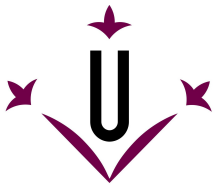

Universitat de Lleida

The Extended Amygdala: Embryonic Origin and Genetic Regulation of its Development

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Universitat de Lleida

Tesis Doctoral

The Extended Amygdala: Embryonic Origin and Genetic Regulation of its Development

realizada por

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The Extended Amygdala: Embryonic Origin and Genetic Regulation of its Development

done by

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Que la Tesis Doctoral titulada "The Extended Amygdala: Embryonic Origin and Genetic Regulation of its Development" ha sido realizada por D. Bupesh Munisamy, bajo la inmediata dirección y supervisión de Dª Loreta M. Medina Hernández, y que el Departamento ha dado su conformidad para que sea presentada ante la Comisión de Doctorado de la Universitat de Lleida.

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La presentación de la Tesis Doctoral titulada "The Extended Amygdala: Embryonic Origin and Genetic Regulation of its Development", realizada por D. Bupesh Munisamy bajo mi inmediata dirección y supervisión, y que presenta para la obtención del grado de Doctor por la Universitat de Lleida.

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Dedicated to my Mentor/Madam

Who introduced me to the field of Neuroscience

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SUMMARY

SUMMARY

Dysfunctions in emotional control and social behavior are behind several human neuropsychiatric disorders, some of which are associated to an alteration of amygdalar development. The control of emotions and social behavior is particularly associated to the so-called extended amygdala, which is a cell corridor of the basal telencephalon extending from the centromedial amygdala to the bed nucleus of the stria terminalis (BST), that constitutes the major output station to effector centers of the hypothalamus and brainstem. However, many aspects of the development of the extended amygdala remain elusive, including the embryonic origin of its different neuron subpopulations. The aim of this Thesis has been to investigate the origin of the neurons of the extended amygdala in mouse embryos (E13.5-E16.5) by using *in vitro* migration assays. The fate mapping results were combined with immunofluorescence for analyzing the phenotype of the neurons that migrated to the amygdala from distinct origins, and were compared with data from immunohistochemistry (to label distinct neuropeptides, proteins or enzymes) and *in situ* hibridization (to detect the mRNA expression of different transcription factors and other proteins) which helped in the delineation of forebrain embryonic domains and extended amygdala subdivisions. In particular, this Thesis deals with studing the embryonic origin of the neurons of the two major subdivisions (or sub-corridors) of the extended amygdala, the medial extended amygdala (Chapter 2) and the central extended amygdala (Chapter 3).

Regarding the medial extended amygdala (Chapter 2), this Thesis provides experimental evidence for a multiple embryonic origin of its principal neurons, including those of the medial amygdala and medial BST. In particular, this study provides novel evidence indicating that a major part of the neurons of the medial amygdala and medial BST derives from the caudoventral part of the medial ganglionic eminence (MGEcv, previously called or included as part of the anterior peduncular area), forming a cell subcorridor with similar molecular features (expression of the transcription factor Lhx6 and the protein calbindin). Comparison to other data indicates that neurons along this MGEcv-related cell subcorridor are interconnected and project to the same hypothalamic targets, which are involved in reproductive behavior. This Thesis also provides novel experimental evidence indicating that the ventral pallium produces some neurons for the medial amygdala, which appear to express the transcription factor Lhx9. The results also confirm that some neurons of the medial extended amygdala originate

in the preoptic area (our results indicate that these cells specifically originate in its commissural subdivision or POC and correlate to expression of the signaling protein Sonic hedgehog) and the supraopto-paraventricular domain of the hypothalamus (or SPV, which derived neurons express the transcription factors Lhx5 and Otp). Similarly to the neurons derived from MGEcv, it is possible that neurons of the medial extended amygdala derived from other embryonic domains also form distinct cell subcorridors related to specific functions.

Regarding the central extended amygdala (Chapter 3), the results of this Thesis show that its major components, including central amygdala and lateral bed nucleus of the stria terminalis (BST), are mosaics formed by different proportions of dorsal lateral ganglionic eminence (LGE)-, ventral LGE- and medial ganglionic eminence (MGE)-derived neurons. Dorsal LGE-derived neurons express the transcription factor Pax6, and primarily populate lateral parts of the central amygdala, but a few also reach the lateral BST. Based on correlation with pre-proenkephalin mRNA and other data, many of these cells are likely enkephalinergic projection neurons. The ventral LGE-derived neurons express the transcription factor Islet1, and primariy populate the central and medial parts of the central amygdala, and part of the lateral BST. Correlation with other studies suggests that these cells represent projection neurons expressing corticotropin-releasing factor. The MGE produces the majority of neurons of lateral BST, but its caudoventral subdivision (MGEcv) also produces an important subpopulation of projection neurons containing somatostatin for medial aspects of the central amygdala. Thus, distinct principal neurons originate in different embryonic domains, but the same domains contribute neurons to most subdivisions of the central extended amygdala, which may explain the similarity in neurochemistry and connections along the corridor. The results, together with other published data, also suggest the existence of at least three subcorridors within the central extended amygdala, each related to a different embryonic origin and involved in the control of a different aspect of fear responses and anxiety.

In conclusion, this study provides important data that clarify relevant aspects on the development and adult organization of the extended amygdala, and helps to set up the foundations for a better understanding of neural control of emotions and social behavior in normal and abnormal conditions.

RESUM

Diversos desordres neuropsiquiàtrics en humans estan relacionats amb una disfunció en el control de les emocions i del comportament social, i alguns d'ells estan associats a una alteració en el desenvolupament de l'amígdala. El control de les emocions i del comportament social estan, en particular, associats a la denominada amígdala estesa, un corredor de cèl·lules del telencèfal basal que s'estén des de l'amígdala centromedial al nucli del llit de l'estria terminal (BST), que constitueix l'estació d'eixida mes important cap a centres efectors de l'hipotàlem i tronc encefàlic. No obstant això, hi ha molts aspectes del desenvolupament de l'amígdala estesa que es desconeixen, incloent l'origen embrionari de les seves diferents subpoblacions de neurones. L'objectiu d'esta Tesi ha sigut investigar l'origen de les neurones de l'amígdala estesa en embrions de ratolí (E13.5-E16.5) per mitjà d'assajos de migració *in vitro*. Els resultats d'estos assajos es van combinar amb inmunofluorescencia per a analitzar el fenotip de les neurones que van migrar a l'amígdala des d'orígens distints, i es van comparar amb dades d'inmunohistoquímica (per a marcar distints neuropèptids, proteïnes o enzims) i hibridació in situ (per a detectar el RNAm de diferents factors de transcripció i altres proteïnes) per a ajudar en la delimitació de distints dominis embrionaris del prosencèfal i distintes subdivisions de l'amígdala estesa. En particular, esta Tesi està dedicada a l'estudi de l'origen embrionari de les neurones de les dos subdivisions (o subcorredors) principals de l'amígdala estesa, l'amígdala medial estesa (Capítol 2) i l'amígdala central estesa (Capítol 3).

En relació a l'amígdala medial estesa (Capítol 2), la present Tesi proporciona evidència experimental de l'origen múltiple de les seves neurones principals, incloent les de l'amígdala medial i les del BST medial. En particular, aquest estudi aporta proves que indiquen que una gran part de les neurones de l'amígdala medial i el BST medial deriva de la part caudoventral de l'eminència ganglionar medial (MGEcv, prèviament coneguda com, o inclosa en, l'àrea peduncular anterior), formant un subcorredor de cèl·lules amb característiques moleculars semblants (expressió del factor de transcripció Lhx6 i la proteïna calbindina). La comparació amb altres dades indica que les neurones d'aquest subcorredor de cèl·lules que deriven de MGEcv estan interconnectades i projecten a les mateixes dianes de l'hipotàlem, involucrades en el control del comportament reproductor. Esta Tesi també aporta dades experimentals que demostren que el pal·li ventral produeix algunes neurones de l'amígdala medial, que pareixen

expressar el factor de transcripció Lhx9. Els resultats també confirmen que algunes neurones de l'amígdala medial estesa s'originen en l'àrea preòptica (les nostres dades indiquen que s'originen específicament en l'àrea preòptica comissural o POC, i aquest origen es correlaciona amb expressió de la proteïna senyalitzadora Shh) i en el domini supraopto-paraventricular de l'hipotàlem (SPV, que dóna lloc a neurones amb expressió postmitòtica dels factors de transcripció Lhx5 i Otp). De forma semblant a les neurones que deriven de MGEcv, és possible que les neurones de l'amígdala medial estesa que deriven d'altres dominis embrionaris també formen distints subcorredors de cèl·lules amb funcions específiques.

En relació a l'amígdala central estesa (Capítol 3), els resultats d'esta Tesi mostren que els seus principals components, incloent l'amígdala central i el BST lateral, són mosaics formats per distintes proporcions de neurones derivades de la part dorsal de l'eminència ganglionar lateral (LGEd), la part ventral de l'eminència ganglionar lateral (LGEv), o l'eminència ganglionar medial (MGE). La neurones derivades de LGEd expressen el factor de transcripció Pax6 i invadeixen preferentment les parts laterals de l'amígdala central, encara que unes poques també arriben al BST lateral. Basant-se en correlació amb el RNAm de pre-proencefalina i altres dades, moltes d'aquestes cèl·lules són probablement neurones de projecció encefalinèrgiques. Les neurones derivades de LGEv expressen el factor de transcripció Islet1 i invadeixen principalment la part central i medial de l'amígdala central, i part del BST lateral. La correlació amb altres estudis suggereix que aquestes cèl·lules són probablement les neurones de projecció que contenen el factor alliberador de la corticotropina. D'altra banda, MGE produeix la majoria de les neurones del BST lateral, però la part caudoventral del MGE (MGEcv) també produeix una important subpoblació de neurones de projecció que expressen somatostatina que invadeixen la part medial de l'amígdala central. Així, distints tipus de neurones de projecció s'originen en distints dominis embrionaris, però els mateixos dominis produeixen neurones per a quasi totes les parts de l'amígdala central estesa, la qual cosa podria explicar la similitud de les neurones en característiques neuroquímiques i connexions al llarg del corredor. Els resultats, junt amb altres dades publicades, també suggereixen l'existència de al menys tres subcorredors de cèl·lules en l'amígdala central estesa, cada un relacionat amb un origen embrionari distint i involucrat en el control d'un aspecte diferent de les respostes de por i ansietat.

En resum, aquest estudi proporciona importants dades que aclareixen aspectes rellevants del desenvolupament i organització adulta de l'amígdala estesa, i ajuda a establir les bases per a una millor comprensió del control neural de les emocions i el comportament social en condicions normals i patològiques.

RESUMEN

Varios desórdenes neuropsiquiátricos humanos están relacionados con una disfunción en el control de las emociones y del comportamiento social, y algunos de ellos están asociados a una alteración en el desarrollo de la amígdala. El control de las emociones y el comportamiento social están, en particular, asociados a la denominada amígdala extendida, un corredor de células del telencéfalo basal que se extiende desde la amígdala centromedial al núcleo del lecho de la estria terminal (BST), que constituye la estación de salida mas importante hacia centros efectores del hipotálamo y tronco encefálico. Sin embargo, existen muchos aspectos del desarrollo de la amígdala extendida que se desconocen, incluyendo el origen embrionario de sus diferentes subpoblaciones de neuronas. El objetivo de esta Tesis ha sido investigar el origen de las neuronas de la amígdala extendida en embriones de ratón (E13.5-E16.5) mediante ensayos de migración *in vitro*. Los resultados de estos ensayos se combinaron con inmunofluorescencia para analizar el fenotipo de las neuronas que migraron a la amígdala desde orígenes distintos, y se compararon con datos de inmunohistoquímica (para marcar distintos neuropéptidos, proteinas o enzimas) e hibridación in situ (para detectar el RNAm de diferentes factores de transcripción y otras proteinas) para ayudar en la delimitación de distintos dominios embrionarios del prosencéfalo y distintas subdivisiones de la amígdala extendida. En particular, esta Tesis está dedicada al estudio del origen embrionario de las neuronas de las dos subdivisiones (o subcorredores) principales de la amígdala extendida, la amígdala medial extendida (Capítulo 2) y la amígdala central extendida (Capítulo 3).

En relación a la amígdala medial extendida (Capítulo 2), la presente Tesis proporciona evidencia experimental del origen múltiple de sus neuronas principales, incluyendo las de la amígdala medial y las del BST medial. En particular, este estudio aporta pruebas que indican que una gran parte de las neuronas de la amígdala medial y el BST medial deriva de la parte caudoventral de la eminencia ganglionar medial (MGEcv, previamente conocida como, o incluida en el área peduncular anterior), formando un subcorredor de células con características moleculares similares (expresión del factor de transcripción Lhx6 y la proteina calbindina). La comparación con otros datos indica que las neuronas de este subcorredor de células que derivan de MGEcv están interconectadas y proyectan a las mismas dianas del hipotálamo, involucradas en control del comportamiento reproductor. Esta Tesis también aporta datos

experimentales que demuestran que el palio ventral produce algunas neuronas de la amígdala medial, que parecen expresar el factor de transcripción Lhx9. Los resultados también confirman que algunas neuronas de la amígdala medial extendida se originan en el área preóptica (nuestros datos indican que se originan específicamente en el área preóptica comisural o POC, y este origen se correlaciona con expresión de la proteina señalizadora Shh) y en el dominio supraopto-paraventricular del hipotálamo (SPV, que da lugar a neuronas con expresión postmitótica de los factores de transcripción Lhx5 y Otp). De forma similar a las neuronas que derivan de MGEcv, es posible que las neuronas de la amígdala medial extendida que derivan de otros dominios embrionarios también formen distintos subcorredores de células con funciones específicas.

En relación a la amígdala central extendida (Capítulo 3), los resultados de esta Tesis muestran que sus principales componentes, incluyendo la amígdala central y el BST lateral, son mosaicos formados por distintas proporciones de neuronas derivadas de la parte dorsal de la eminencia ganglionar lateral (LGEd), la parte ventral de la eminencia ganglionar lateral (LGEv), o la eminencia ganglionar medial (MGE). La neuronas derivadas de LGEd expresan el factor de transcripción Pax6 e invaden preferentemente las partes laterales de la amígdala central, aunque unas pocas también alcanzan el BST lateral. En base a correlación con el RNAm de pre-proencefalina y otros datos, muchas de estas células son probablemente neuronas de proyección encefalinérgicas. Las neuronas derivadas de LGEv expresan el factor de transcripción Islet1 e invaden principalmente la parte central y medial de la amígdala central, y parte del BST lateral. La correlación con otros estudios sugiere que estas células son probablemente las neuronas de proyección que contienen el factor liberador de la corticotropina. Por otro lado, MGE produce la mayoría de las neuronas del BST lateral, pero la parte caudoventral del MGE (MGEcv) también produce una importante subpoblación de neuronas de proyección que expresan somatostatina que invaden la parte medial de la amígdala central. Así, distintos tipos de neuronas de proyección se originan en distintos dominios embrionarios, pero los mismos dominios producen neuronas para casi todas las partes de la amígdala central extendida, lo que podría explicar la similitud de las neuronas en características neuroquímicas y conexiones a lo largo del corredor. Los resultados, junto con otros datos publicados, también sugieren la existencia de al menos tres subcorredores de células en la amígdala central extendida, cada uno relacionado a

un origen embrionario distinto e involucrado en el control de un aspecto diferente de las respuestas de miedo y ansiedad.

En resumen, este estudio proporciona importantes datos que clarifican aspectos relevantes del desarrollo y organización adulta de la amígdala extendida, y ayuda a establecer las bases para una mejor comprensión del control neural de las emociones y el comportamiento social en condiciones normales y patológicas.

CHAPTER 1:
GENERAL INTRODUCTION AND OBJECTIVES

INTRODUCTION

Introduction

During the last century, many studies have been addressed to unravel the mysteries about brain organization and function (reviews in Nieuwenhuys et al., 1998; Striedter, 2005; Butler and Hodos, 2005). However, the last 15 years have been crucial for brain research because of the development of new research techniques involving molecular biology and genetic tools, allowing great advances for understanding brain development and adult organization (Puelles and Rubenstein, 1993, 2003; Marín and Rubenstein, 2001; Briscoe and Ericson, 2001; Ferrán et al., 2007, 2009), the molecular profile of neural pathways controlling behavior (Choi et al., 2005; Bielsky et al., 2004, 2005), and the molecular and cellular basis of dysfunctions producing neurological or neuropsychiatric diseases in humans (see review by Medina and Abellán, 2011).

In general, in all vertebrates the brain can be divided into three large regions, namely from rostral to caudal (Figures 1 and 2): 1) The forebrain or prosencephalon, with the telencephalon rostrodorsally (consisting of the cerebral cortex, the basal ganglia, the amygdala and the septum, as the most important structures), the hypothalamus rostroventrally, and the diencephalon caudally (in prosomeric terms, including the thalamus, epithalamus and pretectum). 2) The midbrain or mesencephalon, including the optic tectum or superior colliculus dorsally, and the tegmentum ventrally. 3) And the hindbrain or rhombencephalon, including rostrally the isthmus, the cerebellum and the pons (the latter two were clasically named metencephalon), and caudally the medulla or classical myelencephalon (Nieuwenhuys et al., 1998; Puelles et al., 2004, 2007; Striedter, 2005; Medina, 2008a).

The forebrain consists of an intricate set of structures that are required for some of the most complex and sophisticated functions of the brain. In different vertebrates it contains centers involved in control of homeostasis and motivated behavior, and responsible of learning, memory, emotions, and control of social behavior; most importantly, in humans it is the reservoir of complex, abstract thoughts and responsible of the highly sophisticated and flexible behavior that characterize our species (reviewed by Striedter, 2005; Medina, 2008a). The malformation and/or dysfunction of forebrain structures lead to various neurological or neuropsychiatric disorders, including Huntington's disease, Alzheimer's disease or other types of dementia, temporal lobe epilepsy, schizophrenia, autism, lysencephaly or mental retardation (for example, Pitkänen et al., 1998; Flames et al., 2004; Kato and Dobyns, 2005; Amaral et al., 2008; Ferrante, 2009). Some of these diseases are related to abnormal development or malformation. For example, schizophrenia, some types of epilepsies, and autism are associated to

abnormal development of the cerebral cortex and/or the amygdala (Kitamura et al., 2002; Flames et al., 2004; Kato and Dobyns, 2005; Amaral et al., 2008; Fazzari et al., 2010). Holoprosencephaly (HPE) is another developmental disorder associated to agenesis of forebrain midline (Lupo et al., 2006). The pathogenesis and the correct identification of the structures affected in these diseases require a closer understanding of normal forebrain development (Xin Geng and Guillermo Oliver 2009). Thus, to better understand the developmental alterations and abnormal formation of the forebrain, it is highly important to study the development of the forebrain in normal conditions. So far, data from research primarily undertaken in several genetic models of different vertebrates, including the teleost zebrafish (*Danio rerio*), the South African clawed frog (*Xenopus laevis)*, the chicken (*Gallus gallus domesticus*), and the mouse (*Mus musculus)*, are beginning to unravel some of the mechanisms involved in early forebrain development and later aspects of its morphogenesis (reviews by Wilson and Rubenstein, 2000; Puelles et al., 2004; Wullimann and Mueller, 2004; Lupo et al., 2006; Moreno et al., 2009; Medina and Abellán, 2009).

In particular, this thesis deals with understanding the development and mature organization of a complex forebrain structure called the extended amygdala, using the mouse as a model. The extended amygdala consists of a cell corridor or continuum that includes the centromedial amygdala (in the caudolateral telencephalon), the bed nuclei of the stria terminalis (rostromedially to the latter) and a dispersed group of cells interposed between both (Alheid and Heimer, 1988; Alheid et al., 1995; de Olmos et al., 2004). The neurons along this cell corridor show similar neurochemical features and connections. The extended amygdala is the major output station of the amygdala, which is a telencephalic center involved in control of emotions and social behaviour (Alheid and Heimer, 1988; Alheid et al., 1995; Swanson, 2000; de Olmos et al., 2004). The extended amygdala has been subdivided into two parallel cell corridors: a central extended amygdala, involved in fear responses and control of ingestion, and a medial extended amygdala, involved in control of reproduction and defense (Alheid and Heimer, 1988; Alheid et al., 1995; de Olmos et al., 2004; for functions see Swanson, 2000). However, embryological support for the existence of these separate corridors was lacking. In recent years, data on expression of developmental regulatory genes (including genes encoding transcription factors and signaling proteins) have revealed that the extended amygdala may be more complex than previously thought, including multiple cell subpopulations of different origin (García-López et al., 2008). The main objective of this thesis is to experimentally analyze the embryonic origin of the cells of the central and medial extended amygdala in the mouse, using an in-vitro fatemap analysis. Since the extended amygdala is a structure located in the rostrodorsal forebrain and appears

to include both telencephalic and extratelencephalic cells (Puelles et al. 2000; García-López et al., 2008), it is mandatory to give a brief summary about some aspects of forebrain development that help to understand its basic divisions and their contribution to the amygdala.

Forebrain organization and its major histogenetic divisions.

The evolution of the vertebrate forebrain and its behavior has been erroneously understood as a linear series of increasing complexity, from "lower" to "higher" groups—fishes, amphibians, reptiles, birds and mammals (reviewed by Striedter, 2005). The supposedly superior organization of the mammalian forebrain would have been reached in evolution with the incorporation of new neural structures, circuits and mechanisms (Kappers et al., 1936; Crosby et al., 1983; Mc Lean, 1990; C. Broglie et al., 2005). However, the brain in different vertebrates is organized according to a common plan, and consists of the same major divisions and subdivisions in fishes, amphibians, sauropsids (including reptiles and birds), and mammals (reviews in Nieuwenhuys et al., 1998; Striedter, 2005; Kaas and Bullock, 2007). Comparably, the brain divisions and subdivisions show different relative sizes and complexities in different groups, and this is often correlated to differences in behavior and ecological adaptations of different vertebrate classes, orders, families and/or species (Striedter, 2005). The molecular, cellular and morphological changes that take place in the anterior or the rostral part of the neural tube during the embryonic development and in evolution lead to the morphological and functional complexity found in the forebrain of different vertebrates (Butler and Hodos, 2005; Striedter 2005).

Data in different vertebrates indicate that during development the neural plate/tube begins to subdivide into distinct longitudinal (dorsoventral or DV) and transversal (rostrocaudal, anteroposterior or AP) subdivisions, under the action of different developmental regulatory genes encoding transcription factors or signaling proteins (reviews by Shimamura et al 1995; Wilson and Rubenstein, 2000; Wilson and Houart, 2004; Puelles et al., 2004; Lupo et al., 2006; Medina, 2008a). The function of some of these developmental regulatory genes has been widely studied, and they have been shown to play important roles in several aspects of development that include patterning and specification of different progenitor zones, neurogenesis, and cellular differentiation (Shimamura et al., 1995; Wilson and Rubenstein, 2000; Wilson and Houart, 2004; Lupo et al., 2006). Mutations in some of these genes in mice (Matsuo et al, 1995; Qiu et al., 1996; Pellegrini et al., 1996; Yoshida et al., 1997) and humans

(Brunelli et al., 1996) result in patterning and morphological abnormalities in specific regions (reviewed by Lupo et al., 2006), and the specific effect depends on where the specific gene is expressed, what downstream genes are affected, and whether its function is redundant with that of other gene(s) (for example, a paralogue gene) that is (are) expressed in the same domain (Shimamura et al 1995; Wilson and Rubenstein, 2000; Wilson and Houart, 2004; Lupo et al., 2006).

The major longitudinal subdivisions along the neural tube (from dorsal to ventral) are called the roof, alar, basal and floor plates, while the main transversal subdivisions are called neuromeres (rhombomeres in the rhombencephalon or hindbrain, and prosomeres in the prosencephalon or forebrain) (Puelles and Rubensetin, 1993, 2003; Shimamura et al 1995; Fraser and Scott-Fraser 1997; Puelles et al., 2004; Wilson and Maden 2005). The parcellation along both AP and DV axis results in a neural tube with multiple subdivisions along the rostrocaudal and dorsoventral axis, each being characterized by: (1) specific location in the bidimensional coordinate diagram of the tube; (2) expression of a specific combination of developmental regulatory genes; and (3) production of specific cell groups, most of which stay within the radial domain they are produced (Puelles and Rubenstein, 1993, 2003; Puelles, 2001a; Puelles et al., 2004, 2007; Medina, 2008a). The divisions thus formed are considered basic units of development, similar to morphogenetic fields or compartments (Puelles and Medina, 2002; Medina, 2007). Importantly, these divisions are comparable across different vertebrates, which share the same building or organization plan (*Bauplan*) for the neural tube (Puelles and Medina, 2002; Medina, 2007).

The forebrain is the most anterior portion of the central nervous system and gives rises to the far most complex territory of the brain. This includes the diencephalon proper (including the thalamus), the hypothalamus, the telencephalon and the optic vesicle (Puelles et al., 2004; Medina, 2008a). In the last two decades, the **prosomeric** model (initially proposed by Puelles and Rubenstein in 1993) has provided a framework that substantially helped for better understanding the development and organization of the forebrain (Puelles and Rubenstein, 1993, 2003), and its evolution (Puelles et al., 2000, 2007; Puelles, 2001a,b). Conceptualy, this model reveals that the forebrain in different vertebrates is subdivided into longitudinal and transverse subdivisions, similarly to the rest of the neural tube, but with the specific consideration that the longitudinal axis is sharply bended in rostral levels of the tube (Fig. 1). This means that the transversal or rostrocaudal subdivisions of the forebrain are going to be located one below the other (and not one in front the other) in frontal and sagittal section planes.

Based on molecular/genetic data and fate map analysis in different vertebrates, the forebrain and its major divisions become specified at the neural plate stage, before they become morphologically visible (Puelles et al., 2004). During development, the forebrain is transversally subdivided into the diencephalic vesicle (or diencephalon proper) caudally, and the secondary prosencephalon rostrally (Fig. 1). As other parts of the neural tube, the forebrain and its major transversal subdivisions are further subdivided into at least four main longitudinal zones, which from dorsal to ventral are named roof, alar, basal and floor plates (Puelles and Rubenstein, 1993, 2003; Puelles, 2001a; Puelles et al., 2004, 2007). Later, the diencephalon proper is additionally subdivided into three prosomeres, called prosomeres 1–3 [p1, p2, p3] from caudal to rostral (Figs. 1, 2) (Puelles and Rubenstein, 1993, 2003; Puelles et al., 2007; Ferran et al., 2007, 2009). Each prosomere contains roof, alar, basal and floor subdivisions. In short, the alar part of p1 includes the pretectum, the alar part of p2 includes the thalamus and epithalamus, and the alar part of p3 includes the prethalamus and the thalamic (or prethalamic) eminence. In the basal/floor parts, the diencephalon includes several prerubral and retromammillary areas ventrally, including the diencephalic parts of the A9 and A10 dopaminergic cell groups (Figs. 1, 2) (Puelles et al., 1987, 2004, 2007; R. García-López et al., 2004; Ferrán et al., 2007, 2009; Smidt and Burbach, 2007; Medina, 2008a,b). On the other hand, at present it is unclear whether the secondary prosencephalon is subdivided into smaller prosomeres, as once proposed (Puelles and Rubenstein, 1993, 2003). In each hemisphere, the secondary prosencephalon is going to produce the hypothalamus ventrally, the optic vesicle laterally, and the telencephalon dorsally. According to the prosomeric model (which is supported by molecular and fate-map data; reviewed by Puelles et al., 2004; Medina, 2008a), the thalamus and prethalamus are located caudal and rostral, respectively, while the hypothalamus is located rostrally to those.

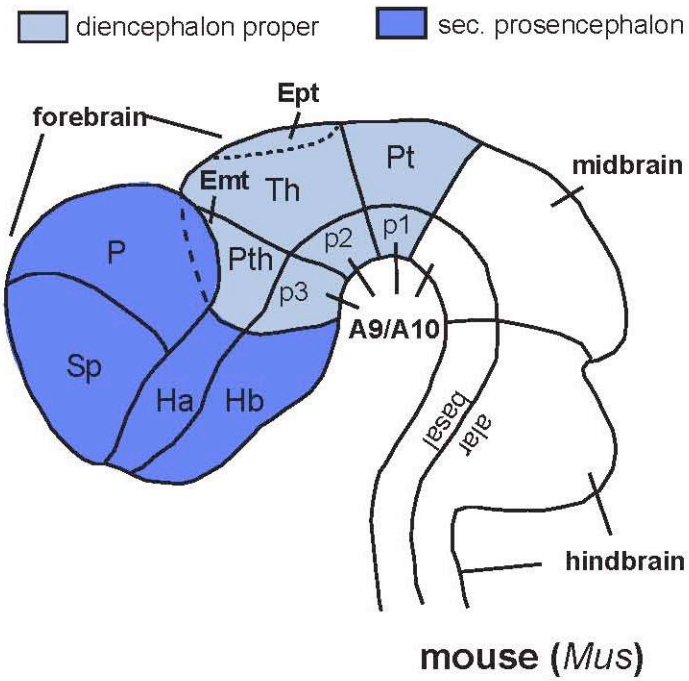


Figure 1. Schematic diagram of a lateral view of the mouse embryonic brain, representing the forebrain and its major divisions and subdivisions. Note that the forebrain shows two major transversal divisions, named the diencephalon proper and the secondary prosencephalon (shown in different colors). The secondary prosencephalon produces the telencephalon dorsally (with pallium and subpallium), the optic vesicle laterally (not represented) and the hypothalamus ventrally (the latter includes alar and basal parts). The amygdala originates in the secondary prosencephalon, and includes pallial, subpallial and alar hypothalamic cell groups. (Courtesy: Medina, Bupesh, Abellán, 2011, Contribution of genoarchitecture for understanting forebrain evolution and development, with particular emphasis in the amygdala, in press). Abbreviations: A9-A10, A9 and A10 dopaminergic cell groups (substantia nigra and ventral tegmental area, respectively); Emt, thalamic or prethalamic eminence; Ha, alar hypothalamus; Hb, basal hypothalamus; p1-p3; prosomeres 1-3; P, pallium; Pt, pretectum; PTh, prethalamus (previously named ventral thalamus); Sp, subpallium; Th, thalamus.

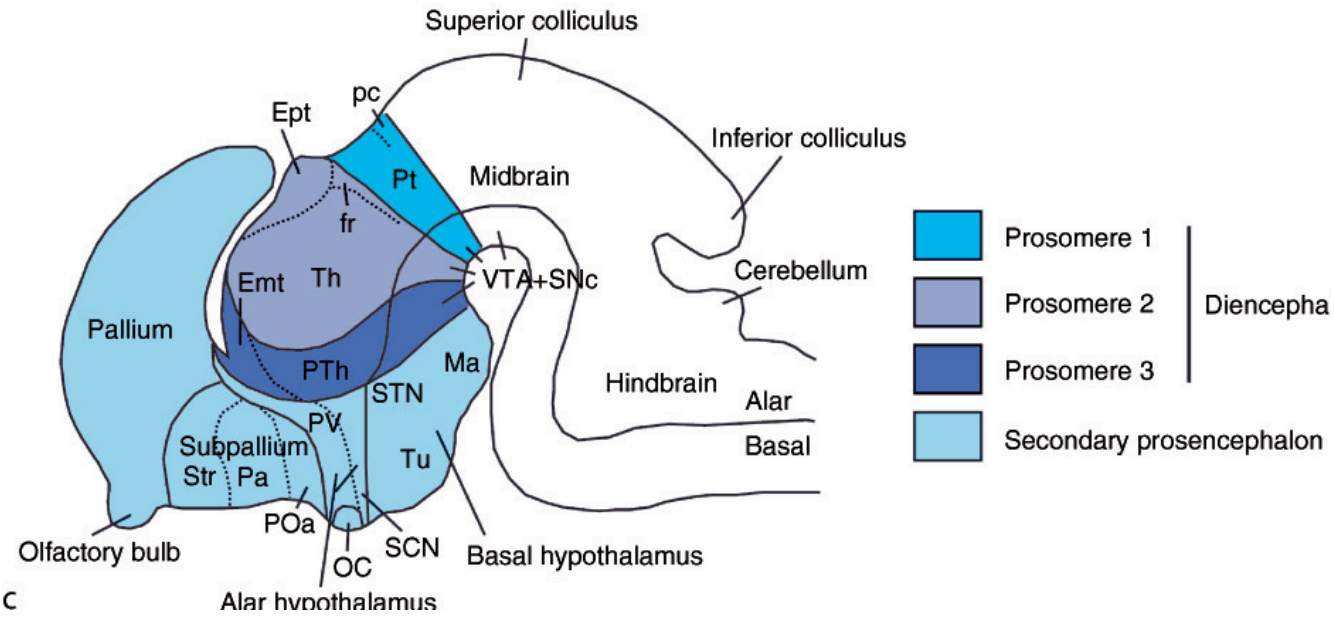


Figure 2:

Sagittal view of a mouse brain during mid-embryonic development, showing the major divisions and subdivisions of the forebrain. The diencephalon proper is subdivided into three major transverse segments, called prosomeres 1, 2 and 3. The dorsal (alar) parts of prosomeres 2 and 3 give rise to the thalamus and prethalamus, respectively. In contrast, the hypothalamus develops from the secondary prosencephalon (i.e., rostral to the diencephalon proper). Since the longitudinal axis is highly bended in the forebrain, the hypothalamus appears located below the prethalamus, and the prethalamus below the thalamus. Courtesy: from a book chapter on the Forebrain, done by L. Medina (2008a), in the Encyclopedia of Neuroscience, Springer-Verlag). Abbreviations: Emt, thalamic or prethalamic eminence; Ept, epithalamus; fr, fasciculus retroflexus; Ha, alar hypothalamus; Hb, basal hypothalamus; Ma, mammillary hypothalamus; OC, optic chiasm; p1-p3; prosomeres 1-3; P, pallium; Pa, pallidum; POa, anterior preoptic area; pc, posterior commissure; Pt, pretectum; PTh, prethalamus (previously named ventral thalamus); PV, paraventricular nucleus and domain; SCN, suprachiasmatic nucleus and domain; SNc, substantia nigra pars compacta (A9 dopaminergic cell group); Sp, subpallium; STN, subthalamic nucleus; Str, striatum; Th, thalamus; Tu, tuberal hypothalamus; VTA, ventral tegmental area (A10 dopaminergic cell group).

The molecular factors involved in the parcellation of the forebrain during early development (from neural plate stage) and the destiny of each subdivision has been analyzed in recent years (Rubenstein et al, 1998; Wilson and Rubenstein, 2000; Cobos et al., 2001; Puelles et al., 2004; Sánchez-Arrones et al., 2009). Fate mapping analysis has shown that all the derivatives of the forebrain originate from the

anterior neural plate (reviewed by Wilson and Rubenstein, 2000). Therefore, mutations in genes that disrupt forebrain development provide a powerful tool for dissecting the mechanisms that regionalize the neural plate, establish fate restrictions, and determine the identities of all its main derivatives including the eyes.

It is known that signals from the prechordal plate play an important role in the patterning and morphogenesis of the overlying forebrain neuroectoderm. For example, the product of the *Shh* gene, produced in the prechordal plate, regulates specification of ventral cell types in the forebrain neuroectoderm (Ericson et al., 1995), and the subdivision of the forebrain into bilateral compartments (Macdonald et al., 1995; Chiang et al., 1996). Morphogenetic signals from the anterior neural ridge (ANR) and from the dorsal and ventral midlines play important and dynamic roles in forebrain and, in particular, telencephalic patterning. These centers serve as the sources of multiple secreted molecules (signaling proteins that act as morphogens) including Shh, Wnts, BMPs, and Fgf8, that impose alone or in concert fate and proliferative decisions to telencephalic progenitors by way of promotion or repression of specific transcription factors (Grove and Fukuchi-Shimogori, 2003; Rubenstein et al., 1998).

In particular, the signals arising from ANR are very important for the rostrocaudal patterning of the forebrain and for later developmental events (Wilson and Houart, 2004; Medina, 2008a; Medina and Abellán, 2009). At an early stage, ANR signals include antagonists of Wnt proteins (secreted Frizzled-related proteins) such as Tlc, which are opposed to Wnt proteins produced in the caudal forebrain (Wilson and Houart, 2004). Wnt proteins are going to caudalize the forebrain (forming the diencephalon proper, sometimes referred as caudal diencephalon), whereas Wnt antagonists produced at the ANR are important for the formation of rostral forebrain, including the telencephalon, eye field and hypothalamus (Backman et al 2005). Therefore, at early stages of CNS development, antagonism of posteriorizing Wnt signals is required for the establishment of the telencephalon (Houart et al., 2002; Mukhopadhyay et al., 2001; Yamaguchi, 2001; Wilson and Houart, 2004).

The rostral forebrain region under the influence of Wnt antagonists starts to express the transcription factor Six3 and will eventually constitute the secondary prosencephalon (reviewed by Medina, 2008a). The functional inactivation of Six3 in mice indicates that Six3 is a crucial regulator of vertebrate head development. In particular, its activity is essential to regulate key molecular events required for the

regional patterning of the anterior neural plate (Lagutin et al., 2003). The generated *Six3* mutant mice lack the rostral forebrain (including telencephalon, eyes and hypothalamus), while the diencephalon proper remains unaltered (Lagutin et al., 2003). In humans, mutations in the *Six3* gene have been associated with holoprosencephaly (a brain disorder in which the forebrain or prosencephalon fails to develop into two hemispheres), which is one of the most common embryologic malformations of the forebrain in humans (Geng and Oliver, 2009). These studies also reaffirm that the diencephalon and secondary prosencephalon constitute two distinct transversal subdivisions of the forebrain, and that the hypothalamus, classically considered part of the diencephalon together with the thalamus, develops in a separate domain rostral to the latter (Puelles et al., 2004; Medina 2008a) (Fig. 1).

Later, Fgf8 is produced in the ANR and even later in the telencephalic midline. Experiments in mouse and zebrafish models suggest that Fgf8, while being very important in anterior signaling (Shimamura and Rubenstein, 1997), is also either directly or indirectly promoting ventral and dorsal fate decisions in the telencephalon (Crossley and Martin, 1995; Gunhaga et al., 2003; Kuschel et al., 2003; Shanmugalingam et al., 2000).

