterior part of pallial amygdala
			Ventrocaudal pallium Posterior part of pallial amygdala and part of amygdalo-hippocampal area

pallium (also expressing *pLhx9*, in addition to *pLhx2* and *pEmx1*) because of its distinct spatiotemporal expression patterns of *pLhx9* and *pTbr1*. At S35, *pLhx9* expands throughout all DLP thickness from the vz to the cortical plate (Figures 4j,l,n and 5b); later in development (from S36), the *pLhx9* expression in the vz and inner plexiform layer diminishes, but the cortical plate continues to show a remarkably strong expression (S36: Figures 7f,h and 8b,d; S37: Figure 9e–j; S38: Figure 10b,c). Regarding *pTbr1*, at S35, it shows a rostrocaudal decreasing expression pattern and, as a consequence, the caudal DLP mantle shows light to extremely light expression of *pTbr1* at this early stage (Figure 2f,h,j), making the caudal part of this pallial area different from the rest at this age; nevertheless, as the rest of the pallium, this division also expresses *pTbr1* at later stages (Figure 6c,e).

Another interesting aspect revealed by the expression of *pLhx9* refers to the formation of the double cortical plate of caudal DLP. At S35, only a single cortical plate is observed in caudal DLP, which expresses *pLhx9* and appears to represent the DC3 area, as it is aligned to the rest of DC (Figure 4l,n). From S36 on, the DLP cortical plate is double with the formation of an outer cell layer representing LCd, which also expresses *pLhx9* (Figures 6h and 7f,h) and *pEmx1* (Figures 4d and 6e,g). This suggests an inside-out pattern of layer formation in DLP, with the DC3 being formed first, followed by LCd.

4. Lateral Pallium (LP). The LP locates between DP (dorsomedially) and VP (ventrally) and gives rise to a dorsal part of the DVR that includes the mesopallium in chicken (Puelles et al., 2000, 2016a). During development, it is characterized by expression of Emx1 in both vz and mantle, expression of Tbr1 and Nr4a2/Nurr1 in part of the mantle (Puelles et al., 2000, 2016a), expression of Lhx2 in the vz, and lack of expression of Lhx9 (Abellán et al., 2009, 2014). Based on its topological position between DP and VP, and its gene expression profile, we tentatively identified the LP at very rostral levels of the brain of *P. algirus* as an area free of *pLhx9* expression in Figures 7b,d (S36) and Figures 9a–c (S37), which shows light *pEmx1* expression (Figure 7a; see Table 2). In Nissl material of adult lizard, this division includes an anterior or rostral part of LC (LCa; Figure 14b,c), as well as an intermediate part of the pallial thickening located deep to it (i in Figure 13; Table 2). In the lizard, it appears that the putative LP does not contact directly the DP, as the rostral extension of DLP interposes between both. As a result, the LP appears as a *pLhx9*-negative area located between two *pLhx9*-positive domains.

5. Ventral Pallium (VP). The VP in chicken locates just above the pallio-subpallial boundary, below LP and DLP, and produces the olfactory bulb and anterior olfactory area, the piriform (olfactory) cortex, and a ventral part of the DVR that includes the nidopallium (Puelles et al., 2000). During development, it is characterized by moderate-to-strong expression of Tbr1 and Lhx9 in the mantle, very light Lhx2 expression in its vz, and because it is free of Emx1 in the vz and most of its mantle, except for its marginal zone; the latter includes immigrant cells from a more dorsal pallial division (Tomioka et al., 2000), some of which appear to express Emx1 based on the gradual invasion of the VP

TABLE 2 Proposed comparison of pallial divisions across amniotes and derivatives of each division

Pallial division	Lizard	Chicken	Mouse	vz[a]	mz
MP	MC, DMC, most DC (DC1 + DC2)	HF (DG, APHm+i/DM, APHl/DL), Ent-m (APHcl/CDL)	HF (DG, CA1–3, Sub), MEC	Emx1 Lhx2 Lhx9 Lef1[b]	Emx1 Lhx2 Lhx9 Lef1[b]
DP	Rostral dorsomedial pallium	Hyperpallium (Wulst)	NCx	Emx1 Lhx2	Emx1 Lhx2
DLP	Caudal DLP (lcs: LCd + DC3) Rostral DLP (rostral LCd, PTd, AOd)	Caudal DLP (dorsal TPO, 'Cluster N') Rostral DLP (Dorsal Mesopallium)	Caudal DLP (LEC, PRh) Rostral DLP (Cl-Ins plus additional rostral areas?)	Emx1 Lhx2 Lhx9	Emx1 Lhx2 Lhx9[c]
LP	LCa, PTi	Ventral Mesopallium	Dorsal Pir, DEn	Emx1 Lhx2	Emx1
VP	LCv, PTv, ADVR, PDVR, NLot, AOm, AOv	Pir, Nidopallium	Ventral Pir, VEn, ACx, most pallial amygdala (L, anterior BM, anterior BL, ACo), LOT1	Lhx2 (weak) Lhx9	Lhx2[d] Lhx9[d]
VCP	LCp, DLA, main part of LA, VPA, Naot, NS	Arcopallium, APir	Caudal part of the pallial amygdala (posterior BL, posterior BM, PCo), APir, LOT2, BAOT	Emx1 Lhx2 Lhx9	Emx1 Lhx2 Lhx9

[a]Expression in the ventricular zone occurs at early developmental stages and, in most cases, is downregulated from middle stages
[b]Lef1 is only available in mouse and chicken (based on Abellán et al., 2014)
[c]Lhx9 is observed in the mantle of rostral DLP in lizards, but not in the putative rostral DLP of chicken and mouse. Their inclusion as part of DLP is based on relative position, expression of Nr4a2 and cerebellin 2, plus other data (see text for discussion)
[d]Mostly restricted to rostral (olfactory-related) and caudal (amygdaloid) levels from intermediate developmental stages
Abbreviations: ACo = anterior cortical amygdalar area; ACx = amygdalo-cortical transition area; ADVR = anterior DVR; AOd = anterior olfactory area- = dorsal part; AOm = anterior olfactory area = medial part; AOv = anterior olfactory area = ventral part; APH = parahippocampal area (chicken); APHcl = caudolateral APH (also called CDL); APHl = lateral APH (also called DL); APHm + i = medial and intermediate parts of APH (also called DM); APir = amygdalo-piriform area; BL = basolateral amygdalar nucleus; BM = basomedial amygdalar nucleus; Cl = claustrum; Cluster N = an area of the avian caudal DLP related to magnetic compass orientation (Heyers et al., 2007); DC = dorsal cortex (lizard); DC1 = medial part of DC; DC2 = intermediate part of DC; DC3 = lateral part of DC; DEn = dorsal endopiriform ncuelus; DG = dentate gyrus; DLA = dorsolateral amygdala (lizard); DLP = dorsolateral pallium; DMC = dorsomedial cortex (lizard); DP = dorsal pallium; DVR = dorsal ventricular ridge; En = endopiriform nucleus; Ent = entorhinal cortex (chicken); HF = hippocampal formation; Ins = insular cortex; L = lateral amygdalar nucleus; LA = lateral amygdala (lizard); LC = lateral cortex (lizard); LCa = anterior part of LC; LCd = dorsal part of LC; LCp = posterior part of LC; LCv = ventral part of LC; lcs = lateral cortical superposition (lizard; includes DC3 and LCd); LEC = lateral entorhinal cortex; LOT = nucleus of the lateral olfactory tract (mouse); LOT1 = layer 1 of LOT; LOT2 = layer 2 of LOT; LP = lateral pallium; Md = dorsal part of msopallium (chicken); MEC = medial entorhinal cortex; MC = medial cortex (lizard); MP = medial pallium; Naot = nucleus of the accessory olfactory tract (lizard); NCx = neocortex; Nlot = nucleus of the lateral olfactory tract (lizard); NS = nucleus sphericus (lizard); PCo = posterior cortical amygdalar area; PDVR = posterior DVR (as described by Lanuza et al., 1998); Pir = piriform or olfactory cortex; PRh = perirhinal cortex; PT = pallial thickening (lizard); PTd = dorsal part of PT; PTi = intermediate part of PT; PTv = ventral part of PT; TPO = temporo-parieto-occipital area (birds; part of it is included in caudal DLP); VEn = ventral endopiriform nucleus; VPA = ventral posterior amygdala (lizard).

marginal zone by such cells during development, together with the lack of VP progenitor cells expressing Emx1 (Abellán et al., 2009; Gorski et al., 2002; Puelles et al., 2000). Based on these features, we identified the VP of *P. algirus* as a division rich in *pLhx9* in vz and mantle at early stages, with light *pLhx2* in the vz, strong expression of *pTbr1* in the mantle (Figures 2d,f,h,j, 3b,d, 4h,j–n, and 5a–d), and poor in *pEmx1* (Figures 4b–e, and 7c,e,g). As in other amniotes, the expression of *pLhx9* in the VP of *P. algirus* is downregulated later in development (Figure 7f,h), becoming restricted mainly to its rostral (olfactory) and caudal poles (Figures 8b,d,f,h and 11a–c,d,e). This pallial division appears to produce most of the lizard anterior and posterior DVR (Figure 10c–f), except for a remarkable caudolateral part related to a distinct pallial sector, the ventrocaudal pallium or VCP. The posterior DVR (PDVR) is considered to be part of the reptilian pallial amygdala (as defined by Lanuza, Belekhova, Martínez-Marcos, Font, & Martínez-García, 1998), and expresses *pLhx9* posteromedially at typical caudal levels (Figures 5b, 8d, 9j–l, and 10e,f), but also in a rostrolateral extension of this area (Figure 9g–i and 11i), which resembles the chicken Lhx9-expressing caudal nidopallium and its rostral continuation (Abellán et al., 2009). The nucleus of the lateral olfactory tract (Nlot), also expressing *pLhx9* (Figures 4l,n and 9g), appears to be at the rostrolateral surface of this whole domain (Figure 11i).

In addition, the VP appears to produce the ventral part of LC (LCv), which shows only light expression of *pLhx9* from intermediate developmental stages (Figures 9d–g and 10c). At more rostral levels, the VP includes a *pLhx9*-expressing area deep to LCv, which we identify as a ventral part of the pallial thickening (PTv, Figures 9b–d and 10a,b). As seen in Figures 9d and 10a, the ventral part of PT or PTv is dorsally continuous with DC3, the deep layer of DLP. The rostral part of DC3 is sometimes included as part of PT, and here we have labeled as dorsal PT (or PTd; Figure 13). Note that PT also includes an intermediate part, just dorsal to PTv, belonging to LP, which is observed at

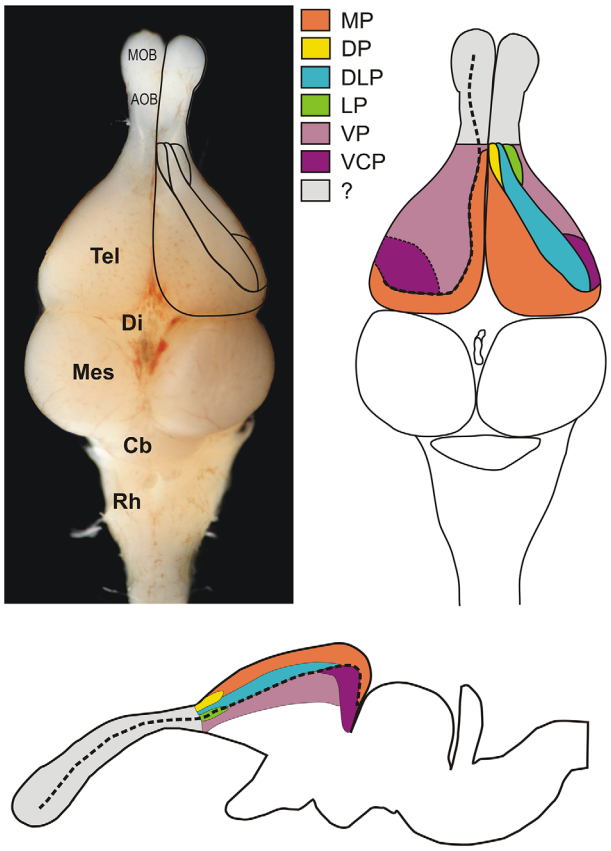

FIGURE 12 Dorsal and lateral views of the brain of the lacertid lizard *P. algirus* at S40 and a colored schematic representation of it showing the pallial divisions, according to the six-part model proposed here. In the right hemisphere of the colored diagram (dorsal view), there is a representation of the six divisions seen from the top, while in the left hemisphere there is a representation of the pallial divisions seen more ventrally. Note the small size of the dorsal pallium (DP), and its position comparable to that of the avian hyperpallium. Also note the presence of two novel divisions, the dorsolateral pallium (DLP, which includes the lateral cortical superposition plus an area rostral to it) and the ventrocaudal pallium (VCP, including the dorsolateral amygdala and nucleus sphericus, among other nuclei). The VCP of the lizard is comparable to the avian arcopallium. The olfactory bulbs (main or MOB, and accessory or AOB) were left in gray, since they may have contributions from the several pallial divisions (perhaps including DLP), in addition to cells from other sources. See Tables 1 and 2 for additional comparisons and see text for more details. As a reference, the major brain divisions are labeled in the figure: telencephalon (Tel), diencephalon (Di), mesencephalon (Mes), and rhombencephalon (Rh) with the cerebellum (Cb).

very rostral levels and is negative for Lhx9 (Figure 9b,c; see Figures 13 and 14b,c to observe the LP and the different parts of PT in an scheme or with Nissl staining, respectively).