Other signals are going to confluence with Fgf8 to pattern the telencephalon, including Wnts and BMPs arising dorsally (from the dorsal midline), retinoid acid laterally, and Shh ventrally (reviews by Campbell, 2003; Lupo et al., 2006; Medina and Abellán, 2009; also Galceran et al., 2000; Lee et al., 2000).

Telencephalic subdivisions and their characterization based on the gene expression patterns.

By way of connections with the brainstem, thalamus, hypothalamus and olfactory epithelium, the telencephalon plays an extremely important role in processing sensory information, in generating the appropiate behavioural responses, and in complex cognitive functions (including learning and memory formation) (Striedter, 2005).

The telencephalic vesicles are paired evaginations in the dorsal part of the anterior forebrain that constitute the most complex and divergent structures in the vertebrate central nervous system. Telencephalic precursors are located rostrally, adjacent to the anterior margin of neural plate, and are

characterized by expression of the transcription factor Foxg1, preiously known as BF1 (Wilson and Rubenstein, 2000; Cobos et al., 2001; Eagleson et al., 1995; Fernandez-Garre et al., 2002). The two major divisions of the telencephalon are the pallium and the subpallium (reviews by Striedter, 1997, 2005; Nieuwenhuys et al., 1998; Butler and Hodos, 2005; see also Puelles et al., 2000, 2004; Medina, 2008a).

The creeping fatemap analysis and the gene expression data reveal that these major telencephalic divisions, and some of their subdivisions are present during embryogenesis in all vertebrates (Wilson and Rubenstein 2000; Puelles et al. 2000, 2004; Wullimann and Mueller, 2004; Medina and Abellán, 2009; Moreno et al., 2009; Mueller and Wullimann, 2009). The pallium gives rise to the cortical regions, claustrum, pallial amygdala, and olfactory bulb in mammals, and to the olfactory bulb and cortical/pallial regions in non-mammals (including the dorsal ventricular ridge in birds and reptiles) (Striedter, 1997; Puelles et al., 2000; Puelles, 2001b; Medina et al., 2004; Abellán et al., 2009; Medina and Abellán, 2009). In different vertebrates, the subpallium is going to produce the basal ganglia, the centromedial-extended amygdala, the cholinergic corticopetal cell groups of the basal telencephalon and most of the septum (Striedter, 1997; Marín et al., 1998; Brox et al., 2003; Puelles et al., 2000; Moreno and González, 2006; Medina, 2008b; Abellán and Medina, 2009; Moreno et al., 2004, 2009, 2010) (Fig. 3a-d).

Molecular and genetic studies in mouse have shown that telencephalic development is regulated by genes expressed from early embryonic stages in either the pallium or subpallium, or specific subdivisions of these (Bulfone et al 1999; Puelles et al 2000, 2001a,b; Marin and Rubenstein, 2001; Campbell 2003; Medina et al. 2004, 2005; Yun et al 2001, 2003) (Figure 3 a,b). In different vertebrates such as mouse and zebrafish, the pallium and the subpallium express different neurogenic genes: *Neurogenin 1* and *NeuroD* in the pallium, and *Mash1/Zash1a* in the subpallium (Wullimann and Mueller, 2004; Medina and Abellan 2009; Osorio et al., 2010) (Figure 3b). These alternative genetic pathways expressed in pallium and subpallium restrict the phenotype of the cells produced in these compartments. For instance, *Neurogenin 1* expressed in pallium is responsible for the production of glutamatergic neurons, whereas the expression of *Mash1* in subpallium is responsible for the production of GABAergic neurons, which are typical in the subpallium (Wullimann and Mueller 2004; Osorio et al., 2010).

Other data have involved additional regulatory genes encoding transcription factors in the differentiation of distinct neuronal subtypes in the pallium or subpallium. For example, *Tbr1* and *Tbr2* (*Eomes* in *Xenopus*) are expressed in the mantle of the pallium in mouse, chicken, turtle and frog (*Xenopus*) (Bulfone et al., 1999; Puelles et al., 2000; Bachy et al 2001, 2002; Brox et al., 2004; Abellán et al., 2009; Moreno et al., 2010), and in the mouse Tbr1 has been shown to play a role in the differentiation of glutamatergic neurons (Hevner et al., 2001). On the other hand, *Dlx1/2* (*Distal-less-4* in *Xenopus*) are expressed in the proliferative zone of the subpallium in mouse, chicken, turtle, frog, zebrafish and lamprey (Fernandez et al., 1998; Puelles et al., 2000; Bachy et al., 2001, 2002; Murakami et al., 2001; Brox et al., 2003; Wullimann and Mueller, 2004), and in the mouse the transcription factor Dlx2 has been shown to be involved in the differentiation of GABAergic neurons (Fig. 3b).

Figure 3 (see legend in next page)

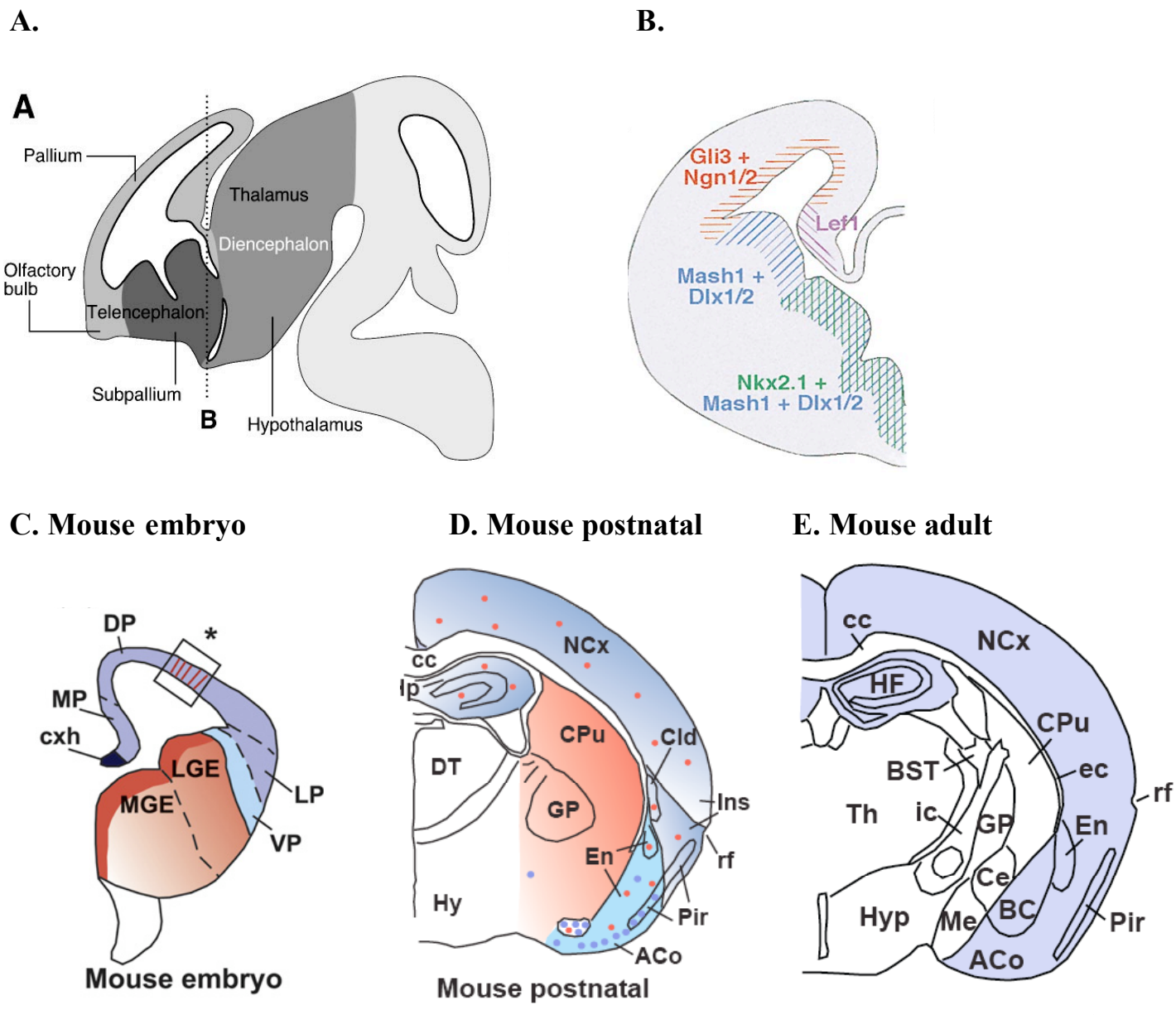


Figure 3:

(A) Schematic drawing of a sagittal section through the brain of an E12.5 mouse showing some of the subdivisions of the forebrain, the diencephalon (in this diagram, the diencephalon is represented in a classical way, and includes the hypothalamus, which really derives from the secondary prosencephalon, but not the diencephaic vesicle) and the telencephalon. In the telencephalon, the pallium is depicted in lighter gray than the subpallium. Courtesy: Marín & Rubenstein, 2003, Annu. Rev. Neurosci.). (B) Schematic drawing of a frontal section through the telencephalon of an E12.5 mouse showing the expression patterns of selected transcription factors in the progenitor zones, which are responsible for regulating telencephalic patterning (Nkx2.1) or distinct aspects of neurogenesis and differentiation (Ngn1/2, Mash1, Dlx1/2) (Courtesy: Marín and Rubenstein, 2003, Annu. Rev. Neurosci.). (C) Schematic diagram of the coronal section of the forebrain in mouse showing the radial units or subdivisions of the pallium and subpallium. Courtesy : Medina and Abellán, 2009, Seminars in Cell & Developmental Biology**.** (D, E) Mouse postnatal and adult telencephalon (in coronal sections), showing the derivatives of the divisions seen in (C). Pallial drivatives are shown in blue, while subpallial derivatives are shown in orange (left) or without colour (right). For abbreviations see Chapter 2.

The emerging data on the combinatorial gene expression patterns of several regulatory genes, including the ones mentioned above, plus *Gsh1/2*, and several LIM-homeodomain genes, in the telencephalon of mouse and chicken show that the subpallium includes at least three major radial subdivisions, whereas the pallium includes at least four radial subdivisions (Puelles et al., 2000, 2004; García-López et al., 2008; Flames et al 2007; Abellán and Medina, 2009).

The three main radial subdivisions of the subpallium are named the striatal subdivision (lateral ganglionic eminence in mouse), the pallidal subdivision (medial ganglionic eminence and the peduncular area in mouse) and the preoptic subdivision (Puelles et al., 2000, 2004; García-López et al., 2008; Flames et al., 2007; Abellán and Medina, 2009; Abellán et al., 2009). The striatal subdivision is characterized by expression of *Gsh2*, *Mash1*, *Dlx1/2*, and *Dlx5*; the pallidal subdivision also expresses *Dlx* and *Mash* genes, but additionally expresses *Nkx2.1*, *Lhx6* and *Lhx7/8*; the preoptic subdivision expresses *Dlx*, *Nkx2.1*, *Lhx6*, *Lhx7/8*, and additionally expresses the gene encoding the signaling protein Sonic hedgehog (Shh) in the ventricular zone (Puelles et al., 2000, 2004; Medina et al., 2004; García-López et al., 2008; Flames et al., 2007; Abellán and Medina, 2009) (Figures 3B, 4B). Since some of these regulatory genes are also expressed in the mantle (for example, *Dlx5*, *Nkx2.1*, *Lhx6* and

Shh), their combinatorial expression patterns, always within the context of the topological framework of the brain, are very useful for analyzing the general derivatives of each division (Puelles et al., 2000; García-López et al., 2008). Importantly, this type of analysis led to the suggestion that different parts of the subpallium give rise to different parts of centromedial extended amygdala (including the centromedial amygdala and the bed nucleus of the stria terminalis or BST) (García-López et al., 2008; see details in section on the amygdala below). However, experimental demonstration was lacking.

Abundant data also exist on the expression of numerous developmental regulatory genes in the pallium, including *Emx1*, *Emx2*, *Lhx2*, *Pax6*, *Tbr1*, *Lmo2*, and *Lmo3*, among other genes, revealing genetic distinctions between subdivisions and the role of some of these genes in patterning and specification, or in cell differentiation (Bulfone et al., 1995; Stoykova et al., 1996; Bishop et al., 2000, 2003; Bulchand et al., 2001; Hevner et al., 2001; Vyas et al., 2003). The pallium was classically subdivided into three main subdivisions namely medial, dorsal and lateral pallia (reviewed by Striedter, 1997), but Puelles and collaborators proposed the existence of four radial subdivisions in the pallium based on the combinatorial expression patterns of several regulatory genes encoding the transcription factors Pax6, Tbr1 and Emx1 (Puelles et al., 2000). In particular, they proposed the subdivision of the classical lateral pallium into two radial domains: a re-defined lateral pallium (smaller than the classical one) and a novel ventral pallium (just adjacent to the pallio-subpallial boundary (Puelles et al., 2000). All pallial subdivisions express *Pax6* in the ventricular zone and *Tbr1* in the mantle (Bulfone et al., 1995, 1999; Puelles et al., 2000). In contrast, *Emx1* is expressed in the ventricular zone and mantle of medial, dorsal and lateral pallium, but is absent in the ventricular zone of the ventral pallium (Puelles et al., 2000; Bishop et al., 2000, 2003). The existence of a ventral pallium has been confirmed by its differential expression of *Dbx1* in the ventricular zone (Yun et al., 2001; Medina et al., 2004), and expression of *Lhx2*, *Lhx9*, *Semaphorin 5A* (*Sema 5A*) and *Neurogenin2* (*Ngn2*) in the mantle (Medina et al., 2004; Tole et al., 2005; García-López et al., 2008; Abellán et al., 2009) (Figure 4A). On the contrary, the ventricular zone of the lateral pallium expresses *Emx1* but lack expression of *Dbx1*, and the mantle expresses *Emx1* and *Cadherin 8* (*Cdh8*), but lacks *Lhx2*, *Lhx9*, *Ngn2* and is poor in *Sema 5A* (Puelles et al., 2000; Medina et al., 2004; García-López et al., 2008; Abellán et al., 2009). Based on the differential expression pattern of *Emx1*, *Cdh8*, *Sema5A*, and *Ngn2*, the lateral and ventral pallial subdivisions have been proposed to give rise to different parts of the claustral complex and pallial amygdala (basal amygdalar complex and cortical amygdalar areas) in the mouse (Puelles et al., 2000; Medina et al 2004). *Lhx2* and *Lhx9* have also turned out to be good markers of the ventral pallial amygdala in the mouse (Tole et al., 2005; García-López et al., 2008), the chicken (Abellán et al., 2009) and the anuran

frog *Xenopus laevis* (Moreno et al., 2004; Moreno and González, 2006). Data on *Lhx9* also suggested that a part of the medial amygdala (usually considered part of the subpallial amygdala) in mouse and chicken derives from the ventral pallium (García-López et al 2008; Abellán et al., 2009). However, experimental demonstration for this was lacking.

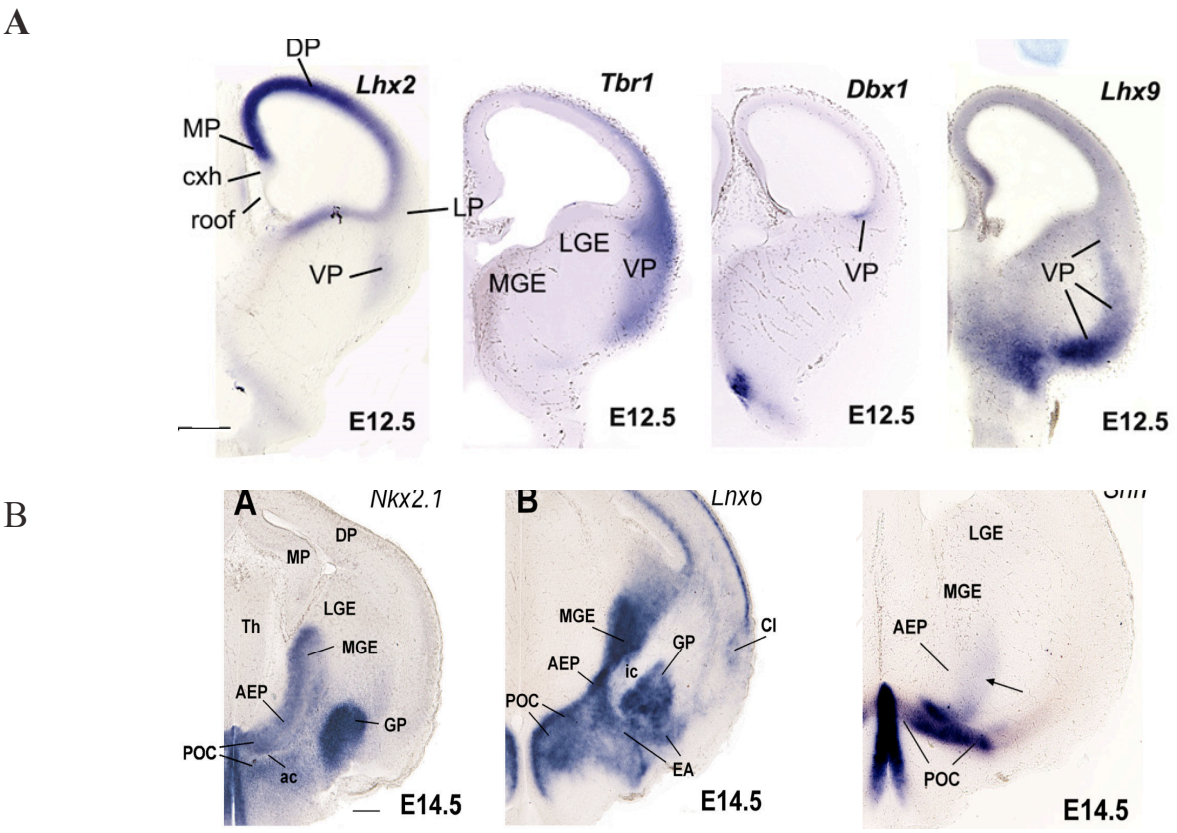


Figure 4 A) Different pallial gene markers showing its distinct expression in the ventral pallial subdivision

B) Expression pattern of several developmental regulatory genes in some of the subpallial subdivisions and their derivatives, including the pallidal subdivision (MGE and AEP; currently, both are named simply MGE) and the POC (García-López et al., 2008). For abbreviations see Chapter 2.

The amygdala and the development of the amygdalar mosaic.

The amygdala is an almond shaped telencephalic structure located in the temporal lobes of the primate cerebral hemispheres, near the hippocampus, and in a corresponding part of the caudolateral telencephalon in rodents (reviewed by Swanson and Petrovich, 1998). In mammals, the amygdala is a highly complex and heterogeneous (mosaic-like) structure, composed of numerous subdivisions that differ in cytoarchitecture, neurochemistry, connections, and gene expression patterns (Price et al., 1987; LeDoux et al., 1990; Amaral et al., 1992; Alheid et al., 1995; Linke et al., 1999; McDonald, 1998; Swanson and Petrovich, 1998; Pitkänen, 2000; Medina et al., 2004; Remedios et al., 2004, García-López et al 2008). As explained below, this heterogeneity is starting to be understood thanks to developmental studies, which are also helping to understand better the amygdala of non-mammals and its evolution in tetrapods (reviews by Moreno and González, 2006; Medina et al., 2011).

Unravelling the development and evolutionary origin of the amygdalar mosaic is essential for trying to understand the high variety and complexity of amygdalar functions, as well as its dysfunctions. In general, the amygdala plays a role in control of fear responses and other basic functions critical for survival (ingestion, reproduction, defense), as well as in arousal and the formulation of emotional memories. It is responsible for the control of homeostasis and mediation of emotional and species-specific social behavior, including several aspects of cognitive functions related to emotion such as attention, recognition of facial expressions of emotion, associative learning like that involved in fear conditioning, and modulation of memory in emotionally arousing events (Adolph et al., 1994; Aggleton, 2000; Davis, 2000; Gallagher, 2000; LeDoux, 1992, 2000). The dysfunctions and/or malformations of the amygdala lead to many psychiatric disorders including temporal lobe epilepsy (Pitkänen et al., 1998), autism (Amaral et al., 2008), schizophrenia (Wolterink et al 2001), and stress-related psychological disorders (Rauch et al., 2006).

Based on morphological criteria, the pioneers in comparative neurobiology Holmgren (1925) and Källén (1951) described the existence of pallial and subpallial divisions, and proposed their derivatives in the telencephalon of different vertebrates. Importantly, they proposed the existence of pallial and subpallial parts of the amygdala, which has been corroborated in the last decades thanks to the substantial data on the expression of developmental regulatory genes and fate mapping analysis (Puelles et al., 2000; Gorski et al., 2002; Medina et al., 2004; García-López et al., 2008; Xu et al., 2008; Hirata et al., 2009; Waclaw et al., 2010; in non-mammals see Puelles et al., 2000, and reviews by

Moreno and González, 2006; Moreno et al., 2009; Medina and Abellán, 2009; Medina et al., 2011). In particular, the results showed nested expression of developmental regulatory genes (including transcription factors and signaling proteins) restricted to the proliferative zone and/or mantle of specific forebrain divisions or subdivisions; these genes were thus considered to be good markers for those specific divisions/subdivisions and their derivatives. The proposed amygdalar derivatives of pallium and subpallium, and their subdivisions based on the combinatorial gene expression patterns are explained in more details in the next section. In addition to these, some data suggested the existence of an extratelencephalic contribution of neurons to the amygdala (Puelles et al., 2000; Puelles and Rubenstein, 2003; Medina et al., 2004; García-López et al., 2008; Bardet et al., 2008; Abellan et al., 2010), and this will also be explained in more detail below. It is important to note that experimental demonstration for many of these proposals was lacking.

Pallial, subpallial, and extra-telencephalic contribution of neurons to the amygdala.

During development the amygdala shows a mosaic-like molecular organization, and includes multiple subpopulations of neurons that express different developmental regulatory genes, such as LIM-containing regulatory genes (Puelles et al., 2000; Medina et al., 2004; García-López et al., 2008; Remedios et al., 2004; Tole et al., 2005; Hirata et al., 2009). These data suggest that different nuclei/areas of the amygdala, or subpopulations of cells within them, appear to derive from several molecularly distinct ventricular sectors of the telencephalon, mainly localized in either the ventrolateral pallium or the subpallium (Puelles et al., 2000; Puelles and Rubenstein, 2003; Medina et al., 2004; García-López et al., 2008).

According to the developmental data based on these regional gene markers, the pallial part of the amygdala includes the basolateral amygdalar complex and some cortical amygdalar areas (Puelles et al., 2000; Medina et al., 2004). This part of the amygdala is rich in glutamatergic/excitatory projection neurons (Swanson and Petrovich, 1998), and this can be explained by their expression during development of *Ngn1/2* and *Tbr1*, which as explained in previous sections are involved in the production or differentiation of this type of neurons (Wullimann and Mueller, 2004; Hevner et al., 2001; reviewed by Medina and Abellán, 2009). In addition, recent findings based on expression of *Tbr1* and the ventral pallial gene marker *Lhx9* suggest that the ventral pallial subdivision also contributes neurons to the medial amygdala (Medina et al., 2004; García-López et al., 2008), which

was considered a subpallial structure based on neurochemistry and connections generally rich in GABA and neuropeptides (Swanson and Petrovich, 1998; Swanson, 2000). Nevertheless, other data also showed the existence of some glutamatergic projections from the medial amygdala (Choi et al., 2005). More developmental data, including fate mapping analysis, were needed to resolved this puzzle. In addition, similarly to other parts of the pallium, the pallial amygdala (basal amygdalar complex and cortical amygdalar areas) appear to contain minor subpopulations of GABAergic interneurons, co-containing different calcium-binding proteins or neuropeptides (Berdel and Morys, 2000; Kemppainen and Pitkänen, 2000; McDonald and Mascagni, 2001; Muller et al., 2003; Legaz et al., 2005), which appear to derive from the subpallium (Marin and Rubenstein, 2001; Xu et al., 2004; 2008; Legaz, 2006; García-López et al., 2008), but more detailed data were needed.

On the other hand, based on the gene expression data, it has been proposed that the subpallium contributes cells to the central and intercalated amygdalar nuclei, to the anterior amygdala and to the major part of the medial amygdala (Puelles et al., 2000; Medina et al., 2004; García-López et al., 2008; Hirata et al., 2009; Soma et al., 2009). As noted above, the subpallial histogenetic subdivisions striatal, pallidal (including the subdomain MGEcv, which is roughly equivalent to the AEP or diagonal domain, as known in different studies; García-López et al., 2008; Bardet et al., 2010) and preoptic, have been proposed to contribute various cell subpopulations to different parts of the subpallial amygdala and to its extension into the bed nuclei of the stria terminalis (the centromedial extended amygdala; see next section and Fig. 5). In particular, based on expression of *Dlx5* and *Lmo4*, the striatal subdivision was proposed to produce most neurons of the central and intercalated nuclei, and the dorsal anterior area of the amygdala (Medina et al., 2004; García-López et al., 2008; see also Puelles et al., 2000, based on expression of Pax6). In contrast, the medial amygdala, proposed by Swanson (Swanson and Petrovich, 1998; Swanson, 2000) to be striatal in nature, did not appeared to derive from the striatal subdivision of the subpallium, but rather from the pallidal and preoptic subdivisions. In particular, the caudoventral part of the pallidal subdivision (MGEcv), previously named or included as part of the the anterior peduncular area (AEP), appears to be the major source for the neurons of the posterodorsal medial amygdala, and many neurons of the anterior medial amygdala (García-López et al., 2008; this subdivision is called diagonal domain by other authors, such as Bardet et al., 2010) (see Figure 5). In contrast, the preoptic area, which is distinguished from the pallidal subdivision by its ventricular expression of both *Nkx2.1* and *Shh* (Flames et al., 2007; García-López et al., 2008), was proposed to produce cells for the anterior and posteroventral parts of the medial amygdala based on the expression

of *Shh* only in these parts of the medial amygdala (García-López et al., 2008). These different proposals were based on gene expression patterns but lacked experimental demonstration.

On the other hand, based on the expression of the transcription factors Otp and Lhx5, the supraoptoparaventricular domain of the alar hypothalamus (SPV domain) was also proposed to contribute a subpopulation of neurons to the medial amygdala (Bardet et al., 2008; García-López et al., 2008; Abellán et al., 2010). Based on this analysis, it was observed that during development some Otp- or Lhx5-expressing neurons appeared to spread tangentialy from this SPV domain to invade the medial amygdala and medial bed nucleus of the stria terminalis (Bardet et al 2008; Abellan et al 2010; see also previous proposal by Puelles and Rubenstein, 2003). However, experimental demonstration for this was lacking.

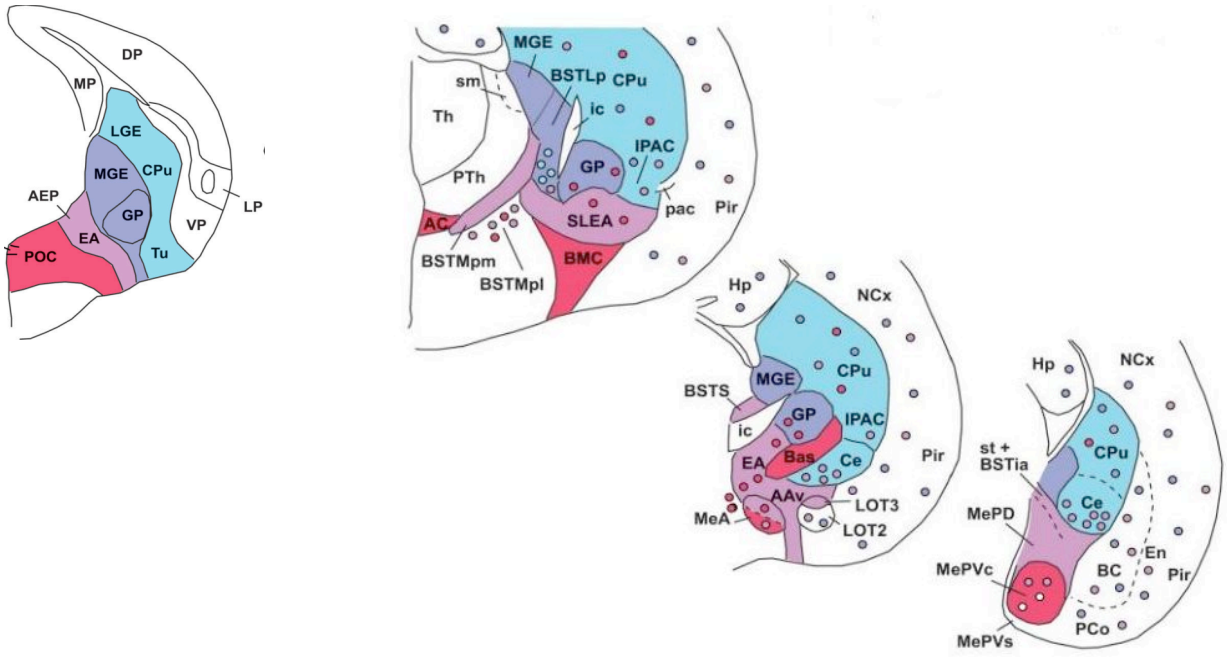


Figure 5:

Schematic diagram of frontal sections through the embryonic telencephalon of mouse, showing the major histogenetic domains of the subpallium (in different colors) and the proposal of their contribution of cells to different amygdalar regions (Courtesy: García-López et al., 2008). For abbreviations see Chapter 2.

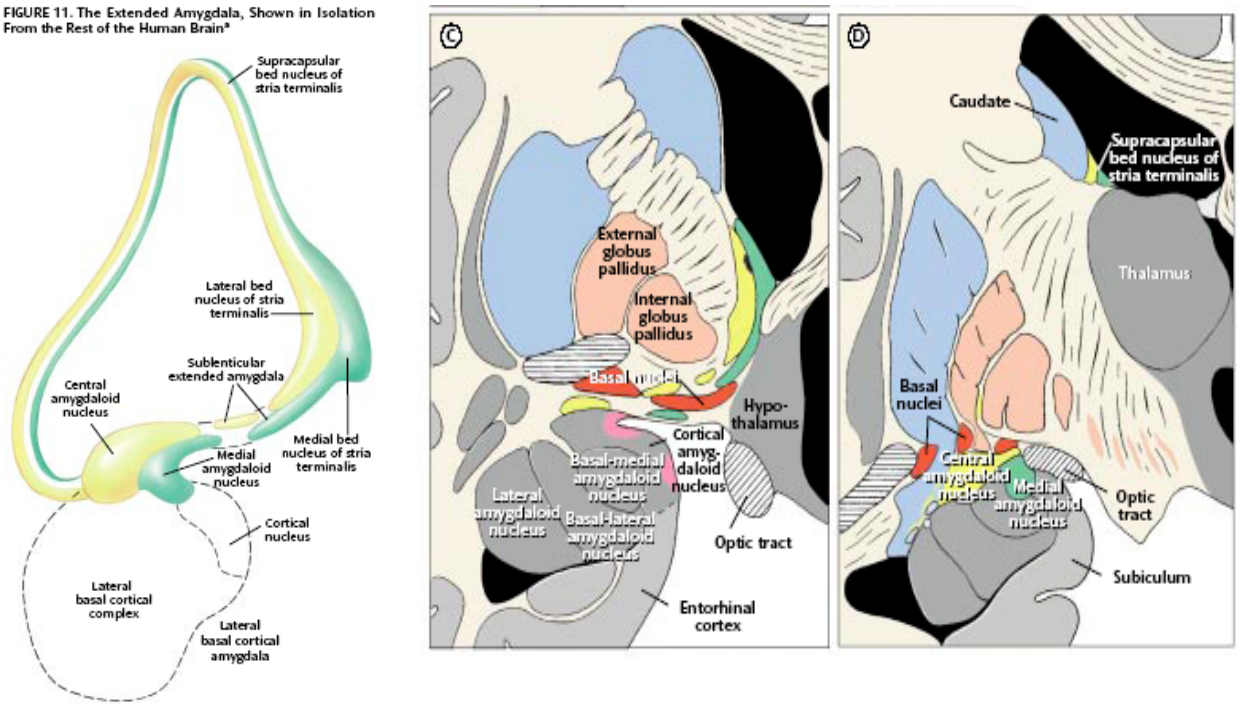
The extended amygdala (central and medial subdivisions).

The concept of *extended amygdala* was first proposed by one of the pioneers in comparative neuroanatomy, J.B Johnston (1923), who suggested the existence of a cell continuum or corridor in the embryonic basal telencephalon of diferent mammals, encompassing and establishing a relationship between the bed nucleus of the stria terminalis and the centromedial amygdaloid nuclei. Almost 50 years later, de Olmos provided evidence supporting the existence of a continuity between the bed nucleus of stria terminalis and the centromedial amygdala even in adult mammals (de Olmos, 1969, 1972). Based on similarity of neurochemistry and connections, de Olmos together with Aheid and Heimer redefined the corridor to make it more concise (de Olmos et al., 1985, 2004; Alheid and Heimer, 1988; Alheid et al 1995; de Olmos and Heimer, 1999; Heimer, 2003; Walker and Davis 2008). They showed that the central (CeA) and medial (MeA) nuclei of the amygdala and the bed nucleus of the stria terminalis (BST or BNST) were connected by columns of cells located throughout the course of the stria terminalis (the fiber tract that connects these amygdala nuclei with the BNST), and throughout the sublenticular part of the basal telencephalon (de Olmos et al., 1985, 2004; Alheid and Heimer 1988; Alheid et al 1995; Heimer, 2003, Walker and Davis 2008) (Figure 6 a,b,c). Moreover, according to this redefined proposal, the extended amygdala comprises two distinct cell corridors, namely the central extended amygdala (dorsally, including the central amygdalar nucleus and the lateral BST or BSTL) and medial extended amygdala (ventrally, including the medial amygdalar nucleus and the medial BST or BSTM). The central extended amygdala (i.e. central amygdala along with BSTL) is involved in fear responses, stress, anxiety and control of ingestion. The medial extended amygdala (i.e., medial amygdala along with BSTM) is responsible for the control of reproduction and defense/aggresive behavior (Alheid and Heimer 1988; Alheid et al., 1995; Heimer 2003; for functions, see also Swanson, 2000).

Various techniques were used by de Olmos, Alheid and Heimer to confirm the existence of these two cell corridors, including Timm's method of heavy metals, cupric silver method of staining argyropillic neurons, immunohistochemical staining, and tract tracing methods (Fig 6 c). However, development data supporting the existence of the extended amygdala were missing.

B

A



C

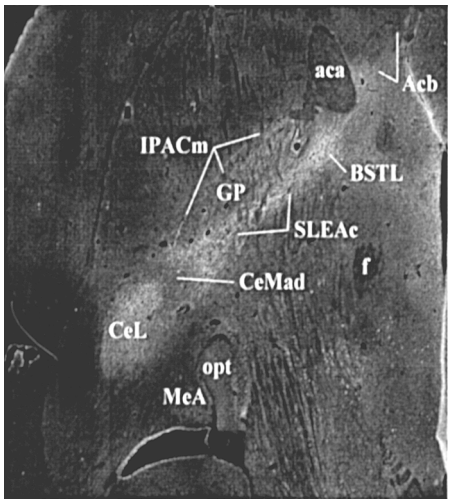


Figure 6:

A. Extended amygdala in human brain, excluding others parts. **B**. The two interrupted cell corridors showing the central (yellow) and medial (green) continuum (courtesy: Heimer, 2003).

C. Cupric silver stained horizontal section in rat showing the cell corridor from the central amygdala to the BST (Courtesy: de Olmos and Heimer, 1999).

OBJECTIVES

Objectives

As noted above, the centromedial extended amygdala plays a key role in control of functions and behaviors that are essential for animal survival, such as fear responses, stress, ingestion, reproduction and defense/aggression. Dysfunctions of the amygdala lead to important neuropsychiatric disorders or diseases, including anxiety/stress disorders, autism or schizophrenia, and some of these dysfunctions appear to be due to a developmental alteration. Thus, developmental studies are essential to understand these alterations, but also to answer key questions that may help to understand better its basic organization, such as: how and where are the different neurons of the extended amygdala produced?; are neurons along the "extended amygdala" corridor (including those of the BST) produced in the same or different embryonic domains? As noted in the Introduction, in recent years, different studies based on gene expression patterns have proposed the putative origin of some parts of the extended amygdala. However, many aspects on the development of the extended amygdala remain elusive, including the experimental fate mapping demonstration of the proposals. Therefore, the aim of this Thesis has been to study the embryonic origin of the neurons of the two cell corridors of the mouse extended amygdala, the central extended amygdala and the medial extended amygdala, by way of *in vitro* experimental fate mapping, combined with immunohistochemistry and/or immunofluorescence (to detect different transcription factors, calcium-binding proteins or neuropeptides) (see more details below). The data were compared with data from gene expression patterns, including data produced for this Thesis as well as data available in the laboratory (on the expression of different transcription factors or signaling proteins), which helped in the delineation of different embryonic subdivisions and parts of the extended amygdala.

Objective 1 (Chapter 2): The first aim of this Thesis was to investigate the origin of the neurons of the medial extended amygdala. Previous work from our laboratory based on the combinatorial expression of developmental regulatory genes (including several transcription factors such as Nkx2.1, Lhx6, and Lhx9, and the signaling protein Shh) and neuronal markers (such as calbindin, neuropeptide Y and somatostatin) suggested that different parts of the medial extended amygdala originate in either the ventral pallium (VP), the caudoventral pallidal subdivision (MGEcv), or a novel domain of the preoptic subdivision called the commissural preoptic area (POC) (García-López et al., 2008). Moreover, other data on the expression of the transcription factors Otp (Bardet et al., 2008) and Lhx5 (Abellán et al., 2010) suggested that an important subpopulation of neurons of the medial extended amygdala originate in the alar hypothalamus, in the SPV domain. The experimental demonstration for all of these

proposals was lacking. One of the objectives of this Thesis was to experimentally investigate these proposals. For that, we prepared organotypic cultures of mouse embryonic forebrain slices, from early to intermediate developmental stages (E12.5 to E16.5), and applied the fluorescent cell tracker CMFDA to label and fate map progenitor cells of several subdivisions, including pallial (VP), subpallial (MGEcv and POC) and hypothalamic (SPV) subdivisions. For helping in the interpretation of the results regarding forebrain compartments and amygdalar subdivisions, we compared these results with data on gene expression patterns (available in the laboratory), and carried out double-labeling for calbindin using immunofluorescence. To try to understand the radial or tangential orientation of cell migrations, we also compared our results with the disposition of radial glial fibers.