More rostrally, the anterior olfactory area shows light expression of *pLhx9* (AO, Figure 9a) as well as *pTbr1* (Figure 1v), with differences between subdivisions: medial, lateral or dorsal. Even more rostrally, the main and accessory olfactory bulbs (MOB and AOB, respectively) express both *pEmx1* and *pLhx9* in the mitral cell layer (Figure 1). However, the AOB only shows expression of *pEmx1* and *pLhx9* rostrally (AOBr, Figure 1g,h,j), but not caudally (Figures 1k,l,o,p,s,t and 11a, b,d), raising doubts on the origin of this latter part. Moreover, the high expression of *pEmx1* in the mitral cell layer of MOB and AOBr makes them depart from the typical molecular profile of VP, raising doubts on their origin and pallial assignation.

6. Ventrocaudal Pallium (VCP). This pallial division was recently defined in chicken and mouse to refer to a sector caudal and lateral with respect to the VP, showing extremely strong expression of *Lhx9*, and moderate expression of *Lhx2* and *Emx1* in vz and mantle, giving rise to the arcopallium in chicken and a posterior part of the pallial amygdala in mouse (Abellán et al., 2014; Medina, Abellán, Vicario, Castro-Robles, & Desfilis, 2017a). A comparable division with the same topological

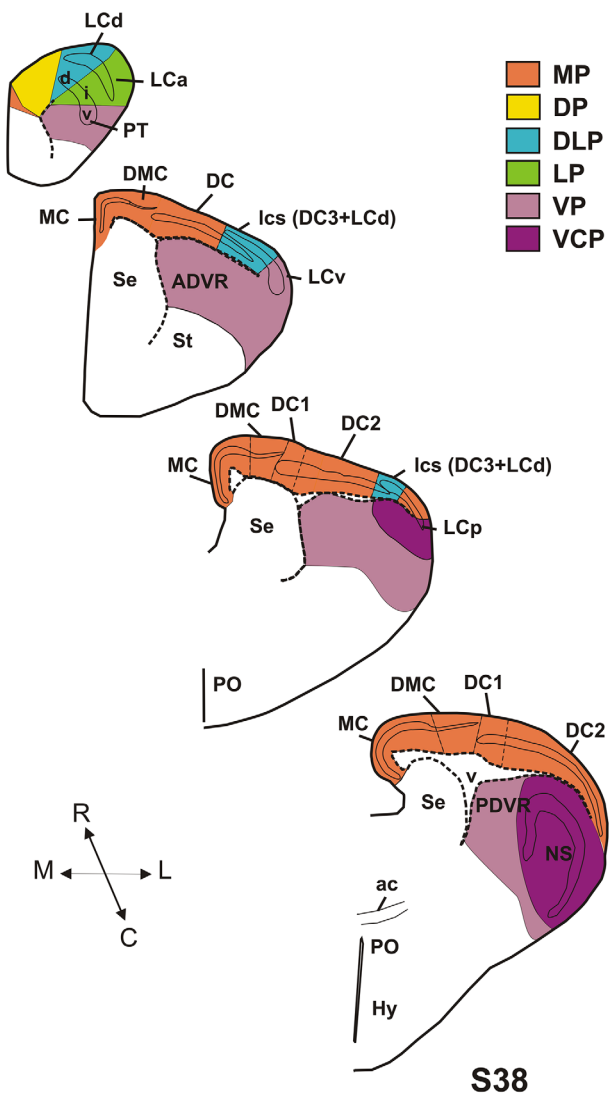

FIGURE 13 Schematic drawings of frontal telencephalic sections of the lacertid lizard *P. algirus* at S38, from rostral (top) to caudal (bottom) levels. The six pallial divisions are distinguished using a color code. Within each division, the main nuclei and areas are labeled using the classical nomenclature. See text for details. For abbreviations see list

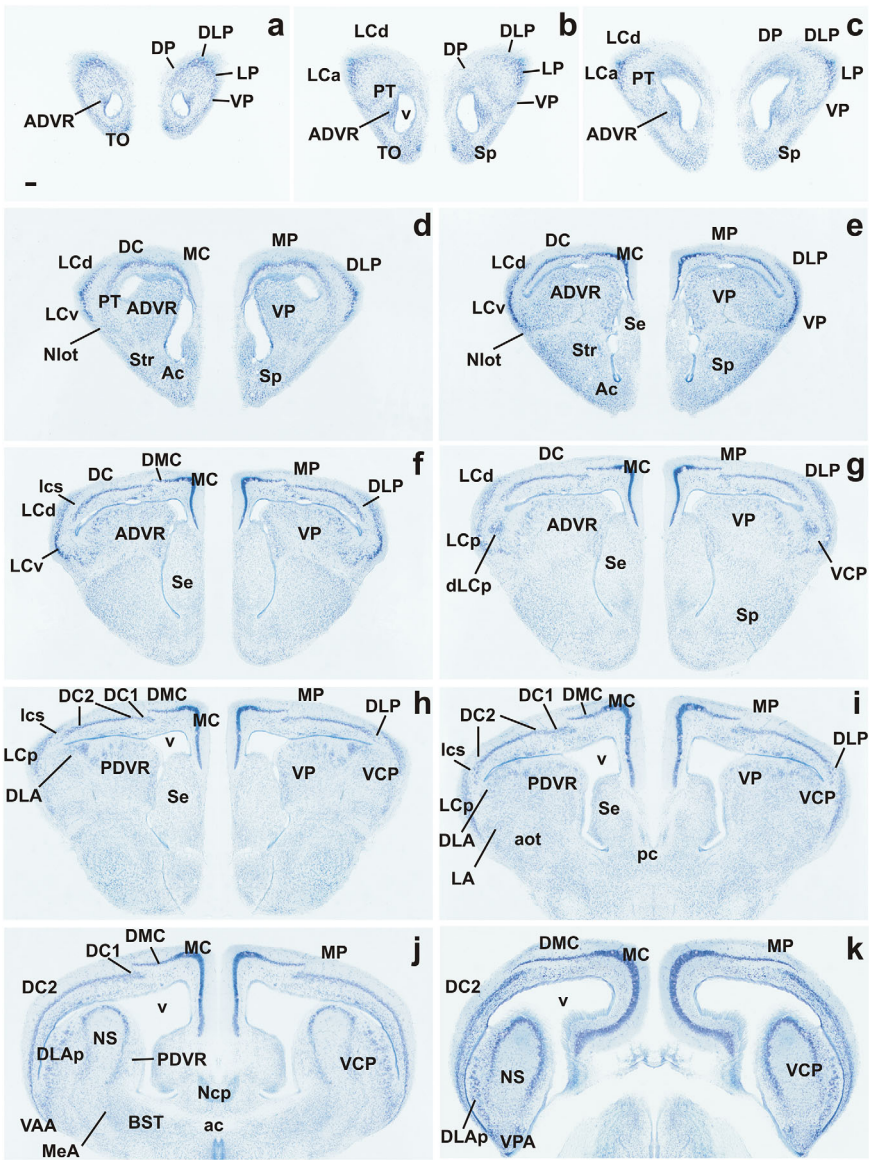

FIGURE 14 Digital images of Nissl-stained frontal telencephalic sections of the lacertid lizard *P. algirus* (adult), from rostral (top) to caudal (bottom) levels. The six pallial divisions (MP, DP, DLP, LP, VP, and VCP) proposed in this study are labeled on the right side of each section, while some of the main nuclei and areas of the telencephalon are labeled on the left (showing a mirror image of the right side) using the classical nomenclature. See text for details. For abbreviations see list. Scale bar (in a, applies to all): 100 μm

location and gene expression profile is present in *P. algirus* (Figures 8b–h, 9j–m, 10d–h, and 11i) and appears to produce a posterior or caudal part of LC (LCp) (Figures 5b, 8b, and 9i,j) and the dorsolateral amygdala (DLA) (Figures 5f,g, 8c–f, 9j–l, and 10e–g). In addition, based on its location and gene expression profile, it also appears to produce the nucleus of the accessory olfactory tract (Naot) (Figures 5c,d, 9j, and 10e) and nucleus sphericus (NS; Figures 5f,g,h, 9m, and 10g,h).

4.4 | Prethalamic eminence and its telencephalic extension

As in other vertebrates, several of the genes expressed in the telencephalon were also expressed in the hypothalamus and the diencephalon proper (including prethalamus and prethalamic eminence in prosomere 3, thalamus/epithalamus in prosomere 2 and pretectum in prosomere 1, according to the prosomeric model), in specific patterns. For example, *pDlx2* was expressed in the suprachiasmatic domain and the prethalamus (Figure 3e,g), while *pTbr1* was expressed in *pDlx2*-negative domains, such as the peduncular subdivision of the supraoptoparaventricular hypothalamic domain (SPV, which includes the paraventricular hypothalamic nucleus; Figure 3h) and the prethalamic eminence (PThE, Figure 3f). In addition, *pLhx2* and/or *pLhx9* were expressed in specific domains of the diencephalon, including part of PThE and some nuclei of the thalamus/epithalamus (Th/Eth) (Figures 5f–h, 6b,d,f,h, 8h, 10g,h, and 11e,g).

At S35 and S36, *pTbr1* expression in PThE was remarkable, allowing clear observation of its telencephalic extension when it contacts the roof plate/choroid tela (Figures 3f and 6). Moreover, as seen in

sagittal sections, the PThE appeared to be continuous rostrally with the compact domain of *pTbr1/pLhx9* expression of the caudal and dorsomedial septum (Figures 6 and 11 see also Figures 3b,d,f and 5d,f), as well as with the cells apparently spread from here into the region of the medial septum and nucleus of the diagonal band (NdB, Figures 2j, 5b,d, 10d, and 11g). As noted above, this PThE-related domain of the caudal and dorsomedial septum with strong expression of *pTbr1* and *pLhx9* appears to give rise to the nucleus of the pallial commissure (Ncp) (Figures 8d,f and 10e,f). In addition, the PThE also appears to extend into a patch of *pTbr1* expression adjacent to the ventral tip of the lateral ventricle, which appears to represent an eminential part of the bed nucleus of the stria terminalis (Bste, Figures 2j and 3b). As seen in sagittal sections (Figure 6c), the PThE-derived cells that apparently extend into the Bste are also continuous with a *pTbr1* expressing area named here septopreoptic area because of its location (SPO, Figure 3b,d,f).