Objective 2 (Chapter 3): The second aim of this Thesis was to investigate the origin of the neurons of the central extended amygdala. Previous data in the mouse based on expression of Dlx5, Pax6, and Islet1, have suggested that the central amygdala originates in the striatal (LGE) subdivision (Puelles et al., 2000; García-López et al., 2008; Waclaw et al., 2010). However, Pax6 and Islet1 are expressed in neurons that derive from either dorsal or ventral subdivisions of LGE, and it was unclear what parts of the central amygdala are populated by each type of neuron. On the other hand, while most neurons of the central amygdala were proposed to originate in the striatal subdivision or LGE, data on Nkx2.1 suggested that the lateral BST originates in the pallidal subdivision or MGE (Puelles et al., 2000; García-López et al., 2008; Xu et al., 2008). Pending confirmation, these data did not provide embryonic support for the central extended amygdala continuum. However, other data in mouse and chicken suggested that the lateral BST receives a subpopulation of immigrant cells from LGE, which express Pax6 or Lmo4 (García-López et al., 2008; Abellán and Medina, 2009). On the other hand, it appeared that the central amygdala include a subpopulation of projection neurons containing somatostatin that immigrate from MGEcv (García-López et al., 2008; Real et al., 2009). Thus, the second aim of the Thesis was to try to resolve all of these uncertainties by way of *in vitro* experimental fate mapping. We followed the same experimental approach as in Objetive 1, and label the progenitor zone of dorsal LGE, ventral LGE or MGEcv in telencephalic slices at E13.5-E16.5. To help in the identification of embryonic domains and the phenotype of migrated cells, and we carried out the double labelling using immunofluorescence for the transcription factors Pax6 or Islet1, for the protein calbindin, and for the neuropeptide somatostatin. We also compared these results with data of mRNA expression of Pax6 and Islet1.

CHAPTER 2:

Medial Extended Amygdala

Multiple Telencephalic and Extratelencephalic Embryonic Domains Contribute Neurons to the Medial Extended Amygdala

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ABSTRACT

Dysfunctions in emotional control and social behavior are behind human neuropsychiatric disorders, some of which are associated with an alteration of amygdalar development. The medial extended amygdala is a key telencephalic center for control of social behavior, but very little is known about its development. We used in vitro migration assays for analyzing the origin of the neurons of the medial extended amygdala in mouse embryos (E13.5–E16.5). We compared the migration assays with immunofluorescence/immunohistochemistry for calbindin and radial glial fibers and with mRNA expression of several genetic markers of distinct forebrain subdivisions. We provide experimental evidence for multiple embryonic origins of the principal neurons of the medial extended amygdala. In particular, we provide novel evidence indicating that a major part of the neurons derives from a caudoventral pallidal subdivision (previously called or included as part of the *anterior peduncular area*), forming a cell corridor with similar molecular features (expression of Lhx6 and calbindin), connectivity, and function, which relates to reproductive behavior. We also provide novel experimental evidence indicating that the ventral pallium produces some neurons for the medial amygdala, which correlates with data from Lhx9 expression. Our results also confirm that some neurons of the medial extended amygdala originate in the preoptic area (our results indicate that these cells specifically originate in its commissural subdivision) and the supraoptoparaventricular domain of the hypothalamus. Our study helps to set up the foundations for a better understanding of medial amygdalar control of behavior in normal and abnormal conditions. J. Comp. Neurol. 519:1505–1525, 2011.

INDEXING TERMS: ventral pallium; medial ganglionic eminence; anterior peduncular area; commissural preoptic area; supraoptoparaventricular hypothalamic domain; cell migrations

The amygdala is a telencephalic structure essential for control of emotions and social behavior (Aggleton, 2000; Davis, 2000; Swanson, 2000; Phelps, 2004; Phelps and LeDoux, 2005), and its dysfunction is associated with several human neuropsychiatric diseases, including temporal lobe epilepsy (Pitkänen et al., 1998), autism (Amaral et al., 2008), and stress-related psychological disorders (Rauch et al., 2006). Some of these diseases are related to an alteration of amygdalar development (for example, autism; Amaral et al., 2008). Thus, studying amygdalar development is extremely important for understanding the pathophysiology of these diseases. The medial amygdala is particularly complex and is part of a cell corridor called the *medial extended amygdala* (Alheid and Heimer, 1988; Alheid et al., 1995; de Olmos et al., 2004), with different subdivisions involved in the control of either reproduction or defense/aggressive behaviors (Swanson and Petrovich, 1998; Swanson, 2000). These different

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functions are controlled by molecularly distinct neuron subpopulations of the medial extended amygdala, which express different transcription factors such as Lhx6, Lhx9, or Lhx5 (Choi et al., 2005). In particular, Lhx6 is expressed in medial extended amygdalar neurons related to the reproduction control pathway (Choi et al., 2005). Similar neuron subpopulations have also been identified in the developing medial extended amygdala of mice (Puelles et al., 2000; Stenman et al., 2003; Remedios et al., 2004; Tole et al., 2005; García-López et al., 2008; Abellán et al., 2010). Based on gene expression patterns, these different neuron subpopulations of the medial extended amygdala have been proposed to originate in different progenitor domains of the forebrain, both telencephalic and extratelencephalic (García-López et al., 2008; Bardet et al., 2008; Abellán et al., 2010; García-Moreno et al., 2010). This suggestion has only partially been shown by different kinds of fate map studies (Xu et al., 2008; Hirata et al., 2009; Soma et al., 2009; García-Moreno et al., 2010). For example, genetic fate mapping with Nkx2.1-cre mice indicates the presence of abundant cells of the Nkx2.1 lineage in the medial amygdala, which originate in the pallidopreoptic region of the subpallium (Xu et al., 2008). Via genetic fate mapping of Dbx1 and in vitro migration assays, the preoptic area has been shown to contribute cells to the medial amygdala (Hirata et al., 2009). However, the preoptic area includes at least two subdivisions (Flames et al., 2007; García-López et al., 2008; Abellán and Medina, 2009), and it is unknown what specific subdivision produces neurons for the medial amygdala. Moreover, it is still unclear whether any specific part of the medial ganglionic eminence produces cells for the medial amygdala. Moreover, it is unknown whether the ventral pallium produces cells for the medial amygdala. Finally, it is also unclear whether any of the above-mentioned forebrain subdivisions contribute cells to other parts of the medial extended amygdala, such as the medial bed nucleus of the stria terminalis. The aim of our study was to investigate these unknown aspects of medial extended amygdalar development by using in vitro fate map assays in mouse forebrain slices. Our resuts provide inequivocal experimental evidence for multiple embryonic origins of the neurons of the medial extended amygdala, including the ventral pallium, the caudoventral part of the medial ganglionic eminence, the commissural part of the preoptic area, and the supraoptoparaventricular hypothalamic domain. Both radial and tangential cell migrations contribute to its formation.

Abbreviations

AAd	anterior amygdala, dorsal part
AAv	ventral part of the anterior amygdala
ACo	anterior cortical amygdalar area
Bas	basal nucleus (Meynert)
BC	basal amygdalar complex
BST	bed nucleus of the stria terminalis
BSTia	intraamygdaloid part of the BST
BSTL	lateral BST
BSTM	medial BST
BSTMpl	posterolateral part of BSTM
BSTMpm	posteromedial part of BSTM
BSTS	supracapsular part of BST
CB	calbindin
Ce	central amygdala
CFDA	green cell tracker (CMFDA)
Co	cortical amygdalar area
CPu	caudoputamen complex
DP	dorsal pallium
EA	extended amygdala
EAme	medial extended amygdala
EMT	prethalamic eminence
En	endopiriform nucleus
fi	fimbria
GP	globus pallidus
HF	hippocampal formation
Hp	hippocampal formation
ic	internal capsule
IPAC	interstitial nucleus of the posterior limb of the anterior commissure
ITC	interstitial amygdalar cells
LGE	lateral ganglionic eminence
lot	lateral olfactory tract
LPO	lateral preoptic area
Me	medial amygdala
MeA	anterior medial amygdala
MePD	posterodorsal medial amygdala
MePV	posteroventral medial amygdala
MePVc	central part of MePV
MePVs	superficial part of MePV
MGE	medial ganglionic eminence
MGEcv	caudoventral part of MGE
MGEd	dorsal part of MGE
MP	medial pallium
NCx	neocortex
PA	pallial amygdala
pac	posterior limb of the anterior commissure
PCo	posterior cortical amygdalar area
Pir	piriform cortex
PO	preoptic area
POB	basal (or ventral) preoptic area
POC	commissural preoptic area
PTh	prethalamus
RC2	radial glial cell marker
rp	roof plate
Se	septum
SFO	subfornical organ
SI/BM	substantia innominata and basal magnocellular complex
SLEA	sublenticular extended amygdala
sm	stria medullaris
SPa	subparaventricular hypothalamic domain
SPV	supraoptoparaventricular hypothalamic domain
Th	thalamus
TR	transmission
Tu	olfactory tubercle
VP	ventral pallium
3V	third ventricle

MATERIALS AND METHODS

Mouse (Swiss) embryos from embryonic day 13.5 (E13.5) until day 16.5 (E16.5) were used in the present study. All animals were treated according to the regulations and laws of the European Union (86/609/EEC) and the Spanish Government (Royal Decree 223/1998; more recently Royal Decree 1021/2005) for care and handling of animals in research. The mouse embryos were obtained from pregnant females, and their brains were processed for preparing organotypic cultures, in situ hybridization, or immunohistochemistry.

TABLE 1.
Primary Antibodies

Antibody	Antigen recognized	Immunogen	Manufacturer, species, mono- vs. polyclonal, catalog or lot No.	Dilution
Anticalbindin	Calbindin D-28k	Recombinant rat calbindin D-28k	Swant (Bellinzona, Switzerland), rabbit polyclonal IgG, catalog No. CB-38a, lot Nos. 9.03 and 18F	1:2,000
RC2	295-kDa intermediate filament-associated protein of radial glial cell cytoskeleton (Ifaprc2)	Cell suspension of rat fetal brain (E14–E17)	Developmental Hybridoma Bank, (University of Iowa), mouse monoclonal IgM	1:100

Organotypic cultures

For the migration assays, we prepared organotypic cultures of forebrain slices following a previously described procedure (Soria and Valdeolmillos, 2002; slightly modified according to Legaz, 2006). Upon extraction, embryos were placed in a standard ice-cold, oxygenated culture medium resembling cerebrospinal fluid (containing 124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4{\cdot}7H_2O$, 26 mM $CHNaO_3$, 2.4 mM $CaCl_2{\cdot}2H_2O$, and 10 mM D(+)-glucose; Soria and Valdeolmillos, 2002), in which brains were dissected out. The brains were sectioned at 300 μm in the frontal or horizontal plane using a vibratome (Leica VT 1000S), and the slices were mounted onto porous culture plate inserts (Millicell-CM, 0.4 μm pore diameter; 30 mm insert diameter; Millipore, Molsheim, France; Soria and Valdeolmillos, 2002) and placed in culture medium (DMEM F-12; Gibco, Grand Island, NY; supplemented with 5% fetal bovine serum, 0.1 mM glutamine, 6.5 mg/ml D-glucose, 1% supplement N2, and 1% penicilline; Soria and Valdeolmillos, 2002; Legaz, 2006). Slices were allowed to recover in a CO_2 incubator (5% CO_2; 37°C) for 1 hour before application of the tracer dye. After that, tungsten particles coated with the fluorescent dye CMFDA (Cell Tracker green 5-chloromethylfluorescein diacetate; Invitrogen-Molecular Probes, Paisley, United Kingdom; excitation peak 490 nm, emission peak 514 nm; Alifragis et al., 2002) or CMTMR (Cell Tracker orange 4-chloromethyl benzoyl amino tetramethyl rhodamine; excitation peak 546 nm, emission peak 574 nm; Molecular Probes, Eugene, OR; Alifragis et al., 2002) were applied to the ventricular/subventricular zone of the ventral pallium (VP), the medial ganglionic eminence (in particular, its caudoventral subdivision, previously named or included as part of the *anterior peduncular area*; Puelles et al., 2000; García-López et al., 2008), the preoptic subdivision, or the supraoptoparaventricular hypothalamic domain. The slices were then transferred to culture medium (Neurobasal; Gibco; supplemented with 5% fetal bovine serum, 0.1 mM, glutamine, 6.5 mg/ml D-glucose, 1% supplement B27; Gibco; and 1% penicillin; Soria and Valdeolmillos, 2002; Legaz, 2006) and incubated in a CO_2 incubator (5%, 37°C) for 48 hours. After incubation, the slices were fixed with phosphate-buffered 4% paraformaldehyde (pH 7.4) for 8 min and then rinsed and stored in phosphate buffer (0.1 M, pH 7.4) containing 0.1% sodium azide until microscopic observation. The labeling was analyzed and images were captured with a confocal scanner microscope (Leica TCS SP2 or Olympus FV500).

Immunohistochemistry and immunofluorescence

To understand better the results of the migration assays, some embryonic brains were sectioned in frontal or horizontal planes and processed for immunohistochemistry to detect calbindin (rabbit anticalbindin) or radial glial fibers (mouse anti-RC2). Moreover, selected slices from the migration experiments were processed (directly or after resectioning at 40–50 μm thick) for immunofluorescence to detect calbindin. See Table 1 and section below for details on the primary antibodies. The primary antibodies were diluted in PBS containing 0.3% Triton X-100, and the tissue was incubated for 2–3 days at 4°C, under constant gentle agitation.

After this incubation and standard washes in PBS-Triton, the sections were incubated in a secondary antiserum for either 1 hour (for immunohistochemistry) or 2 hours (for immunofluorescence) at room temperature. For immunohistochemistry, the secondary antisera used were either biotinylated goat anti-rabbit (for calbindin) or biotinylated goat anti-mouse (for RC2). Both secondary antisera were purchased from Vector (Burlingame, CA) and were diluted at 1:200. After washing, the sections were incubated in the avidin-biotin complex (ABC kit; Vector; 0,003% dilution) for 1 hour at room temperature. The immunolabeling was revealed by 0.05% diaminobenzidine (DAB; Sigma-Aldrich, Steinheim, Germany) in 0.05 M Tris (pH 7.6), containing 0.03% H_2O_2. For immunofluorescence, we used Alexa-conjugated donkey anti-rabbit secondary antiserum (Alexa 488 for CMTMR experiments or Alexa 568 for CMFDA experiments; Molecular Probes). After incubation, the sections were rinsed and stored (at

4°C, in the darkness) until analysis with a confocal microscope. To check the specificity of our secondary antisera, some sections were processed omitting the primary antibody.

Antibody characterization

See Table 1 for a list of all primary antibodies used. The calbindin D-28k antibody recognized a single band of 28 kD m.w. on Western blots of rat brain (manufacturer's data sheet) and stained a pattern of cellular morphology and distribution in the mouse brain that is identical to previous reports on localization of the mRNA of the same protein using in situ hibridization histochemistry (Sequier et al., 1990).

The RC2 antibody recognized a band of 295 kD m.w. on Western blots of mouse brain, spinal cord, and skeletal muscle, and ultrastructure analysis revealed that the antibody stained an epitope of the cytoskeleton on radial glial cells in the mouse brain (Chanas-Sacré et al., 2000). This antibody produces a staining pattern of radial glial cells in the mouse brain identical to that reported previously (Misson et al., 1988; Toresson et al., 1999; Chanas-Sacré et al., 2000; Soma et al., 2009).

In situ hybridization

For a better understanding of the results of the migration assays, frontal or horizontal embryonic brain sections were processed for in situ hybridization using digoxigenin-labeled riboprobes, following a procedure previously described (Medina et al., 2004; García-López et al., 2008; Abellán and Medina, 2008, 2009). The riboprobes were synthesized from cDNAs of the following mouse genes: *Lhx5* (plasmid obtained from Dr. Westphal's laboratory; Zhao et al., 1999; bp 1–2226; Genbank accession No. U61155.1), *Lhx6* (Grigoriu et al., 1998; 410 bp fragment, bp 699–1110; Genbank accession No. NM_008500; primary identifier: BC065077.1), *Lhx9* [Rétaux et al., 1999; bp 1–1016 (full length); Genbank accession No. AF134761], and *Sonic hedgehog* (Shh; A.P. McMahon; Shimamura and Rubenstein, 1997; bp 1–2727; Genbank accession No. NM_009170).

We used PCR to obtain the DNA template employed for synthesizing the riboprobe. We synthesized the antisense digoxigenin-labeled riboprobes by using Roche Diagnostics (Mannheim, Germany) protocols for the genes mentioned above. Before hybridization, the sections were treated with proteinase K (10 μg/ml; Roche Diagnostics) in PBS containing 0.1% Tween-20 (PBT 1×) for 1 minute. After that, sections were abundantly washed and postfixed with phosphate-buffered 4% paraformaldehyde containing 0.06% glutaraldehyde for 20 minutes. The sections were washed again, prehybridized in hybridization buffer (HB) for 2 hours at 58°C, and then hybridized in HB containing the ribprobe overnight at 58°C (0.5–1 μg/ml, depending on the probe and embryo size). The hybridization buffer contained 50% deionized formamide, 1.3× standard saline citrate (SSC; pH 5), 5 mM ethylenediaminetetraacetic acid (EDTA; pH 8.0; Sigma-Aldrich), 1 mg/ml yeast tRNA (Sigma-Aldrich), 0.2% Tween-20, and 100 μg/ml heparin (Sigma-Aldrich), completed with water (free of RNAase and DNAase; Sigma-Aldrich). After hybridization, the sections were washed with a mix 1:1 of MABT 1× (1.2% maleic acid, 0.8% NaOH, 0.84% NaCl and 0.1% Tween-20) and HB at 58°C during 20 minutes and washed abundantly at room temperature with MABT 1× (about 2 hours). After this, the sections were blocked with a solution containing blocking reagent (Roche), MABT 1× and sheep serum (Sigma) for 4 hours at room temperature, then incubated in an antibody against digoxigenin (alkaline phosphatase-coupled antidigoxigenin, diluted 1:3,500; Roche Diagnostics) overnight at 4°C and later were washed with MABT 1× and finally revealed with BM purple (Roche Diagnostics). Sections were then mounted on glycerol gelatin (Sigma).

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, whereas serial images from fluorescent material were taken using the confocal microscope (Leica TCS SP2 or Olympus FV500). Selected digital images were adjusted for brightness/contrast in Adobe Photoshop, and figures were mounted and labeled in FreeHand 10.

Identification of cell masses and nomenclature

For identification of forebrain cell masses during development, we used an atlas of developing mouse (Jacobowitz and Abbot, 1997) and rat (Paxinos et al., 1994; Foster, 1998) brain as well as our own publications on the subject (especially Legaz et al., 2005b; García-López et al., 2008; Abellán et al., 2010). For BST and amygdalar subdivisions, we followed the brain atlas by Paxinos and colleagues (Paxinos et al., 1999, and Paxinos and Franklin, 2004; which follow the scheme of Alheid et al., 1995).

RESULTS

The ventral pallium produces cells for the basal amygdalar complex and cortical areas and also for the medial amygdala

Our results from migration assays in telencephalic slice cultures of E13.5–E15.5 mice indicate that the ventral pallium (VP) produces abundant cells that invade the piriform cortex, basal amygdalar complex, and cortical

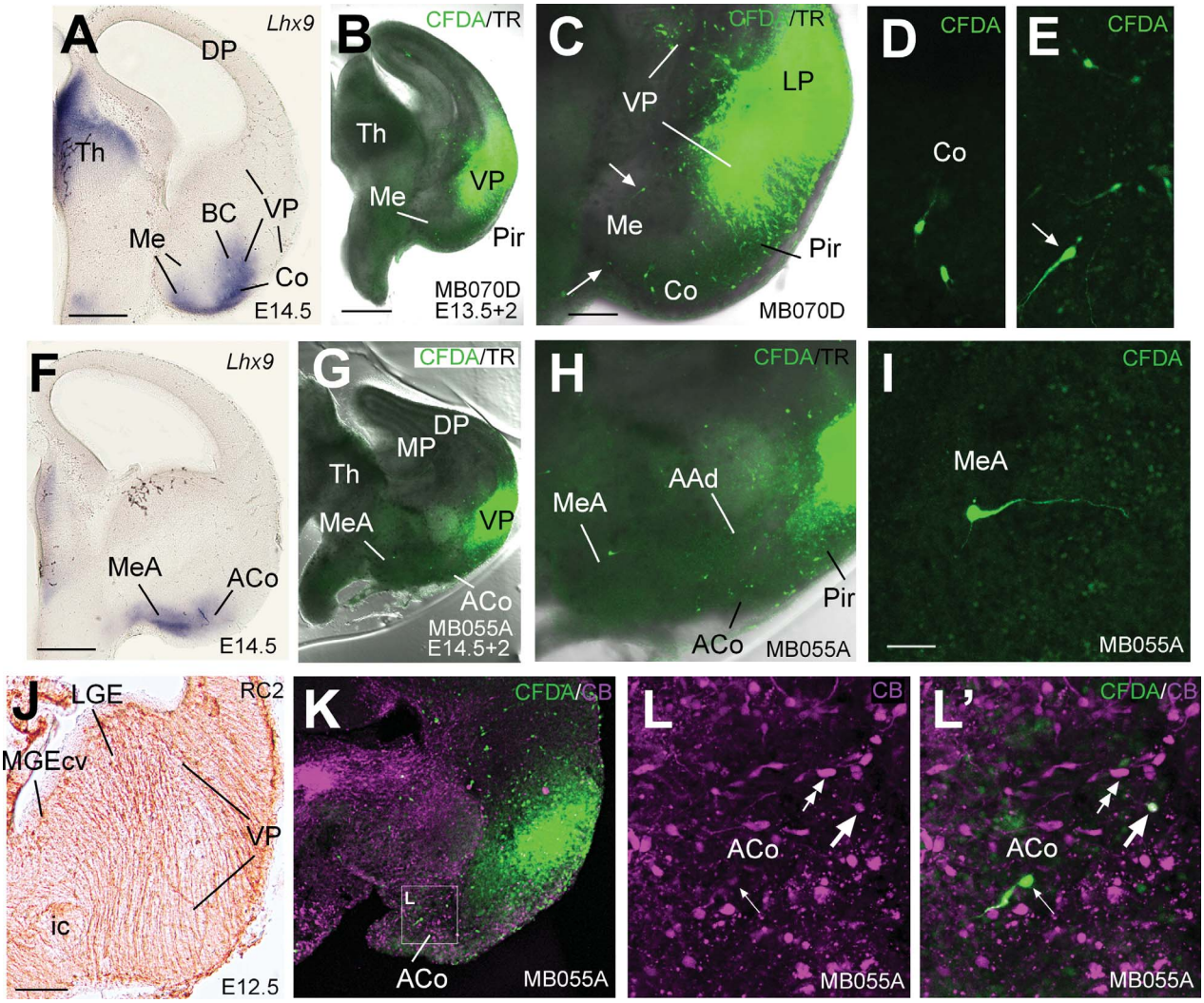

Figure 1. A–L′: Digital images from two different organotypic cultures of telencephalic frontal slices of mouse embryo (MB070D and MB055A), in which the fluorescent cell tracker CMFDA (green) was centered in the ventral pallium (VP), without involving the subpallium. The results were compared with Lhx9 mRNA expression (A,F) and radial glial fiber distribution (RC2, J), and the slices were immunolabeled to detect calbindin (CB; shown in magenta; see K,L,L′). L,L′: Detail of CMFDA-labeled cells in the anterior cortical amygdalar area (ACo). In L,L′, the thin arrow points to a CMFDA cell that is not double-labeled for CB, and the thick arrow points to a double-labeled cell, whereas the double-headed arrow points to a CB cell that is not labeled for CMFDA. Note a few CMFDA-labeled cells in the medial amygdala (Me, MeA; C,E,H; arrows). See text for more details. For abbreviations see list. Scale bars = 0.4 mm in A,F; 0.5 mm in B (applies to B,G); 0.2 mm in C (applies to C,H,K); 0.05 mm in I (applies to D,E,I,L,L′); 0.1 mm in J.

amygdalar areas (Figs. 1, 2). This agrees with previous reports based on gene expression patterns (Puelles et al., 2000; Medina et al., 2004; García-López et al., 2008), in utero electroporation (Soma et al., 2009), and genetic fate mapping of Dbx1-lineage neurons (Hirata et al., 2009; Waclaw et al., 2010). Our results are shown in three selected cases in which the cell tracker CMFDA was applied to the ventral pallium (panoramic views in Figs. 1B,G, 2A). In two cases, CMFDA application involved the subventricular zone, ventral migratory stream, and part of the ventral pallial mantle (MB070D and MD097A in Figs. 1C, 2C, respectively); in one case, CMFDA involved the ventral migratory stream and part of the ventral pallial mantle (MB055A in Fig. 1K). None of these cases involved the subpallium. The location of the cell tracker was inferred from the comparison with the transcription factor Lhx9 (considered a good ventral pallial marker; Tole et al., 2005; García-López et al., 2008) and calbindin (Figs. 1A,F,K, 2C). Two of these cases are represented in schematic drawings in Figure 2E,F. One novel finding of our study is that a few of the ventral pallium-derived cells of the piriform cortex, basal amygdalar

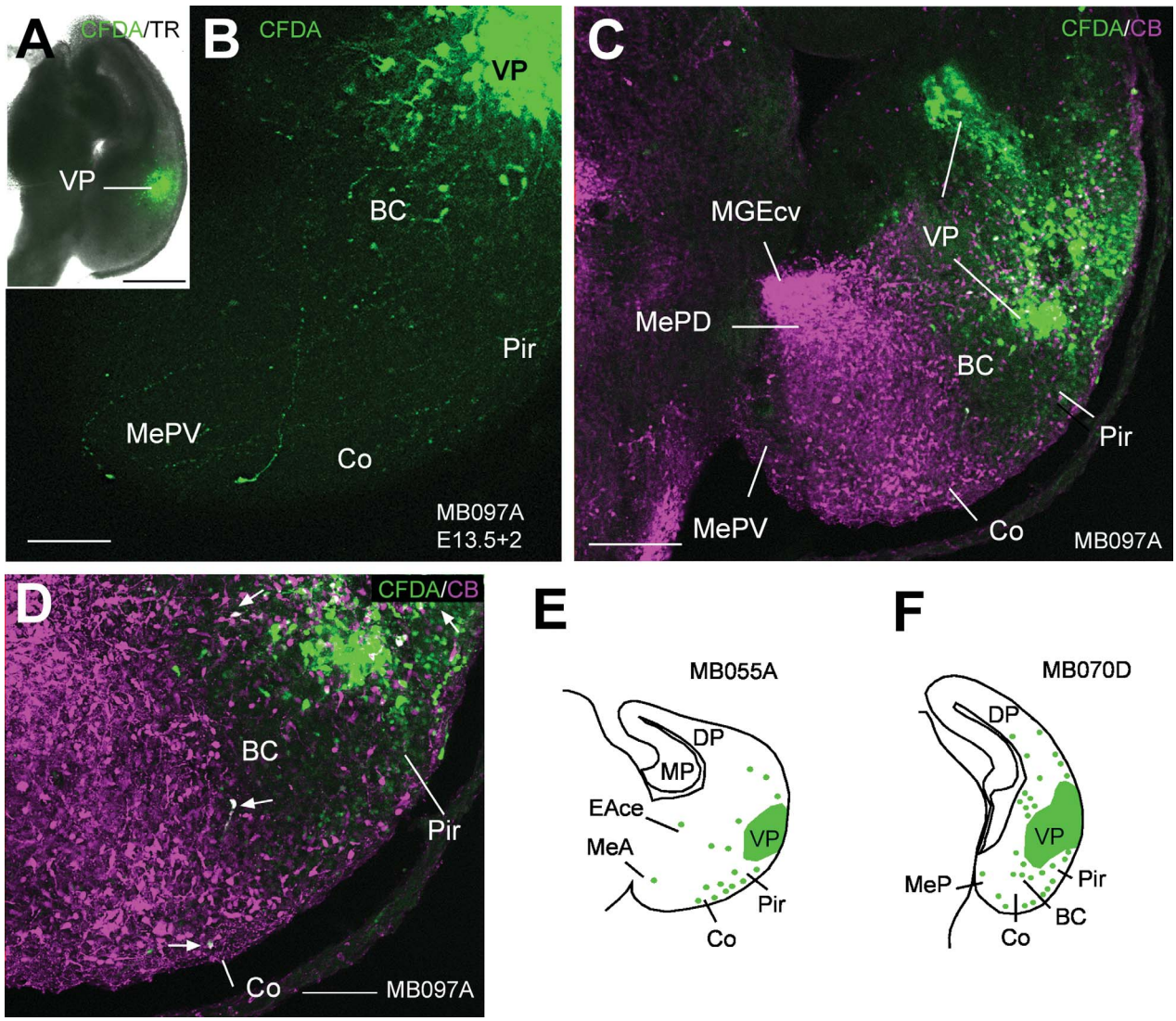

Figure 2. **A–D:** Digital images from one organotypic culture of a telencephalic frontal slice of mouse embryo (MB097A), in which the fluorescent cell tracker CMFDA (green) was centered in the caudal ventral pallium (VP). The slice was immunolabeled to detect calbindin (CB; shown in magenta; see C,D). Abundant CMFDA-labeled cells were observed in the piriform cortex (Pir) and pallial amygdala. D shows a detail of CMFDA-labeled cells in the pallial amygdala, some of which are double labeled for CB (arrows). A few CMFDA-labeled cells were also present in the medial amygdala (Me, B). **E,F:** Schematic drawings of the results obtained in two migration assays in which CMFDA was applied to the ventral pallium. See text for details. For abbreviations see list. Scale bars = 0.5 mm in A; 0.2 mm in B,C; 0.1 mm in D.

complex, and cortical amygdalar areas contain calbindin, as shown by double labeling using immunofluorescence (Figs. 1L,L′, 2D).

In addition to this, herein we provide experimental evidence of another novel finding: that the ventral pallium also produces cells for the medial amygdala. Notably, in all cases of ventral pallial labeling, a few labeled cells were also detected in the medial amygdala (Figs. 1C,H,I, 2C,E,F). The location of the medial amygdala was confirmed by comparison with calbindin or Lhx9 (Figs. 1A,F,K, 2C). When the cell tracker was placed in relatively more rostral parts of the ventral pallium, labeled cells were observed in the anterior medial amygdala (MeA; Fig. 1H). In contrast, when the cell tracker involved more caudal aspects of the ventral pallium, labeled cells were observed in the posterior medial amygdala (for example, Figs. 1C, 2B). In the latter cases, a few cells were observed in both dorsal and ventral subdivisions of the posterior medial amygdala (MePD, MePV; Figs. 1C, 2B), although there was a trend for labeled cells to locate superficially in MePV (Fig. 2B).

Comparison to the disposition of radial glial fibers suggested that cells that populate the basal amygdalar complex and cortical amygdalar areas following application of

the cell tracker in the ventral pallium primarily migrate radially (Fig. 1J). In contrast, cells that populate the medial amygdala in these experiments appear to follow a tangential migration.

The caudoventral part of the pallidal subdivision is a preferential source of cells for specific nuclei of the medial extended amygdala

Previous data have identified a distinct domain in the caudoventral aspect of the pallidal subdivision that was suggested to produce abundant neurons expressing Lhx6, calbindin, and the neuropeptides somatostatin and NPY for the medial extended amygdala (García-López et al., 2008), but experimental evidence for this claim was lacking. This domain was referred to as the *anterior peduncular area* (García-López et al., 2008) and is part of a larger region previously called the *anterior entopeduncular area* or *AEP* (Puelles et al., 2000; Marín and Rubenstein, 2001). To avoid confusion with these terms, here we refer to this domain as the *caudoventral pallidal subdivision* or *caudoventral medial ganglionic eminence* (MGEcv), which is in line with its pallidal-like molecular features and its position (Flames et al., 2007; García-López et al., 2008). In contrast to the preoptic subdivision, the MGEcv does not express Sonic hedgehog in the ventricular zone (Flames et al., 2007; García-López et al., 2008; see next section). Here we tried to analyze whether MGEcv produces cells for the medial extended amygdala by using in vitro migration assays. Our results provide novel experimental evidence indicating that this is the case, as shown in six selected cases in Figures 3–5 (four of them are represented in schematic drawings in Fig. 5F–I). Figures 3 and 4 show three cases in which the cell tracker CMFDA was applied in the MGEcv in frontal telencephalic slices (E13.5–E15.5 mice), at different rostrocaudal levels. The location of the cell tracker application site was checked by comparison with calbindin and Lhx6, both enriched in the MGEcv domain (Figs. 3A–C,H, 4B,C). A large amount of labeled cells extended toward the surface, overlapping a stream of calbindin-immunopositive neurons that extends laterally from the periventricular sector [where the medial bed nucleus of the stria terminalis (BSTM) forms], along the sublenticular part of the medial extended amygdala (below the globus pallidus; Fig. 3D,F,H, double-arrow, K) and, more caudally, reaching the anterior medial amygdala (Fig. 4B–D). Comparison with radial glial fibers indicated that cells along this pathway follow a radial migration (Fig. 3G). In agreement with this, the leading process of most of these labeled cells was parallel to the radial dimension and oriented toward the pial surface.

In addition, numerous labeled cells also invaded, presumably by tangential migration, dorsal aspects of the pallidum (including the globus pallidus and lateral bed nucleus of the stria terminalis), the striatum, and the pallium (including the cerebral cortex, the piriform cortex, the claustral complex, and the pallial amygdala; Figs. 3F,H–L, 4B,D). The leading processes of these labeled cells were either orthogonal to the radial dimension (Fig. 3I,J) or had different orientations (Fig. 3I,L). Double labeling indicated that some of the MGEcv-derived cells in the dorsal pallidum (at the level of the lateral bed nucleus of the stria terminalis; Fig. 3I–I″), olfactory tubercle, and pallium were immunopositive for calbindin (Fig. 3J–J″), and may at least partially represent interneurons. Thus, the MGEcv appears to be a distinct source of cells that migrate tangentially to other parts of the pallidum, the striatum, and the pallium/cortex, and at least part of the latter appear to represent calbindin-positive interneurons. In addition, the MGEcv also appears to produce a few labeled cells that migrate tangentially to the preoptic area (Fig. 3K,M).

The migration assays using frontal slices did not allow us to determine whether MGEcv also produces cells for the posterior medial amygdala, because this part of the complex is located caudally to the MGEcv ventricular zone. To overcome this difficulty, we prepared organotypic cultures of horizontal forebrain slices, because this plane was previously observed to be good for visualization of a Lhx6-expressing and calbindin-positive cell corridor extending from the MGEcv ventricular zone into the posterior medial amygdala. Figures 4 and 5 include three cases of horizontal slice cultures in which the cell tracker was applied to (or involved) the MGEcv. The location site of the tracker was checked by comparison with Lhx6 or calbindin (Figs. 4E,F, 5A,B). In these cases, numerous labeled cells extended from the cell tracker application site in MGEcv (rostromedially) into the caudolateral telencephalon, invading the posterodorsal part of the medial amygdala (Figs. 4H–J, 5B–C′). This corridor of labeled cells overlapped a calbindin-positive cell corridor showing the same disposition, extending from the rostromedial, periventricular MGEcv into the posterodorsal medial amygdala. Double labeling showed that most of the MGEcv-derived cells along this cell corridor were immunopositive for calbindin (Fig. 5D,E). In these experiments, a number of labeled cells abandoned the main corridor to enter the globus pallidus, different parts of the striatal division, the pallial amygdala, and the piriform cortex (Figs. 4H–J, 5C,C′,F–I).

The commissural preoptic area produces some septal and preoptic cell populations but also produces some cells for the medial extended amygdala

The preoptic area of the subpallium has been shown to produce neurons for the medial amygdala (Hirata et al., 2009), but it is unclear what specific preoptic subdivision

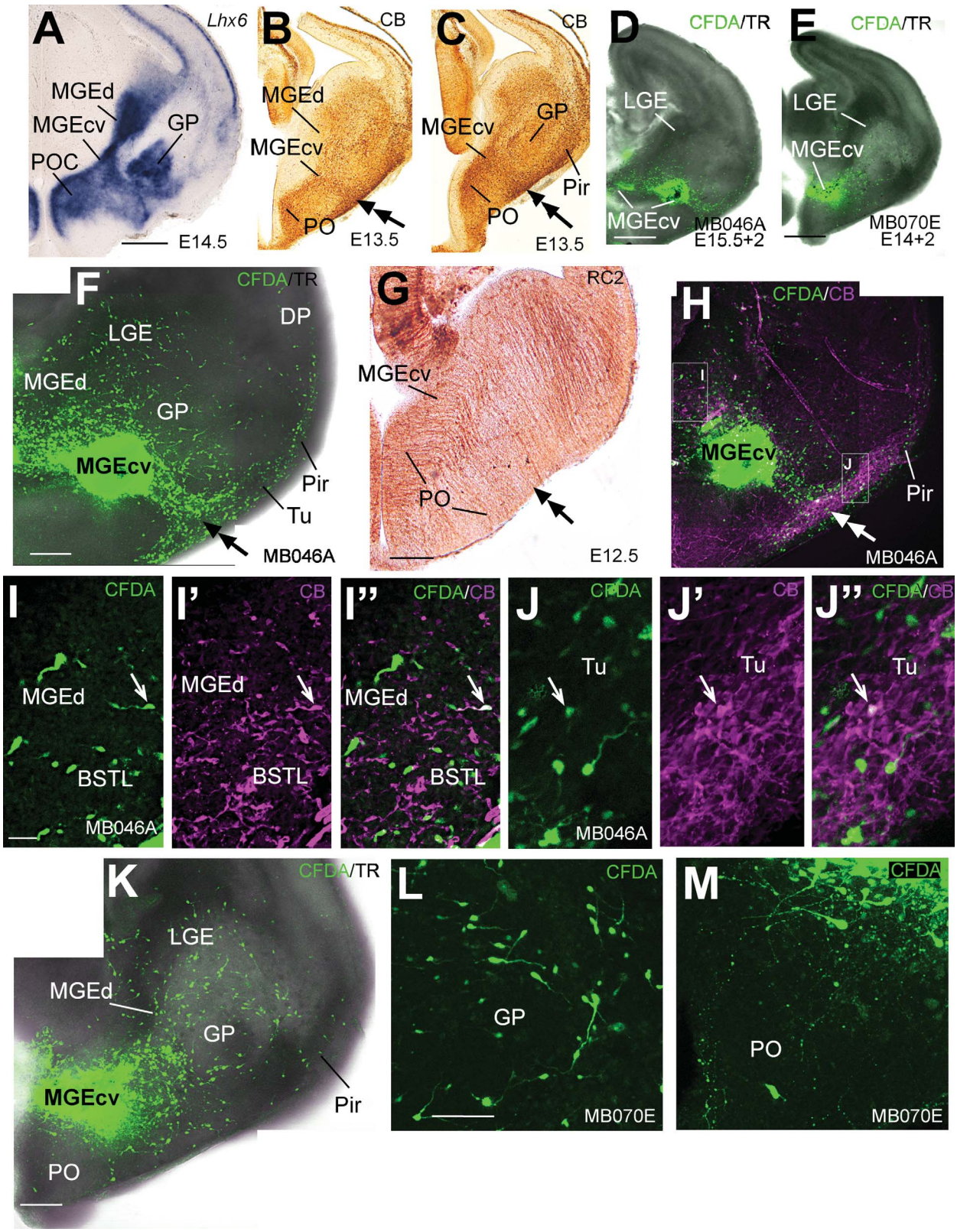

Figure 3. **A–M:** Digital images from two different organotypic cultures of telencephalic frontal slices of mouse embryo (MB046A and MB070E), in which the fluoresencent cell tracker CMFDA (green) was applied in the caudoventral part of the medial ganglionic eminence (MGEcv). The results were compared with expression of Lhx6 mRNA (A) or calbindin protein (CB; B,C). In addition, selected slices were immunolabeled to detect CB (shown in magenta; see H,I′–J′′). I–I′′ and J–J′′ are details from the boxed areas in H, in which double-labeled cells are pointed with an arrow. See text for details. For abbreviations see list. Scale bars = 0.4 mm in A (applies to A–C); 0.5 mm in D,E; 0.2 mm in F (applies to F,H); 0.2 mm in G,K; 0.02 mm in I (applies to I–J′′); 0.1 mm in L (applies to L,M).

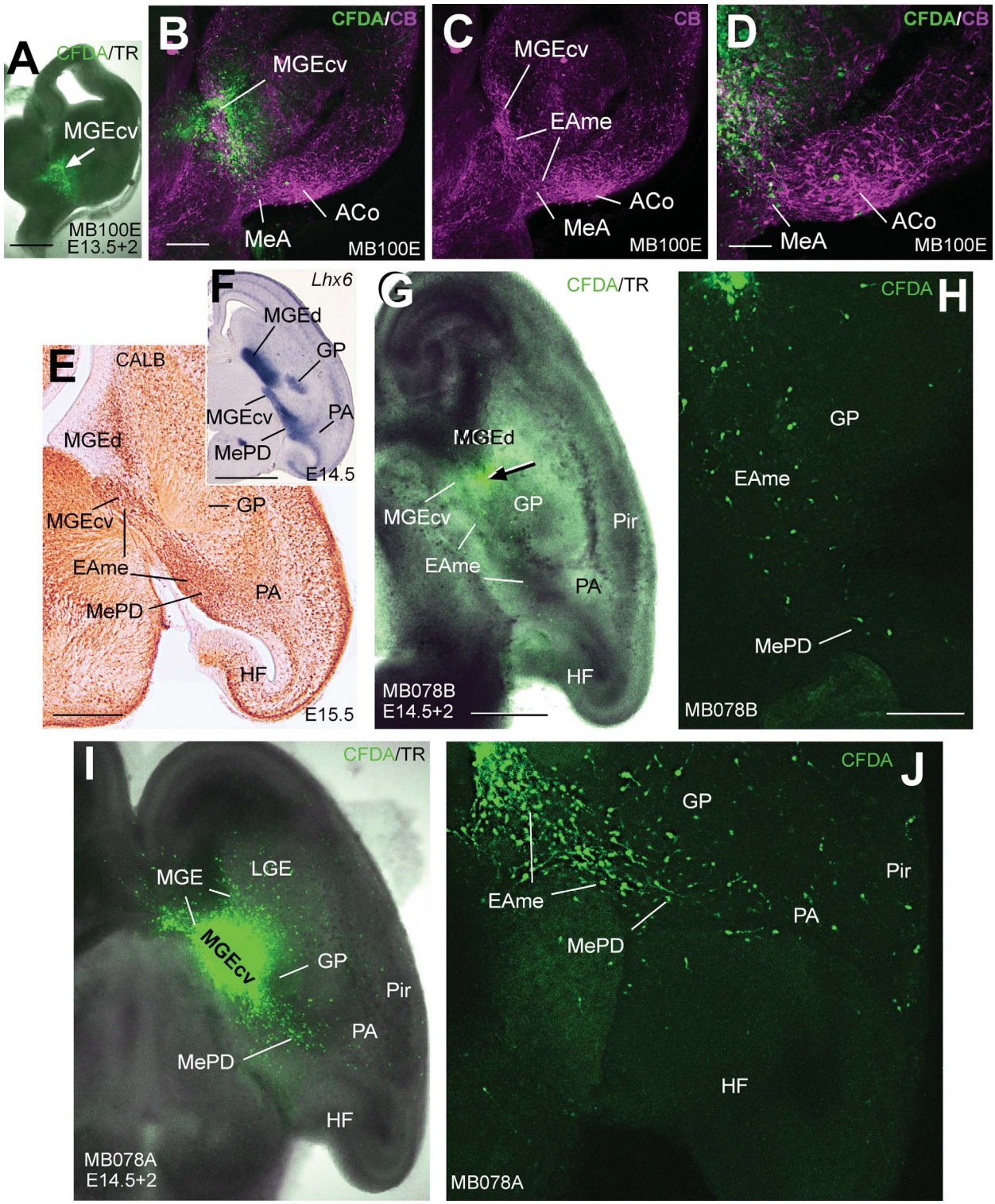

Figure 4. A–J: Digital images from three different organotypic cultures of telencephalic frontal (MB100E) or horizontal (MB078B and MB078A) slices of mouse embryo, in which the fluoresencent cell tracker CMFDA (green) was applied in the caudoventral part of the medial ganglionic eminence (MGEcv) The results were compared with expression of Lhx6 mRNA (see F) or calbindin protein (CB; E). In addition, selected slices were immunolabeled to detect CB (shown in magenta; see B–D). Abundant CMFDA-labeled cells were observed to invade the medial extended amygdala (EAme), overlapping a similar corridor of CB-positive cells (B,C,E). See text for more details. For abbreviations see list. Scale bars = 0.5 mm in A; 0.2 mm in B (applies to B,C); 0.1 mm in D; 0.4 mm in E; 1 mm in F; 0.5 mm in G (applies to G,I); 0.2 mm in H (applies to H,J).

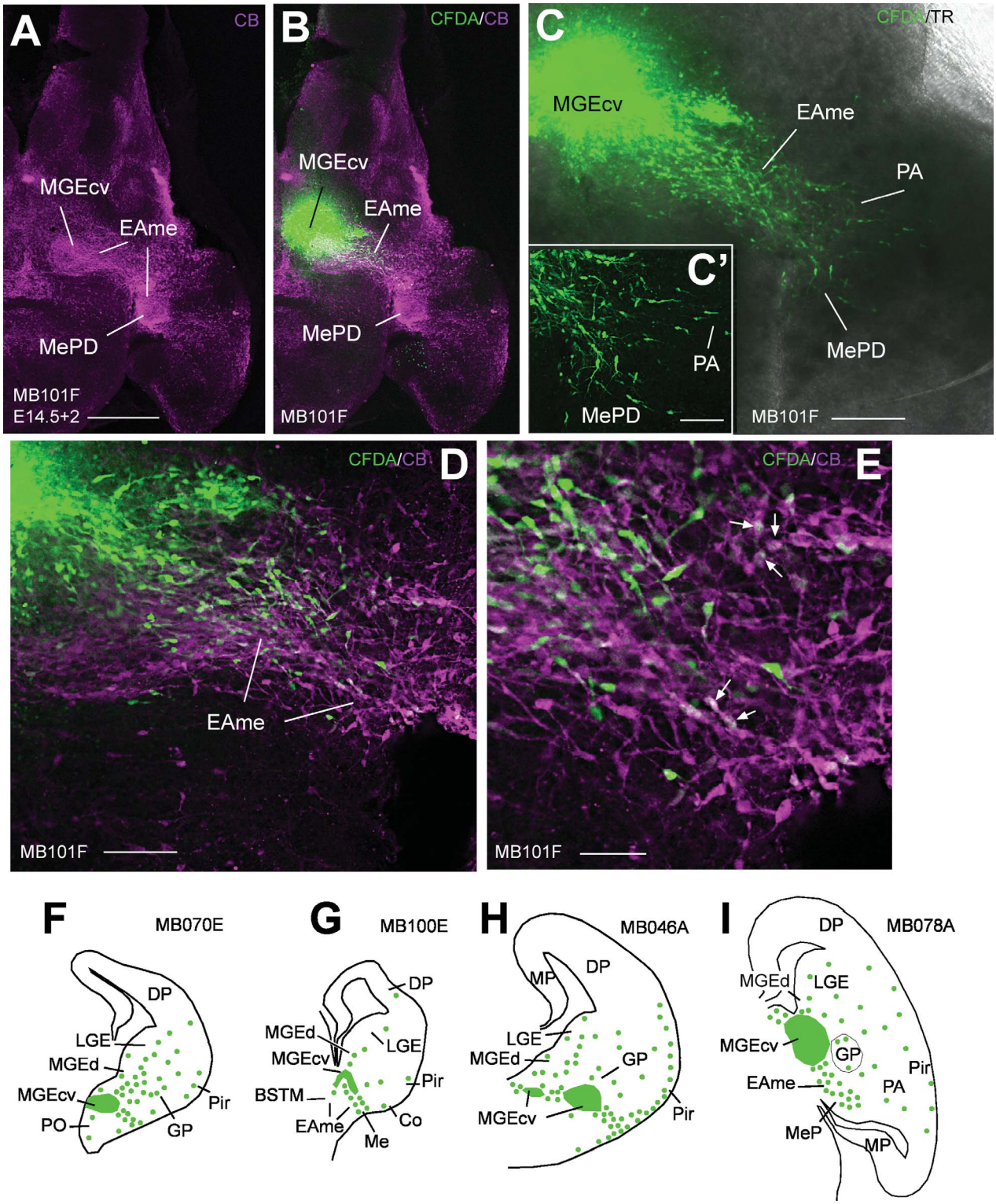

Figure 5. **A–E:** Digital images from one organotypic culture of a telencephalic horizontal slice of mouse embryo (MB101F), in which the fluoresencent cell tracker CMFDA (green light emission) was applied in the caudoventral part of the medial ganglionic eminence (MGEcv). The results were compared with expression of Lhx6 mRNA (see Fig. 4F) and the slice was immunolabeled to detect calbindin protein (CB; shown in magenta; see B,D,E). Abundant CMFDA-labeled cells were observed to invade the medial extended amygdala (EAme), overlapping a similar corridor of CB-positive cells (A,B,D). **F–I:** Schematic drawings of four slice cultures (three frontal and one horizontal), in which CMFDA was applied to the MGEcv. See text for more details. For abbreviations see list. Scale bars = 0.5 mm in A (applies to A,B); 0.2 mm in C; 0.1 mm in C′,D; 0.05 mm in E.

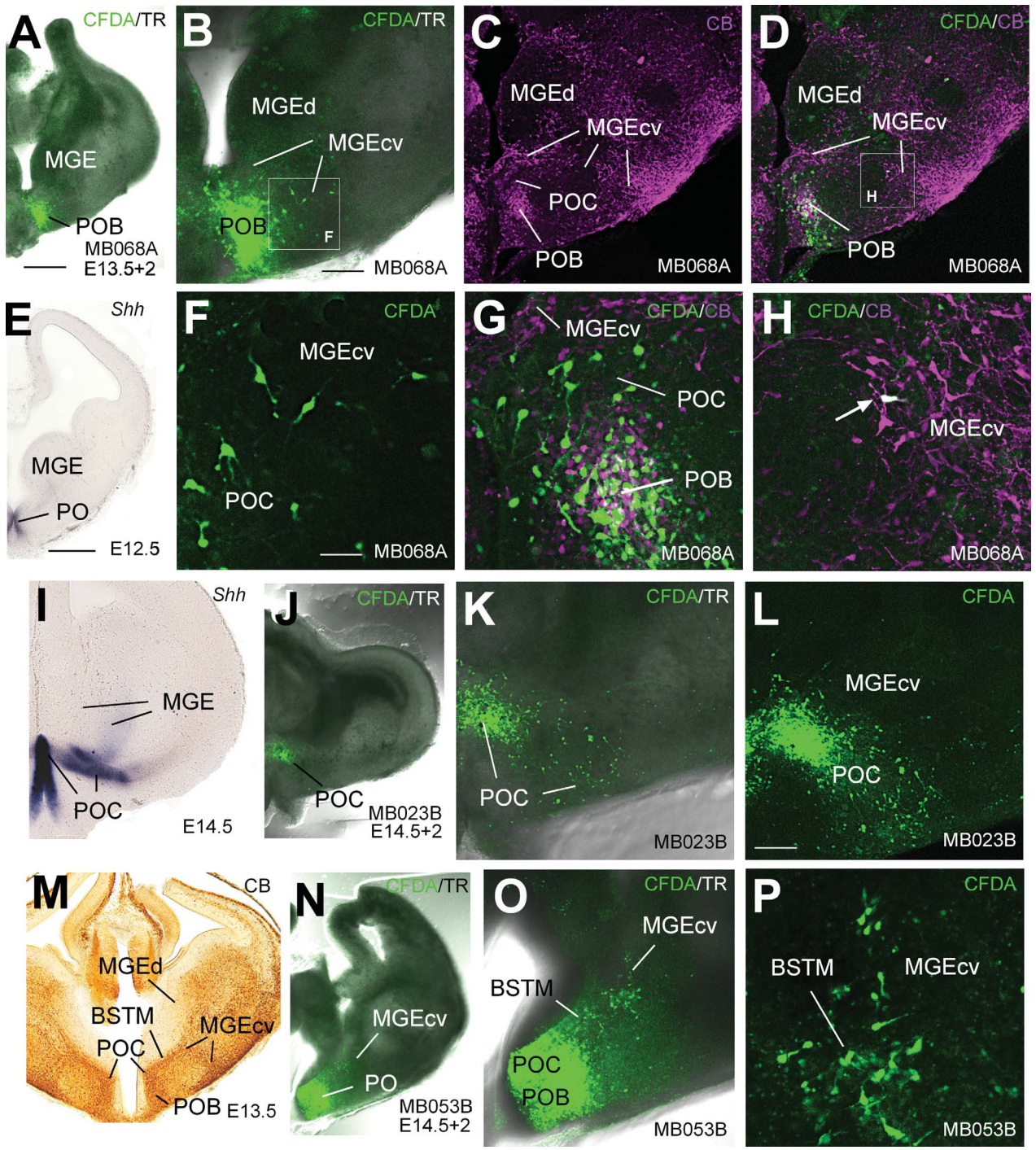

Figure 6. **A–P:** Digital images from three different organotypic cultures of telencephalic frontal slices of mouse embryo (MB068A, MB023B, and MB053B), in which the fluoresencent cell tracker CMFDA (green) was applied in the commissural preoptic area (POC) or the basal preoptic area (POB). The results were compared with the expression of Sonic hedgehog mRNA (Shh; see E,I) or calbindin protein (CB; M). In addition, selected slices were immunolabeled to detect CB (shown in magenta; see C,D,G,H). See text for details. For abbreviations see list. Scale bars = 0.5 mm in A (applies to A,J,N); 0.2 mm in B (applies to B–D,K,O); 0.4 mm in E (applies to E,I,M); 0.05 mm in F (applies to F–H,P); 0.1 mm in L.

produces these cells, and whether preoptic-derived cells also invade other parts of the medial extended amygdala. Here we tried to analyze this by in vitro migration assays. Our results are shown in seven selected cases in which the fluorescent cell tracker CMFDA was applied to the commissural (POC) and/or the basal (POB) preoptic subdivisions of E13.5–E15.5 mice (Figs. 6, 7). The location of the cell tracker was deduced by comparison with Shh (a

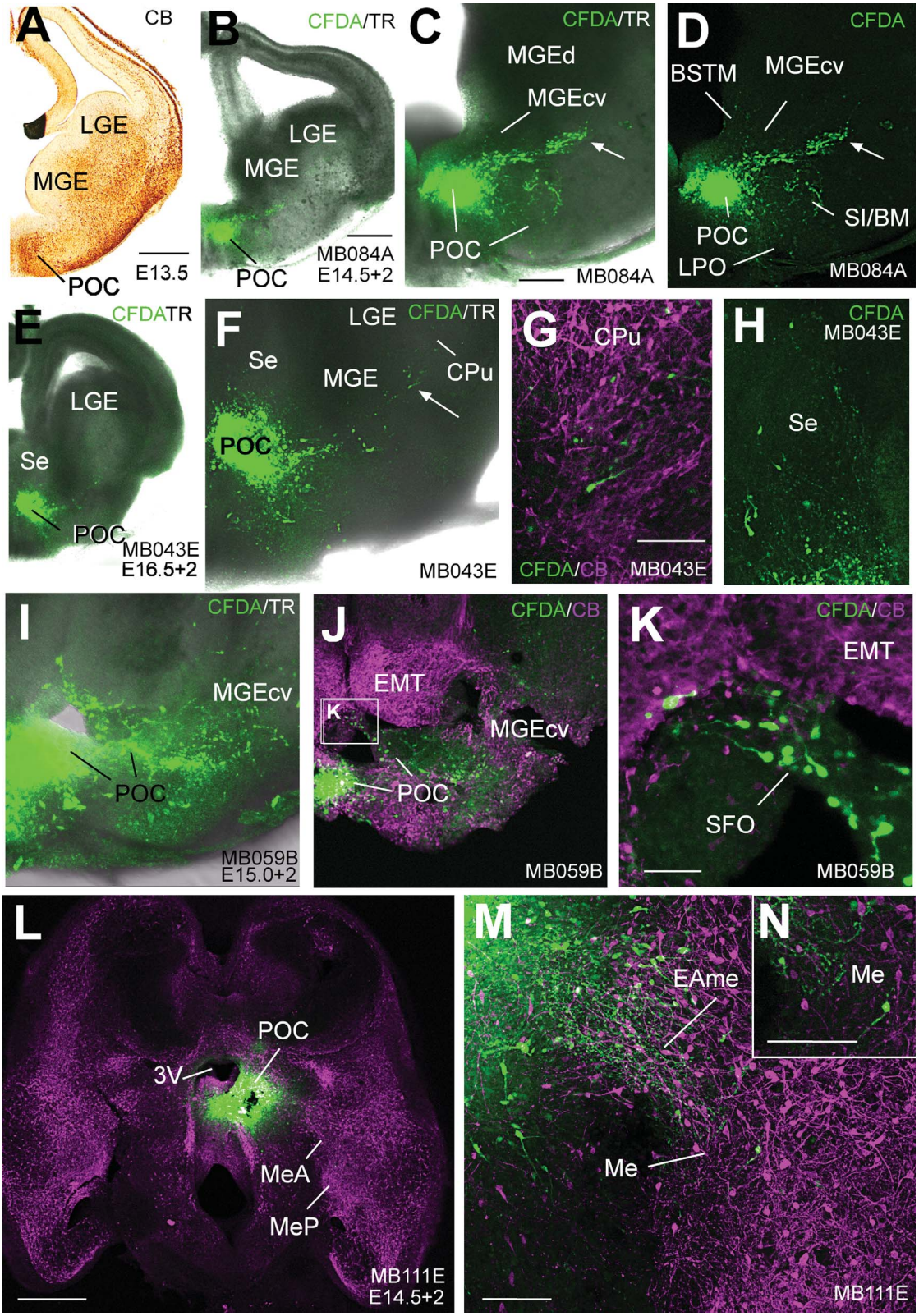

Figure 7. A–N: Digital images from four different organotypic cultures of telencephalic frontal or horizontal slices of mouse embryo (frontal slices: MB084A, MB043E, and MB059B; horizontal: MB111F), in which the fluoresencent cell tracker CMFDA (green) was applied in the commissural preoptic area (POC). The results were compared with calbindin (CB; A). In addition, selected slices were immunolabeled to detect CB (shown in magenta; see G,J–M). See text for details. For abbreviations see list. Scale bars = 0.4 mm in A; 0.5 mm in B (applies to B,E); 0.2 mm in C (applies to C,D,F,I,J); 0.1 mm in G (applies to G,H); 0.05 mm in K; 0.5 mm in L; 0.1 mm inv M,N.

good preoptic marker; Flames et al., 2007; García-López et al., 2008) or calbindin (Figs. 6C–E,I,M, 7A,J,L). Our results provide novel evidence indicating that the POC is the major source of preoptic cells for the medial extended amygdala and other areas of the basal telencephalon, including the septum (Figs. 6, 7). When the cell tracker was restricted to the POB, only few labeled cells were observed outside the ventromedial preoptic region, and extremely few of these cells invaded the caudoventral medial ganglionic eminence (Fig. 6A–H; some of these cells were calbindin positive). In contrast, when the cell tracker involved the POC, in addition to cells invading the radial preoptic domain (Figs. 6J–L, 7F; see radial glial fiber disposition in Fig. 3G), abundant cells were observed to invade tangentially the medial bed nucleus of the stria terminalis and other areas of the basal telencephalon (Figs. 6L,N–P, 7C,F), including the area along the posterior limb of the anterior commissure (Fig. 7B–D). In addition, some POC-derived cells appeared to migrate tangentially into the striatum (Fig. 7F,G) and the ventromedial septum (Fig. 7F,H), including the presumptive subfornical organ (Fig. 7I–K). None of these labeled cells was found to be calbindin immunopositive (Fig. 7G,K). In frontal slices involving the POC, the medial amygdalar nucleus could not be observed. To overcome this problem, we carried out migration assays in horizontal slices of embryonic forebrain (Fig. 7L–N). In these experiments, when the cell tracker involved the POC, labeled cells were observed to invade the medial amygdalar nucleus and other aspects of the medial extended amygdala (Fig. 7M,N). In these experiments, POC-derived cells did not invade the posterodorsal subdivision of the medial amygdala.

The supraoptoparaventricular hypothalamic domain is also a source of cells for the medial extended amygdala

Our migration assays also show that the supraoptoparaventricular domain of the alar hypothalamus (SPV) produces some cells for the medial extended amygdala, including the medial bed nucleus of the stria terminalis and the medial amygdalar nucleus (Figs. 8, 9). This agrees with previous reports based on gene expression (Bardet et al., 2008; Abellán et al., 2010) and fate-map analysis (García-Moreno et al., 2010). Figures 8 and 9 show several cases in which the cell tracker was applied to rostral or caudal levels of the SPV (schematic drawings of three cases are shown in Fig. 9G–I). The location of the cell tracker was checked by comparison with Lhx5 (Fig. 8A for rostral SPV; Fig. 8I for more caudal levels of SPV), and the location of the labeled cells was also deduced by comparing with calbindin immunostaining (Figs. 8F–H, 9C,D–D″,E). In these cases, numerous labeled cells were observed to extend toward the surface (Figs. 8C,J,K, 9B), following the radial dimension as deduced by comparison with radial glial fibers (Fig. 9F). The labeled cells located along the radial dimension likely produce the paraventricular nuclear complex (PVN) and the supraoptic nucleus (SON) of the hypothalamus, as previously shown based on data from Otp knockout (Wang and Lufkin, 2000). In addition, abundant labeled cells followed a tangential migration into the MGEcv area of the basal telencephalon, invading part of the medial extended amygdala, such as part of the medial bed nucleus of the stria terminalis and the medial amygdala (Figs. 8C–H,K,M,N, 9C). A novel finding of our study is that some of these SPV-derived cells were calbindin immunopositive (Fig. 9D–D″). In addition, we also provide novel experimental evidence for a tangential migration of a few SPV-derived cells into the subparaventricular domain of the alar hypothalamus (SPa; Figs. 8K,L, 9B,H,I).

DISCUSSION

Pallial, subpallial, and hypothalamic embryonic domains contribute cells to the medial extended amygdala

Our results from in vitro migration assays show that the medial extended amygdala includes different cell subpopulations that originate in the ventral pallium (VP), caudoventral pallidum (MGEcv), commissural preoptic area (POC), and suparoptoparaventricular hypothalamic domain (SPV; Fig. 10). Although this was suggested previously based on gene expression patterns (García-López et al., 2008; see also Puelles et al., 2000; Puelles and Rubenstein, 2003; Bardet et al., 2008; Abellán et al., 2010; for SPV contribution), the experimental evidence for the VP, MGEcv, and POC contributions was lacking (VP, MGEcv) or was imprecise (preoptic area). Therefore, this is the first comprenhensive experimental study in which the neurons of the medial extended amygdala, including both the medial amygdalar nucleus and the medial bed nucleus of the stria terminalis, have been shown to originate from multiple sources in the pallium, subpallium, and hypothalamus. We discuss the details of each contribution to the medial extended amygdala under separate subheadings below.

Ventral pallium

An important finding of our study is that VP produces some cells for the anterior and posterior parts of the medial amygdala. This was suggested previously based on Lhx9 expression (García-López et al., 2008), but experimental demostration was lacking. It is unlikely that the presence of labeled cells in the medial amygdala in our VP experiments is due to contamination of the lateral

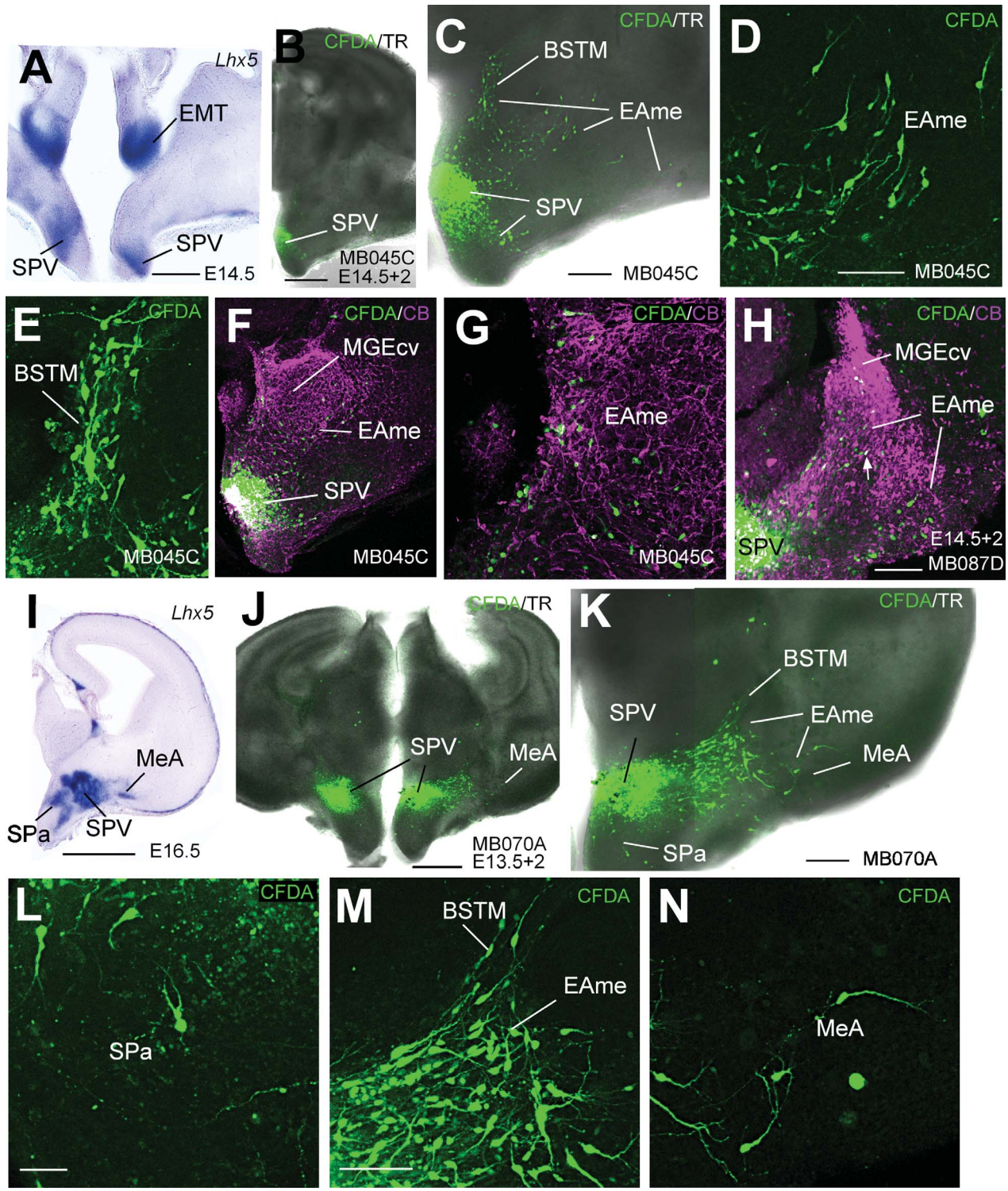

Figure 8. **A–N:** Digital images from three different organotypic cultures of forebrain frontal slices of mouse embryo (MB045C, MB087D, and MB070A), in which the fluoresencent cell tracker CMFDA (green) was applied to or involved the rostral, intermediate, or caudal parts of the supraoptoparaventricular domain of the alar hypothalamus (SPV). The results were compared with expression of Lhx5 mRNA (a genetic marker of SPV; see A,I) and the slices were immunolabeled to detect calbindin (CB; shown in magenta; see F–H). See text for details. For abbreviations see list. Scale bars = 0.4 mm in A; 0.5 mm in B; 0.2 mm in C (applies to C,F); 0.1 mm in D (applies to D,E,G); 0.2 mm in H,K; 1 mm in I; 0.5 mm in J; 0.05 mm in L; 0.1 mm in M (applies to M,N).

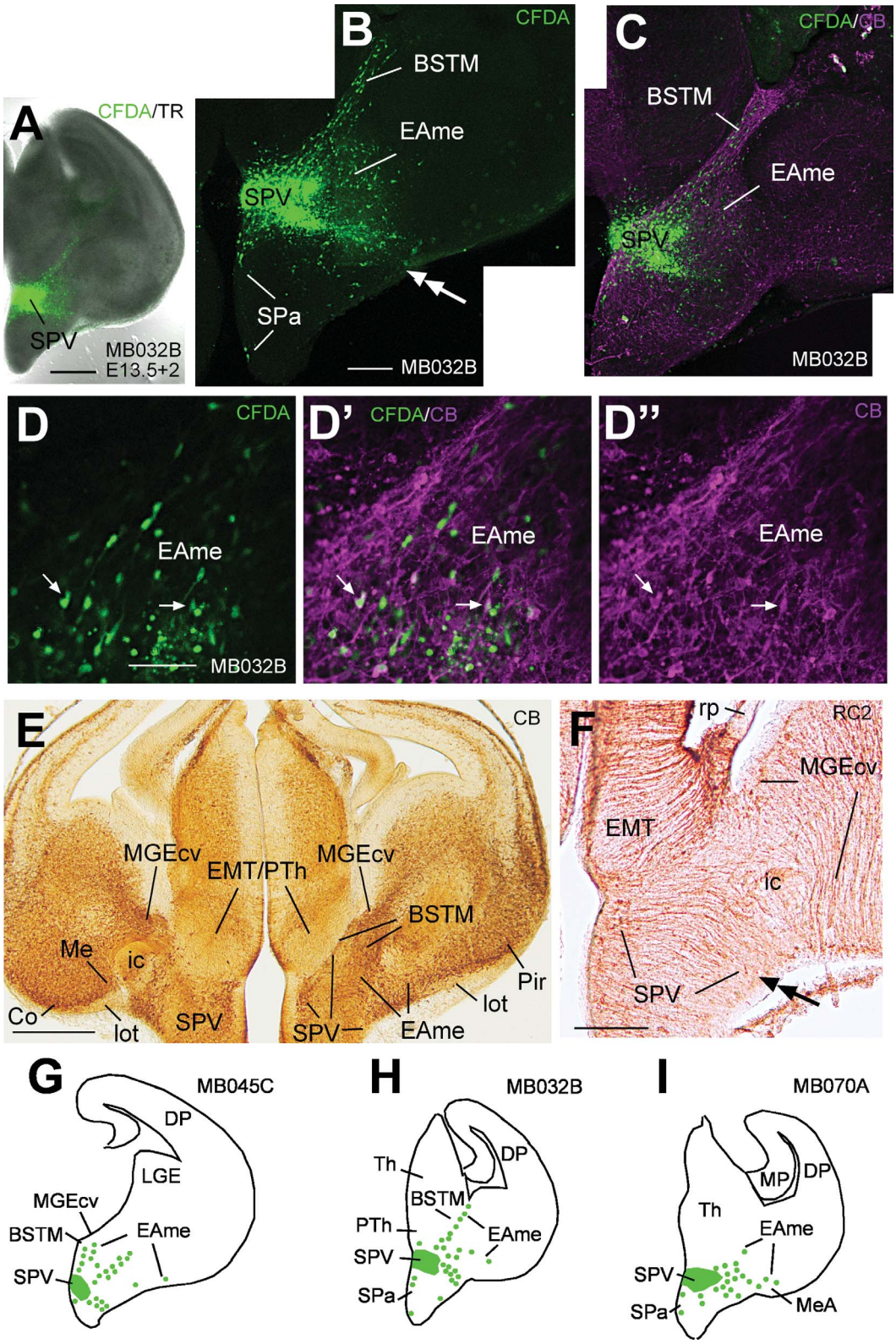

Figure 9. **A–F:** Digital images from one organotypic culture of a forebrain frontal slice of mouse embryo (MB032B), in which the fluorescent cell tracker CMFDA (green) was applied to the intermediate part of the supraoptoparaventricular domain of the alar hypothalamus (SPV). The results were compared with expression of calbindin (CB; see E) and radial glial fiber disposition (RC2; F), and the slice was immunolabeled to detect CB (see C,D–D″; shown in magenta). SPV surface is indicated with a double-headed arrow in B,F. Some of the CMFDA-labeled cells in the BSTM and other parts of EAme were calbindin positive (arrows in D–D″). **G–I:** Schematic drawings of three different experiments, in which CMFDA was applied to the SPV at rostral, intermediate, or caudal levels. See text for more details. For abbreviations see list. Scale bars = 0.5 mm in A; 0.2 mm in B (applies to B,C); 0.1 mm in D (applies to D–D″); 0.4 mm in E; 0.2 mm in F.

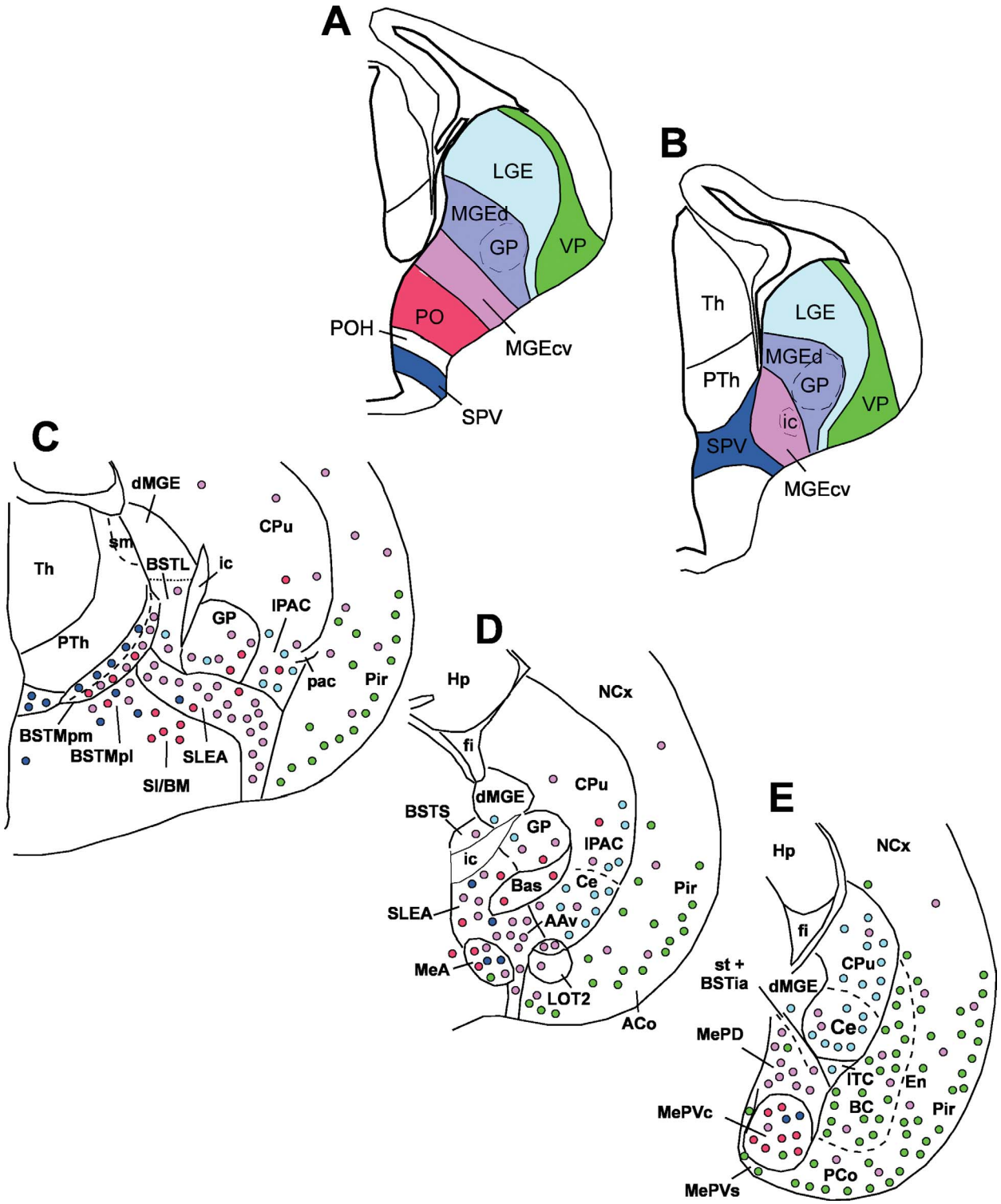

Figure 10. A–E: Schematic drawings of frontal sections through the embryonic forebrain, showing the major embryonic divisions (in different colors) involved in the formation of the amygdala, and their contribution of cells (colored circles) to the different mature amygdalar nuclei/areas. The schemes are based primarily on data from the present migration assay study (focussed on the medial extended amygdala) but also include data on the amygdala published recently by other authors (for example, Hirata et al., 2009, for the preoptic and VP derivatives; Waclaw et al., 2010, for LGE and VP derivatives; and García-Moreno et al., 2010, for SPV derivatives). Note that in the schemes we are not including dorsal MGE derivatives (such as part of the cells of the globus pallidus), because we are focussed on the amygdala. See text for details. For abbreviations see list.

ganglionic emience, because, in these experiments, we observed abundant labeled cells in known ventral pallial derivatives such as the piriform cortex and pallial amygdala, but the striatal mantle remained either nearly or completely free of labeling. In the cases in which the striatum was totally devoid of labeled cells, we still could see a few cells in the medial amygdala. On the other hand, a minor migration of neurons from pallium to subpallium has been previously observed in mouse (via genetic fate mapping of a subset of Emx1-lineage pallial cells that invades the striatum; Cocas et al., 2009) and chicken (via expression of pallial marker genes; Abellán and Medina, 2009). In the posterior medial amygdala, VP-derived cells show a trend to locate in a superficial band, a domain previously observed to express several pallial marker genes, such Lhx9 (García-López et al., 2008) and Tbr1 (Medina et al., 2004). As with other pallial-derived neurons, it is expected that VP-derived cells of the medial amygdala are glutamatergic. In agreement with this, the transcription factor Tbr1 (as noted above, expressed in the superficial domain of the medial amygdala where VP-derived cells accumulate) is involved in the differentiation of cortical/pallial glutamatergic neurons (Hevner et al., 2001). Moreover, some of the projections of the medial amygdala are known to be excitatory and, likely, glutamatergic (Choi et al., 2005).