5 | DISCUSSION

5.1 | Comparison of pallial divisions in tetrapods based on topology and gene expression

The field of comparative neuroembryology, under the prism of the evolutionary developmental biology (evo-devo) and the help of combinatorial expression patterns of developmental regulatory genes, has meant a rebirth for studies of brain evolution, and research using such focus is becoming fundamental for understanding the pallium (Bachy et al., 2002; Brox, Ferreiro, Puelles, & Medina, 2004; Kuratani, 2009; Medina & Abellán, 2009; Mueller & Wullimann, 2009; Murakami et al., 2001; Puelles et al., 2000; Striedter, 2005). The evo-devo approach is not only useful for comparative purposes, but also for extrapolating evolutionary trends and identify possible mechanisms of evolution (Medina, 2007a; Striedter, 2017). One important criterion used by most comparative neuroembryologists is that they analyze data (gene expression, fate mappings, results from experimental ablations or transplants, effects of gene mutations, and any other) in the context of the topological framework (*Bauplan* or building plan) of the neural tube (reviewed by Ferrán, 2017; Medina, 2007a; Nieuwenhuys and Puelles, 2016; Nieuwenhuys, 1998, 2009; see also Puelles and Medina, 2002; Striedter, 2005). These principles used by comparative neuroembryologists are very important to try to deduce the plan of fundamental units that are common to the brain of all vertebrates; these include recognizing the relative position of each unit according to internal coordinates of the neural tube, which is invariant independently of deformations due to the differential growth of these units during development and/or during evolution. These fundamental units are better distinguished during early development, and genes involved in their early specification and morphogenesis are very useful for comparative purposes because, when used in combinations, allow both visualization and tracking of derivatives of such units throughout development (Medina, 2007a,b; Medina & Abellán, 2009; Medina, Abellán, & Desfilis, 2013; Nieuwenhuys & Puelles, 2016; Puelles & Medina, 2002).

The study of the combinatorial expression of these developmental regulatory genes, in the context of the brain topological framework, has been very fruitful for both identifying and comparing pallial divisions between mouse, chicken, and the anuran *Xenopus laevis* (Abellán et al., 2009, 2014; Bachy et al., 2002; Brox et al., 2004; Medina & Abellán, 2009; Moreno & González, 2006; Moreno et al., 2004; Puelles et al., 2000, 2007, 2017). However, when the expression of genes is used to compare brain divisions or cells without considering the topological framework (Chen, Winkler, Pfenning, & Jarvis, 2013) and/or if the comparison is based not on early genes involved in specification and morphogenesis of brain units, but on genes related to mature functional features of nonconserved structures such as the pallium (as done, e.g., by Dugas-Ford, Rowell, & Ragsdale, 2012; Jarvis et al., 2013; Suzuki and Hirata, 2014; Suzuki, Kawasaki, Gojobori, & Hirata, 2012), some of the similarities found should be considered as functional analogies, but not as homologies (commented by Medina et al., 2013; and by Puelles et al., 2017).

The first comparative data on expression of transcription factors in the embryonic telencephalon of mouse and chicken led to a change of paradigm regarding pallial divisions found in all vertebrates, passing from three (tripartite model, with medial, dorsal, and lateral divisions) to four divisions (tetrapartite model, with medial, dorsal, lateral and ventral divisions; Puelles et al., 2000, 2017; Table 1). The tetrapartite model was clearly advantageous with respect to the classic tripartite model because it explained better the patterns of genoarchitecture and chemoarchitecture of the pallium, including the expression of early regulatory genes during development (Puelles et al., 2000); it also explained better the results from fate mapping studies (for instance, Cobos, Shimamura, Rubenstein, Martínez, & Puelles, 2001b); and it was additionally better for comparison across species (reviewed by Puelles et al., 2017). For this reason, it soon became widely used by most comparative embryologists studying the telencephalon and, in particular, the pallium of different vertebrates, including mammals, birds, amphibians, and different fishes (Abellán et al., 2009; Bachy et al., 2002; Brox et al., 2003, 2004; González, Morona, López, Moreno, & Northcutt, 2010; Medina & Abellán, 2009; Medina et al., 2004; Moreno et al., 2004, 2012; Mueller & Wullimann, 2009; Mueller, Dong, Berberoglu, & Guo, 2011; Quintana-Urzainqui et al., 2015; Redies, Medina, & Puelles, 2001).

The tetrapartite model has recently been updated to accommodate new data, implying a redefinition of the lateral pallium, which is now thought to include the claustro-insular complex (the mesopallium in birds), but does not give rise to any part of the pallial amygdala (Puelles, 2014; Table 1); as a consequence, the ventral pallium was also reconsidered and produces the piriform cortex, the endopirifom nuclei (or part of them) and most of the pallial amygdala in mammals, and the piriform cortex, nidopallium and arcopallium in birds (Puelles et al., 2016a,2017; Puelles, 2014). In the revisited tetrapartite model, the dorsal pallium did not change, and gives rise to the neocortex in mammals and hyperpallium in birds (Puelles et al., 2000, 2007, 2017). Regarding the medial pallium, in the latest version of the revisited tetrapartite model it is considered to produce the hippocampal formation plus the entorhinal cortex (Puelles et al., 2017), partially following the

suggestion of Abellán et al. (2014) based on Lef1 and other genes (as discussed below).

The promoters of the tetrapartite model have recently made proposals on the location of the four pallial divisions in the brain of reptiles, and have tentatively inferred how the pallium was organized in stem amniotes from which modern mammals and sauropsids evolved (Puelles et al., 2017). However, data on the expression of transcription factors in the embryonic brain of reptiles are quite scarce, lack enough detail and are mostly limited to turtles (Fernández et al., 1998; Moreno, Morona, López, & González, 2010, 2012), which according to molecular phylogeny appear to be phylogenetically closer to the archosaurs (birds and crocodiles) than to other reptiles as lizards and snakes (Crawford et al., 2015). In fact, most recent data on detailed gene expression in the embryonic brain of sauropsids were from species in one single clade, the Archelosauria (including birds, crocodiles, and turtles). Therefore, there was an urgent need to obtain detailed gene expression data in the embryonic brain from species of the other sauropsidian clade, Lepidosauria (including lizards, snakes, and the tuatara), in order to infer how the pallium was organized in basal amniotes, and from here deduce possible evolutionary trends of each pallial division in different lineages. For this reason, we chose the lacertid lizard *P. algirus* for cloning and studying the expression of several developmental regulatory genes known to play key roles in the development of different pallial divisions in other amniotes, including those encoding the transcription factors Lhx2, Lhx9, Emx1, and Tbr1 (Abellán et al., 2009, 2014; Bachy et al., 2002; Brox et al., 2004; Moreno et al., 2004; Puelles et al., 2000). All these genes were expressed in the pallium of *P. algirus*, showing patterns comparable to those of their orthologues in mouse and chicken (Puelles et al., 2000; Abellán et al., 2009, 2014), and highly similar to those of their orthologues in *X. laevis* (Bachy et al., 2002; Brox et al., 2004; Moreno et al., 2004).

5.2 | Toward a new model of six pallial divisions?

Although the tetrapartite model has been very useful for the progress in our understanding of the pallium and its evolution, when comparing mouse and chicken we observed difficulties for explaining the expression patterns of an increasingly large list of developmental regulatory genes and for comparing pallial structures such as the entorhinal cortex and caudal parts of the pallial amygdala across species. This led us to propose a modification of the tetrapartite model, including not four but six pallial divisions (hexapartite model; Abellán et al., 2014; Medina et al., 2017a), which is at present under evaluation (Tables 1 and 2). Based on their relative positions, these divisions are the medial, dorsal, dorsolateral (dorsolateral-caudal in Abellán et al., 2014), lateral, ventral, and ventrocaudal (ventrolateral-caudal in Abellán et al., 2014) pallia (Abellán et al., 2014; Medina et al., 2017a).

Our data on the expression of transcription factors in the embryonic brain of the lizard *P. algirus* fit well when interpreted using the hexapartite model (Figures 12 and 13), and the pallial divisions can easily be compared to those in chicken and mouse (Lhx2, Lhx9, and Tbr1: Abellán et al., 2009, 2014; Emx1 and Tbr1: Puelles et al., 2000; Table 2). Similarly, to those of mouse and chicken, each pallial division in *P. algirus* shows a unique topological position and distinct combinatorial and spatiotemporal gene expression pattern, as follows:

- Three pallial sectors, the medial pallium (MP), the dorsolateral pallium (DLP), and the ventrocaudal pallium (VCP), show expression of all Lhx2, Lhx9, and Emx1 in the vz and mantle at early embryonic stages, although each shows a particular pattern as explained in the following sections.
- The ventral pallium (VP) shows light expression of Lhx2 in the vz (lighter than adjacent pallial sectors), expresses Lhx9 moderate to strongly in the vz and mantle (later in development, the expression becomes restricted to parts of VP), and is devoid of Emx1 in the vz and most of the mantle.
- Two additional pallial divisions, the dorsal pallium (DP) and the lateral pallium (LP), appear at very rostral levels (just behind the anterior olfactory area) interposed between the rostral extension of the previous divisions, and show moderate to strong expression of Lhx2 and Emx1 in the mantle, but no expression of Lhx9; that is, the DP and the LP appear as Lhx9-negative gaps interposed between Lhx9-positive domains, as follows: the DP appears interposed between MP and the rostral extension of DLP, and at early stages shows expression of both Lhx2 and Emx1 (ventricular zone and mantle; Table 2), although the latter is soon downregulated; DP also shows transient mantle expression of Lhx9 at very early stages, which—as explained below—may be comparable to the transient expression of this transcription factor by a subset of preplate cells described in the mouse dorsal pallium (Rétaux et al., 1999; Yamashaki et al., 2004); the LP is seen at similar rostral levels interposed between the rostral extension of DLP and VP. At intermediate and caudal levels, DP and LP are no longer observed, and DLP contacts MP medially and VP ventrally (and VCP ventrocaudally).

Like in mouse, chicken and turtle (Moreno et al., 2010; Puelles et al., 2000), during development the mantle of these pallial divisions in the lizard *P. algirus* also expresses Tbr1, a transcription factor involved in the differentiation of glutamatergic neurons (Hevner et al., 2001). As in other vertebrates (Abellán et al., 2009; Brox et al., 2004), the expression of Tbr1 is not constant throughout development, as it is not expressed until differentiation starts and it becomes stronger during the peaks of differentiation, a pattern that is different for each pallial sector. For example, in the lizard *P. algirus*, at S35 the expression of Tbr1 is very strong in DP, VP and rostral levels of MP, but it is weak or negligible in DLP and caudal parts of MP, which is likely due to a delay in the formation of the latter areas. In agreement with this, the caudal parts of DLP and MP start to express Tbr1 from S36 (Figure 6).

The relative positions of these six pallial sectors of *P. algirus* and their molecular profile (at least regarding the transcription factors studied here) show the highest resemblance to those of their respective pallial divisions in chicken, which facilitates inference of how the pallium was likely organized in ancestral sauropsids. Notably, our data suggest that both the DP and the LP in *P. algirus* are very small and only restricted to the rostral pole. If this were the ancestral condition in sauropsids, it would mean that these pallial sectors underwent an

expansion in the lineages of archosaurs (particularly in birds) and mammals. We discussed data on each pallial division in separate sections next, starting by the divisions with high expression of Lhx9 (see also Table 2 and Figures 12–14).

5.3 | Medial pallium

The medial pallium is defined as the sector having a topological position adjacent to the choroid tela and cortical hem (revised by Nieuwenhuys, 2009; Medina et al., 2017b; Puelles et al., 2017), and the embryonic expression of the transcription factors Lhx2, Lhx9, and Lef1 (Abellán et al., 2014). In mouse, it gives rise to the hippocampal formation, but also to some parahippocampal areas as the medial entorhinal cortex, and possibly the pre- and parasubiculum (Abellán et al., 2014). In chicken, in addition to previously recognized parts of the hippocampal complex (including the ventral hippocampus, the V-field area and medial and lateral parts of the parahippocampal areas [APH], Atoji and Wild, 2004; Reiner et al., 2004), the medial pallium also includes a dorsolateral corticoid area (CDL), also named caudolateral parahippocampal area (APHcl) due to its relation to the rest of the hippocampal complex based on similar cytoarchitecture and gene expression profile during development (Abellán et al., 2014; Puelles, Martinez de-la-Torre, Paxinos, Watson, & Martinez, 2007; Redies et al., 2001). In agreement with its medial pallial nature, the avian APHcl/CDL is densely interconnected with other parts of the avian hippocampal complex, and was suggested to be part of the avian limbic cortex (Atoji & Wild, 2005). At caudal levels, the avian APHcl/CDL is laterally continuous with an olfactory bulb-recipient area previously called piriform cortex (Atoji & Wild, 2014; Reiner & Karten, 1985) or entorhinal cortex (Abellán et al., 2009, 2014). The APHcl/CDL itself also receives olfactory bulb input at its lateral part (Atoji & Wild, 2014; Reiner & Karten, 1985). Based on their contiguity, gene expression patterns during development and connections, both APHcl/CDL and this olfacto-recipient area have been considered to represent a single sector of the medial pallium, comparable to the medial entorhinal cortex of mammals (discussed by Abellán et al., 2014; Medina et al., 2017b; see more below).