Caudoventral pallidum

A previous study reported the existence of abundant neurons of the Nkx2.1 lineage in the medial amygdala (Xu et al., 2008), but this finding did not allow distinguishing whether these cells came from the MGE or the preoptic area, because both subpallial subdivisions express Nkx2.1 during development (Marín et al., 2000; Puelles et al., 2000; Flames et al., 2007; García-López et al., 2008). Our study provides inequivocal experimental evidence that the caudoventral MGE (previously called or included as part of the *anterior peduncular area*, or as part of the fifth MGE subdivision; Flames et al., 2007; García-López et al., 2008) is a major source of neurons for the medial extended amygdala, including the medial bed nucleus of the stria terminalis (BSTM) and the anterior and posterodorsal parts of the medial amygdala. Other subdivisions of MGE only appear to contribute few cells (if any) to the medial extended amygdala (not shown). Moreover, our study indicates that the MGEcv-derived cells constitute a cell corridor along the medial extended amygdala (extending from the BSTM to the anterior and posterodorsal medial amygdala), rich in expression of the transcription factor Lhx6 and in calbindin-positive neurons. The Lhx6-expressing neurons of this cell corridor are interconnected and show inhibitory projections to hypothalamic centers involved in reproduction control (Choi et al., 2005; for review see García-López et al., 2008). Altogether, it appears that neurons with the same embryonic origin in the medial extended amygdala share many molecular, connectivity, and functional features. This highlights the relevance of embryonic studies for understanding the functional organization of the brain.

Commissural preoptic area

A recent genetic and experimental fate map has shown that the preoptic area produces a subpopulation of neurons of the medial amygdala (Hirata et al., 2009). However, it was unclear what specific subdivision of the preoptic area produces cells for the medial amygdala and whether the preoptic area also produces cells for other parts of the medial extended amygdala. Our migration assay data indicate that the POC appears to be the major source of preoptic neurons for the medial extended amygdala, because POB produce only extremely few cells for the basal telencephalon. This agrees with a previous suggestion based on Shh expression (García-López et al., 2008). Moreover, our experimental results showed that POC-derived cells invade both medial and lateral parts of the medial extended amygdala (such as the BSTM, the sublenticular extended amygdala, and part of the medial amygdalar nucleus) and other parts of the basal telencephalon (including the striatum and septum). By using genetic fate map, Hirata and colleagues (2009) reported that the Dbx1-lineage neurons of the medial amygdala are all GABAergic and have a preoptic origin. Moreover, according to these authors, most Dbx1-lineage neurons of the medial amygdala are nitrergic (Hirata et al., 2009), which is a subtype of projection neuron of the medial amygdala (Tanaka et al., 1997). Insofar as the POB does not seem to contribute many cells to the basal telencephalon, the Dbx1-lineage neurons of the medial amygdala reported by Hirata et al. (2009) may originate specifically or primarily in the POC.

Supraoptoparaventricular hypothalamic domain

Our data show that the hypothalamic SPV produces cells for the medial extended amygdala, including the BSTM and the medial amygdala. This agrees with previous suggestions based on expression of the transcription factors Otp (Bardet et al., 2008) and Lhx5 (Abellán et al., 2010), and recent fate map demonstration via in utero tracing injections and analysis of Otp knockout mice (García-Moreno et al., 2010). In contrast to the latter study, our study showed that some SPV-derived neurons of the medial extended amygdala are calbindin positive. Based on correlation with the vesicular glutamate transporter 2, SPV-derived cells of the medial extended amygdala appear to be glutamatergic (Abellán et al., 2010; García-Moreno et al., 2010) and contribute to the excitatory projections of the medial amygdala. The SPV radial domain is

rich in vasopressin (AVP)- and oxytocin (OT)-containing neurons (Wang and Lufkin, 2000), and some AVP containing neurons are also observed in the BSTM and medial amygdala of some mammals, such as rat and mouse (De Vries and Buijs, 1983; Caffé et al, 1987; Wang and De Vries, 1995; Wang et al., 1997; Wang and Lufkin, 2000). It appears that the abundance of AVP-containing neurons in the medial amygdala and their projections to the septum, which have been correlated with parental care and monogamous behavior (Wang et al., 1994), are highly variable among mammalian species (Wang et al., 1997). If these cells were shown to derive from SPV, it would be important to investigate what developmental mechanisms are behind their differential presence in the medial amygdala of different mammals.

Finally, one novel finding of our study is that SPV also produces cells that migrate tangentially to the subparaventricular area. Given that the transcription factor Otp (expressed in SPV-derived neurons) is essential for the development of dopaminergic neurons of the hypothalamus (Ryu et al., 2007), and the periventricular location of the SPV-derived cells observed in the SPa (present study), it is possible that these cells belong to the A14 dopaminergic cell group.

The principal neurons that conform the medial extended amygdala follow radial or tangential migration pathways

Our results show that MGEcv is a major source of neurons of the medial extended amygdala, although at least three other telencephalic and extratelencephalic embryonic subdivisions also contribute neurons to this structure. This raises many questions regarding the developmental mechanisms that lead these different cells to migrate and intermingle or lie close together in the medial amygdala and on the evolutionary origin of these different subpopulations. One important aspect to evaluate is whether the neurons from each embryonic source undergo radial or tangential migration to reach the medial extended amygdala, because evolutionary variations in the extent of such migrations may produce the same cells to be located in distinct topographic positions in different species. Here we have tried to understand what type of migration is followed by the cells that make up the medial extended amygdala of the mouse by comparison with the disposition of radial glial fibers in E12.5–E14.5 embryos. This comparison suggests that MGEcv-derived neurons of the medial extended amygdala follow a primarily radial migration. In contrast, neurons derived from VP, POC, and SPV appear to reach the medial extended amygdala by way of tangential migrations.

Another important observation of our study is that principal (projection) neurons migrate to the medial extended amygdala by both radial and tangential migrations. Thus, Lhx6-expressing neurons of the medial amygdala, which appear to arrive from MGEcv by radial migration (present data), show GABAergic projections to hypothalamic centers involved in reproduction control (Choi et al., 2005). On the other hand, nitrergic neurons of the medial amygdala, shown to arrive from the preoptic subdivision (Hirata et al., 2009) possibly by tangential migration, are known to project to the paraventricular hypothalamic nucleus (Tanaka et al., 1997), which controls neuroendocrine functions by way of the hypothalamohypophyseal axis. In addition, glutamatergic neurons of the medial amygdala, which appear to arrive from VP and SPV by tangential migration (present data and García-Moreno et al., 2010), were shown to project to hypothalamic centers involved in defense control (Choi et al., 2005). Recently, the principal neurons of the globus pallidus were also shown to reach the nucleus by both radial and tangential migration processes (Nóbrega-Pereira et al., 2010). Therefore, tangential migration in the telencephalon is not only restricted to interneurons but also used by subpopulations of principal neurons (as shown for the medial extended amygdala and globus pallidus).

The caudoventral pallidum is the preferential, but not the only, source of the calbindin-positive neurons of the medial extended amygdala

Our double-labeling results indicate that the majority of the MGEcv-derived cells that form part of the medial extended amygdala are calbindin positive. However, although a preferential source, MGEcv is not the only source of the calbindin-positive cells of the medial extended amygdala. Our results indicate that the ventral pallium and especially SPV also produce some calbindin-positive neurons of the medial extended amygdala. This is particularly evident for the BSTM, which includes two distinct calbindin-rich subdivisions, one primarily derived from MGEcv and another one primarily (if not entirely) derived from the SPV (Figs. 9E, 10C). Although calbindin neurons of the medial extended amygdala derived from MGEcv are likely GABAergic (as expected of most subpallium-derived neurons), calbindin cells derived from the VP or the SPV are likely glutamatergic.

Calbindin cells in the pallial amygdala and piriform cortex include both interneurons and principal neurons

Our results indicate that MGEcv is a source of numerous cells that migrate tangentially to the pallium, having

as a preferential destiny its ventrolateral part, including the piriform cortex, the claustral complex, and the pallial amygdala. Some of these MGEcv-derived cells of the ventrolateral pallium are calbindin positive and likely represent interneurons, as shown previously for other MGE-derived cells of the cerebral cortex and other parts of the pallium (Lavdas et al., 1999; Marin et al., 2000; Pleasure et al., 2000; Marin and Rubenstein, 2001; Anderson et al., 2001; Nery et al., 2002; Legaz et al., 2005a). However, our results also indicate that some calbindin-positive neurons of the piriform cortex and pallial amygdala originate in the ventral (or ventrolateral) pallium. This is in agreement with data indicating that about 25% of the calbindin neurons of the basal amygdalar complex of the rat do not contain GABA and show a pyramidal-like morphology (Kemppainen and Pitkänen, 2000). Calbindin-positive pyramidal-like neurons have also been observed in the mouse basal amygdalar complex (Legaz et al., 2005b). These non-GABAergic, calbindin-positive neurons of the pallial amygala likely derive from the ventrolateral pallium and may represent a subtype of principal glutamatergic neuron. Therefore, calbindin cannot be considered a general marker of pallial/cortical interneurons, because this is not so in the ventrolateral pallium.

LITERATURE CITED

Abellán A, Medina L. 2008. Expression of cLhx6 and cLhx7/8 suggests a pallido-pedunculo-preoptic origin for the lateral and medial parts of the avian bed nucleus of the stria terminalis. Brain Res Bull 75:299–304.

Abellán A, Medina L. 2009. Subdivisions and derivatives of the chicken subpallium based on expression of LIM and other regulatory genes and markers of neuron subpopulations during development. J Comp Neurol 515:465–501.

Abellán A, Vernier B, Rétaux S, Medina L. 2010. Similarities and differences in the forebrain expression of Lhx1 and Lhx5 between chicken and mouse: Insights for understanding telencephalic development and evolution. J Comp Neurol 518:3512–3528.

Aggleton JP. 2000. The amygdala: a functional analysis, 2nd ed. Oxford: Oxford University Press.

Alheid GF, Heimer L. 1988. New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience 27: 1–39.

Alheid GF, de Olmos J, Beltramino CA. 1995. Amygdala and extended amygdala. In: Paxinos G, editor. The rat nervous system. San Diego: Academic Press. p 495–578.

Alifragis P, Parnavelas JG, Nadarajah B. 2002. A novel method of labeling and characterizing migrating neurons in the developing central nervous system. Exp Neurol 174: 259–265.

Amaral DG, Schumann CM, Nordahl CW. 2008. Neuroanatomy of autism. Trends Neurosci 31:137–145.

Anderson SA, Marín O, Horn C, Jennings K, Rubenstein JLR. 2001. Distinct cortical migrations from the medial and lateral ganglionic eminences. Development 128:353–363.

Bardet SM, Martinez-de-la-Torre M, Northcutt RG, Rubenstein JL, Puelles L. 2008. Conserved pattern of OTP-positive cells in the paraventricular nucleus and other hypothalamic sites of tetrapods. Brain Res Bull 75:231–235.

Caffé AR, van Leeuwen FW, Luiten PG. 1987. Vasopressin cells in the medial amygdala of the rat project to the lateral septum and ventral hippocampus. J Comp Neurol 261: 237–252.

Chanas-Sacre G, Rogister B, Moonen G, Leprince P. 2000. Radial glia phenotype: origin, regulation, and transdifferentiation. J Neurosci Res 61:357–363.

Choi GB, Dong HW, Murphy AJ, Valenzuela DM, Yancopoulos GD, Swanson LW, Anderson DJ. 2005. Lhx6 delineates a pathway mediating innate reproductive behaviors from the amygdala to the hypothalamus. Neuron 46: 647–660.

Cocas LA, Miyoshi G, Carney RS, Sousa VH, Hirata T, Jones KR, Fishell G, Huntsman MM, Corbin JG. 2009. Emx1-lineage progenitors differentially contribute to neural diversity in the striatum and amygdala. J Neurosci 29: 15933–15946.

Davis M. 2000. The role of the amygdala in conditioned and unconditioned fear and anxiety. In: Aggleton JP, editor. The amygdala: a functional analysis, 2nd ed. Oxford: Oxford University Press. p 213–287.

de Olmos JS, Beltramino CA, Alheid G. 2004. Amygdala and extended amygdala of the rat: A cytoarchitectonical, fibroarchitectonical, and chemoarchitectonical survey. In: Paxinos G, editor. The rat nervous system, 3rd ed. Amsterdam: Elsevier-Academic Press. p 509–603.

De Vries GJ, Buijs RM. 1983. The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum. Brain Res 273: 307–317.

Flames N, Pla R, Gelman DM, Rubenstein JL, Puelles L, Marín O. 2007. Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. J Neurosci 27:9682–9695.

Foster GA. 1998. Chemical neuroanatomy of the prenatal rat brain. A developmental atlas. Oxford: Oxford University Press.

García-López M, Abellán A, Legaz I, Rubenstein JL, Puelles L, Medina L. 2008. Histogenetic compartments of the mouse centromedial and extended amygdala based on gene expression patterns during development. J Comp Neurol 506:46–74.

García-Moreno F, Pedraza M, Di Giovannantonio LG, Di Salvio M, López-Mascaraque L, Simeone A, De Carlos JA. 2010. A neuronal migratory pathway crossing from diencephalon to telencephalon populates amygdala nuclei. Nat Neurosci 13:680–689.

Grigoriu M, Tucker AS, Sharpe PT, Pachnis V. 1998. Expression and regulation of Lhx6 and Lhx7, a novel subfamily of LIM homeodomain encoding genes, suggests a role in mammalian head development, Development 125: 2063–2074.

Hevner RF, Shi L, Justice N, Hsueh Y, Sheng M, Smiga S, Bulfone A, Goffinet AM, Campagnoni AT, Rubenstein JLR. 2001. Tbr1 regulates differentiation of the preplate and layer 6. Neuron 29:353–366.

Hirata T, Li P, Lanuza GM, Cocas LA, Huntsman MM, Corbin JG. 2009. Identification of distinct telencephalic progenitor pools for neuronal diversity in the amygdala. Nat Neurosci 12:141–149.

Jacobowitz DM, Abbott LC. 1997. Chemoarchitectonic atlas of the developing mouse brain. Boca Raton, FL: CRC Press.

Kemppainen S, Pitkänen A. 2000. Distribution of parvalbumin, calretinin, and calbindin-D (28k) immunoreactivity in the

rat amygdaloid complex and colocalization with gamma-aminobutyric acid. J Comp Neurol 426:441–467.

Lavdas AA, Grigoriou M, Pachnis V, Parnavelas JG. 1999. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J Neurosci 19:7881–7888.

Legaz I. 2006. Caracterización genética y origen de las neuronas de la región claustroamigdalina en ratón. PhD Dissertation, University of Murcia.

Legaz I, García-López M, Medina L. 2005a. Subpallial origin of part of the calbindin-positive neurons of the claustral complex and piriform cortex. Brain Res Bull 66:470–474.

Legaz I, Olmos L, Real MA, Guirado S, Dávila JC, Medina L. 2005b. Development of neurons and fibers containing calcium-binding proteins in the pallial amygdala of mouse, with special emphasis in those of the basolateral amygdalar complex. J Comp Neurol 488:492–513.

Marín O, Rubenstein JLR. 2001. A long, remarkable journey: tangential migration in the telencephalon. Nat Rev Neurosci 2:780–790.

Marín O, Anderson SA, Rubenstein JLR. 2000. Origin and molecular specification of striatal interneurons. J Neurosci 20: 6063–6076.

Medina L, Legaz I, González G, de Castro F, Rubenstein JLR, Puelles L. 2004. Expression of Dbx1, neurogenin 2, semaphorin 5A, cCadherin 8, and Emx1 distinguish ventral and lateral pallial histogenetic divisions in the developing claustroamygdaloid complex. J Comp Neurol 474:504–523.

Misson JP, Edwards MA, Yamamoto M, Caviness VS Jr. 1988. Identification of radial glial cells within the developing murine central nervous system: studies based upon a new immunohistochemical marker. Brain Res Dev Brain Res 44: 95–108.

Nery S, Fishell G, Corbin JG. 2002. The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. Nat Neurosci 5:1279–1287.

Nóbrega-Pereira S, Gelman D, Bartolini G, Pla R, Pierani A, Marín O. 2010. Origin and molecular specification of globus pallidus neurons. J Neurosci 30:2824–2834.

Paxinos G, Franklin KBJ. 2004. The mouse brain in stereotaxic coordinates, 2nd Ed. San Diego: Academic Press.

Paxinos G, Ashwell KWS, Törk I. 1994. Atlas of the developing rat nervous system, 2nd ed. San Diego: Academic Press.

Paxinos G, Kus L, Ashwell KWS, Watson C. 1999. Chemoarchitectonic atlas of the rat forebrain. San Diego: Academic Press.

Phelps EA. 2004. Human emotion and memory: interactions of the amygdala and hippocampal complex. Curr Opin Neurobiol 14:198–202.

Phelps EA, LeDoux JE. 2005. Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron 48:175–187.

Pitkänen A, Tuunanen J, Kälviäinen R, Partanen K, Salmenperä T. 1998. Amygdala damage in experimental and human temporal lobe epilepsy. Epilepsy Res 32:233–253.

Pleasure SJ, Anderson S, Hevner R, Bagri A, Marin O, Lowenstein DH, Rubenstein JLR. 2000. Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. Neuron 28:727–740.

Puelles L, Rubenstein JLR. 2003. Forebrain gene expression domains and the evolving prosomeric model. Trends Neurosci 26:469–476.

Puelles L, Kuwana E, Puelles E, Bulfone A, Shimamura K, Keleher J, Smiga S, Rubenstein JLR. 2000. Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. J Comp Neurol 424: 409–438.

Rauch SL, Shin LM, Phelps EA. 2006. Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research—past, present, and future. Biol Psychiatry 60:376–382.

Real MA, Heredia R, Labrador MC, Dávila JC, Guirado S. 2009. Expression of somatostatin and neuropeptide Y in the embryonic, postnatal, and adult mouse amygdalar complex. J Comp Neurol 513:335–348.

Remedios R, Subramanian L, Tole S. 2004. LIM genes parcellate the embryonic amygdala and regulate its development. J Neurosci 24:6986–6990.

Rétaux S, Rogard M, Bach I, Failli V, Besson MJ. 1999. Lhx9: a novel LIM-homeodomain gene expressed in the developing forebrain. J Neurosci 19:783–793.

Ryu S, Mahler J, Acampora D, Holzschuh J, Erhardt S, Omodei D, Simeone A, Driever W. 2007. Orthopedia homeodomain protein is essential for diencephalic dopaminergic neuron development. Curr Biol 17:873–880.

Sequier JM, Hunziker W, Andressen C, Celio MR. 1990. Calbindin D-28k protein and mRNA localization in the rat brain. Eur J Neurosci 2:1118–1126.

Shimamura K, Rubenstein JLR. 1997. Inductive interactions direct early regionalization of the mouse forebrain. Development 124:2709–2718.

Soma M, Aizawa H, Ito Y, Maekawa M, Osumi N, Nakahira E, Okamoto H, Tanaka K, Yuasa S. 2009. Development of the mouse amygdala as revealed by enhanced green fluorescent protein gene transfer by means of in utero electroporation. J Comp Neurol 513:113–128.

Soria JM, Valdeolmillos M. 2002. Receptor-activated calcium signals in tangentially migrating cortical cells. Cereb Cortex 12:831–839.

Stenman J, Yu RT, Evans RM, Campbell K. 2003. Tlx and Pax6 co-operate genetically to establish the pallio-subpallial boundary in the embryonic mouse telencephalon. Development 130:1113–1122.

Swanson LW. 2000. Cerebral hemisphere regulation of motivated behavior. Brain Res 886:113–164.

Swanson LW, Petrovich GD. 1998. What is the amygdala? Trends Neurosci 21:323–331.

Tanaka M, Ikeda T, Hayashi S, Iijima N, Amaya F, Hisa Y, Ibata Y. 1997. Nitrergic neurons in the medial amygdala project to the hypothalamic paraventricular nucleus of the rat. Brain Res 777:13–21.

Tole S, Remedios R, Saha B, Stoykova A. 2005. Selective requirement of Pax6, but not Emx2, in the specification and development of several nuclei of the amygdaloid complex. J Neurosci 25:2753–2760.

Toresson H, Mata de Urquiza A, Fagerström C, Perlmann T, Campbell K. 1999. Retinoids are produced by glia in the lateral ganglionic eminence and regulate striatal neuron differentiation. Development 126:1317–1326.

Waclaw RR, Ehrman LA, Pierani A, Campbell K. 2010. Developmental origin of the neuronal subtypes that comprise the amygdalar fear circuit in the mouse. J Neurosci 30: 6944–6953.

Wang W, Lufkin T. 2000. The murine Otp homeobox gene plays an essential role in the specification of neuronal cell lineages in the developing hypothalamus. Dev Biol 227: 432–449.

Wang Z, De Vries GJ. 1995. Androgen and estrogen effects on vasopressin messenger RNA expression in the medial amygdaloid nucleus in male and female rats. J Neuroendocrinol 7:827–831.

Wang Z, Ferris CF, De Vries GJ. 1994. Role of septal vasopressin innervation in paternal behavior in prairie voles (*Microtus ochrogaster*). Proc Natl Acad Sci U S A 91:400–404.

Wang Z, Young LJ, Liu Y, Insel TR. 1997. Species differences in vasopressin receptor binding are evident early in development: comparative anatomic studies in prairie and montane voles. J Comp Neurol 378:535–546.

Xu Q, Tam M, Anderson SA. 2008. Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. J Comp Neurol 506: 16–29.

Zhao Y, Sheng HZ, Amini R, Grinberg A, Lee E, Huang S, Taira M, Westphal H. 1999. Control of hippocampal morphogenesis and neuronal differentiation by the LIM homeobox gene Lhx5. Science 284:1155–1158.

CHAPTER 3:

Central Extended Amygdala

Genetic and Experimental Evidence Supports the Continuum of the Central Extended Amygdala and a Mutiple Embryonic Origin of Its Principal Neurons

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ABSTRACT

The central extended amygdala is the major output center for telencephalic control of ingestion, fear responses, stress, and anxiety. In spite of the abundant data supporting the similarity in neurochemistry, connections, and function along the extended amygdala, embryological support for this continuum is lacking. By using a combination of in vitro migration assays, in situ hybridization, and immunostaining, here we show that its major components, including central amygdala and lateral bed nucleus of the stria terminalis (BST), are mosaics formed by different proportions of dorsal lateral ganglionic eminence (LGE)-, ventral LGE-, and medial ganglionic eminence (MGE)-derived principal neurons. The dorsal LGE produces Pax6-expressing neurons that primarily populate lateral parts of the central extended amygdala, including the capsular and part of lateral central amygdala, but also produces a few cells for the lateral BST. Based on correlation with preproenkephalin, many of these cells are likely enkephalinergic. The ventral LGE produces Islet1-expressing neurons that populate primarily the central and medial parts of the central amygdala but also produces numerous neurons for the lateral BST. Correlation with corticotropin-releasing factor suggests that these neurons express this neuropeptide. The MGE produces the majority of neurons of the lateral BST, but its ventrocaudal subdivision also produces an important subpopulation of projection neurons containing somatostatin for medial aspects of the central amygdala. Thus, distinct principal neurons originate in different embryonic domains, but the same domains contribute neurons to most subdivisions of the central extended amygdala, which may explain the similarity in neurochemistry and connections along the corridor. J. Comp. Neurol. 519:3507–3531, 2011.

INDEXING TERMS: lateral ganglionic eminence; medial ganglionic eminence; Pax6; Islet1; enkephalin; CRF; somatostatin; calbindin; tyrosine hydroxylase; cell migrations

The extended amygdala is a cell corridor or continuum located in the basal telencephalon that is involved in control of basic behaviors essential for survival, and its dysfunction appears to be related to some neuropsychiatric disorders (Alheid and Heimer, 1988; Alheid et al., 1995; Heimer, 2003). Although initially proposed by Johnston (1923), the extended amygdala was redefined by de Olmos, Alheid, and Heimer (de Olmos et al., 1985, 2004; Alheid and Heimer, 1988; Alheid et al., 1995), and distinguished from other basal telencephalic cell groups of the "substantia innominata" on the basis of its distinct neurochemistry and connectivity patterns. The latter authors proposed the existence of two subdivisions of the extended amygdala: 1) the central extended amygdala, related to the central amygdala and lateral bed nucleus of the stria terminalis (BST), involved in control of ingestion and fear responses, and 2) the medial extended amygdala, related to the medial amygdala and medial BST, involved in control of reproduction and/or defense (de Olmos et al., 1985, 2004; Alheid and Heimer, 1988; Alheid et al., 1995; see also Swanson, 2000; Heimer, 2003; Choi et al., 2005; Wilensky et al., 2006; Walker and Davis, 2008; Petrovich et al., 2009). In spite of the

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abundant data on the neurochemistry, connections, and function of the extended amygdala of adult rodents, information on its development is scarce. Thus, embryological support for the existence of the two cell corridors, or for explaining the striking similarity in neurochemistry and connections along each of the corridor cells of the extended amygdala, is absent. Recent genetic and fate mapping data indicate that an important part of the cell continuum of the medial extended amygdala, characterized by Lhx6 expression, share an identical embryonic origin in a ventrocaudal part of the medial ganglionic eminence (MGEvc; García-López et al., 2008; Bupesh et al., 2011). This part of the continuum is rich in calbindin-immunoreactive cells (García-López et al., 2008; Bupesh et al., 2011), receives odor information related to reproduction, and projects to hypothalamic centers involved in reproduction control (Choi et al., 2005). However, it has also become clear that the medial extended amygdala contains multiple neuron subpopulations that originate in distinct progenitor domains of the pallium and subpallium and outside the telencephalon (Puelles et al., 2000; García-López et al., 2008; Hirata et al., 2009; Soma et al., 2009; García-Moreno et al., 2010; Bupesh et al., 2011). Thus, instead of a single cell continuum, the medial extended amygdala may consist of several parallel (and perhaps partially intermingled) cell corridors, each one related to a different origin and possibly a different function (García-

Abbreviations

AAd	anterior amygdala, dorsal part
AAv	ventral part of the anterior amygdala
ACo	anterior cortical amygdalar area
BC	basal amygdalar complex
BST	bed nucleus of the stria terminalis
BSTia	intraamygdaloid part of the BST
BSTL	lateral BST
BSTLd	dorsal part of BSTL
BSTLp	posterior part of BSTL
BSTLv	ventral part of BSTL
BSTM	medial BST
Ce	central amygdala
CeC	capsular central amygdala
CeCvm	ventromedial part of CeC
CeL	lateral central amygdala
CeL (c)	central part of CeL
CeL (l)	lateral part of CeL
CeL (m)	medial part of CeL
CeM	medial central amygdala
Co	cortical amygdalar area
CPu	caudoputamen complex
DP	dorsal pallium
EA	extended amygdala
EAce	central extended amygdala
EAme	medial extended amygdala
ec	external capsule
EMT	prethalamic eminence
En	endopiriform nucleus
GP	globus pallidus
ic	internal capsule
IPAC	interstitial nucleus of the posterior limb of the anterior commissure
ITC	interstitial amygdalar cells
LGE	lateral ganglionic eminence
LGEd	dorsal part of LGE
LGEv	ventral part of LGE
lot	lateral olfactory tract
MGE	medial ganglionic eminence
MGEd	dorsal part of MGE
MGEvc	ventrocaudal part of MGE
Me	medial amygdala
MeP	posterior medial amygdala
MP	medial pallium
NCx	neocortex
nsp	nigrostriatal projection
p3	prosomere 3
ot	optic tract
PA	pallial amygdala
pac	posterior limb of the anterior commissure
Pir	piriform cortex
PVN	paraventricular hypothalamic nucleus
st	stria terminalis
PTh	prethalamus
Th	thalamus
TR	transmission
Tu	olfactory tubercle
ve	Ventricle

López et al., 2008; Abellán and Medina, 2008, 2009). Regarding the central extended amygdala, some data indicate an origin in MGE for the lateral BST (Swanson and Petrovich, 1989; Puelles et al., 2000; Xu et al., 2008; García-López et al., 2008), and an origin in the lateral ganglionic eminence (LGE) for the central amygdala and other striatal amygdalar cell groups, such as the intercalated cells (Puelles et al., 2000; Medina et al., 2004; García-López et al., 2008; Kaoru et al., 2010; Waclaw et al., 2010). In this panorama, the existence of a cell continuum for the central extended amygdala is difficult to support on an embryological basis, because current data appear to support the existence of only a "striatopallidal" type functional organization (as defined by Alheid and Heimer, 1988; and Swanson, 2000). However, based on Lmo4 expression in mouse, a LIM-only gene typical in the striatum, it was suggested that some cells of the lateral BST may originate in LGE (García-López et al., 2008). This was also proposed for the chicken lateral BST based on expression of Pax6, a transcription factor expressed in postmitotic cells derived from dorsal LGE (Abellán and Medina, 2009). It is unclear whether the lateral BST of the mouse includes Pax6-expressing cells of dorsal LGE origin, although it appears that such cells are found in the central amygdala (Puelles et al., 2000). In addition, it appears that many neurons of the central amygdala in mouse derive from Islet1-expressing progenitors of the ventral LGE (Waclaw et al., 2010), although it is unclear what exact subdivisions of the mature central amygdala contain dorsal LGE (Pax6 lineage)-derived or ventral LGE (Islet1 lineage)-derived cells. Also, it is unknown whether cells from the ventral LGE (Islet1) domain populate the lateral BST. Moreover, some of the somatostatin-containing projections neurons of the central amygdala have been proposed to originate in MGEvc (García-López et al., 2008; Real et al., 2009). If these proposals are true, this would mean that the central extended amygdala continuum proposed by Olmos, Alheid, Heimer and collaborators may indeed have embryological support and consists primarily of a mixture of striatal-like and pallidal-like cells. However, as noted above, many crucial aspects of central extended amygdala development remain unknown. Therefore, the aim of this study has been to analyze these questions of central extended amygdala development using the mouse as a model. Our results provide embryonic support for the central extended amygdala continuum and for the similarity of neurochemistry and connections of cells in this whole area.

MATERIALS AND METHODS

Mouse (Swiss) embryos from embryonic day 13.5 (E13.5) until day 18.5 (E18.5) and neonates (P0) were used in the present study. All animals were treated according to the regulations and laws of the European Union (86/609/EEC) and the Spanish Government (Royal Decree 1021/2005) for care and handling of animals in research. The protocols used were approved by the Committee for handling and care of research animals of the University of Lleida. The mouse embryos were obtained from pregnant females, and their brains were processed for in situ hybridization, for immunohistochemistry, or for preparing organotypic cultures for migration assays.

In situ hybridization

Frontal or horizontal embryonic brain sections of E13.5–E18.5 mice were processed for in situ hybridization using digoxigenin-labeled riboprobes, following a procedure previously described (Medina et al., 2004; García-López et al., 2008; Abellán and Medina, 2008, 2009). The riboprobes were synthesized from cDNAs of mouse *Pax6* (bp 1–260; Genbank accession no: NM_013627), mouse *Islet1* (bp 1–459; Genbank accession no: W42403; Research Genetics Inc.; plasmid kindly provided by Dr. Elisa Marti), and rat *preproenkephalin* (ppENK; bp 1–938; Genbank accession no: Y07503; cDNA kindly provided by Dr. G. Drolet; Yoshikawa et al., 1984; Poulin et al., 2006; the hybridization pattern observed in the mouse telencephalon here is identical to that reported in the rat by Yoshikawa et al., 1984, and Poulin et al., 2006, 2008).

We used PCR to obtain the DNA template employed for synthesizing the riboprobe. We synthesized the antisense digoxigenin-labeled riboprobes using Roche Diagnostics's (Mannheim, Germany) protocols for the genes mentioned above. Before hybridization, the sections were treated with proteinase K (10 μg/ml; Roche Diagnostics) in PBS containing 0.1% Tween-20 (1× PBT) for 1 minute. After that, sections were abundantly washed and postfixed with phosphate-buffered 4% paraformaldehyde containing 0.06% glutaraldehyde for 20 minutes. The sections were washed again, prehybridized in hybridization buffer (HB) for 2 hours at 58°C, and then hybridized in HB containing the ribprobe overnight at 58°C (0.5–1 μg/ml, depending on the probe and embryo size). The hybridization buffer contained 50% deionized formamide, 1.3× standard saline citrate (SSC; pH 5), 5 mM ethylenediaminetetraacetic acid (EDTA; pH 8.0; Sigma-Aldrich, Steinheim, Germany), 1 mg/ml yeast tRNA (Sigma-Aldrich), 0.2% Tween-20, 100 μg/ml heparin (Sigma-Aldrich), completed with water (free of RNAase and DNAase; Sigma-Aldrich). After hybridization, the sections were washed with a mix 1:1 of 1× MABT (1.2% maleic acid, 0.8% NaOH, 0.84% NaCl, and 0.1% Tween-20) and HB at 58°C for 20 minutes and washed abundantly at room temperature with 1× MABT (about 2 hours). After this, the sections were blocked with a solution containing blocking reagent (Roche Diagnostics), 1× MABT, and sheep serum (Sigma) for 4 hours at

TABLE 1.
Primary Antibodies

Antibody	Antigen recognized	Immunogen	Manufacturer, species, mono- vs. polyclonal, catalog or lot No.	Dilution
Anticalbindin	Calbindin D-28k	Recombinant rat calbindin D-28k	Swant (Bellinzona, Switzerland); rabbit polyclonal IgG; catalog No. CB-38a, lot No. 9.03 and 18F	1:2,000
Anti-Islet1	Islet1/Islet2	C-terminal residues 178–349 of rat Islet1	Developmental Hybridoma Bank, (Univ. of Iowa, Iowa); mouse monoclonal IgG2b; catalog No. 39.4D5	1:200
Anti-Pax6	Pax6	Recombinant protein containing amino acids 1–223 of chick Pax6, made in *E. coli*	Developmental Hybridoma Bank, (Univ. of Iowa, Iowa); mouse monoclonal IgG1; catalog No. Pax6	1:200
Antisomatostatin	Somatostatin	Synthetic peptide LELQRSANSNPAMAPRERK mapping amino acids 84–102 of human SOM	Santa Cruz Biotechnology, Santa Cruz, CA; goat polyclonal; catalog No. SC-7819; lot No. L0209	1:1,000
Antityrosine hydroxylase	Tyrosine hydroxylase	Denatured tyrosine hydroxylase from rat pheochromocytoma	Millipore, Temecula, CA; rabbit polyclonal; catalog No. AB152; lot No. NG1723896	1:1,000

room temperature, then incubated in an antibody against digoxigenin (alkaline-phosphatase coupled antidigoxigenin; diluted 1:3,500; Roche Diagnostics) overnight at 4°C, later washed with 1× MABT, and finally revealed with BM purple (Roche Diagnostics). Sections were then mounted on glycerol gelatin (Sigma).