In reptiles, there is general agreement that the medial pallium includes both the medial (MC) and dorsomedial (DMC) cortices, and that these cortical areas are homologous to parts of the hippocampal complex of birds and mammals (Bruce, 2007; Bruce & Bradford, 2009; Striedter, 1997). MC has been proposed to be homologous to the mammalian dentate gyrus and to the avian ventral hippocampus plus the ventral part of the V-shaped area (Hi1 of the atlas by Puelles et al., 2007; see discussion in Abellán et al., 2014; López-García et al., 1992; Medina et al., 2017b). DMC has been compared to the mammalian CA3 and to the dorsal part of the avian V-shaped area (or hippocampal sector 2—Hi2—as defined in the chicken brain atlas by Puelles et al., 2007; see discussion in López-García et al., 1992; Medina et al., 2017b). However, there is a great controversy regarding the homologies of the reptilian dorsal cortex (Butler, 1994; Bruce & Neary, 1995a; Bruce & Bradford, 2009; Dugas-Ford et al., 2012; López-García et al., 1992; Reiner, 1993).

Our data in *P. algirus* suggest that the medial pallium in lizards encompasses not only the medial (MC) and dorsomedial (DMC) cortices, but also the vast majority of the dorsal cortex, including its medial (DC1) and intermediate (DC2) parts, with the only exception of its lateralmost part (corresponding to DC3), which relates to the lateral cortical superposition (lcs) and according to our results belongs to a different pallial sector, the DLP. Similarly, to birds, the lcs (including DC3) is no longer present at caudal levels, and the remaining DC sector extends laterally. The inclusion of MP, DMC, and most DC (DC1 and DC2) as parts of the medial pallium and therefore as components of the reptilian hippocampal complex agrees with the proposals by Martínez-Guijarro, Berbel, Molowny, and López-García, (1984, 1990), Olucha, Martínez-García, Poch, Schwerdtfeger, and López-García, (1988), Hoogland and Vermeulen-VanderZee (1989, 1993), López-García et al. (1992), and Bruce and Neary (1995), which were based on the medial position of these cortices in the pallium, their three-layered cytoarchitecture, their strong Timm-positive staining (enriched in the hippocampal mossy fiber system in mammals), and their connectivity patterns (including extensive connections between them, and projections to the lateral septum, limbic part of the striatum, amygdala and hypothalamus; see further discussion below and also the reviews by López-García et al., 1992, and by Medina et al., 2017b; for details on the connections of the hippocampal complex in mammals see van Strien, Cappaert, & Witter, 2009; Witter & Amaral, 2004; Witter, 2012).

Our data show a mediolateral decreasing gradient of Lhx9 expression in the vz of the medial pallium, from MC to DC2, that abruptly stops when reaching the vz of DC3 (i.e., the vz related to the lateral cortical superposition), which agrees with the idea that the lcs does not belong to the medial pallium. The Timm-positive staining also decreases its intensity when reaching the lateral cortical superposition, and shows a distinct pattern in this area (Olucha et al., 1988; Pérez-Clausell, 1988). In the cortex, the Timm staining relates to the zinc-containing glutamatergic projections of MC/dentate gyrus-like neurons (López-García et al., 1992; Martínez-Guijarro et al., 1984, 1990; Olucha et al., 1988), suggesting that the weak Timm-staining in lcs may relate to a lighter input from MC (Hoogland & Vermeulen-VanderZee, 1993; discussed by Medina et al., 2017b).

From S36, Lhx9 starts to be downregulated in parts of the medial pallium. From S37, MC is devoid of expression of this transcription factor in the cortical plate, although its vz still maintains expression. Moreover, DMC and DC1 show very light or negligible expression of Lhx9 in both vz and mantle. From S37, Lhx9 expression basically remains with moderate to strong expression only in the cortical plate of the intermediate sector of the dorsal cortex (DC2). The distinctive gene expression profile of each DC subdivision in lizard helps to clarify their extension in both mediolateral and rostrocaudal dimensions; for example, based on these profiles, DC2 does not reach the caudalmost pole of the medial pallium, and DC1 (practically devoid of Lhx9 expression in vz and mantle) is the only part of the dorsal cortex present at the caudalmost levels.

The Lhx9 expression pattern in lizard at middle/late developmental stages resembles the situation in the medial pallium in chicken at late

prehatching stages (Abellán et al., 2009, 2014), and together with other data allows one-to-one comparisons between subdivisions of chicken and lizard. In fact, the expansion of the medial pallium along the cortical plate above the lateral horn of the ventricle, as well as the series of subdivisions found are remarkably similar between chicken, lizards and other sauropsids (Medina et al., 2017b). The only difference is that while in reptiles the lateral expansion of the ventricle is seen from rostral levels, in birds this is observed only at caudal levels. This may be due to an expansion of the rostral structures in birds (hyperpallium and mesopallium, which belong to the dorsal and lateral pallia, respectively).

At late prehatching stages of chicken, the APHcl/CDL is the only area of the medial pallium that remains with strong expression of Lhx9 (Abellán et al., 2009, 2014), and appears directly comparable and likely homologous to the lateral part of lizard DC2 (DC2-lateral), which also maintains strong Lhx9 expression throughout development (present results). In addition to their high similarity in terms of embryonic origin, relative position above the lateral horn of the ventricle and genetic profile, the avian APHcl/CDL and lizard DC2-lateral are also similar regarding their connections: extensive reciprocal connections with medial parts of the hippocampal complex, and projections to septum, amygdala and hypothalamus (Atoji and Wild, 2005; Bruce & Bulter, 1984; Hoogland & Vermeulen-VanderZee, 1989, 1993). In addition, both the avian APHcl/CDL and the lizard DC2 project to the olfactory bulb (birds: Atoji & Wild, 2014 and Reiner & Karten, 1985; and lizards: Hoogland & Vermeulen-VanderZee, 1989; Martínez-García, Olucha, Teruel, Lorente, & Schwerdtfeger, 1991).

As noted above, the avian APHcl/CDL and its lateral extension (an olfactory bulb-recipient area, which also keeps strong Lhx9 expression) were suggested to be comparable to the medial entorhinal cortex of mouse (Abellán et al., 2014; see also Redies et al., 2001; Suárez, Dávila, Real, Guirado, & Medina, 2006). The lizard DC2 has also been proposed to be comparable to the medial entorhinal cortex (Medina et al., 2017b) based on its projections to the MC (dentate gyrus homologue), which resemble part of the perforant pathway of mammals (López-García et al., 1992), that is, the projection from the entorhinal cortex to the dentate gyrus (Canto, Wouterlood, & Witter, 2008; van Strien et al., 2009; Witter, 2012; Witter & Amaral, 2004; Witter, Kleven, & Flatmoen, 2017). In birds, the APHcl/CDL also projects to the dentate gyrus homologue (including the V-shaped area; Atoji & Wild, 2005; the dentate gyrus homology is based on topological position and expression of the transcription factor Prox1: Abellán et al., 2014; Atoji, Sarkar, & Wild, 2016; Medina et al., 2017b). This suggests that this part of the perforant pathway was likely present in the common ancestor of amniotes (Medina et al., 2017b). As in mammals (references above), the putative perforant pathway of sauropsids does not only reach the dentate gyrus homologue, but also medial pallial sectors comparable to the CA fields (including the reptilian DMC and DC1, and the avian Hi2-sector and medial/intermediate APH; Medina et al., 2017b).

In addition to the previous projections, another typical feature of the mammalian entorhinal cortex is that it receives direct olfactory bulb input, but—in contrast—the lizard DC2 does not appear to receive direct input from the olfactory bulb (Lohman & Smeets, 1993; Martínez-García et al., 1991; Ubeda-Bañon et al., 2011). However, projections from the olfactory bulb reach the lateral entorhinal cortex, but do not reach the medial entorhinal cortex in most placental mammals and marsupials, with the exception of rats, where some olfactory bulb axons target a lateral part of this cortex (Kosel, Van Hoesen, & West, 1981; Martínez-Marcos & Halpern, 2006). Nevertheless, processed olfactory information also reaches the medial entorhinal cortex (and its homologues in sauropsids) by way of input from the piriform cortex and lateral entorhinal cortex (including the lcs in reptiles; Atoji and Wild, 2005; Gnatkovsky, Uva, & de Curtis, 2004; Hoogland & Vermeulen-van-der-Zee, 1995; Martínez-Marcos et al., 1999; van Strien et al., 2009; see discussion on the lateral entorhinal cortex in next section). As noted above, the sauropsidian APHcl/CDL and DC2 project to the olfactory bulbs (Atoji & Wild, 2014; Martínez-García et al., 1991; Lohman & Smeets, 1993). In mammals, most studies reported that only the lateral entorhinal cortex projects to the olfactory bulbs (de Olmos, Hardy, & Heimer, 1978; Mohedano-Moriano et al., 2012; Shipley & Adamek, 1984; Witter, 2012). Among sensory systems, olfaction is considered the most universal for navigation and for emotional/social memories (Jacobs, Arter, Cook, & Sulloway, 2015). Based on their connections and known functions, the medial and lateral entorhinal cortices (and their homologues in sauropsids), in association with the hippocampal formation and other structures as the amygdala (Gnatkovsky et al., 2004; Medina et al., 2017a,b), are possibly involved in the formation of olfactory memories and the olfactory-mediated orientation and navigation described in different vertebrates, including sea turtles (Manton, Karr, & Ehrenfeld, 1972), homing pigeons (Gagliardo, 2013; Papi, 1991), hamsters (Tomlinson & Johnston, 1991), and humans (Jacobs et al., 2015).

In contrast to the APHcl/CDL, the lateral subdivision of the parahippocampal area (APHl) maintains light to moderate expression of Lhx9 at late prehatching stages in chicken (Abellán et al., 2009, 2014). Based on this and its relative position, it appears comparable to the medial part of lizard DC2 (DC2-m) (Medina et al., 2017b). This particular sector of the medial pallium is rich in expression of Nr4a2/Nurr1 in chicken (Puelles et al., 2016a), and based on this and other features, including connections, it has been compared to the mammalian subiculum (Medina et al., 2017b). Regarding the other three subdivisions of the lizard medial pallium (DC1, DMC, MC), they seem globally comparable to the parts of the chicken medial pallium that also downregulate Lhx9 expression at late developmental stages (i.e., medial parts of APH, V-shaped area and ventral hippocampus; Abellán et al., 2014). However, our data do not allow one-to-one comparisons. In a previous study, we use combinatorial expression of Prox1 and other regulatory genes in chicken and mouse in order to identify comparable areas of the medial pallium (Abellán et al., 2014). Based on these patterns, we proposed that the avian dentate gyrus (expressing Prox1) includes the ventral hippocampus and ventral part of the V-shaped area (Hi1; Abellán et al., 2014; Hi1 is the hippocampal sector 1, as defined in the chicken brain atlas by Puelles et al., 2007). Moreover, we suggested that the avian CA3 may include the dorsal part of the V-shaped area (Hi2; Abellán et al., 2014; Hi2 is the hippocampal sector 2, as defined

in the chicken brain atlas by Puelles et al., 2007). We would need more results on gene expression (including Prox1) in order to unambiguously identify comparable sectors in reptiles. Nevertheless, based on position, chemoarchitecture, and connections, we recently proposed that the lizard DC1 is comparable to the avian medial/intermediate parts of APH, the DMC to the dorsal part of the avian V-shaped area (or Hi2) and the MC to the avian ventral hippocampus plus the ventral part of the V-shaped area (Hi1; see discussion in Medina et al., 2017b).

5.4 | Dorsolateral pallium (DLP)

The dorsolateral pallium (DLP) was initially described in chicken and mouse as a caudal sector expressing Lhx9, Lhx2, and Emx1 during development (Abellán et al., 2014; for Emx1 expression refer to Puelles et al., 2000). The results of this study suggest that DLP is larger than previously thought and extends from rostral to caudal levels (Figures 12–14; Table 2). In the lizard, the rostral DLP appears as a Lhx9/Emx1 expressing area interposed between DP and LP (both negative for Lhx9). This rostral pole of DLP includes a distinct cortical plate (Figure 14a) that has sometimes been included as part of the rostral LC (Martínez-Marcos et al., 1999) or as part of the anterior olfactory area (Martínez-García et al., 1991). The caudal part of the DLP of lizard is larger (representing the main part) and includes the lateral cortical superposition (lcs). In the lizard *P. algirus* the lcs was shown to form a radial unit as the two overlapping cell plates (DC3 and LCd) were traversed by the same radial glial fibers (Guirado & Dávila, 2002). Guirado and Dávila (2002) proposed that the lcs, with its double cell plate and ipsilateral inputs from the dorsolateral anterior thalamic nucleus (comparable to the anterior thalamic complex), might be the reptilian precursor of the neocortex. Our data, however, show that this pallial sector shows a remarkably strong expression of Lhx9 (from deep to superficial layers) throughout development, which disagrees with its putative dorsal pallial nature. Note that although the dorsal pallium in mouse shows transient expression of Lhx9 at early stages (Rétaux et al., 1999; Abellán et al., 2009), this is located in the preplate and relates to transient expression by Cajal-Retzius cells (Yamashaki et al., 2004), which are known to originate outside the dorsal pallium (García-Moreno, López-Mascaraque, & De Carlos, 2007). More recently, the lcs has been proposed to be part of the reptilian lateral pallium (Puelles et al, 2016a,2017). However, its strong expression of Lhx9 throughout development also disagrees with this proposal.

Based on its topological position and gene expression profile, we consider the lizard lcs directly comparable to the chicken caudal DLP, which also occupies a dorsolateral position in the pallium and expresses Lhx2, Lhx9, and Emx1 during development (Abellán et al., 2014). The caudal DLP of chicken includes a dorsal part of the so-called temporo-parieto-occipital area or TPO (as referred to in other articles; for instance, Atoji & Wild, 2005), and was included as part of the "pallium externum" by other authors (Veenman et al., 1995). However, since the TPO includes more areas than the histogenetic domain producing the caudal DLP, we prefer to use this more specific term. Puelles et al. (2017) refer to the caudal DLP as the caudal hyperpallium because they consider it a caudal part of the dorsal pallium, but the fact that DLP expresses Lhx9 throughout development disagrees with this idea. Moreover, according to our interpretation, the caudal DLP is not only behind (caudal to) DP, but also behind LP (Figure 12).

In addition, like in the lizard, the chicken DLP may also include a rostral extension located lateral to DP (interposed between DP and LP) that reaches retrobulbar levels. This is based on the rostral continuation of the expression of some regulatory genes that are also present in the caudal DLP. For example, based on expression of Nr4a2/Nurr1 (Puelles et al., 2016a), the chicken caudal DLP appears rostrally continuous with a subdomain of the dorsal part of the mesopallium (Md). A similar conclusion can be withdrawn when analyzing the expression of cerebellin 2, present in the caudal DLP and a subdomain of Md (Reiner, Yang, Cagle, & Honig, 2011). Nevertheless, Nr4a2/Nurr1 and cerebellin 2 do not appear to overlap in Md, but they occupy adjacent subdomains: expression of Nr4a2/Nurr1 in Md (Puelles et al., 2016a) is dorsal to that of cerebellin 2 (Reiner et al., 2011). Overall, these results suggest that: (a) avian Md and caudal DLP may represent rostral and caudal parts of the same pallial division, namely DLP; and (2) avian Md and caudal DLP may be comparable to the lizard rostral and caudal parts of the DLP, respectively. However, while the caudal part of chicken DLP, as the lizard lcs, expresses both Lhx9 and Emx1, the putative rostral extension in chicken (Md) expresses Emx1 but not Lhx9 (Abellán et al., 2009), making it different from the rostral DLP of lizard. It is unclear whether Md primordium transiently expresses Lhx9 at very early stages. More studies are needed to further evaluate our proposals.

Similarly to the rostral DLP of lizards (see Martínez-García et al., 1991), the avian Md extends to the retrobulbar region and is reciprocally connected with the olfactory bulb (Atoji and Wild, 2014; note that Md is erroneously identified as densocellular hyperpallium or HD in this article; based on its position between the medial and the lateral vallecula, the region reciprocally connected with the olfactory bulb is Md, since the true HD—as the rest of the hyperpallium—is located medially to the medial vallecula; Puelles et al., 2007; Reiner et al., 2004, 2011). In the lizard, the reciprocal connection with the olfactory bulb is also a feature of lcs (Martínez-García et al., 1991). However, it is unclear whether this is so with the caudal (main) part of the DLP in birds, since the latter includes a superficial cortical plate that extends ventrally, which may have been included as part of an area reciprocally connected with the olfactory bulb, identified as the piriform cortex (Atoji & Wild, 2014; discussed by Medina et al., 2017b, and below).

The homologue of the DLP pallial division in mammals is more difficult to identify due to pallial divergence, including a huge enlargement of the dorsal pallium in mammals, which pushed ventralwards the pallial sectors located medially (i.e., the hippocampal formation) and laterally (including the claustroinsular complex, the endopiriform-piriform complex, and the pallial amygdala). However, the relative position of the DLP should remain invariant in all amniotes: this is, lateral to DP and MP (in direct relation with MP when DP disappears), and medial to or above LP (rostrally), VP (at intermediate levels, when LP disappears) or VCP (at caudal levels). Based on these considerations, we tentatively proposed that the mammalian caudal DLP may produce the lateral entorhinal cortex or LEC (Abellán et al., 2014; also discussed in Medina

et al., 2017b). Moreover, we previously suggested that the lizard lcs (derived from caudal DLP) is comparable to the lateral entorhinal cortex or LEC (Medina et al., 2017b). In addition to their similarity in terms of topological position and genetic profile, the general cytoarchitecture of the reptilian lcs, with its double cortical plate, partially resembles that of the mammalian LEC, with superficial and deep cell layers separated by a cell poor lamina dissecans (Witter & Amaral, 2004). Moreover, some of the connections of the reptilian lcs are similar to those of the LEC of mammals, including their topography with respect to deep or superficial cell layers. As described for the mammalian LEC (Canto et al., 2008), the reptilian lcs receives olfactory bulb input (ending at the superficial plexiform layer of LCd) and projects back to the bulb (the axons arising from cells located in the deep cell plate, i.e., in DC3) (Martínez-García et al., 1991).

Moreover, like the mammalian LEC (Canto et al., 2008; Witter & Amaral, 2004), the reptilian lcs projects to the dentate gyrus (MC), and this projection originates from cells located in the superficial cell plate (i.e., LCd; Bruce & Butler, 1984; López-García et al., 1992). Such a projection from LCd to MC of lizards was considered comparable to part of the perforant pathway of mammals (the other part being the projection from DC2, which as discussed above is comparable to mammalian medial entorhinal cortex or MEC) (López-García et al., 1992; also discussed by Medina et al., 2017b). In mammals, the projections from LEC to DG originate from superficial layer II cells (reviewed by Witter, 2012; Witter & Amaral, 2004; Witter et al., 2017), which express reelin at least in some mammals as the ferret (Ramos-Moreno, Galazo, Porrero, Martínez-Cerdeño, & Clascá, 2006) and the mouse (Allen Developing Mouse Brain Atlas). The presence of such reelin-containing cells densely grouped in layer II is characteristic of the entorhinal cortex in mammals, as they are not observed in other neighboring cortical areas (containing dispersed reelin cells mostly related to interneurons; the neocortex also contains reelin-positive projection neurons but located in layer 5), with the exception of the piriform/olfactory cortex (Ramos-Moreno et al., 2006; Allen Developing Mouse Brain Atlas). In the ferret, such cells are seen in layer II of LEC but not MEC (see Figure 7 in Ramos-Moreno et al., 2006). Notably, the superficial cell plate of the reptilian main part of DLP (i.e., dorsal LC) is distinctly populated by a compact group of reelin-positive cells (Goffinet et al., 1999), highly resembling those of the superficial layer II of mammalian LEC.

As noted above, the reptilian lcs (and perhaps the mammalian LEC) seems comparable to avian caudal DLP. However, the avian caudal DLP is quite large and complex, and possibly contains newly evolved cell groups (without homologue in the DLP of reptiles and mammals) as well as other cell groups that might have evolved from similar ones present in the DLP of the common ancestor (found also in other amniotes). In order to investigate these possibilities, it would be necessary to study the genetic profile (at embryonic and mature ages), neurochemical features and connections of the different cell subpopulations of avian DLP. Regarding the connections, since a typical feature of the reptilian lcs and the mammalian LEC is the direct input from the main olfactory bulb (see references above), it is important to investigate whether the superficial cortical plate of caudal DLP receives this type of input. Nevertheless, throughout the adaptive process that took place during avian evolution, the olfactory input to DLP may have been diminished, in benefit of other inputs (and other functional specialization) carrying information relevant for navigation during flight. In this respect, it is interesting to note the presence of an area within avian caudal DLP, called "Cluster N," which is involved in magnetic compass orientation (Heyers, Manns, Luksch, Güntürkün, & Mouritsen, 2007). Another typical feature that needs to be evaluated is the presence of reelin-positive neurons in avian caudal DLP projecting to the dentate gyrus homologue. During development, the avian caudal DLP (often included as part of the hyperpallium at early embryonic stages) includes reelin positive cells at its surface (Bernier, Bar, D'Arcangelo, Curran, & Goffinet, 2000), resembling those of LEC and reptilian lcs, but their presence in the adult avian DLP and their connections are unknown.

Another interesting observation of the present study is that the cell layers of the lizard lcs appear to originate following an inside-out pattern (as explained in the Results section): that is, based on the first visualization and relative position of these Lhx9-expressing layers during development, the deep layer (DC3) appears to be formed earlier than the superficial layer (LCd). If confirmed by experimental neurogenesis studies (using BrdU or other markers of neurogenesis), this would be the first evidence for an inside-out pattern of pallial layer formation (or cell aggregation) in reptiles, in which the outside-in pattern is the general rule and this is considered to be the ancestral condition in tetrapods (Goffinet, 1983; Medina & Abellán, 2009; Moreno & González, 2017). In contrast, in the mammalian pallium, the prevalent temporal order of layer formation follows a birthdate-dependent inside-out pattern, which is observed in the neocortex and the entorhinal cortex, but —in contrast to previous suggestions—this pattern is absent or less clear in hippocampal formation subdivisions as the subiculum (Wyss, Sripanidkulchai, & Hickey, 1983), the CA fields and the dentate gyrus (Hayashi, Kubo, Kitazawa, & Nakajima, 2015; Xu et al., 2014). These observations are interesting in the context of pallial evolution, as it now appears that the layer formation in the hippocampal formation does not follow the inside-out pattern either in lizards (and other sauropsids) or in mammals, but—in contrast—such inside-out pattern may be found in the lizard lcs as well as in the mammalian LEC. If confirmed in reptiles and in the avian DLP, this would mean that the inside-out pattern of cell aggregation was already present in the DLP pallial sector of stem amniotes.