Organotypic cultures

For the migration assays, we prepared organotypic cultures of forebrain slices from E13.5 to E16.5 mice following a previously described procedure (Soria and Valdeolmillos, 2002; slightly modified according to Legaz, 2006). Upon extraction, embryos were placed in a standard ice-cold, oxygenated culture medium artificially resembling cerebrospinal fluid (containing 124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4 \cdot 7H_2O$, 26 mM $CHNaO_3$, 2.4 mM $CaCl_2 \cdot 2H_2O$, and 10 mM D(+)-glucose; Soria and Valdeolmillos, 2002), in which brains were dissected out. The brains were sectioned at 300 μm in the frontal or horizontal plane using a vibratome (Leica VT 1000S), and the slices were mounted onto porous culture plate inserts (Millicell-CM, 0.4 μm pore diameter; 30 mm insert diameter; Millipore, Molsheim, France; Soria and Valdeolmillos, 2002) and placed in culture medium DMEM F-12 (Gibco, Grand Island, NY; supplemented with 5% fetal bovine serum, 0.1 mM glutamine, 6.5 mg/ml D-glucose, 1% supplement N2, and 1% penicillin; Soria and Valdeolmillos, 2002; Legaz, 2006). Slices were allowed to recover in a CO_2 incubator (5% CO_2; 37°C) for 1 hour before application of the tracer dye. After that, tungsten particles coated with the fluorescent dye CMFDA (Cell Tracker Green 5-chloromethylfluorescein diacetate; Invitrogen-Molecular Probes, Paisley, United Kingdom; excitation peak 490 nm; emission peak 514 nm; Alifragis et al., 2002) were applied to the ventricular/subventricular zone of either the LGE (at intermediate or caudal levels) or the MGE (in particular, its caudoventral subdivision, previously named or included as part of the anterior peduncular area [AEP]; Puelles et al., 2000; García-López et al., 2008). The slices were then transferred to culture medium Neurobasal (Gibco; supplemented with 5% fetal bovine serum, 0.1 mM, glutamine, 6.5 mg/ml D-glucose, 1% supplement B27 [Gibco], and 1% penicillin; Soria and Valdeolmillos, 2002; Legaz, 2006) and incubated in a CO_2 incubator (5%; 37°C) for 24–48 hours. After incubation, the slices were fixed with phosphate-buffered 4% paraformaldehyde (pH 7.4) for 8 minutes, and then rinsed and stored in phosphate buffer (0.1 M, pH 7.4) containing 0.1% sodium azide until microscopic observation. The labeling was analyzed and images were captured with a confocal scanning microscope (Olympus FV500).

Immunohistochemistry and immunofluorescence

Some embryonic brains were sectioned in frontal or horizontal planes and processed for immunohistochemistry to detect tyrosine hydroxylase (TH; rabbit antityrosine hydroxylase; Millipore, Temecula, CA) to help in the identification of the central extended amygdala (Fallon et al., 1978; Alheid et al., 1995), and somatostatin (SOM; goat antisomatostatin; Santa Cruz Biotechnology, Santa Cruz, CA) to analyze in detail the location of SOM-immunoreactive cells in the mature central extended amygdala. Moreover, selected slices from the migration experiments were processed (directly or, more often, after resectioned at 40–50 μm thick) for immunofluorescence to detect calbindin (CB; rabbit anticalbindin;

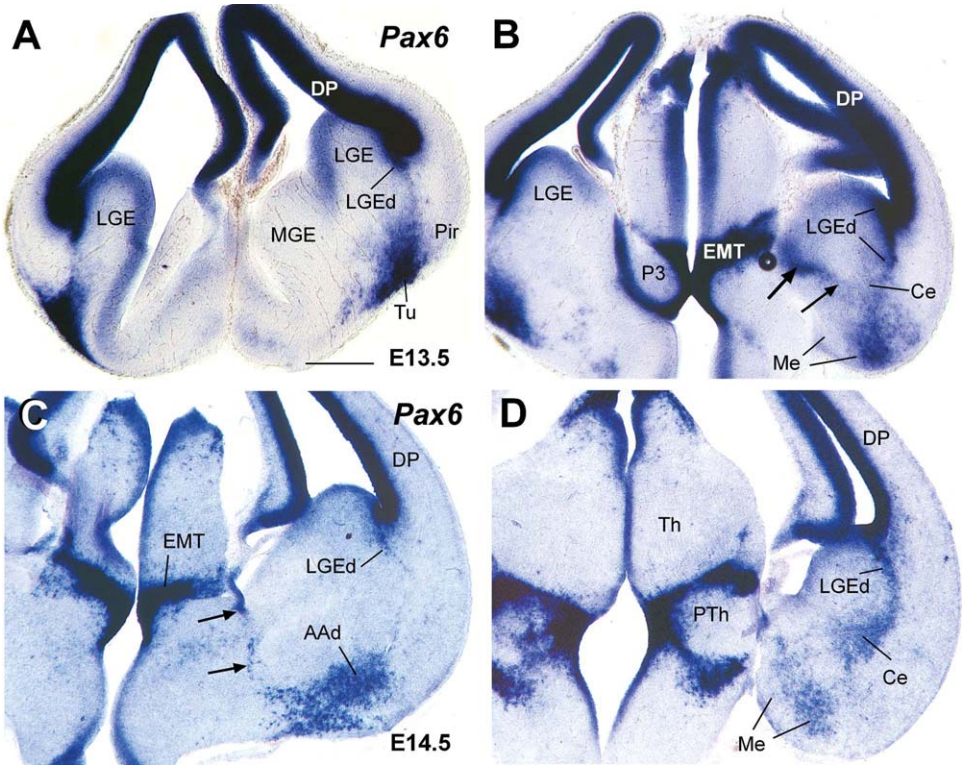

Figure 1. **A–D**: Digital images of frontal sections through the embryonic mouse telencephalon at E13.5–E14.5 showing the expression of *Pax6* mRNA in the amygdalar primordium (in situ hybridization). Note the stream of Pax6-expressing cells that appears to originate in the dorsal LGE and extends radially into the surface. At caudal levels, these cells invade the central amygdala (Ce; B,D). The arrows in B and C point to another stream of Pax6-expressing cells, which appear to originate in the prethalamic eminence (EMT) and invade parts of the amygdala. For abbreviations see list. Scale bar = 0.4 mm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Swant, Bellinzona, Switzerland), Pax6 (mouse anti-Pax6, Developmental Hybridoma Bank, University of Iowa), Islet1 (mouse anti-Islet1; Developmental Hybridoma Bank, University of Iowa), or SOM (goat antisomatostatin; Santa Cruz Biotechnology). See Table 1 and section below for details on the primary antibodies. The primary antibodies were diluted in PBS containing 0.3% Triton X-100, and the tissue was incubated for 2–3 days at 4°C under constant and gentle agitation. To block nonspecific binding of the secondary antisera, 10% normal goat serum (Sigma) and/or 10% normal horse serum (Sigma) was added to the solution containing the Pax6 and Islet1 primary antibodies.

After this incubation and standard washes in PBS-Triton, the sections were incubated in a secondary antiserum for either 1 hour (for immunohistochemistry) or 2 hours (for immunofluorescence) at room temperature. For immunohistochemistry, the secondary antisera used were either biotinylated goat anti-rabbit (for CB and TH) or biotinylated rabbit anti-goat (for SOM). Both secondary antisera were purchased from Vector (Burlingame, CA) and were diluted at 1:200. After washing, the sections were incubated in the avidin-biotin complex (ABC kit; Vector; 0,003% dilution) for 1 hour at room temperature. The immunolabeling was revealed by 0.05% diaminobenzidine (DAB; Sigma-Aldrich) in 0.05 M Tris (pH 7.6), containing 0.03% H_2O_2. For immunofluorescence, we used the following secondary antisera: donkey anti-rabbit (for CB), donkey anti-mouse (for Pax6 and Islet1), and donkey anti-goat (for SOM), all conjugated to Alexa 568 (Molecular Probes) and diluted 1:500. After incubation, the sections were rinsed and stored (at 4°C, in the darkness) until analysis with a confocal microscope. To check the specificity of our secondary antisera, some sections were processed omitting the primary antibody. After this, no labeling was observed.

Antibody characterization

See Table 1 for a list of all primary antibodies used. The rabbit anticalbindin D-28k antibody recognized a single band of 28 kD m.w. on Western blots of rat brain (manufacturer's data sheet) and stained a pattern of cellular morphology and distribution in the mouse brain identical to that in previous reports on localization of the

mRNA of the same protein via in situ hibridization histochemistry (Sequier et al., 1990).

The mouse anti-Islet1 antibody was raised against the C-terminal residues 178–349 of rat Islet-1, produces a comparable staining pattern in rat and chicken brain, and recognizes both Islet1 and Islet2 (Thor et al., 1991; Varela-Echavarría et al., 1996; Abellán and Medina, 2009). In the chicken, staining with this antiserum is colocalized with the mRNA distribution of Islet-1 protein by in situ hibridization histochemistry (Varela-Echavarría et al., 1996). The staining pattern obtained with this antibody in the developing mouse forebrain (Elshatory and Gan, 2008; present results) is identical to that obtained with in situ hybridization (Stenman et al., 2003a; present results: compare Fig. 3 and Fig. 7).

The mouse anti-Pax6 antibody was raised against a recombinant protein containing amino acids 1–223 of chick Pax6, made in *Escherichia coli* (Kawakami et al., 1997). The staining pattern obtained with this antibody in the developing mouse neural tube (Cameron et al., 2009), including the forebrain (present results), is identical to that obtained with in situ hybridization (Ericson et al., 1997; Stoykova et al., 2000; Puelles et al., 2000; present results: compare Fig. 1 and Fig. 10).

The goat antisomatostatin antibody was raised against the synthetic peptide LELQRSANSNPAMAPRERK mapping amino acids 84–102 of human SOM (Santa Cruz Biotechnology). Its specificity has been checked by Western blotting in mouse brain homogenates, producing a single band of the expected molecular size corresponding to the synthetic peptide (Fig. 1 in Real et al., 2009). With this antibody we obtained a staining pattern in the forebrain identical to that reported previously with the same or different antibodies against SOM (García-López et al., 2008; Real et al., 2009).

The rabbit antityrosine hydroxylase antibody was raised against denatured TH from rat pheochromocytoma, and by Western blotting it recognizes a band of approximately 62 kDa on PC12 lysates, which corresponds to the enzyme TH (manufacturer's data sheet). In the developing mouse brain, it produces a staining pattern identical to that described in previous reports in mouse, rat, and other mammals (Smeets and Reiner, 1994; Jacobowitz and Abbot, 1997), and the distribution of immunoreactive perikarya seen with this antibody is identical to that observed via in situ hybridization in the mouse brain (Marín et al., 2005; unpublished data from our laboratory).

Digital photographs and figures

Digital photographs from hybridized and immunostained sections were taken with a Leica microscope (DMR HC) equipped with a Zeiss Axiovision digital camera, and serial images from fluorescent material were taken with the confocal microscope (Olympus FV500). Selected digital images were adjusted for brightness and contrast in Adobe PhotoShop, and figures were mounted and labeled in FreeHand 10.

Identification of cell masses and nomenclature

For identification of forebrain cell masses during development, we used atlases of developing mouse (Jacobowitz and Abbot, 1997) and rat (Paxinos et al., 1994; Foster, 1998) brain as well as our own publications on the subject (especially Legaz et al., 2005b; García-López et al., 2008; Abellán et al., 2010). For BST and amygdalar subdivisions, we followed the brain atlas by Paxinos and colleagues (Paxinos et al., 1999; Paxinos and Franklin, 2004), which follow the scheme of Alheid et al. (1995).

RESULTS

Characterization of major cell subpopulations and subdivisions of the developing mouse central extended amygdala based on Pax6, Islet1, enkephalin, SOM, and TH

To understand the developmental origin and final distribution of distinct cell subpopulations within the central extended amygdala, we first analyzed the expression of the transcription factors Pax6 and Islet1 by in situ hybridization throughout development (from E13.5 to prenatal stages and birth; Figs. 1–3), which allowed distinction of cells derived from dorsal LGE and ventral LGE, respectively (Puelles et al., 2000; Stenman et al., 2003a; Waclaw et al., 2010). This was compared with the distribution of cells expressing the neuropeptides enkephalin (preproenkephalin or ppENK; by in situ hybridization) or SOM (by immunohistochemistry) at middle (E16.5) and prenatal (E17.5, E18.5; Fig. 2) stages, which label distinct subpopulations of neurons in the central extended amygdala of adult rodents (Moga and Gray, 1985; Moga et al., 1989; Poulin et al., 2006, 2008). Moreover, SOM-containing cells of the central amygdala were proposed to originate in MGEvc (García-López et al., 2008; Real et al., 2009). To help in the localization of the central extended amygdala, we compared our results with Pax6, Islet1, enkephalin, and SOM with the immunohistochemical expression of the rate-limiting catecholamine enzyme TH, which is present at high levels in fibers and varicosities of the central extended amygdala in adult rodents, allowing its distinction from other parts of the extended amygdala (Fallon et al., 1978; Alheid et al., 1995; Paxinos and Franklin, 2004). As shown in Figure 2, TH-immunoreactive innervation was abundant in the central extended

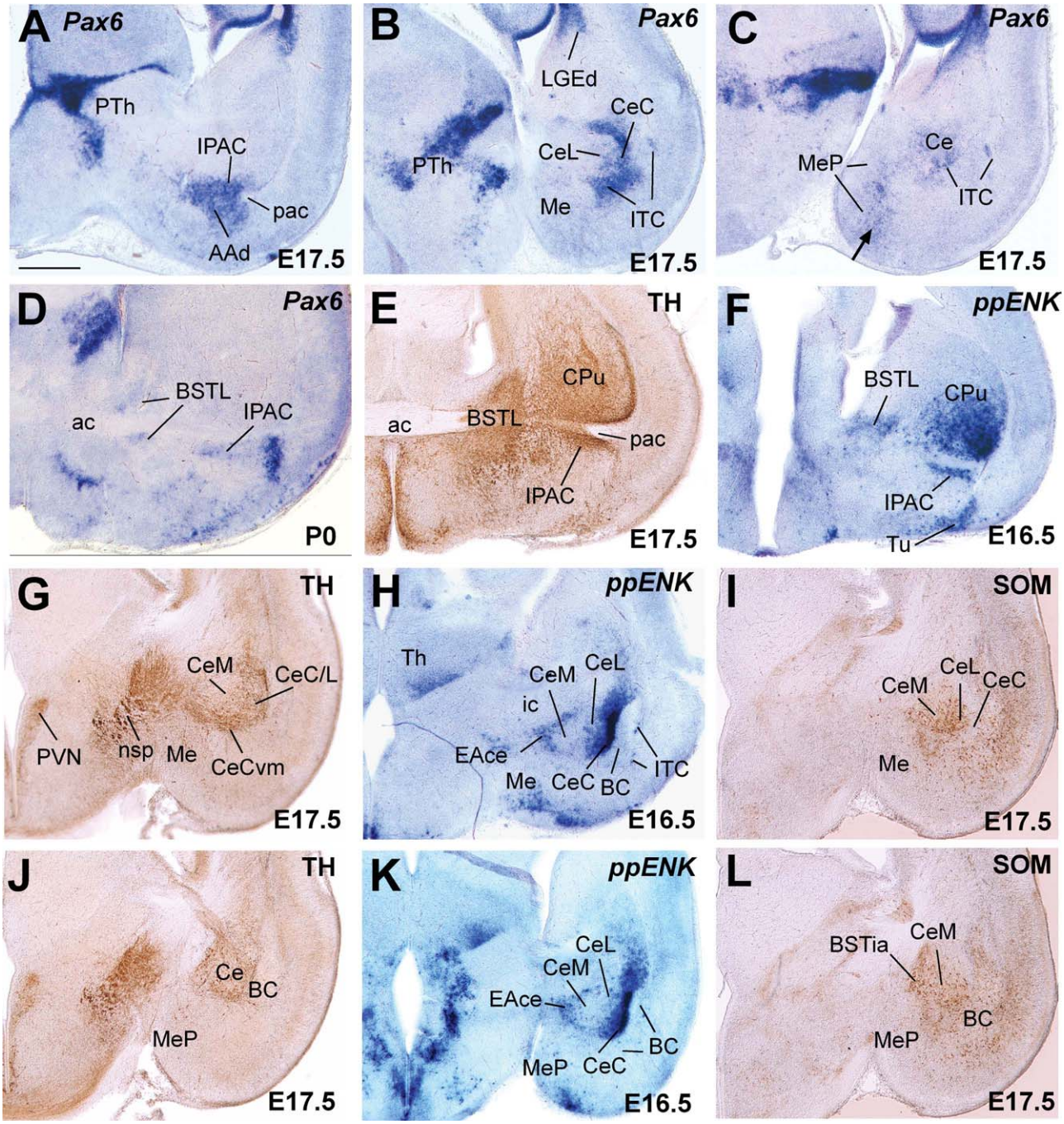

Figure 2. A–D: Digital images of frontal sections through the developing mouse telencephalon at prenatal embryonic stages (E17.5) or at birth (P0), showing the expression of *Pax6* mRNA in the central extended amygdala. E–L: Frontal sections through the telencephalon of mouse E16.5–E17.5, showing the expression of the enzyme tyrosine hydroxylase (TH; by immunohistochemistry), *preproenkephalin* mRNA (*ppENK*, by in situ hybridization), and the neuropeptide somatostatin (SOM; by immunohistochemistry) in levels of the central extended amygdala similar to those shown for *Pax6*. Note the similarity in the distribution of *ppENK*- and *Pax6*-expressing cells in the central amygdala, both enriched in the capsular/lateral parts of the central amygdala. In contrast, somatostatin cells are more abundant in the medial part of the central amygdala (medial and part of the lateral subnuclei). The arrow in C points to Pax6-expressing cells in the medial amygdala. For abbreviations see list. Scale bar = 0.5 mm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

amygdala at E17.5, including the lateral BST, the interstitial nucleus of the posterior limb of the anterior commissure (IPAC), the anterior amygdala (in particular its dorsal part), and the central amygdala (Ce; Fig. 2E,G,J). A part (but not all) of the intercalated cells of the amygdala (ITC) also showed dense innervation by TH-immunoreactive fibers and varicosities at E17.5.

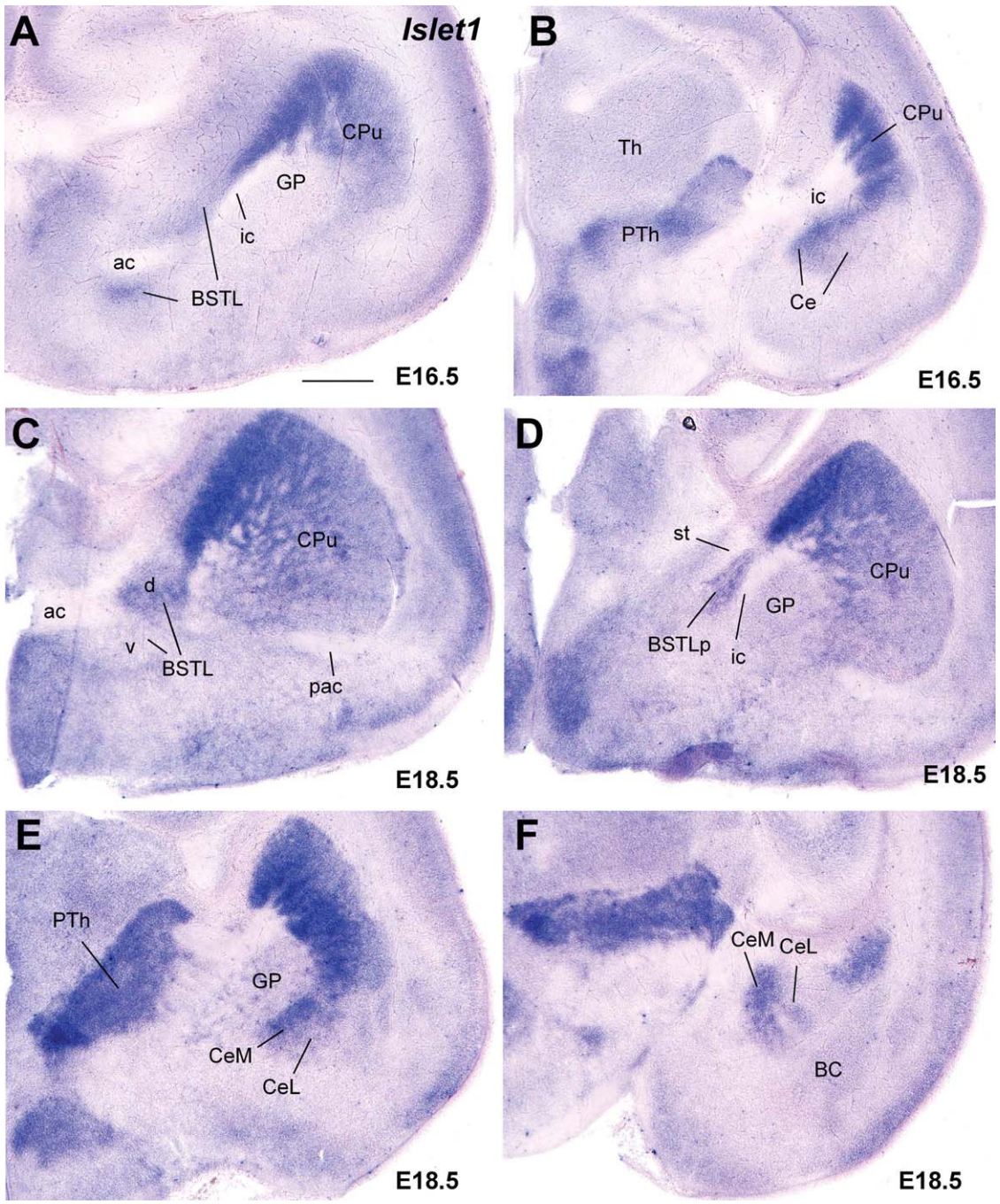

Figure 3. Digital images of frontal sections through the embryonic mouse telencephalon at E16.5–E18.5 showing the expression of *Islet1* mRNA (in situ hybridization) in the central extended amygdala. In the central amygdala, the expression is high in the medial part (encompassing the medial subnucleus or CeM, and the adjacent part of the lateral subnucleus or CeL). Note the expresion in the lateral BST (BSTL). See text for more details. For abbreviations see list. Scale bar = 0.4 mm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Pax6

This transcription factor has previously been noted to be expressed in the ventricular zone of both the pallium and the LGE (Stoykova et al., 2000; Puelles et al., 2000; Toresson et al., 2000; Flames et al., 2007) but not in the MGE or more ventral parts of the subpallium (Fig. 1A,B). These results indicated that this marker is excellent for the distinction of the dorsal LGE and its derived cells in the striatum, central amygdala, and olfactory bulb, which, in contrast to those of ventral LGE, maintain postmitotic expression of Pax6 (Puelles et al., 2000; Flames et al.,

2007). However, the exact distribution of Pax6-expressing cells in the central amygdala subdivisions was unclear, and it was unknown whether Pax6-lineage cells invade other parts of the central extended amygdala continuum. Our study provides novel and relevant details on the expression of Pax6 in the central extended amygdala from E13.5 to prenatal stages. At E13.5–E14.5, a stream of postmitotic cells expressing Pax6 extended radially from the ventricular/subventricular zone of dorsal LGE into the mantle, reaching the surface at the olfactory tubercle region, just ventral to the piriform cortex (Fig. 1A,B). This radial stream of Pax6-expressing cells was observed from rostral to caudal levels. At caudal levels, abundant Pax6-expressing cells were observed along the lateral margin of the developing striatum and central extended amygdala (adjacent to the palliosubpallial boundary), being concentrated in the dorsal anterior amygdala (AAd) and central amygdala (Ce) primordia (Fig. 1B,C). A few cells were also observed to separate from dorsal LGE and invade tangentially the pallidal region medial to the globus pallidus, where the lateral BST develops (not shown). Curiously, at E13.5–E14.5, another stream of Pax6-expressing cells reached the amygdala, apparently originating in the prethalamic eminence (EMT, arrows in Fig. 1B,C). At E13.5–E14.5, abundant Pax6-expressing cells were also observed in part of the medial amygdala, but their exact embryonic origin was unclear, perhaps in dorsal LGE, in EMT, or in both (Fig. 1B,D). In addition, some postmitotic Pax6-expressing cells were observed in the pallium.

At prenatal stages (E17.5–E18.5) and at birth (P0), the central extended amygdala already showed a relatively mature organization in terms of subdivisions, cell types, and main connections (such as innervation by TH-immunoreactive fibers), and the distribution of Pax6-expressing cells in the different subdivisions was more clear (Fig. 2). Pax6-expressing cells were present in the lateral BST, interstitial nucleus of the anterior commissure (IPAC, mostly its lateral part), dorsal anterior amygdala (AAd), intercalated amygdalar cells (ITC, including paracapsular cells and main masses), and central amygdala (Fig. 2A–D). However, differences in the abundance of Pax6-expressing cells in distinct central extended amygdala subdivisions were observed. Pax6-expressing cells were very abundant and densely packed in IPAC, AAd, and ITC (Fig. 2A–C). In the central amygdala, Pax6-expressing cells also were very abundant and densely packed in the capsular subdivision (CeC, except for its ventromedial part; Fig. 2B). In addition, a moderately abundant subpopulation of Pax6 cells was present in the lateral subdivision (CeL, mainly its lateralmost zone), but the medial subdivision (CeM) and the ventromedial aspect of the CeC contained only very few, scattered Pax6 cells (Fig. 2B,C). On the other hand, the lateral BST contained a small group of Pax6-expressing cells, which appear to locate in the dorsal and posterior subnuclei (which correspond to the oval and rhomboid subnuclei, respectively, of the anterolateral BST complex of Swanson and collaborators; Dong et al., 2001a; Fig. 2D). With regard to the medial amygdala, the number of Pax6-expressing cells present in this structure at prenatal stages was relativey low, and these cells were mostly concentrated (showing at times cell aggregates) in the lateral aspects of the posterior medial amygdala (arrow in Fig. 2C).

Islet1

During early and intermediate embryonic stages of development, this transcription factor is known to be expressed by the proliferating cells of the subventricular zone of ventral LGE and by its derivatives in the striatal mantle (including principal neurons of the caudate-putamen; Stenman et al., 2003a,b) and the central amygdala (Waclaw et al., 2010). However, the exact subdivisions of the central amygdalar nucleus that are occupied by Islet1-lineage cells remained unclear, as did whether these cells occupy other parts of the central extended amygdala. Our data on Islet1 mRNA expression during prenatal stages showed that the distribution of Islet1-expressing in the central amygdala is not even (Fig. 3). At E16.5–E18.5 mice, Islet1-expressing cells were concentrated in the medial and at least part of the lateral subdivisions, whereas the capsular subdivision (very rich in Pax6-expressing cells, as noted above) was very poor in and primarily remained free of Islet1 cells (Fig. 3B,E,F). Moreover, at E15.5–E16.5, a stream of Islet1-expressing cells appeared to extend from the ventral LGE into the lateral BST primordium, just medial to the internal capsule (Fig. 3A). At prenatal stages (E18.5), numerous Islet1-expressing cells were concentrated in the dorsal and posterior subnuclei of lateral BST (which correspond to the oval and rhomboid subnuclei, respectively, of the anterolateral BST complex of Swanson and colleagues; Dong et al., 2001a), although some cells were also present in the ventral subnucleus (including the fusiform and subcommissural subnuclei of the anterolateral BST complex of Swanson and colleagues; Dong et al., 2001a; Fig. 3C,D).

ppENK

An important subpopulation of enkephalinergic neurons is known to be present in the central extended amygdala of adult rodents (Moga and Gray, 1985; Moga et al., 1989; Poulin et al., 2006, 2008). To understand its location with respect to that of the Pax6- or Islet1-expressing cells, we studied its distribution at middle (E16.5) and prenatal (E17.5, E18.5) stages of mouse using in situ hybridization. Interestingly, the distribution

of ppENK-expressing neurons in the central extended amygdala at these stages greatly resembled that of Pax6-expressing cells, although ppENK-expressing cells were more numerous. In the central amygdala, ppENK-expressing cells were very abundant and densely packed in the capsular subdivision (CeC, excepting its ventromedial part), and many cells were also present in the lateral subdivision (CeL, mainly its lateralmost zone; Fig. 2H,K). Densely packed ppENK-expressing cells were also observed in other parts of the central extended amygdala, including the intercalated cells, the interstitial nucleus of the posterior limb of the anterior commissure, a part of the substantia innominata (labeled EAce in the figures), and dorsal and posterior parts of the lateral BST (Fig. 2F,H,K). In most of these nuclei, the abundance of ppENK-expressing cells at prenatal stages was similar to that of Pax6-expressing cells. The only exception was the lateral BST, where ppENK-expressing cells were far more abundant than Pax6-expressing cells.

SOM

This neuropeptide is present in a subpopulation of projection neurons of the central extended amygdala of adult rodents (Moga and Gray, 1985; Moga et al., 1989), and previous developmental studies suggested that SOM-containing (SOM^+) cells of the central amygdalar nucleus may immigrate from the MGEvc (García-López et al., 2008; Real et al., 2009). However, the exact location of these cells with respect to Pax6- and Islet1-expressing cells of the central amygdala was unknown. Our data on immunohistochemistry indicate that, at middle and prenatal stages, SOM^+ cells of the mouse central amygdala appear to include two groups: 1) a prominent group of large cells (likely projection neurons), associated with an intensely SOM^+ neuropile, located in the Pax6-poor sector of the central amygdala, encompassing the medial subnucleus (CeM) and the adjacent part of the lateral subnucleus (CeL-medial zone), and 2) a minor group of scattered SOM^+ cells (likely interneurons), associated with the Pax6-rich sector and SOM-poor neuropile of the central amygdala, which encompasses the capsular subnucleus (CeC) and the adjacent part of the lateral subnucleus (CeL-lateral zone; Fig. 2I,L).

Cell migration from mouse LGE to the central extended amygdala

Although genetic fate mapping of Islet1 progenitors has shown that ventral LGE is a specific source of neurons for the central amygdala (Waclaw et al., 2010), experimental/genetic fate mapping from dorsal LGE into the amygdala was lacking. Moreover, it was unknown whether dorsal LGE and ventral LGE also produce cells for the lateral BST or other parts of the central extended amygdala continuum. To investigate these uncertainties, we carried out migration assays of telencephalic slices at E13.5–E16.5 and labeled the ventricular and/or subventricular zone of either dorsal or ventral LGE progenitor subdomains at intermediate or caudal levels, using the fluorescent cell tracker CMFDA. Our results from migration assays indicated that both LGE subdomains give rise to abundant cells that migrate radially into the striatal mantle, also invading lateral parts of the central extended amygdala (Figs. 4–7). Whereas the ventral LGE produced cells that invaded primarily medial aspects of the caudate-putamen (Figs. 4, 5), the dorsal LGE produced cells that invaded radially dorsal and lateral aspects of the striatum, reaching the olfactory tubercle and, more caudally, the IPAC and the anterior amygdala (Figs. 6, 7). At caudal levels, the ventral LGE-derived cells preferentially invaded lateral-medial aspects of the central amygdala (Fig. 5), and the dorsal LGE produced cells that invaded primarily the capsular-lateral aspects of the central amygdala (Figs. 6, 7). The location of the central amygdala was precisely verified by immunofluorescence for Islet1 (Fig. 5C,D), Pax6 (Fig. 7B), or CB (Fig. 6B,C; CB is a very useful landmark for distinct amygdalar subdivisions in adult and developing rodents; McDonald, 1997; Kemppainen and Pitkänen, 2000; Legaz et al., 2005b). Importantly, by immunofluorescence, cells in the central amygdala and other parts of the mantle derived from ventral LGE were often double labeled for Islet1, but never for Pax6 (Fig. 5E–E″). On the contrary, cells in the central amygdala and other parts of the mantle derived from dorsal LGE were often double labeled for Pax6, but never for Islet1 (Fig. 7C–C″). Some of the cells in the central amygdala derived from both dorsal LGE and ventral LGE were double labeled with CB (Fig. 6D–D″).

Notably, in addition to radial migrations, both dorsal LGE and ventral LGE also produced abundant cells that migrated tangentially into ventral aspects of the striatum, and some of these cells appeared to reach pallidal territories, including the globus pallidus and other pallidal areas, which could be confirmed by comparison with Islet1, Pax6, and CB (Figs. 4B–D; 5B,D,E′, 6E,F, 7D,E). Of relevance for our study, some cells derived from both ventral LGE and dorsal LGE were observed to invade the lateral BST (Figs. 4B–D, 6E,F), which is considered the pallidal part of the central extended amygdala (Swanson and Petrovich, 1998; Puelles et al., 2000; Xu et al., 2008; García-López et al., 2008). Similarly to those of the central amygdala, some cells of the lateral BST derived from ventral LGE were double labeled for Islet1, but never for Pax6 (Fig. 4E–E′). On the contrary, some

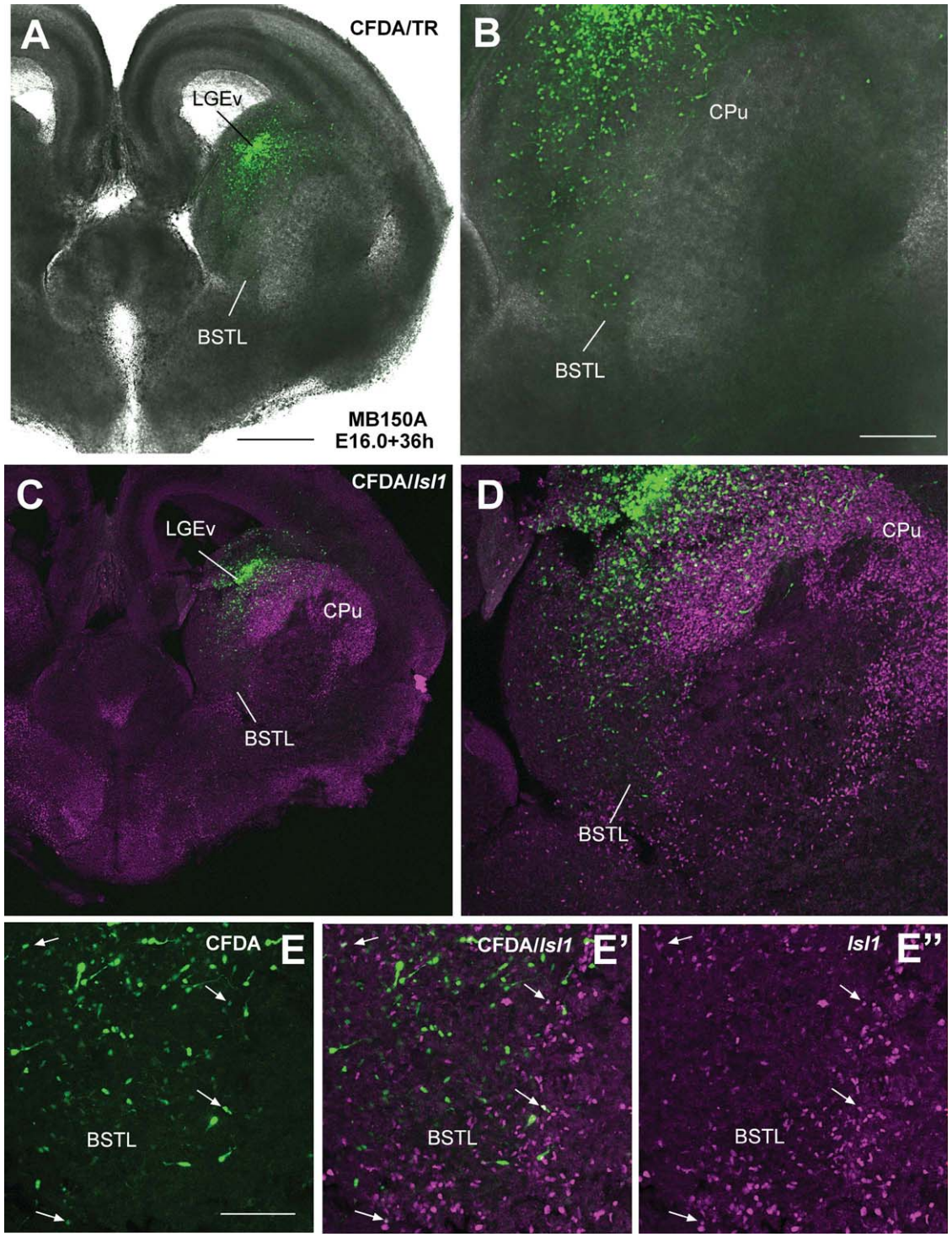

Figure 4. A–E″: Digital images from one representative organotypic culture of a telencephalic frontal slice of mouse embryo (MB150A), at an intermediate telencephalic level, in which the fluorescent cell tracker CMFDA (green) was centered in the ventral LGE. The slice was resectioned and immunolabeled to detect Islet1 (C–E″, shown in magenta). In addition to cells migrating to the striatal mantle (CPu), some cells were observed to migrate tangentially to more ventral, pallidal regions, and invaded the lateral BST (BSTL). Like those in the striatum, ventral LGE-derived cells of the lateral BST were often double labeled for Islet1 (arrows in E–E″). Scale bars = 0.5 mm in A (applies to A,C); 0.2 mm in B (applies to B,D); 0.1 mm in E (applies to E–E″).