According to Puelles et al. (2017), a problem with the proposal that caudal DLP produces LEC in mammals is that when LEC starts to be seen in frontal sections, it is interposed between the perirhinal cortex (a proposed caudal extension of the lateral pallium based on Nr4a2/Nurr1 expression) and the piriform cortex (which is part of the ventral pallium; Puelles et al., 2017). However, if Nr4a2/Nurr1 expression does not only define the LP, but also the DLP (in fact, according to our argument, the earliest and strongest expression of this gene may be related to DLP, instead of LP), the perirhinal cortex may not be part of LP but DLP. Moreover, like in lizard and chicken, the DLP of

mammals likely extends more rostrally, and—like in the chicken—the expression of Nr4a2/Nurr1 as well as cerebellin 2 may help to identity the DLP at more rostral levels in mammals. Based on the strong expression of these genes from early stages, the DLP in mouse may also include at least part of (if not all) the claustro-insular complex (for Nr4a2/Nurr1 see Puelles, 2014; Puelles et al., 2016a; for cerebellin 2 see the Allen Developing Mouse Brain Atlas). Notably, while Nr4a2/Nurr1 is primarily expressed in the claustrum proper or insular claustrum (i.e., in the deep stratum of the complex), cerebellin 2 is mostly expressed in the overlying insular cortex (Allen Developing Mouse Brain Atlas). Thus, based on its relative position and early expression of both Nr4a2/Nurr1 and cerebellin 2, this complex seems comparable to the dorsal mesopallium of birds. Based on the observation in chicken (explained above; Reiner et al., 2011; Puelles et al., 2016a), these two different cell lineages (expressing Nr4a2/Nurr1 or cerebellin 2) appear to originate in distinct vz sectors of the rostral or intermediate DLP (and perhaps it also occurs at caudal levels). In chicken and mouse, they mostly remain segregated, although showing a different organization: while they occupy adjacent dorsal and ventral sectors in chicken, in mammals they occupy deep and superficial strata. Combining our argument with that exposed by Puelles (Puelles et al., 2016a; Puelles, 2014), perhaps most if not all the claustrum/insular cortex (expressing both Nurr1 and cerebellin 2) is part of DLP at intermediate rostrocaudal levels and is comparable to avian dorsal mesopallium, while the dorsal endopirifom nucleus (expressing Nurr1 but not cerebellin 2) together with the overlying dorsal part of the piriform cortex is part of LP and comparable to the ventral mesopallium (Table 1). The claustro-insular complex can be followed rostrally into the orbitofrontal cortex, and relates medially to the dorsal peduncular cortex, at retrobulbar levels (Franklin & Paxinos, 2008). Curiously, the dorsal peduncular cortex, together with the taenia tecta, is one of the very few areas of the rostral telencephalon of mammals reciprocally connected with the olfactory bulb (Shipley, Ennis, & Puche, 2004; this area is dorsally adjacent to the taenia tecta and is often included as part of the prefrontal cortex; De Carlos, López-Mascaraque, & Valverde, 1989), thus resembling in its relative position and its reciprocal olfactory bulb connection the avian Md and the lizard rostral DLP. Moreover, the dorsal peduncular cortex is reciprocally connected with the granular/dysgranular part of the insular cortex, and both project to the same brainstem targets (Akhter et al., 2014). Perhaps they represent rostral and intermediate parts of the mammalian DLP (Table 1). Regarding the orbitofrontal cortex, it is remarkable that this area differs from the neocortex because of its strong olfactory inputs from the piriform (olfactory cortex) and the entorhinal cortex (Rolls, 2004), resembling the rostral parts of DLP in birds (Md; Atoji & Wild, 2005; Bingman, Casini, Nocjar, & Jones, 1994; note that at rostral levels Md is mislabeled as HD in these articles) and lizards (Hoogland & Vermeulen-VanderZee, 1995). Like the insular cortex, the orbitofrontal cortex includes agranular and dysgranular parts (with only 3 or 4 layers, respectively, instead of 6), which are considered limbic cortices due to their connections (Barbas & Zikopoulos, 2006). These agranular/dysgranular parts may be part of the DLP, instead of the DP.

In summary, if our suggestions are confirmed, it would mean that the DLP of mammals might extend from retrobulbar rostral levels to very caudal (entorhinal) levels, as suggested for the same division of lizard and chicken (and, by extension, of all sauropsids). More research is needed in embryos of different species to know whether our suggestion is true.

5.5 | The ventral pallium and the new ventrocaudal pallium

With respect to the ventral pallium (VP) and the ventrocaudal pallium (VCP) of the lizard *P. algirus*, these comprise different parts of the DVR and show gene expression profiles identical to DVR divisions of chicken that occupy similar relative positions to those in lizard (Abellán et al., 2009, 2014). In chicken and lizard, the distinction between VP and VCP is based on: (a) their relation to different ventricular sectors and their different gene expression profile in vz and mantle during development (Abellán et al., 2009, 2014; present results); and (b) the existence of a cell free lamina separating both pallial sectors (Abellán et al., 2009; present results).

In chicken, VP produces the nidopallium and VCP the arcopallium (Abellán et al., 2014). Both pallial regions are divided by the dorsal arcopallial lamina (Reiner et al., 2004), which is also observed during development, separating their vz respective sectors (Abellán et al., 2009). Moreover, they differ in their expression of developmental regulatory genes such as Lhx9, Lhx2, and Emx1 (Puelles et al., 2000; Abellán et al., 2009, 2014). While the arcopallium is rich in expression of all three genes in the vz and mantle, the nidopallium shows only moderate expression of Lhx9, which becomes restricted to caudal parts of its mantle from intermediate developmental stages, and does not express Emx1 in the vz and most of the mantle at any time. In addition, the vz of both sectors differ regarding Lhx2 expression, as this is very weak in VP, but it is strong in VCP (Abellán et al., 2009, 2014).

We also identified two major sectors in the DVR of the lizard *P. algirus*, which based on their topological position and different expression of Lhx9, Lhx2, and Emx1 appear comparable to the avian VP-derived nidopallium or the VCP-derived arcopallium. During development, the VP sector of lizard (vz and corresponding DVR mantle) expresses Lhx9, but this is gradually downregulated and becomes restricted to the mantle of the posterior pole encompassing the lizard PDVR (as defined by Lanuza et al., 1998; see Figures 13–14); thus, the lizard VP would include the ADVR, comparable to the anterior and intermediate parts of the avian nidopallium, and the PDVR comparable to the avian caudal nidopallium. Like the chicken VP, the vz of the VP sector of lizard shows only light expression of Lhx2, and its vz and most of the mantle is devoid of Emx1. In contrast, a posterolateral part of the lizard DVR that expresses moderate- to strongly Lhx9, Lhx2, and Emx1 in the vz and mantle appears to represent the lizard VCP, comparable to the avian arcopallium. Based on this expression profile, the VCP division of lizard encompasses the nucleus sphericus, the so-called dorsolateral amygdala, as well as other pallial amygdalar nuclei (as explained in Results).

These two different DVR sectors comparable to VP and VCP of other vertebrates have also been identified in the DVR of the green iguana, and were called anterior and posterior hyperstriatum, respectively, by Northcutt (1967). Interestingly, both regions are separated by a cell free lamina (Northcutt, 1967), resembling the lamina separating nidopallium and arcopallium in birds. Our data on gene expression in the DVR in *P. algirus* agree with the description of Northcutt. On the one hand, the posterior hyperstriatum described by Northcutt (1967) includes the dorsolateral amygdala (DLA) and nucleus sphericus (NS), which according to our results appear to derive from VCP, being comparable to the avian arcopallium (Puelles et al., 2000; Abellán et al., 2009, 2014). In the green iguana, this region relates to a distinct ventricular ridge clearly observed at caudolateral DVR levels (in Figures 7–9 of Northcutt, 1967). In the lacertid lizard *P. algirus*, the ventricular ridge associated to the VCP is less prominent, but still visible at least during development (see our Figures 9l, 10e, and 11i). This different sector of the posterolateral DVR is also observed in the microsmatic lizard *Anolis carolinensis* (Greenberg, 1982), but its organization resembles more that of the avian arcopallium due to almost complete absence of NS in *Anolis* (Armstrong, Gamble, & Goldby, 1953), the major target of the accessory olfactory tract in other lizards (Lohman & Smeets, 1993; Martínez-Marcos et al., 1999). In turtles, another group of microsmatic reptiles that lacks the NS (Reiner & Karten, 1985), this sector is also clearly distinguished and resembles the chicken arcopallium based on its strong expression of the transcription factor ER81/Etv1 (Dugas-Ford et al., 2012). In turtle and chicken, ER81/Etv1 is expressed in the rest of the DVR but at much lighter levels than those in the caudolateral (VCP) part of the DVR (Dugas-Ford et al., 2012). Moreover, based on the relative (topological) position with respect to DLA and NS, as well as the expression of Lhx9, Emx1, and Lhx2, it appears that the nucleus of the accessory olfactory tract (Naot), the lateral amygdala (LA) and the posterior ventral amygdala (VPA), as identified in some lizards and snakes (Lanuza et al., 1998; Martínez-Marcos et al., 1999; Smeets, Hoogland, & Lohman, 1986), are all part of the VCP. In mammals, the VCP also produces a posterior part of the amygdala, including posterior parts of the basal complex and cortical amygdalar areas, the accessory olfactory nucleus, layer 2 of nucleus of the olfactory tract, as well as the amygdalohippocampal complex (or part of it; Medina et al., 2017a).

Conversely, the anterior hyperstriatum identified by Northcutt (1967) in the green iguana includes a large part of the DVR, which according to our data appears to derive from VP. As noted above, this region of the lizard DVR derived from VP is comparable to the avian nidopallium.

As discussed by us in a previous review on the amygdala (Medina et al., 2017a), the lizard VP-derived PDVR (Figures 13–14) contains medial and lateral subdivisions (described by Martínez-García, Novejarque, & Lanuza, 2007), which might be comparable to the avian caudomedial and caudolateral nidopallium (NCL), respectively, based on their similar origin and relative position within the caudal VP (present results; Abellán et al., 2009; Medina & Abellán, 2009). On the one hand, the medial part of the lizard PDVR and medial part of the avian caudal nidopallium (NCM) receive sensory input from the collothalamus (Kröner & Güntürkün, 1999; Lanuza et al., 1998). The medial part of the lizard PDVR also receives visceral input from the parabrachial nucleus and projects to the hypothalamus, and may be comparable to the VP-derived anterior parts of the lateral/basomedial amygdala of mammals (Bruce & Neary, 1995b; Lanuza et al., 1998; reviewed by Martínez-García et al., 2007; Medina et al., 2017a).

Conversely, the lizard PDVR and the NCL show ample connections with other parts of the telencephalon, turning them as highly associative centers (Lanuza et al., 1998; Medina et al., 2017a; Martínez-García, Martínez-Marcos, & Lanuza, 2002, 2007). In particular, like the lizard PDVR (Lanuza et al., 1998), the avian NCL receives input from sensory and associative areas of the ventral pallium (avian nidopallium), dorsal pallium (avian hyperpallium or Wulst) and hippocampal formation (Atoji & Wild, 2005; Kröner & Günturkün, 1999), and projects to the centromedial extended amygdala and the basal ganglia, including its viscerolimbic part (Kröner and Güntürkün, 1999; Veenman et al., 1995 also discussed by Medina et al., 2017a). This highly associative center has been considered functionally analogous to the prefrontal cortex (Kröner & Güntürkün, 1999), but it is likely homologous to part of the basal amygdalar complex of mammals based on its VP origin and gene expression profile (it expresses Lhx9 during development; Abellán et al., 2009; discussed by Medina, Bupesh, & Abellán, 2011, 2017a). In particular, it seems comparable to the VP-derived anterior basolateral nucleus due to its ample associative connections with other parts of the pallium and projections to the extended amygdala and viscerolimbic part of the basal ganglia (Lanuza et al., 1998; Swanson & Petrovich, 1998; reviewed by Medina et al., 2017a), although this needs be taken with caution due to evolutionary divergence.

Like in the nidopallium, most radial glial fibers in the lizard DVR show an oblique trajectory from caudomedial to rostrolateral, as evidenced by accidental labeling of tanycytes following an axonal tracker injection in part of the PDVR (Figure 2B,C in Martínez-García et al., 2002). This means that, when seen in frontal sections, the pial surface of the PDVR, as that of the avian caudal nidopallium (Abellán et al., 2009; Medina et al., 2017a; Redies et al., 2001), is located at more rostral levels. This is important for understanding the tridimensional organization of cell populations, chemo- and geno-architecture, and connections, as well as for comparative purposes. This oblique radial dimension is also appreciated when analyzing Lhx9 expression in frontal and sagittal sections: the Lhx9-expression domain seen caudomedially in the PDVR (Figure 9l) shows a rostral continuation that ends at intermediate and superficial (lateral) levels of the DVR (Figure 9f,g); this is an area usually considered part of the ADVR, but according to our interpretation it represents a rostral extension of the PDVR, different from the Lhx9-poor ADVR. This whole domain of the DVR expressing Lhx9 appears to have at its surface the nucleus of the lateral olfactory tract (Nlot), which expresses Lhx9 from early stages (Figures 4n and 9g), but does not appear to express Emx1, in agreement with its VP origin. This gene expression profile is similar to that of mouse VP at very early stages, although some parts of VP mantle including the piriform cortex and the anterior basal amygdalar complex (rich from very early

stages in Dbx1-linage cells with VP origin; Puelles et al., 2016b) receive Emx1-lineage immigrant cells at later stages of development (Gorski et al., 2002; Puelles et al., 2000). Based on its gene expression profile (expressing Lhx9 but not Emx1) and olfactory bulb input, this reptilian nucleus appears comparable to layer 1 of the nucleus of the olfactory tract of mammals, also with VP origin (García-López et al., 2008; Gorski et al., 2002).

A feature typical of VP is the presence of the olfactory tract and the olfactory (piriform) cortex at its surface (Puelles, 2001; Puelles et al., 2007, 2017; Striedter, 1997). In birds, the olfactory cortex is observed at the surface of the nidopallium (Abellán et al., 2009; Atoji & Wild, 2014; Medina et al., 2011; Puelles et al., 2007; Reiner & Karten, 1985; Striedter, Marchant, & Beydler, 1998). The avian olfactory cortex is organized in several areas from rostral to caudal levels, including a prepiriform cortex at very rostral levels, a piriform cortex at intermediate levels, and several amygdalar-related olfactory areas at caudal levels (usually included as part of the piriform cortex; Abellán et al., 2009; Atoji & Wild, 2014; Puelles et al., 2007; Reiner & Karten, 1985). Moreover, the lateral olfactory tract appears to have two main branches, a dorsal branch related to Md (i.e., the putative rostral part of DLP) and a ventral branch at the surface of the nidopallium (i.e., at the surface of VP; Atoji & Wild, 2014; note the Md is labeled as HD in this article). This may also be so in reptiles and other vertebrates.

In reptiles, the olfactory lateral cortex (LC) is also described as containing several subdivisions, dorsal, ventral and posterior (Lohman & Smeets, 1993; Martínez-García et al., 1991; Martínez-Marcos et al., 1999; Reiner & Karten, 1985). However, according to our data, not all of them belong to the VP. This would agree with differences found in the connections of the different subdivisions of the reptilian LC (Hoogland & Vermeulen-VanderZee, 1995). As noted above, the dorsal part of LC (LCd) relates to the lateral cortical superposition and belongs to the DLP, instead of VP. The posterior part of LC appears to belong to VCP (LCp, Figures 9i,j and 14h,i). We also identified an anterior part of LC, negative for Lhx9, related to LP (LCa, Figures 9b,c and 14b,c).

Regarding the ventral part of LC (LCv), this seems to derive from VP, being thus comparable to the VP-derived piriform cortex of mammals and birds. In lizards of different families, the LCv is located at the surface of a dorsolateral part of the ADVR and/or a ventral part of the pallial thickening (PT; *P. algirus* [Guirado, Dávila, Real, & Medina, 2000; present results], *Podarcis hispanica* [Martínez-García et al., 1991]; *Gekko gecko* lizard [Smeets et al., 1986]; *Tupinambis nigropunctatus* [Curwen, 1937]). In fact, in the lizard *T. nigropunctatus*, the vz related to the ventral part of PT and LCv presents a small ridge, which is located above that related to the main part of the ADVR (Curwen, 1937). Based on radial glial fiber distribution (from Yanes, Monzon-Mayor, Ghandour, de Barry, & Gombos, 1990; Guirado et al., 2000) and our own observations (Figures 2c–f, 9b–d, and 10a,b), it appears that the LCv derives from the neuroepithelium covering the lateral end of the ventricle (sometimes named sulcus lateralis) and the vz sector immediately below. LCv expresses Lhx9 at early stages (Figure 4j,l), but later downregulates it (Figure 9a–d), and does not express Emx1 (Figure 4b–d). The vz producing LCv appears to be the same sector that also produces a dorsolateral part of ADVR and, at rostral levels, a part of the pallial thickening (PT) that lies deep to LCv (Figures 2d 9b–d, and 13). Our data in *P. algirus* suggest that this sector may represent a dorsal and rostral subdivision of VP, based on its relative position with respect to LCv and because the ADVR and this part of PT also express Lhx9 at early stages, but not Emx1 (Figure 4a–c,i,j).

Similarly to LC, the PT is quite complex and appears to contain at least three different subdivisions, which change in size in different reptiles (Figure 13): (1) a dorsal part (PTd) that relates to DLP and expresses both Lhx9 and Emx1 (Figures 4c,d,j, 9d, and 13); in lizards this division is sometimes identified as rostral DC (in fact, it is caudally continuous with DC3) and is deep to rostral LCd, but the same division is identified as part of PT in turtles (Johnston, 1915; see also Powers & Reiner, 1980); (2) an intermediate part (PTi) related to LP (interposed at rostral levels between the DLP and VP parts), lacking Lhx9 (Figures 7b, d and 9c); and (3) a ventral part (PTv) related to VP (deep to LCv), expressing Lhx9 but not Emx1 (Figures 4b,c,i,j and 9d). Since the reptilian LP is only present at very rostral levels, the DLP (PTd or rostral DC3) and VP parts of PT (PTv) soon become contiguous (Figures 9d, 10a, and 13). However, due to the complexity and high interspecies variation of the region containing PT, it is necessary to carry out more detailed studies of regulatory gene expression in the embryonic telencephalon of different reptiles in order to identify homologous subdivisions, find the basic pattern of pallial fields common to all sauropsids, and deduce how these divisions were organized in the last common ancestor. Only when this is clear, we will be able to extract reliable conclusions from the comparison with the pallium of mammals.

5.6 | Origin of the olfactory bulbs and anterior olfactory areas

The VP in other vertebrates has also been proposed to give rise to the olfactory bulbs and the anterior olfactory area (Puelles et al., 2000, 2017; see also Medina & Abellán, 2009). In the lizard *P. algirus*, the VP also appears to extend to at least the retrobulbar region, giving rise to medial and lateral parts of the anterior olfactory area (at some levels separated by the ventricle) that express Lhx9, but not Emx1. However, in the dorsal part of the anterior olfactory area and in the main olfactory bulb there is expression of Emx1 from early stages, which is not consistent with a putative VP origin. In the main olfactory bulb, the mitral cell layer (containing most glutamatergic projection cells of the olfactory bulbs, the others being the tufted cells) express both Emx1 (very strongly) and Lhx9 in lizard (Figure 1), as well as in mouse and chicken (Abellán et al., 2009; Mallamaci et al., 1998; Puelles et al., 2000). It is unclear whether the expression of these transcription factors occurs in the same or different mitral/tufted cells, as these appear to be heterogeneous (Mallamaci et al., 1998). This raises doubts on the origin of the Emx1 cells, since the VP is not a source of such cells. One possibility is that the olfactory bulbs contain a part derived from DLP, thus explaining the expression of both Emx1 and Lhx9 by mitral/tufted cells. This would agree with the course of a dorsal branch of the lateral olfactory tract and the finding of extensive reciprocal connections of the olfactory bulb with the rostral part of DLP in birds and reptiles (as

discussed above). Other possibilities (compatible with the previous one) are that the olfactory bulbs contain a part derived from LP, or that the LP and/or DLP give rise to a massive tangential migration of Emx1 cells to the bulbs. The olfactory bulbs of frogs express Emx1 (Brox et al., 2004) but not Lhx9 (Moreno et al., 2004). However, in teleost fishes the olfactory bulb contains mitral cells expressing Emx1 and Lhx9 (Alunni et al., 2004). Thus, it is likely that mitral cells expressing Emx1 and/or Lhx9 were already present in early jawed-vertebrates, and Lhx9 expression was lost in frogs. In mouse and zebrafish, the olfactory placode and the pioneer axons of the olfactory nerve express Emx1 (Briata et al., 1996; Whitlock & Westerfield, 1998). Remarkably, in zebrafish these Emx1 expressing pioneer axons of the olfactory nerve establish first contact with an Emx1 expressing domain of the telencephalic pallium that represents the primordium of the olfactory bulb (Whitlock & Westerfield, 1998). These data together suggest that Emx1 cells of the olfactory bulb are indeed evolutionarily ancient and represent a fundamental early feature of this structure instead of being related to a secondary event of immigration of cells. Moreover, based on its expression of both Emx1 and Lhx9 in fishes and other vertebrates, this data favor the idea that the olfactory bulb may include at least a part derived from DLP. In agreement with this, at early stages, cerebellin 2 (rich in a ventral subdivision of chicken Md and a part of the mouse claustro-insular complex, Reiner et al., 2011; Allen Developing Mouse Brain Atlas) is strongly expressed in the mitral/tufted cells of the olfactory bulb (Reiner et al., 2011; Allen Developing Mouse Brain Atlas). Thus, the olfactory bulb may have contributions from several pallial sectors (including at least DLP and VP), which may help to explain the heterogeneity of the mitral/tufted cells. Moreover, cells with different origin and genetic profile may give rise to different branches of the olfactory tract, and these may travel through the same pallial division and/or preferentially target cells showing the same or a highly similar molecular profile (as suggested in the amygdala and the hippocampal complex; Medina et al., 2017a,b). In mouse, different subsets of mitral cells appear to express either Emx1 or Emx2, and these transcription factors show segregated expression in at least some targets of the olfactory projection, such as the amygdala (Mallamaci et al., 1998). In zebrafish, different subsets of olfactory bulb mitral cells also may express Emx1, Emx2, or Emx3, and these different cells may target different pallial subdomains, where the expression of these transcription factors is mostly segregated: Emx3 mainly in the dorsomedial pallium or Dm, which includes the proposed VP-related pallial amygdala (Northcutt, 2006; Wullimann & Mueller, 2004); Emx2 in the dorsocentral pallium or Dc, an associative center with dispute homology (Mueller et al., 2011; Northcutt, 2011); and Emx1 in the dorsoposterior pallium or Dp, which was previously compared to the olfactory cortex of amniotes but, based on its position adjacent to the taeniae and its high expression of Emx1, it rather seems to be a distinct part of the medial pallium, as already noted by Nieuwenhuys (2009; for gene expression and further discussion see Ganz et al., 2015). This prompts to investigate the embryonic origin and molecular profile of different subsets of mitral/tufted cells in different vertebrates, as well as revise the axonal trajectory and targets of the olfactory projections that arise in each mitral/tufted cell subset.

Our data also show that the accessory olfactory bulb (AOB) contains two distinct parts, a rostral AOB with strong expression of both Emx1 and Lhx9, and a caudal part free of both, although all parts express Tbr1 in the mitral cell layer (Figures 1 and 11). This agrees with a recent study showing that the caudal part of AOB originates in the prethalamic eminence (at least the mitral cells that express the transcription factor Lhx5; Huilgol et al., 2013), and with a report showing partially different connections of the rostral and caudal parts of AOB (Martínez-Marcos & Halpern, 1999; see also Shipley et al., 2004). Lhx5 expression has also been described in mitral cells of the AOB in frogs (Moreno et al., 2004) and mouse (Abellán, Vernier, Rétaux, & Medina, 2010), and it would be interesting to know whether this cell population is also present in lizards.

As noted above, in the lizard *P. algirus*, as in other vertebrates, the olfactory bulbs also contain a large amount of Dlx2 expressing cells in the granule cell layer (present results; Brox et al., 2003; Puelles et al., 2000; Quintana-Urzainqui et al., 2015). These appear to be immigrant cells that originate in the subpallium, which reach the bulbs from early stages following the rostral migratory stream, and give rise to granular and periglomelular interneurons (Marín & Rubenstein, 2001; Quintana-Urzainqui et al., 2015). The "rostral migratory stream" has also been described in lizards and appears to retain cells with mitotic activity throughout life, being described as a neurogenic niche in the adult brain (Alvarez-Buylla & Garcia-Verdugo, 2002; Font et al., 2001).