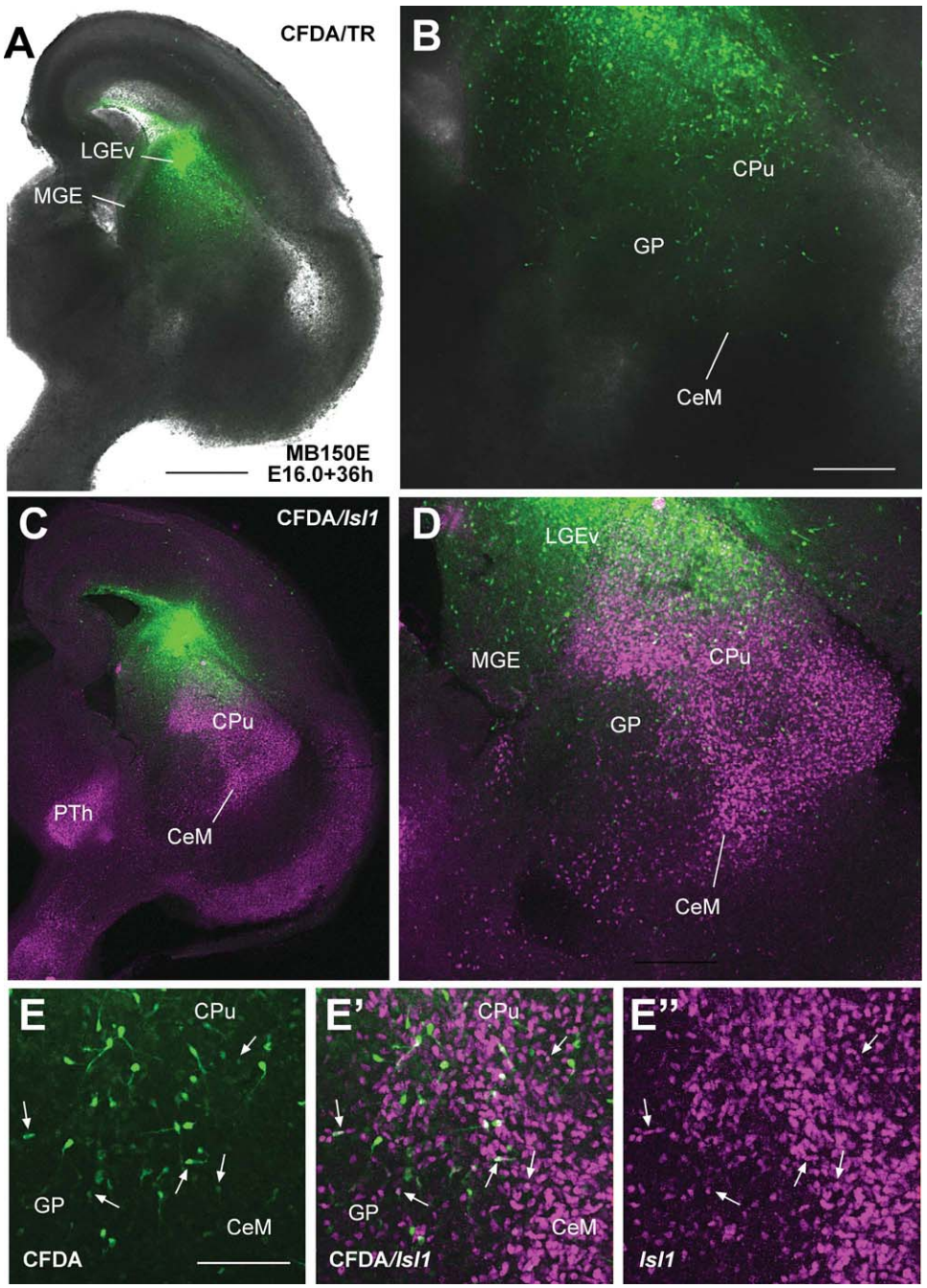

Figure 5. A–E″: Digital images from one representative organotypic culture of a telencephalic frontal slice of mouse embryo (MB150E), at a caudal telencephalic level, in which the fluorescent cell tracker CMFDA (green) was involving the ventral LGE. The slice was resectioned and immunolabeled to detect Islet1 (C–E″, shown in magenta). Many cells migrated into the striatal mantle (CPu) and the medial part of the central amygdala (CeM), overlapping the Islet1-expressing zone. In addition, some cells were observed to migrate tangentially to more ventral, pallidal regions and to invade the globus pallidus (GP) and more medial pallidal regions. In all of these places, cells were often double labeled for Islet1 (arrows in E–E″). Scale bars = 0.5 mm in A (applies to A,C); 0.2 mm in B (applies to B,D); 0.1 mm in E (applies to E–E″).

cells of the lateral BST derived from dorsal LGE were double labeled for Pax6, but never for Islet1 (Fig. 7D,E). Some of the cells in the lateral BST derived from either dorsal LGE or ventral LGE were double labeled with CB (Fig. 6G). A few cells from dorsal LGE also reached the medial BST (Fig. 6F) and, at caudal levels, the intraamygdaloid BST (Fig. 6A,B). No cells derived from dorsal LGE or ventral LGE were observed to invade the medial amygdala (Fig. 6A,B).

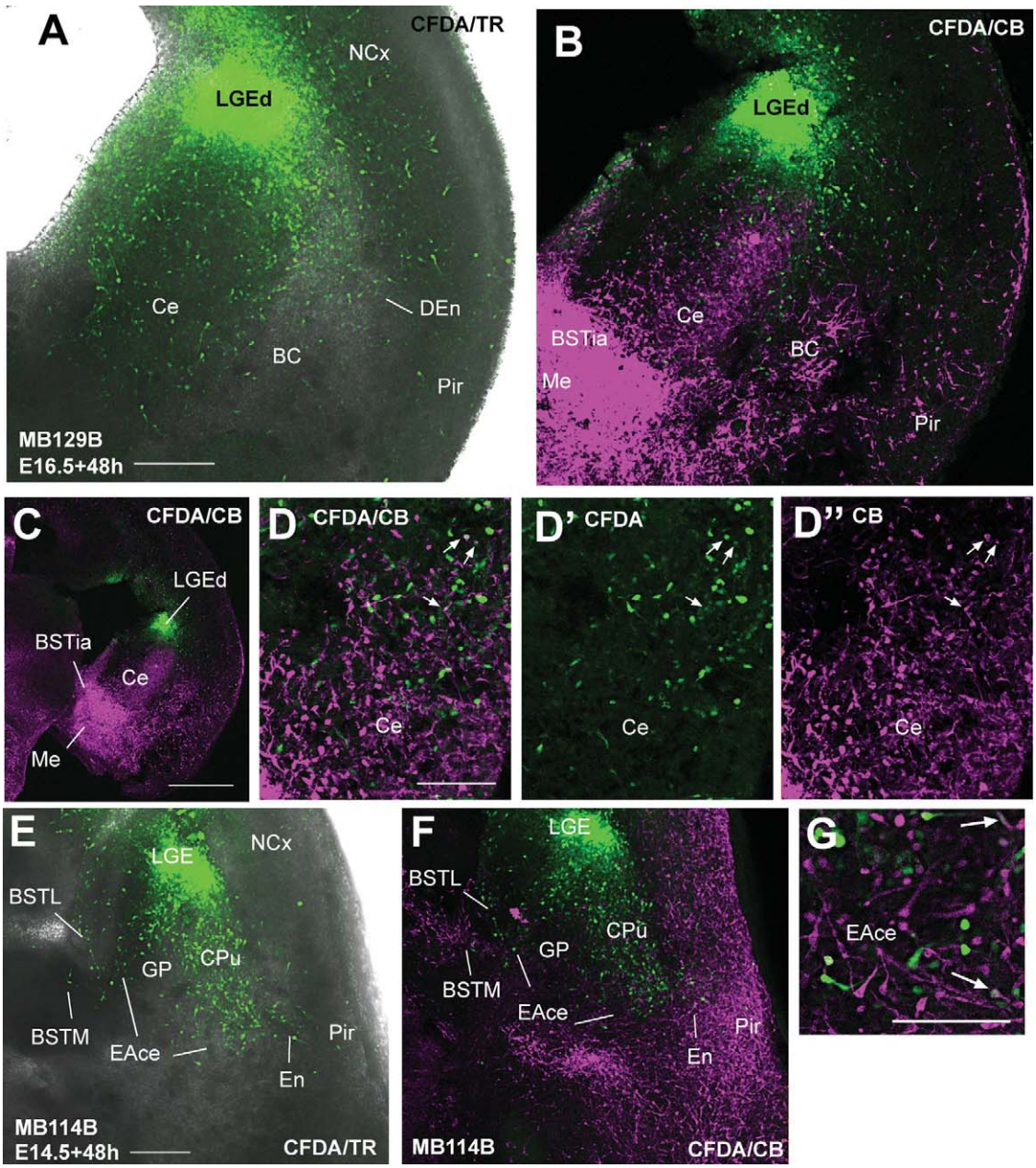

Figure 6. Digital images from two representative organotypic cultures of telencephalic frontal (MB129B, **A–D″**) or oblique horizontal (MB114B, **E–G**) slices of mouse embryo, in which the fluorescent cell tracker CMFDA (green) either was centered in dorsal LGE or was involving dorsal LGE and part of ventral LGE. The slices were immunolabeled to detect calbindin (CB; B–D″,F,G; shown in magenta), for helping in the identification of cell groups. Numerous cells migrated into the striatal mantle (CPu), the central amygdala (Ce), and other lateral parts of the central extended amygdala (EAce). In addition, some cells were observed to migrate tangentially to more ventral, pallidal regions and to invade the lateral BST (BSTL). Some cells in the central amygdala and other parts of the continuum were double labeled for calbindin (arrows in D–D″,G). See text for more details. For abbreviations see list. Scale bars = 0.2 mm in A (applies to A,B); 0.5 mm in C; 0.1 mm in D (applies to D–D″); 0.2 mm in E (applies to E,F); 0.1 mm in G.

Cell migration from mouse MGE to the central extended amygdala

Although the central amygdala derives primarily from the LGE (as noted above), it has been suggested that it also may contain a subpopulation of principal neurons containing SOM that originates in the ventrocaudal MGE or MGEvc (García-López et al., 2008; Real et al., 2009). To investigate this possibility, we carried out migration assays of telencephalic slices at E13.5–E16.5 and labeled the ventricular and/or subventricular zone of MGEvc with the fluorescent cell tracker CMFDA,

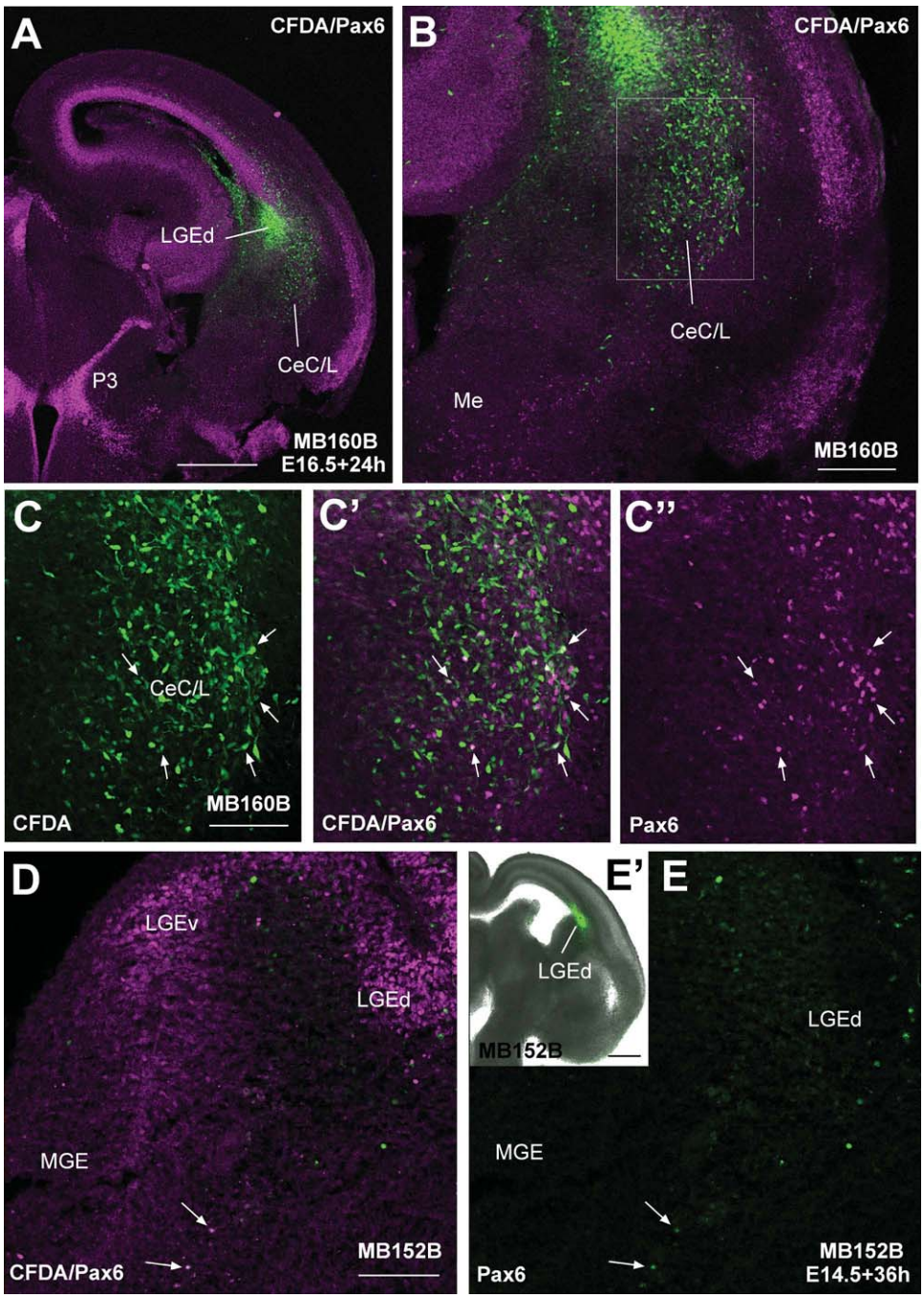

Figure 7. A–E′: Digital images from two representative organotypic cultures of telencephalic frontal slices of mouse embryo (MB152B and MB160B), at a caudal telencephalic level, in which the fluorescent cell tracker CMFDA (green) was centered in the dorsal LGE. The slices were resectioned and immunolabeled to detect Pax6 (A–D, magenta). Many cells migrated into the capsular/lateral part of the central amygdala (CeC/L), overlapping in this location Pax6-expressing cells (B; detail of the square area in C–C″). Some of the cells in CeC/L were double labeled for Pax6 (arrows in C–C″). In addition, a few cells were observed to migrate tangentially to more ventral, pallidal regions (medial to the globus pallidus) and appeared to invade posterior parts of the BST (D,E). Some of these cells were labeled for Pax6 (arrows in D,E). Scale bars = = 0.5 mm in A,E′; 0.2 mm in B; 0.1 mm in C (applies to C–C″); 0.2 mm in D (applies to D,E).

followed by immunofluorescence for SOM. To follow MGEvc cells into the amygdala, we labeled MGEvc progenitor domain either in frontal slices at caudal levels (Fig. 8) or in horizontal slices (Figs. 9, 10). After these experiments, numerous MGEvc-derived cells were observed in the medial extended amygdala (Fig. 8A,B,D,E, 9C,D, 10B,C), as expected (Bupesh et al., 2011). In addition, abundant cells were observed to

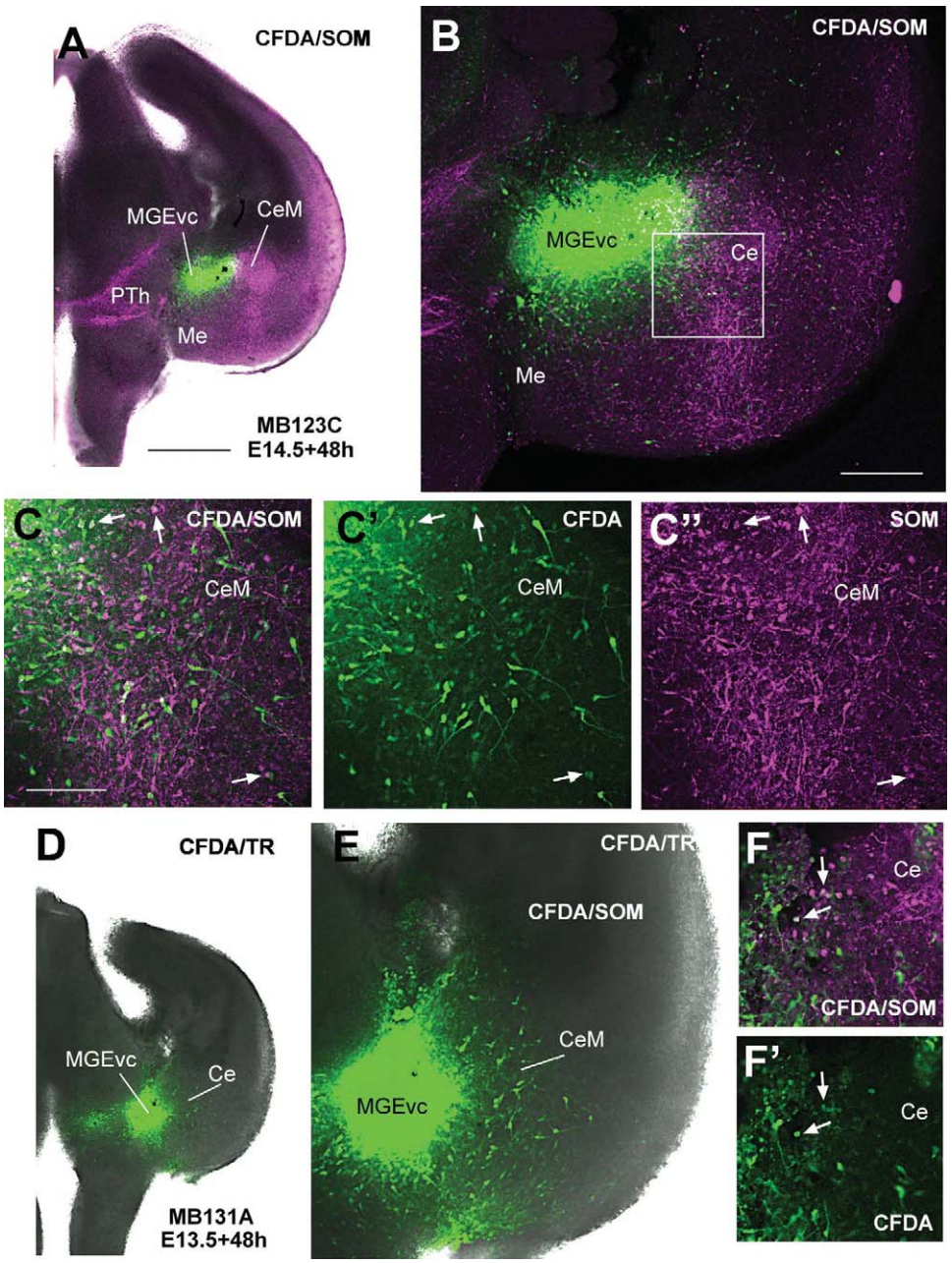

Figure 8. A–F″: Digital images from two representative organotypic cultures of telencephalic frontal slices of mouse embryo (MB123C in A–C″; and MB 131A, in D–F′), in which the fluorescent cell tracker CMFDA (green) was centered in the ventrocaudal MGE (MGEvc). The slices were resectioned and immunolabeled to detect somatostatin (SOM; B–C″,F, magenta). Many cells migrated into the medial part of the central amygdala (CeM), overlapping in this location many SOM-expressing cells (B; details of the boxed area are shown in C–C″). Some of the cells in CeM were double labeled for somatostatin (arrows in C–C″, and in F,F″). Scale bars = 0.5 mm in A (applies to A,D); 0.2 mm in B (applies to B,E); 0.1 mm in C (applies to C–C″,F,F′).

migrate tangentially to striatal and pallial territories (Figs. 8B,E, 9D, 10C), many of which likely constitute specific interneuron subtypes (Bupesh et al., 2011). Moreover, an important group of MGEvc-derived cells was observed to invade tangentially the central extended amygdala (Figs. 8B, 9C,D, 10C), including the SOM-rich part of the lateromedial part of the central amygdala (Figs. 9B, 10B,C). Immunofluorescence for SOM showed that many of these MGEvc-derived cells located in the central amygdala were double labeled (Figs. 8C–C″,F,F′, 10D–D″). On the other hand, some MGEvc-derived cells also invaded via tangential

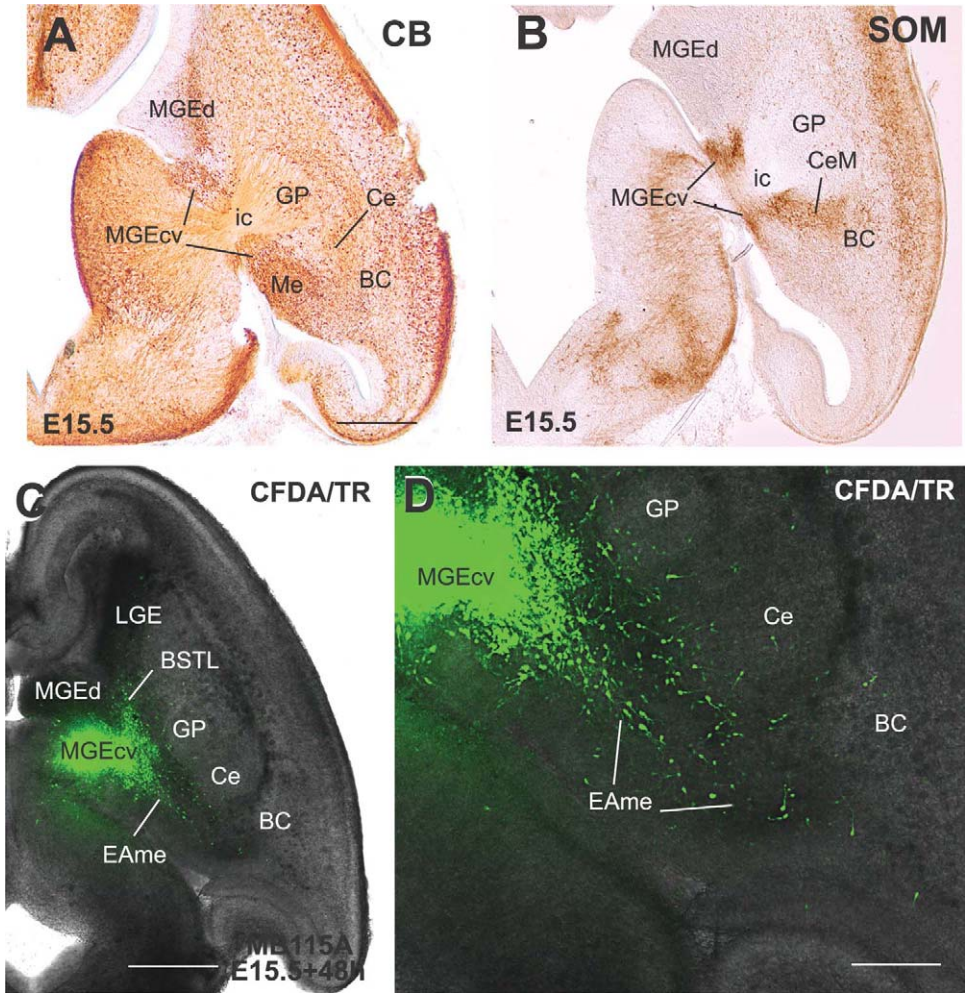

Figure 9. A,B: Digital images of horizontal sections through the telencephalon at the level of the central amygdala, which have been immunostained for either calbindin (CB; A) or somatostatin (SOM; B). The medial part of the central amygdala is rich in SOM^+ cells and fibers, which appear to originate from the ventrocaudal part of MGE (MGEvc). C,D: Digital images from a representative organotypic culture of a telencephalic horizontal slice of mouse embryo (MB115A), at a level roughly comparable to that shown in A,B, in which the fluoresencent cell tracker CMFDA (green) was centered in the ventrocaudal MGE (MGEvc). Many cells migrate through the medial extended amygdala corridor (as previously reported; Bupesh et al., 2011). In addition, some cells migrated tangentially and invaded the central amygdala, the striatum, and several pallial areas. A few cells also invaded the lateral BST and the globus pallidus. Scale bars = 0.4 mm n A (applies to A,B); 0.5 mm in C; 0.2 mm in D.

migration dorsal parts of the pallidum, including the globus pallidus and the lateral BST (Fig. 9C,D).

DISCUSSION

The central extended amygdala is the major output center for telencephalic control of ingestion, fear responses, and anxiety (Alheid et al., 1995; Heimer, 2003; Walker and Davis, 2008). By using a combination of migration assays, in situ hybridization, and immunohistochemistry/immunofluorescence, we have shown that its major components, the central amygdala and lateral BST, are mosaics formed by different proportions of dorsal LGE-, ventral LGE-, and MGE-derived neurons (this excludes the interneurons), as follows (Fig. 11, Table 2): 1) the capsular part of the central amygdala (excepting its ventromedial aspect) is formed primarily by Pax6-expressing cells derived from dorsal LGE and is generally poor in or free of Islet1-expressing cells derived from ventral LGE and SOM^+ cells derived from MGEvc; 2) the lateral part of the central amygdala contains moderately important subpopulations of Pax6-expressing cells derived from dorsal LGE (mostly in its lateralmost zone), Islet1-expressing cells derived from ventral LGE (mostly in its central and medial zones), and SOM^+ cells derived from MGEvc (mostly in its medial zone); 3) the medial part of the central amygdala contains only a minor subpopulation of Pax6-expressing cells derived from dorsal LGE

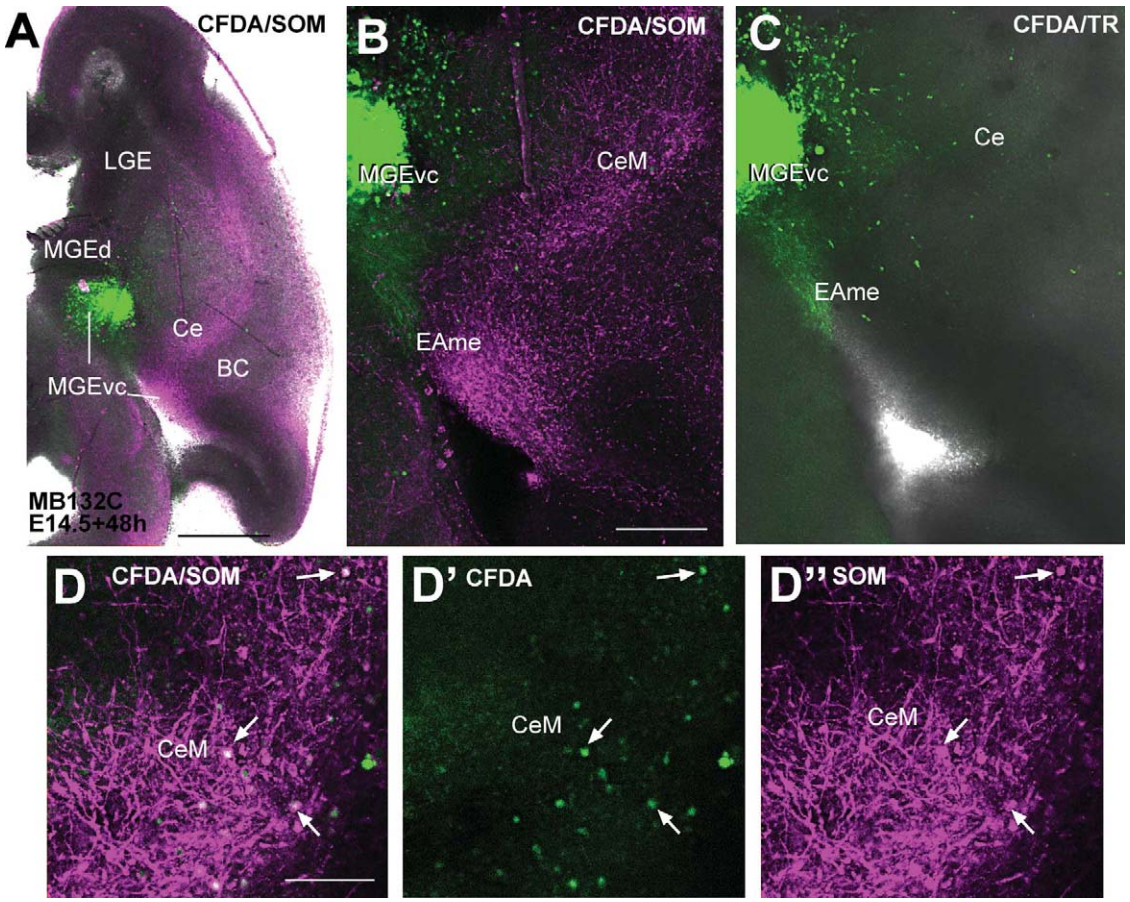

Figure 10. A–D″: Digital images from a representative organotypic culture of a telencephalic horizontal slice of mouse embryo (MB132C), in which the fluorescent cell tracker CMFDA (green) was centered in the ventrocaudal MGE (MGEvc). The slice was immunolabeled to detect somatostatin (SOM; magenta). Many cells migrated into the medial part of the central amygdala (CeM), overlapping in this location many SOM-expressing cells. Many of the cells in CeM were double labeled for somatostatin (arrows in D–D″). Scale bars = 0.5 mm in A; 0.2 mm in B (applies to B,C); 0.1 mm in D (applies to D–D″).

but includes major subpopulations of Islet1-expressing cells derived from ventral LGE and SOM^+ cells derived from MGEvc; and 4) the lateral BST, in addition to Nkx2.1-expressing neurons derived from MGE (Puelles et al., 2000; Xu et al., 2008; García-López et al., 2008), includes subpopulations of Pax6- and Islet1-expressing cells, which derive from either dorsal LGE or ventral LGE, respectively. Moreover, the strong correlation between Pax6 and ppENK in the central extended amygdala during development suggests that the dorsal LGE-derived cells that populate this continuum are mostly enkephalinergic. Nervertheless, ppENK-expressing cells are more abundant than Pax6-expressing cells (especially in the lateral BST), so it is likely that some of the enkephalinergic cells of the continuum (at least part of those in lateral BST) originate elsewhere. Finally, another novel finding of our study is that the medial amygdala includes a minor subpopulation of Pax6-expressing neurons, which does not originate in dorsal LGE but instead may immigrate from the prethalamic eminence. We discuss these results in separate sections below.

Distinct principal neurons of the central amygdala originate in dorsal LGE, ventral LGE, and MGEvc

A recent genetic fate mapping study using cre/LoxP transgenic mice has shown that projection neurons of the central amygdala derive, at least in part, from Islet1-expressing progenitors of ventral LGE (Waclaw et al., 2010), but it was unclear what specific subdivisions were populated by these cells. In addition, previous studies in mouse and chicken suggested that Pax6-expressing cells from dorsal LGE invade the central amygdala (Puelles et al., 2000; Tole et al., 2005; Abellán and Medina, 2009), but experimental demonstration was lacking. Our results on migration assays combined with immunofluorescence for Islet1 or Pax6 corroborate those of Waclaw et al.

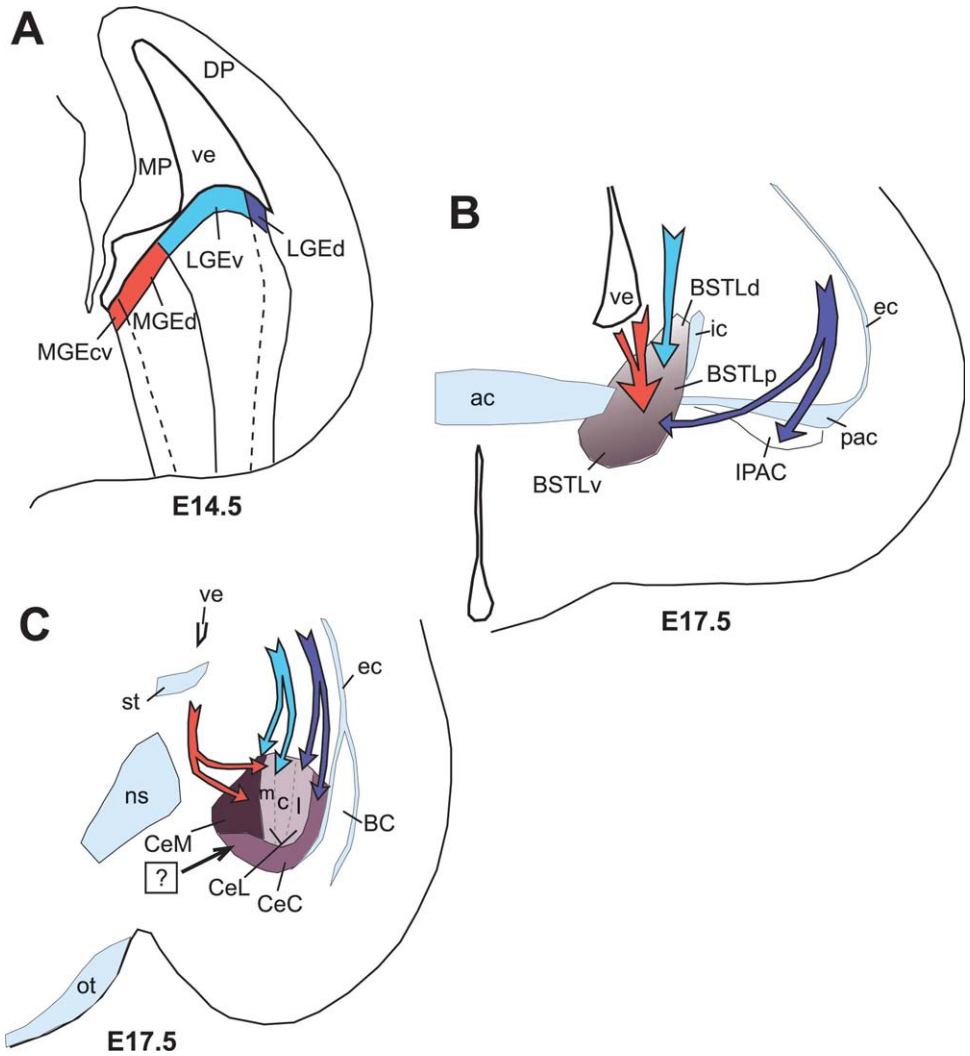

Figure 11. Schematic drawings of frontal sections of the embryonic telencephalon at early and prenatal stages, showing the major subdivisions of LGE and MGE and their contribution to the formation of the different parts of the central amygdala and BST. The question mark in C indicates a ventromedial part of the capsular central amygdala, which is rich in TH immunoreactivity but appears to contain only few cells expressing Pax6, ppENK, Islet1, or somatostatin (as noted in Figs. 2, 3). See text for other details. For abbreviations see list.

(2010) on ventral LGE and provide a novel experimental demonstration supporting the proposal of Puelles and other authors on dorsal LGE as an additional source of neurons for the central amygdala. Moreover, our results provide novel details on the distribution of dorsal LGE- vs. ventral LGE-derived cells in the central amygdala (Fig. 11). Dorsal LGE-derived cells express Pax6 and preferentially invade the capsular and lateralmost zone of the lateral subnuclei of the central amygdala, whereas ventral LGE-derived cells express Islet1 and populate primarily the centromedial zones of the lateral subnucleus and the medial subnucleus of the central amygdala. The strong correlation between Pax6 and ppENK in the capsular/lateral subnuclei of the central amygdala (present results; for ppENK, see also Poulin et al., 2006, 2008) suggests that most if not all enkephalinergic neurons of the central amygdala originate in dorsal LGE (Table 2). ppENK-expressing cells are very abundant in the capsular-lateral part of the central amygdala (present results in mouse, in agreementt with those in the rat by Poulin et al., 2006, 2008), so it is likely that they represent projection neurons and are involved in the output from these subdivisions to the substantia innominata, lateral BST, and lateral hypothalamus (Dong et al., 2001a; Petrovich et al., 2001; see also Poulin et al., 2008). In fact, a large part of the enkephalinergic innervation of the lateral BST has been shown to originate in the central amygdala (Rao et al., 1987). Therefore, projection neurons of the central amygdala derive at least from two distinct progenitor zones: ventral LGE (as indicated by Waclaw et al., 2010) and dorsal LGE (as proposed here). In addition to specific transcription factors and neuropeptides (see more on neuropeptides below), our data indicate that both

TABLE 2.

Derivatives of Dorsal LGE, Ventral LGE, and MGE[1] in the Central Amygdala

Progenitor domain	Transcription factor expressed by derived cells	Subdivisions populated by derived cells	Possible phenotype of derived neurons
LGE (dorsal)	Pax6	CeC	ENK^+
		CeL (l)	Some CB^+
LGE (ventral)	Islet1	CeL (c)	CRF^+ (some co contain NT and/or DYN), mainly in CeL (c)
		CeL (m)	CB^+, mainly in CeL (m)
		CeM	
MGE (ventrocaudal)	Nkx2.1, Lhx6	CeM	SOM^+ (some cocontain substance P)
		CeL (m)	

[1]Refers only to projection neurons, because MGE is also known to produce interneurons for the central amygdala (containing calbindin and/or some neuropeptides).

dorsal LGE and ventral LGE also produce CB-containing neurons for the central amygdala. According to previous studies in the rat, CB-immunoreactive cells are present in all subdivisions of the central amygdala, although they are particularly abundant in a dorsal part of the capsular central amygdala and in the medial one-third of the lateral central amygdala (also called the *intermediate central amygdala*) and may include projection neurons (McDonald, 1997; Kemppainen and Pitkäanen, 2000). Nevertheless, some CB cells in the central amygdala likely represent interneurons originating in the MGE (Bupesh et al., 2011).

In addition, we provide novel experimental data combined with immunofluorescence data indicating that MGEvc also produces many SOM-containing neurons of the medial (SOM-rich) part of the central amygdala (Fig. 11, Table 2), confirming similar suggestions previously made by García-López et al. (2008) and Real et al. (2009). The MGE (including its MGEvc subdivision, previously included as part of the AEP) is known to produce distinct classes of interneurons for the striatum and pallium/cortex, such as those described for the striatal and pallial parts of the amygdala (the central amygdala and basal amygdalar complex, respectively), which contain distinct calcium-binding proteins such as CB and/or neuropeptides such as SOM (Lavdas et al., 1999; Anderson et al., 2001; Marín and Rubenstein, 2001; Nery et al., 2002; Xu et al., 2004, 2008; Legaz et al., 2005a,b; García-López et al., 2008; Real et al., 2009; Bupesh et al., 2011). However, the SOM^+ cells of the medial aspect of the central amygdala, shown here to immigrate from MGEvc, possess a remarkably large perikaryon and do not resemble the typical peptidergic interneurons found in other parts of the amygdala (such as the interneurons cocontaining SOM and neuropeptide Y; McDonald, 1989) but rather resemble a subtype of projection neuron described in the central amygdala (Higgins and Schwaber, 1983; Moga and Gray, 1985; McDonald, 1987; discussion in García-López et al., 2008; and Real et al., 2009). Other centers of the telencephalon, such as the globus pallidus and the medial amygdala, include distinct subpopulations of projections neurons that originate in different progenitor domains, reaching their final destination either by radial or tangential migration (Nóbrega-Pereira et al., 2010; Bupesh et al., 2011). This appears to be part of a general mechanism of central nervous system development, by way of which different neuronal subtypes can be produced in molecularly distinct progenitor domains and/or at different times in the same domain (Briscoe and Ericson, 2001; Marín and Rubenstein, 2001; Flames et al., 2007). In initial formulations regarding the telencephalon, it was thought that, as a general rule, principal neurons of any given center reach the final destination by radial migration, whereas interneurons often reach the center by tangential migration (Marín and Rubenstein, 2001). As noted above, recent data indicate that this proposed rule is not so general, at least in telencephalon, inasmuch as distinct principal neurons in the globus pallidus (Nobrega-Pereira et al., 2010) and the extended amygdala (Bupesh et al., 2011; present data) appear to reach their final destination by radial and tangential migrations. Thus, at least in the telencephalon, the latter situation is more common that previously thought.