5.7 | Where are the dorsal pallium and the lateral pallium of reptiles?

The search of a sauropsidian pallial region homologous to the mammalian dorsal pallium (giving rise to the neocortex) has been object of long and passionate scientific debate. Three main proposals have been done regarding the structures homologous to the mammalian neocortex in the pallium of reptiles and other sauropsids: (a) the neocortex homologue is solely the reptilian dorsal cortex (hyperpallium of birds) or parts of it (pallial thickening and/or lateral superposition; Aboitiz et al., 2003; Bruce, 2007; Medina, 2007b; Puelles, 2001); (b) the neocortex homologue is found in the reptilian dorsal cortex/PT and avian hyperpallium (superior neocortex) plus the DVR or part of it (temporal neocortex; Butler, 1994; Reiner, 1993); (c) different cell layers (or cells with different connections) of the neocortex are found distributed in the dorsal cortex/hyperpallium and in different nuclei of the DVR (Dugas-Ford et al., 2012; Karten, 1997). However, as explained above, our developmental data do not agree with any of these proposals. Considering this, one obvious question is where the reptilian dorsal pallium is located, even raising doubts on its presence at all in reptiles (see discussions on this topic by Northcutt & Kaas, 1995). We thought that, before comparing to mammals, it might be useful to compare to other sauropsids as birds. In birds, the dorsal pallium is located at rostromedial levels of the telencephalic hemisphere, and produces a bulge on the top (hence the German term Wulst to refer to it; reviewed by Reiner et al., 2004). The very rostral pole of the telencephalon of some reptiles, including turtles (Figures 25-27 in Johnston, 1915) and crocodiles (Figure 5 in Crosby, 1917), also shows a bulge at the dorsomedial surface. In turtles

and crocodiles, this bulge is larger and laterally delimited by the presence of a furrow at the surface, which seems comparable to the avian medial vallecula and/or the mammalian rhinal sulcus (Medina & Reiner, 2000; Puelles et al., 2007; Reiner et al., 2004). A vallecula-like furrow is not seen in most lizards and snakes, although a comparable pallial area is also observed at very rostral levels in these reptiles (e.g., see Figure 6 in Curwen, 1937; and Figure 13A in Martínez-Marcos et al., 1999; see also our Figure 14a-c), which is sometimes identified as part of the general pallium (Curwen, 1937), or is left unlabeled (Martínez-Marcos et al., 1999), but more often, the rostral area including the putative DP in reptiles is ignored. The territory related to this dorsal bulge in reptiles includes at its medial edge the hippocampal primordium, and is laterally flanked by the rostral extension of DLP. Our data show that this particular bulge in the lizard *P. algirus* shows expression of Emx1, Lhx2, and Tbr1, but lack Lhx9 during development. Thus, this seems comparable in relative position and gene expression profile to the chicken Wulst, representing dorsal pallial sectors homologous between them and with the mammalian dorsal pallium (producing the neocortex). Notably, the dorsal pallium of reptiles was correctly depicted in a rostral position comparable to that of the avian hyperpallium in Figure 6 of Puelles et al. (2017); the illustration shown in this figure was taken from Stingelin, 1958), although these authors did not identified or mentioned this rostral pallial area in other parts of their work. Unfortunately, the connections of this pallial sector in reptiles are unknown. Nevertheless, if our proposal is confirmed, it would mean that the dorsal pallium in reptiles, particularly in lepidosaurs, is much smaller than previously thought (Medina & Reiner, 2000; Medina, 2007b). As a consequence, many data from previous studies should be reanalyzed to explore this very rostral area here proposed to include the reptilian dorsal pallium. In addition, the definition of the so-called "visual cortex" of turtles, which receives dorsal geniculate input (Heller & Ulinski, 1987) and contains cells that respond to visual stimuli (Kriegstein, 1987; Senseman, 1999; Ulinski, 2007), should also be considered with caution and possibly reinterpreted, as this corresponds to dorsal parts of the reptilian cortex now considered to belong to pallial divisions different from the dorsal pallium (as explained above). As discussed above, the reptilian dorsal cortex appears comparable to different parts of the mammalian hippocampus (DC1) or entorhinal cortex (DC2, DC3); the latter is an associative center where multimodal and highly processed unimodal sensory information converge, and acts as a gateway to the hippocampus (Canto et al., 2008). Notably, in mammals many cells of the hippocampus and entorhinal cortex respond selectively to visual stimuli and are involved in object and place discrimination and memory, which is important for representation of the external world and for navigation (Kreiman, Koch, & Fried, 2000; Suzuki, Miller, & Desimone, 1997; Zhu, Brown, & Aggleton, 1995). Thus, in our opinion the turtle/reptilian dorsal cortex resembles in its capacity to respond to visual stimuli the hippocampus/entorhinal cortex, rather than the visual cortex. Similarly to the entorhinal cortex, the DC in turtles and lizards receives input from the anterior dorsolateral thalamic nucleus (Bruce & Bulter, 1984; Hall, Foster, Ebner, & Hall, 1977), a multimodal center of the thalamus (Desfilis, Font, Belekhova, & Kenigfest, 2002). One intriguing difference is that the DC in turtles (but not lizards) also receives direct input from the dorsal geniculate nucleus (Hall et al., 1977; Heller & Ulinski, 1987; note that in lizards the geniculate projection does not reach DC, but only more ventral parts of PT; Bruce & Butler, 1984; Kenigfest et al., 1997), which in mammals targets the visual cortex (Wilson & Cragg, 1967). Thus, we cannot discard the possibility that DP of turtles (as noted above, evolutionarily closer to archosaurs based on molecular phylogeny) is larger than that of lizards/snakes.

Regarding the LP of reptiles, at present we lacked enough data to be able to unequivocally identify this pallial sector. In chicken, the LP produces the mesopallium, while in mouse it gives rise to the claustro-insular complex, and this sector has been claimed to be the first in the pallium to express Nr4a2/Nurr1 at very early developmental stages (Puelles, 2014; Puelles et al., 2016a). However, as explained above, the area with the strongest and earliest expression of Nr4a2/Nurr1 may represent part of a different pallial division, namely DLP (including its rostral extension, which in birds appears to be a dorsal part of the mesopallium or Md). Unfortunately, there are not detailed data on Nr4a2/Nurr1 in reptiles.

Other regulatory genes expressed in LP at early stages include Emx1 (vz and mantle), Lhx2 (vz), and Tbr1 (mantle), but not Lhx9 (Abellán et al., 2009, 2014; Puelles et al., 2000). Based on these features and its relative position between DLP and VP, we tentatively identified a sector at very rostral levels of the pallium of *P. algirus* that might represent the LP (Figures 7d and 9a-c). As noted above, this sector appears to include an intermediate part of the PT, as also proposed by Bruce (2007) and Puelles et al. (2017), although our interpretation differs in considering the PT as a complex region encompassing DLP, LP, and VP divisions. The LP also appears to include a subdivision of the LC located at the surface of the LP-part of PT. Again, more data are needed to evaluate this proposal.

5.8 | The contribution of the prethalamic eminence (PThE) to the telencephalon

The PThE appears to contribute subpopulations of cells to the telencephalon in different vertebrates, and these cells express Pax6, Eomes (Tbr2) and/or Lhx5 (Abellán et al., 2009, 2010; Abellán & Medina, 2009; Huilgol et al., 2013; Medina et al., 2017a; Puelles et al., 2000; Vicario, Abellán, Desfilis, & Medina, 2014, 2015, 2017). In mouse, chicken and zebra finch, some of these populations appear to invade the extended amygdala (Abellán et al., 2009, 2010, 2013; Abellán & Medina, 2009; Medina et al., 2017a; Vicario et al., 2014, 2015, 2017). In the lizard *P. algirus*, the PThE appears to produce an eminential subdivision the bed nucleus of the stria terminalis (Bste), similarly to other vertebrates (Vicario et al., 2014, 2015).

In addition, the PThE in mouse also produces Cajal-Retzius cells for the lateroventral parts of the pallium (Abellán et al., 2010; Cabrera-Socorro, Hernandez-Acosta, Gonzalez-Gomez, and Meyer, 2007; Meyer, Soria, Martínez-Galán, Martín-Clemente, & Fairén, 1998; Soriano & Del Río, 2005). Moreover, in mouse and frog, some PThE cells were shown to reach the AOB (as explained previously; Huilgol et al., 2013). With our material in the lizard *P. algirus*, we could not see

a continuation of such cells to the AOB. However, our results using sagittal sections suggested a continuity of the PThE with the pallial septum, from where cells distributed to invade the medial septum and diagonal band (Figure 11g). Our results, together with those in mouse and chicken (Abellán et al., 2010; Medina & Abellán, 2012), also show that a caudal part of the previously considered pallial septum may have a PThE origin, being thus different from the pallial part of the septum observed more rostrally. These two parts of the septum (PThE and pallial) differ in their expression of Lef1 (Abellán et al., 2014). Thus, according to our interpretation, the septum appears to include at least two different dorsal areas: (1) an intermediate sector expressing Lhx9, Lhx5, and Lef1 and perhaps related to the medial pallium; and (2) a caudal part, expressing Lhx9, Lhx5 but not Lef1 (Abellán et al., 2009, 2010, 2014), that perhaps represents an extension of the PThE, which produces the nucleus of the pallial commissure, as well as cells that migrate to the medial septum and diagonal band nucleus. Our suggestion requires further evaluation.

5.9 | Concluding remarks

Our data support the existence of at least six pallial divisions in the telencephalon of lizards, which are comparable to similar ones present in other reptiles, birds, and mammals. At early developmental stages, each pallial sector is characterized by a unique topological position and gene expression profile that are highly conserved across species. We took advance of the higher similarity between lizards and chicken regarding the size and topographic displacements of the pallial sectors to identify homologous pallial sectors during development, also having the support of distinctive gene expression profiles. Our aim was to better understand the organization of the pallium in stem sauropsids, for later comparing to that of mammals. In both chicken and lizards, the MP occupies most of the cortical plate as the ventricle expands laterally, including most of the DC in lizards and a comparable corticoid area in chicken. The lizard lateral cortical superposition (including DC3 and LCd) belongs to a distinct dorsolateral pallium (DLP), and it appears comparable to a caudal sector of the DLP of chicken. In lizard, the DLP includes a rostral extension, which reaches retrobulbar regions and is reciprocally connected with the olfactory bulb. This seems comparable to avian Md. Regarding the dorsal pallium, it appears located at rostromedial levels and relates to a dorsomedial bulge in both lizard and chicken (i.e., the avian hyperpallium or Wulst). The lateral pallium (LP) is positioned between the rostral extension of DLP and the ventral pallium, and appears to be very small and located only at the rostral pole of the pallium in lizards. In chicken and lizards, the DVR derives from two different pallial sectors, the ventral pallium (VP) and the ventrocaudal pallium (VCP). The VP produces a large part of the lizard DVR comparable to the avian nidopallium. The VCP produces the arcopallium in chicken and a similar caudolateral part of the DVR that includes the dorsolateral amygdala and nucleus sphericus in lizard. These six pallial divisions appear to be present in all amniotes, and are homologous as fields, but their relative size changes considerably between different species of reptiles, birds and mammals. This proposal involves a change of paradigm regarding organization and homology of different pallial divisions in reptiles with consequences for understanding the organization and evolution of the mammalian pallium, not only affecting the neocortex, but also the septum, pallial amygdala, the entorhinal cortex and the olfactory bulb. This proposal also raises questions regarding the existence of some of these divisions in anamniotes. For example, considering the very small size of the dorsal pallium in lizards, its presence in anamniotes seems unlikely, which is in agreement with the conclusions of Northcutt (2011). In contrast, our data turns the DLP as a likely candidate to be found in the pallium of amphibians and, perhaps, fishes. Finally, the new paradigm opens a new venue for exploring the mechanisms behind the differential evolution of each pallial sector in different species.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTIONS

All authors contributed significantly to the research that led to preparation of this article. ED and LM designed the study. VS cloned the genes. ED and AA performed the experiments. ED, LM and AA analysed and interpreted data. LM and ED drafted the manuscript and the figures, and all authors revised and approved the manuscript.

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