Like those of the striatum proper, projection neurons of the central amygdala and other striatal-like amygdalar nuclei (such as the intercalated cells) are GABAergic and cocontain one or more neuropeptides, such as enkephalin, corticotropin-releasing factor (CRF), SOM, neurotensin, substance P, and dynorphin (Moga and Gray, 1985; Rao et al., 1987; Shimada et al., 1989; Paré and Smith, 1994; Alheid et al., 1995; Swanson and Petrovich, 1998; Poulin et al., 2008; Reyes et al., 2008; Panguluri et al., 2009). These principal neurons can be grouped into three major subtypes, which show a trend to locate in separate domains of the central amygdala (Shimada et al., 1989; Poulin et al., 2006, 2008; Marchant et al., 2007),

although considerable overlap exists (Cassell et al., 1986). These three major neuron subtypes are enkephalinergic (ENK^+) cells (Rao et al., 1987; Poulin et al., 2006, 2008), neurons containing CRF (CRF^+) that cocontain neurotensin and/or dynorphin (Shimada et al., 1989; Marchant et al., 2007), and neurons that contain SOM (SOM^+) and substance P (Shimada et al., 1989). Note that these are the major subtypes, but there are minor subpopulations containing only one of the neuropeptides mentioned above. It appears that ENK^+ neurons are enriched in the capsular central amygdala and the lateral one-third of the lateral central amygdala (Poulin et al., 2006, 2008; present results); CRF^+ neurons cocontaining neurotensin and/or dynorphin are concentrated primarily in the central portion of the lateral central amygdala (Marchant et al., 2007); and SOM^+ neurons locate primarily in the medial central amygdala and the adjacent medial one-third of the lateral central amygdala (Shimada et al., 1989; present results). Based on their differential distribution and the developmental data presented here, it appears that SOM^+ cells derive from MGEvc (Table 2). Moreover, based on the high correlation between Pax6 and ppENK, it is likely that ENK^+ cells of the central amygdala originate mostly in the dorsal LGE (Table 2). Finally, based on the correlation between CRF/dynorphin and Islet1 (but not Pax6, ppENK, or SOM), it is likely that CRF^+ cells originate in ventral LGE (Table 2). Thus, three distinct progenitor domains of the subpallium may produce the three major principal neurons of the central amygdala.

The three major neuron subtypes of the central amygdala mentioned above appear to play different roles in fear responses and anxiety, suggesting a relationship between embryonic origin and cellular function. Thus, neurons of the medial subnucleus of the central amygdala (including SOM^+/substance P^+ cells) project to lateral hypothalamic and brainstem centers (central gray, parabrachial nucleus, dorsal vagal complex) that mediate fear behavior (Gray and Magnuson, 1992) and are particularly involved in short-term fear responses or phasic fear (defined as "a generally adaptive state of apprehension that begins rapidly and dissipates quickly once the threat is removed"; Walker and Davis, 2008; Davis et al., 2010). In contrast, the lateral subnucleus of the central amygdala, in particular its CRF^+ cells, which give rise to descending projections to the same lateral hypothalamic and brainstem centers targetted by the medial subnucleus (Gray and Magnuson, 1992; Gray, 1993), are involved in initiating a slower developing but sustained (long-lasting) fear response, akin to anxiety (produced by "less specific and less predictable threats, or by those that are physically or psychologically more distant"; Walker and Davis, 2008; Davis et al., 2010). Both CRF^+ cells and SOM^+ cells of the central amygdala projecting to the lateral hypothalamus and brainstem also appear to play a role in taste processing and control of ingestion behavior (Panguluri et al., 2008). The fact that the same circuits control fear responses and ingestion is not surprising, because fear, stress, and anxiety are linked to suppression of eating and anorexia nervosa (Petrovich et al., 2009, and references therein). On the other hand, it appears that ENK^+ neurons of the central amygdala do not project directly to the brainstem (Moga and Gray, 1985; Gray and Magnuson, 1987, 1992) but appear to be involved in projections to the lateral BST, being responsible of part of the enkephalinergic innervation in this center (Rao et al., 1987). Central amygdalar ENK^+ projections may modulate directly or indirectly the role of BSTL in stress and anxiety-like behaviors (Walker et al., 2009; Kung et al., 2010), although they also appear to play a role in the context-specific behavioral effects of psychostimulants (Day et al., 2001). Moreover, because ENK^+ cells are so abundant in the capsular subnucleus of the central amygdala (Poulin et al., 2008, present results), it is likely that these cells are involved in other known projections of this subdivision, such as the intranuclear projections to the medial subdivision of the central amygdala (Petrovich and Swanson, 1997; Jolkkonen and Pitkänen, 1998) and the projections to the lateral hypothalamus (Petrovich et al., 2001). The capsular and medial parts of the central amygdala show a light projection to the parvocellular/autonomic part of the paraventricular hypothalamic nucleus (Gray et al., 1989; Petrovich et al., 2001). This part of the nucleus contains cells that are both innervated by ENK^+ axons (perhaps including axon terminals from ENK^+ cells of the central amygdala and lateral BST) and activated by noxious (stressful) stimulation (Pretel and Piekut, 1991) and contains ENK^+ neurons that project to a zone of the parabrachial nucleus different from that targeted by the central amygdala (Moga et al., 1989). This part of the parabrachial nucleus may include nociceptive neurons, reported to be activated by noxious stimuli and to project to the central amygdala, being part of a pathway involved in emotional responses to noxious events (Bernand and Besson, 1988). Thus, there appears to be a distinct ENK-related descending pathway from the central extended amygdala, which may modulate specific aspects of stress and anxiety in relation to nociception (such as stress-induced analgesia; see Poulin et al., 2006, and references therein). In addition, the central amygdala plays an important role in associative learning, including aversive learning (Petrovich et al., 2009) and conditional fear (Paré et al., 2004; Wilensky et al., 2006; Jimenez and Maren, 2009; Ciocchi et al., 2010), and it appears that ENK levels in neurons of the central

amygdala are involved in the modulation of learned fear responses (Petrovich et al., 2000).

The IPAC, the AAd, and the ITC derive primarily from dorsal LGE but include cells from MGE

Previous data based on expression of Dlx5 compared with either pallial (such as Tbr1) or pallidal (such as Nkx2.1, Lhx6) marker transcription factors indicated that the IPAC, the AAd, and the ITC derive from LGE and, together with the central amygdala, are part of the "striatal amygdala" (Medina et al., 2004; García-López et al., 2008). In agreement with their subpallial origin, the IPAC, AAd, and ITC are rich in GABAergic neurons and poor in glutamatergic cells (Paré and Smith, 1994; Alheid et al., 1995; Swanson, 2000; Poulin et al., 2008). Our results on migration assays and Pax6 expression confirm the striatal nature of these cell groups and indicate that, in particular, most cells of the IPAC, AAd, and ITC derive from dorsal LGE. Similarly to the cells of the central amygdala that derive from dorsal LGE (see previous section), most neurons of IPAC (its lateral part) and ITC are enkephalinergic, and these may include projection neurons (present results; Poulin et al., 2008). Nevertheless, IPAC and ITC also receive a subpopulation of neurons from MGEvc (García-López et al., 2008; present results based on migration assays), but it is unclear whether these cells are only interneurons (Bupesh et al., 2011) or also include projection neurons. Some of these MGE-derived cells in the IPAC were immunoreactive for SOM, thus resembling the situation found in the medial part of the central amygdala. Our data agree with results from recent studies showing that ITC (including the main intercalated masses and the paracapsular cells) derive from dorsal LGE, based on expression of several transcription factors, such as Foxp2, Meis2, and Pax6 (Kaoru et al., 2010; Waclaw et al., 2010), and on results from Sp8 and Gsx2 conditional inactivation (Waclaw et al., 2010). Together with the central amygdala and lateral BST, the ITC, IPAC (in particular, its medial subdivision), and part of the substantia innominata are considered part of the central extended amygdala system, showing chemoarchitecture, connections, and function similar to those of other parts of the continuum (Alheid et al., 1995, 1999; Shammah-Lagnado et al., 1999, 2001; Paré et al., 2004). Among these groups, the ITC are the best studied, because their GABAergic cells constitute an interface that appears to gate the connections between the basal amygdalar complex and the medial part of the central amygdala (Paré et al., 2004), and their contribution is essential for extinction of conditioned fear responses (Jüngling et al., 2008; Likhtik et al., 2008).

The lateral BST contains neurons from MGE but also from both dorsal LGE and ventral LGE

Different developmental studies based on expression of the transcription factors Nkx2.1 and Lhx6 (Puelles et al., 2000; García-López et al., 2008) and genetic fate map of Nkx2.1 progenitor cells in the telencephalon (Xu et al., 2008) have indicated that the BST derives primarily from MGE. This supports previous claims suggesting that the BST, including lateral BST and medial BST, represents the pallidal part of the extended amygdala (Swanson, 2000) or at least contains a pallidal-like part (Alheid and Heimer, 1988; Alheid et al., 1995). However, recent data based on migration assays and expression of transcription factors and other regulatory genes indicate that this simple scheme has to be modified, because the medial BST, similarly to the medial amygdala, also includes neuron subpopulations derived from the preoptic area and the alar hypothalamus (García-López et al., 2008; Bupesh et al., 2011). In addition, it is likely that these cell groups belonging to the medial extended amygdala also include some cells derived from the prethalamic eminence, as proposed by Puelles and colleagues (2000). Our present data on Pax6 combined with migration assays suggest that this may be the case. As noted in Results, in addition to the stream of Pax6-expressing cells derived from dorsal LGE, we detected another stream of Pax6 cells reaching the amygdala that appears to extend from and originate in the prethalamic eminence. These Pax6 cells appear to invade and constitute a small neuron subpopulation in the medial amygdala (as seen in prenatal stages) and do not appear to originate in dorsal LGE, insofar as our migration assays discarded this possibility. A contribution of Pax6-expressing cells from the prethalamic eminence to the extended amygdala is also present in the chicken (Abellán and Medina, 2009).

With regard to the lateral BST, previous data based on Pax6 expression in the chicken suggested that, in addition to MGE-derived cells, it may include a subpopulation of cells derived from the dorsal LGE (Abellán and Medina, 2009). A similar suggestion was made for the mouse based on expression of Lmo4, an LIM-only gene typically expressed in the striatal mantle but not the pallidum during development (García-López et al., 2008). Alheid and colleagues (Alheid and Heimer, 1988; Alheid et al., 1995) also suggested, based on cell morphology and connections, that the lateral BST may include a striatal-like neuron subpopulation or subdivision. Our data on migration assays and Pax6 confirmed that dorsal LGE produces a small subset of cells for the lateral BST in the mouse, some of which express Pax6 (some also express CB). In addition, our results on migration assays and Islet1

indicate that ventral LGE also produces many cells for the lateral BST, which accounts for at least part of the Islet1-expressing cells seen in this nucleus (which are numerous in the dorsal and posterior subnuclei and scarce in the ventral subnucleus). Thus, the lateral BST includes neurons from the same progenitor domains as the central amygdala, i.e., dorsal LGE, ventral LGE, and MGE. Moreover, our migration assays indicate that lateral BST may include cells from both dorsal and ventrocaudal parts of MGE (see also García-López et al., 2008; and Bupesh et al., 2011). As noted above, a similar situation of multiple origins in LGE and several MGE subdomains has been reported recently for the principal neurons of the globus pallidus (Nobrega-Pereira et al., 2010). The lateral BST also includes subpopulations of projection neurons similar to those described for the central amygdala: neurons coexpressing CRF and neurotensin in the dorsal subnucleus and neurons expressing SOM and substance P in the ventral and posterior subnuclei (Gray and Magnuson, 1987, 1992; Shimada et al., 1989; the posterior subnucleus also contains neurons containing neurotensin alone: Moga et al., 1989). These neurons of lateral BST are contacted by axons from "homologous" cells of the central amygdala and appear to project to the same targets in the lateral hypothalamus and brainstem as those (Gray and Magnuson, 1987, 1992; see above). It appears that the central amygdala (primarily its medial part) and lateral BST projections play complementary roles for control of either phasic or sustained fear, respectively (Walker and Davis, 2008; Walker et al., 2009; Davis et al., 2010). Similarly to those of the central amygdala, CRF^+ cells of the lateral BST may originate in ventral LGE, whereas those expressing SOM may derive from MGEvc, but additional studies are needed to investigate this possibility. On the other hand, like the central amygdala, the lateral BST contains neurons expressing enkephalin (Gray and Magnuson, 1987, 1992; Poulin et al., 2008; present results), but these neurons do not appear to project to the brainstem (Gray and Magnuson, 1987, 1992; Moga et al., 1989), although they are involved in the reciprocal projections from lateral BST to the central amygdala (Poulin et al., 2006) and perhaps in the projections to the hypothalamus (Dong et al., 2001b). As noted above, based on the high correlation between Pax6 and ppENK, most enkephalinergic neurons of the central amygdala, IPAC, and ITC appear to derive from dorsal LGE, and this may be true for at least some of those of the lateral BST. However, the number of ppENK-expressing cells in the lateral BST is far larger that that of Pax6-expressing cells. Therefore, many ppENK-expressing cells of the lateral BST likely have a different origin.

The central extended amygdala continuum

The concept of the extended amygdala was rescued and redefined by de Olmos, Alheid, and Heimer, who subdivided it into two separate cell corridors that differ in neurochemistry and connections, the central extended amygdala and the medial extended amygdala (de Olmos et al., 1985, 2004; Alheid and Heimer, 1988; Alheid et al., 1995; Heimer, 2003). As noted above, the central extended amygdala includes the ITC, central amygdala, IPAC, part of the substantia innominata, and lateral BST and is essential for control of ingestion and for fear, stress, and anxiety responses. Numerous publications show the similarity in the neurochemistry and connections of the cells along this cell corridor (some of them are mentioned in previous sections). However, developmental data of the nuclei forming the central extended amygdala were scarce until recently, and they have provided information on the distinct embryonic origin of different parts of the corridor, either in LGE (ITC, central amygdala; Puelles et al., 2000; Medina et al., 2004; Kaoru et al., 2010; Waclaw et al., 2010) or in MGE (lateral BST; Puelles et al., 2000; Xu et al., 2004; García-López et al., 2008). Moreover, according to those data, striatal parts of the amygdala, such as ITC and central amygdala, appeared to derive primarily from separate dorsal or ventral sectors of LGE, respectively (Kaoru et al., 2010; Waclaw et al., 2010). Therefore, these data did not support a uniformity along the cell corridor in terms of embryonic origin and could not explain the similarity of their cells regarding neurochemistry and connectivity. Here we provide novel results that helps to resolve this puzzle. Thus, the nuclei that form the central extended amygdala, particularly the central amygdala and the lateral BST, are formed by different proportions of neurons derived from dorsal LGE, ventral LGE, or MGE. Based on the data discussed in previous sections, it appears that many cells derived from dorsal LGE are enkephalinergic and populate primarily the ITC, the capsular central amygdala, the lateral one-third of the lateral central amygdala, and the IPAC, and a few also appear to reach the lateral BST. On the other hand, cells derived from ventral LGE may include CRF^+ neurons (often cocontaining neurotensin and/or dynorphin) and locate primarily in the central part of the lateral central amygdala and the dorsal and (less often) posterior subnuclei of the lateral BST (for location of CRF-, neurotensin-, and/or dynorphin-containing neurons see Moga et al., 1989, and Marchant et al. 2007). Finally, cells derived from MGEvc appear to contain SOM^+ and substance P and locate primarily in the medial part of the central amygdala and in the ventral and posterior subnuclei of the lateral BST (present results; for lateral BST see Moga et al., 1989). MGE-derived SOM^+ cells

also invade other parts of the central extended amygdala, such as the IPAC. Based on the data presented above, there appears to be a trend for cells with the same neurochemical profile to be connected with "homologous" cells along the corridor and for homologous cells to project to similar targets in the hypothalamus/brainstem (see references in previous sections). Thus, our data provide embryonic support for the existence of the central amygdala corridor and for the similarity in terms of neurochemistry and connections. Our data also indicate that distinct cells of the corridor originate in separate progenitor domains. Finally, our data also support, at least partially, the suggestion that capsular/lateral parts of the central amygdala are primarily striatal-like, whereas the medial part of the central amygdala and the lateral BST are primarily pallidal-like but also include some striatal-like cells (Alheid and Heimer, 1988). Although these subdivisions contain a mixture of LGE- and MGE-derived cells, most cells of the capsular/lateral parts of the central amygdala derived from LGE, whereas many or most neurons in the medial part of the central amygdala and lateral BST appear to derived from MGE.

LITERATURE CITED

Abellán A, Medina L. 2008. Expression of cLhx6 and cLhx7/8 suggests a pallidopedunculopreoptic origin for the lateral and medial parts of the avian bed nucleus of the stria terminalis. Brain Res Bull 75:299–304.

Abellán A, Medina L. 2009. Subdivisions and derivatives of the chicken subpallium based on expression of LIM and other regulatory genes and markers of neuron subpopulations during development. J Comp Neurol 515:465–501.

Abellán A, Vernier B, Rétaux S, Medina L. 2010. Similarities and differences in the forebrain expression of Lhx1 and Lhx5 between chicken and mouse: Insights for understanding telencephalic development and evolution. J Comp Neurol 518:3512–3528.

Alheid GF, Heimer L. 1988. New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience 27: 1–39.

Alheid GF, de Olmos J, Beltramino CA. 1995. Amygdala and extended amygdala. In: Paxinos G, editor. The rat nervous system. San Diego: Academic Press. p 495–578.

Alheid GF, Shammah-Lagnado SJ, Beltramino CA. 1999. The interstitial nucleus of the posterior limb of the anterior commissure: a novel layer of the central division of extended amygdala. Ann N Y Acad Sci 877:645–654.

Alifragis P, Parnavelas JG, Nadarajah B. 2002. A novel method of labeling and characterizing migrating neurons in the developing central nervous system. Exp Neurol 174: 259–265.

Anderson SA, Marín O, Horn C, Jennings K, Rubenstein JLR. 2001. Distinct cortical migrations from the medial and lateral ganglionic eminences. Development 128:353–363.

Bernard JF, Besson JM. 1988. Convergence of nociceptive information on the parabrachio-amygdala neurons in the rat. C R Acad Sci III 307:841–847.

Briscoe J, Ericson J. 2001. Specification of neuronal fates in the ventral neural tube. Curr Opin Neurobiol 11:43–49.

Bupesh M, Legaz I, Abellán A, Medina L. 2011. Multiple telencephalic and extratelencephalic embryonic domains contribute neurons to the medial extended amygdala. J Comp Neurol 519:1505–1525.

Cameron DA, Pennimpede T, Petkovich M. 2009. Tulp3 is a critical repressor of mouse hedgehog signaling. Dev Dyn 238:1140–1149.

Cassell MD, Gray TS, Kiss JZ. 1986. Neuronal architecture in the rat central nucleus of the amygdala: a cytological, hodological, and immunocytochemical study. J Comp Neurol 246:478–499.

Choi GB, Dong HW, Murphy AJ, Valenzuela DM, Yancopoulos GD, Swanson LW, Anderson DJ. 2005. Lhx6 delineates a pathway mediating innate reproductive behaviors from the amygdala to the hypothalamus. Neuron 46:647–660.

Ciocchi S, Herry C, Grenier F, Wolff SB, Letzkus JJ, Vlachos I, Ehrlich I, Sprengel R, Deisseroth K, Stadler MB, Müller C, Lüthi A. 2010. Encoding of conditioned fear in central amygdala inhibitory circuits. Nature 468:277–282.

Davis M, Walker DL, Miles L, Grillon C. 2010. Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. Neuropsychopharmacology 35: 105–135.

Day HE, Badiani A, Uslaner JM, Oates MM, Vittoz NM, Robinson TE, Watson SJ Jr, Akil H. 2001. Environmental novelty differentially affects c-fos mRNA expression induced by amphetamine or cocaine in subregions of the bed nucleus of the stria terminalis and amygdala. J Neurosci 21: 732–740.

de Olmos JS, Alheid GF, Beltramino CA. 1985. Amygdala. In: Paxinos G, editor. The rat nervous system. Sydney: Academic Press. p 223, 334.

de Olmos JS, Beltramino CA, Alheid G. 2004. Amygdala and extended amygdala of the rat: a cytoarchitectonical, fibroarchitectonical, and chemoarchitectonical survey. In: Paxinos G, editor. The rat nervous system, 3rd ed. Amsterdam: Elsevier-Academic Press. p 509–603.

Dong HW, Petrovich GD, Swanson LW. 2001a. Topography of projections from amygdala to bed nuclei of the stria terminalis. Brain Res Brain Res Rev 38:192–246.

Dong HW, Petrovich GD, Watts AG, Swanson LW. 2001b. Basic organization of projections from the oval and fusiform nuclei of the bed nuclei of the stria terminalis in adult rat brain. J Comp Neurol 436:430–455.

Elshatory Y, Gan L. 2008. The LIM-homeobox gene *Islet-1* is required for the development of restricted forebrain cholinergic neurons. J Neurosci 28:3291–3297.

Ericson J, Rashbass P, Schedl A, Brenner-Morton S, Kawakami A, van Heyningen V, Jessell TM, Briscoe J. 1997. Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. Cell 90:169–180.

Fallon JH, Koziell DA, Moore RY. 1978. Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal cortex and entorhinal cortex. J Comp Neurol 180:509–532.

Flames N, Pla R, Gelman DM, Rubenstein JL, Puelles L, Marín O. 2007. Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. J Neurosci 27:9682–9695.

Foster GA. 1998. Chemical neuroanatomy of the prenatal rat brain. A developmental atlas. Oxford: Oxford University Press.

García-López M, Abellán A, Legaz I, Rubenstein JL, Puelles L, Medina L. 2008. Histogenetic compartments of the mouse centromedial and extended amygdala based on gene expression patterns during development. J Comp Neurol 506:46–74.

García-Moreno F, Pedraza M, Di Giovannantonio LG, Di Salvio M, López-Mascaraque L, Simeone A, De Carlos JA. 2010. A

neuronal migratory pathway crossing from diencephalon to telencephalon populates amygdala nuclei. Nat Neurosci 13:680–689.

Gray TS. 1993. Amygdaloid CRF pathways. Role in autonomic, neuroendocrine, and behavioral responses to stress. Ann N Y Acad Sci 697:53–60.

Gray TS, Magnuson DJ. 1987. Neuropeptide neuronal efferents from the bed nucleus of the stria terminalis and central amygdaloid nucleus to the dorsal vagal complex in the rat. J Comp Neurol 262:365–374.

Gray TS, Magnuson DJ. 1992. Peptide immunoreactive neurons in the amygdala and the bed nucleus of the stria terminalis project to the midbrain central gray in the rat. Peptides 13:451–460.

Gray TS, Carney ME, Magnuson DJ. 1989. Direct projections from the central amygdaloid nucleus to the hypothalamic paraventricular nucleus: possible role in stress-induced adrenocorticotropin release. Neuroendocrinology 50: 433–446.

Heimer L. 2003. A new anatomical framework for neuropsychiatric disorders and drug abuse. Am J Psychiatry 160: 1726–1739.

Higgins GA, Schwaber JS. 1983. Somatostatinergic projections from the central nucleus of the amygdala to the vagal nuclei. Peptides 4:657–662.

Hirata T, Li P, Lanuza GM, Cocas LA, Huntsman MM, Corbin JG. 2009. Identification of distinct telencephalic progenitor pools for neuronal diversity in the amygdala. Nat Neurosci 12:141–149.

Jacobowitz DM, Abbott LC. 1997. Chemoarchitectonic atlas of the developing mouse brain. Boca Raton, FL: CRC Press.

Jimenez SA, Maren S. 2009. Nuclear disconnection within the amygdala reveals a direct pathway to fear. Learn Mem 16: 766–768.

Johnston JB. 1923. Further contributions to the study of the evolution of the forebrain. J Comp Neurol 35:337–481.

Jolkkonen E, Pitkänen A. 1998. Intrinsic connections of the rat amygdaloid complex: projections originating in the central nucleus. J Comp Neurol 395:53–72.

Jüngling K, Seidenbecher T, Sosulina L, Lesting J, Sangha S, Clark SD, Okamura N, Duangdao DM, Xu YL, Reinscheid RK, Pape HC. 2008. Neuropeptide S-mediated control of fear expression and extinction: role of intercalated GABAergic neurons in the amygdala. Neuron 59:298–310.

Kaoru T, Liu FC, Ishida M, Oishi T, Hayashi M, Kitagawa M, Shimoda K, Takahashi H. 2010. Molecular characterization of the intercalated cell masses of the amygdala: implications for the relationship with the striatum. Neuroscience 166:220–230.

Kawakami A, Kimura-Kawakami M, Nomura T, Fujisawa H. 1997. Distributions of PAX6 and PAX7 suggest their involvement in both early and late phases of chick brain development. Mech Dev 66:119–130.

Kemppainen S, Pitkänen A. 2000. Distribution of parvalbumin, calretinin, and calbindin-D(28k) immunoreactivity in the rat amygdaloid complex and colocalization with gamma-aminobutyric acid. J Comp Neurol 426:441–467.

Kung JC, Chen TC, Shyu BC, Hsiao S, Huang AC. 2010. Anxiety- and depressive-like responses and c-fos activity in preproenkephalin knockout mice: oversensitivity hypothesis of enkephalin deficit-induced posttraumatic stress disorder. J Biomed Sci 17:29.

Lavdas AA, Grigoriou M, Pachnis V, Parnavelas JG. 1999. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J Neurosci 19:7881–7888.

Legaz I. 2006. Caracterización genética y origen de las neuronas de la región claustroamigdalina en ratón. PhD Dissertation, University of Murcia, Spain.

Legaz I, García-López M, Medina L. 2005a. Subpallial origin of part of the Calbindin-positive neurons of the claustral complex and piriform cortex. Brain Res Bull 66:470–474.

Legaz I, Olmos L, Real MA, Guirado S, Dávila JC, Medina L. 2005b. Development of neurons and fibers containing calcium-binding proteins in the pallial amygdala of mouse, with special emphasis in those of the basolateral amygdalar complex. J Comp Neurol 488:492–513.

Likhtik E, Popa D, Apergis-Schoute J, Fidacaro GA, Paré D. 2008. Amygdala intercalated neurons are required for expression of fear extinction. Nature 454:642–645.

Marchant NJ, Densmore VS, Osborne PB. 2007. Coexpression of prodynorphin and corticotrophin-releasing hormone in the rat central amygdala: evidence of two distinct endogenous opioid systems in the lateral division. J Comp Neurol 504:702–715.

Marín O, Rubenstein JLR. 2001. A long, remarkable journey: tangential migration in the telencephalon. Nat Rev Neurosci 2:780–790.

Marín F, Herrero MT, Vyas S, Puelles L. 2005. Ontogeny of tyrosine hydroxylase mRNA expression in mid- and forebrain: neuromeric pattern and novel positive regions. Dev Dyn 234:709–717.

McDonald AJ. 1987. Somatostatinergic projections from the amygdala to the bed nucleus of the stria terminalis and medial preoptic-hypothalamic region. Neurosci Lett 75: 271–277.

McDonald AJ. 1989. Coexistence of somatostatin with neuropeptide Y, but not with cholecystokinin or vasoactive intestinal peptide, in neurons of the rat amygdala. Brain Res 500:37–45.

McDonald AJ. 1997. Calbindin-D28k immunoreactivity in the rat amygdala. J Comp Neurol 383:231–244.

Medina L, Legaz I, González G, de Castro F, Rubenstein JLR, Puelles L. 2004. Expression of Dbx1, neurogenin 2, semaphorin 5A, cadherin 8, and Emx1 distinguish ventral and lateral pallial histogenetic divisions in the developing claustroamygdaloid complex. J Comp Neurol 474:504–523.

Moga MM, Gray TS. 1985. Evidence for corticotropin-releasing factor, neurotensin, and somatostatin in the neural pathway from the central nucleus of the amygdala to the parabrachial nucleus. J Comp Neurol 241:275–284.

Moga MM, Saper CB, Gray TS. 1989. Bed nucleus of the stria terminalis: cytoarchitecture, immunohistochemistry, and projection to the parabrachial nucleus in the rat. J Comp Neurol 283:315–332.

Nery S, Fishell G, Corbin JG. 2002. The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. Nat Neurosci 5:1279–1287.

Nóbrega-Pereira S, Gelman D, Bartolini G, Pla R, Pierani A, Marín O. 2010. Origin and molecular specification of globus pallidus neurons. J Neurosci 30:2824–2834.

Panguluri S, Saggu S, Lundy R. 2009. Comparison of somatostatin and corticotrophin-releasing hormone immunoreactivity in forebrain neurons projecting to taste-responsive and non-responsive regions of the parabrachial nucleus in rat. Brain Res 1298:57–69.

Paré D, Smith Y. 1994. GABAergic projection from the intercalated cell masses of the amygdala to the basal forebrain in cats. J Comp Neurol 344:33–49.

Paré D, Quirk GJ, Ledoux JE. 2004. New vistas on amygdala networks in conditioned fear. J Neurophysiol 92:1–9.

Paxinos G, Franklin KBJ. 2004. The mouse brain in stereotaxic coordinates, 2nd ed. San Diego: Academic Press.

Paxinos G, Ashwell KWS, Törk I. 1994. Atlas of the developing rat nervous system, 2nd ed. San Diego: Academic Press.

Paxinos G, Kus L, Ashwell KWS, Watson C. 1999. Chemoarchitectonic atlas of the rat forebrain. San Diego: Academic Press.

Petrovich GD, Swanson LW. 1997. Projections from the lateral part of the central amygdalar nucleus to the postulated fear conditioning circuit. Brain Res 763:247–254.

Petrovich GD, Scicli AP, Thompson RF, Swanson LW. 2000. Associative fear conditioning of enkephalin mRNA levels in central amygdalar neurons. Behav Neurosci 114:681–686.

Petrovich GD, Canteras NS, Swanson LW. 2001. Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. Brain Res Brain Res Rev 38: 247–289.

Petrovich GD, Ross CA, Mody P, Holland PC, Gallagher M. 2009. Central, but not basolateral, amygdala is critical for control of feeding by aversive learned cues. J Neurosci 29: 15205–15212.

Poulin JF, Chevalier B, Laforest S, Drolet G. 2006. Enkephalinergic afferents of the centromedial amygdala in the rat. J Comp Neurol 496:859–876.

Poulin JF, Castonguay-Lebel Z, Laforest S, Drolet G. 2008. Enkephalin co-expression with classic neurotransmitters in the amygdaloid complex of the rat. J Comp Neurol 506: 943–959.

Pretel S, Piekut DT. 1991. ACTH and enkephalin axonal input to paraventricular neurons containing c-fos-like immunoreactivity. Synapse 8:100–106.

Puelles L, Kuwana E, Puelles E, Bulfone A, Shimamura K, Keleher J, Smiga S, Rubenstein JLR. 2000. Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. J Comp Neurol 424: 409–438.

Rao ZR, Yamano M, Shiosaka S, Shinohara A, Tohyama M. 1987. Origin of leucine-enkephalin fibers and their two main afferent pathways in the bed nucleus of the stria terminalis in the rat. Exp Brain Res 65:411–420.

Real MA, Heredia R, Labrador MC, Dávila JC, Guirado S. 2009. Expression of somatostatin and neuropeptide Y in the embryonic, postnatal, and adult mouse amygdalar complex. J Comp Neurol 513:335–348.

Reyes BA, Drolet G, Van Bockstaele EJ. 2008. Dynorphin and stress-related peptides in rat locus coeruleus: contribution of amygdalar efferents. J Comp Neurol 508:663–675.

Séquier JM, Hunziker W, Andressen C, Celio MR. 1990. Calbindin D-28k protein and mRNA localization in the rat brain. Eur J Neurosci 2:1118–1126.

Shammah-Lagnado SJ, Alheid GF, Heimer L. 1999. Afferent connections of the interstitial nucleus of the posterior limb of the anterior commissure and adjacent amygdalostriatal transition area in the rat. Neuroscience 94:1097–1123.

Shammah-Lagnado SJ, Alheid GF, Heimer L. 2001. Striatal and central extended amygdala parts of the interstitial nucleus of the posterior limb of the anterior commissure: evidence from tract-tracing techniques in the rat. J Comp Neurol 439:104–126.

Shimada S, Inagaki S, Kubota Y, Ogawa N, Shibasaki T, Takagi H. 1989. Coexistence of peptides (corticotropin releasing factor/neurotensin and substance P/somatostatin) in the bed nucleus of the stria terminalis and central amygdaloid nucleus of the rat. Neuroscience 30:377–383.

Smeets WJAJ, Reiner A. 1994. Phylogeny and development of catecholamine systems in the CNS of vertebrates. Cambridge: Cambridge University Press.

Soma M, Aizawa H, Ito Y, Maekawa M, Osumi N, Nakahira E, Okamoto H, Tanaka K, Yuasa S. 2009. Development of the mouse amygdala as revealed by enhanced green fluorescent protein gene transfer by means of in utero electroporation. J Comp Neurol 513:113–128.

Soria JM, Valdeolmillos M. 2002. Receptor-activated calcium signals in tangentially migrating cortical cells. Cereb Cortex 12:831–839.

Stenman J Toresson H, Campbell K. 2003a. Identification of two distinct progenitor populations in the lateral ganglionic eminence: implications for striatal and olfactory bulb neurogenesis. J Neurosci 23:167–174.

Stenman J, Yu RT, Evans RM, Campbell K. 2003b. Tlx and Pax6 co-operate genetically to establish the pallio-subpallial boundary in the embryonic mouse telencephalon. Development 130:1113–1122.

Stoykova A, Treichel D, Hallonet M, Gruss P. 2000. Pax6 modulates the dorsoventral patterning of the mammalian telencephalon. J Neurosci 20:8042–8050.

Swanson LW. 2000. Cerebral hemisphere regulation of motivated behavior. Brain Res 886:113–164.

Swanson LW, Petrovich GD. 1998. What is the amygdala? Trends Neurosci 21:323–331.

Thor S, Ericson J, Brännström T, Edlund T. 1991. The homeodomain LIM protein Isl-1 is expressed in subsets of neurons and endocrine cells in the adult rat. Neuron 7:881–889.

Tole S, Remedios R, Saha B, Stoykova A. 2005. Selective requirement of Pax6, but not Emx2, in the specification and development of several nuclei of the amygdaloid complex. J Neurosci 25:2753–2760.

Toresson H, Potter SS, Campbell K. 2000. Genetic control of dorsal-ventral identity in the telencephalon: opposing roles for Pax6 and Gsh2. Development 127:4361–4371.

Varela-Echavarría A, Pfaff SL, Guthrie S. 1996. Differential expression of LIM homeobox genes among motor neuron subpopulations in the developing chick brain stem. Mol Cell Neurosci 8:242–257.

Waclaw RR, Ehrman LA, Pierani A, Campbell K. 2010. Developmental origin of the neuronal subtypes that comprise the amygdalar fear circuit in the mouse. J Neurosci 30: 6944–6953.

Walker DL, Davis M. 2008. Role of the extended amygdala in short-duration vs. sustained fear: a tribute to Dr. Lennart Heimer. Brain Struct Funct 213:29–42.

Walker DL, Miles LA, Davis M. 2009. Selective participation of the bed nucleus of the stria terminalis and CRF in sustained anxiety-like vs. phasic fear-like responses. Prog Neuropsychopharmacol Biol Psychiatry 33:1291–1308.

Wilensky AE, Schafe GE, Kristensen MP, LeDoux JE. 2006. Rethinking the fear circuit: The central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. J Neurosci 26: 12387–12396.

Xu Q, Cobos I, De la Cruz E, Rubenstein JLR, Anderson SA. 2004. Origins of cortical interneuron subtypes. J Neurosci 24:2612–2622.

Xu Q, Tam M, Anderson SA. 2008. Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. J Comp Neurol 506: 16–29.

Yoshikawa K, Williams C, Sabol SL. 1984. Rat brain preproenkephalin mRNA. cDNA cloning, primary structure, and distribution in the central nervous system. J Biol Chem 259: 14301–14308.

CHAPTER 4: GENERAL DISCUSSION

Discussion

As early as 1925, Holmgren proposed that the amygdala of mammals included some nuclei and areas derived from the pallium (the basal amygdalar complex and cortical areas), and some nuclei derived from the subpallium (the centromedial amygdala). At about the same time, Johnston (1923) proposed the existence of a cell continuum in the basal telencephalon of mammals, extending from the subpallial amygdala to the bed nucleus of the stria terminalis, and including additional olfactory-related cell groups. These early proposals have been corroborated and refined in the last decades thanks to novel data obtained by the use of modern techniques for fate mapping, for analyzing gene product expression (mRNA or protein), and for carrying out tract-tracing studies (for example, Alheid and Heimer, 1988; Alheid et al., 1995; Swanson and Petrovich, 1998; Puelles et al., 2000). Modern developmental data have been crucial for a better understanding of the amygdala. The proposal of Holmgren (1925) on the pallial and subpallial parts of the amygdala has been supported by expression of the mRNA of different transcription factors typical of either the pallium (Tbr1, Emx1) or the subpallium (Dlx2, Dlx5, Nkx2.1, Lhx6) during development (Puelles et al., 2000; Medina et al., 2004; García-López et al., 2008), and by genetic or experimental fate mapping (Gorski et al., 2002; Stühmer et al., 2002; Xu et al., 2008; Soma et al., 2009; Hirata et al., 2009; Waclaw et al., 2010). These data also agree with (and helped to explain) the high abundance of GABAergic or glutamatergic neurons in either the subpallium or pallium, respectively (Swanson and Petrovich, 1998; Swanson, 2000). These data also gave support to the proposal that the centromedial amygdala and BST are subpallial, but additionally raised some new questions and concerns. For example, the medial amygdala was intriguing because it appears to express both pallial and subpallial transcription factors, and some data suggested it may contain cells of extratelencephalic origin (Puelles et al., 2000; Medina et al., 2004; García-López et al., 2008). Moreover, since the pallium and subpallium contain several subdivisions, many researchers further detailed the amygdalar derivatives of these subdomains (for example, García-López et al., 2008). This raised additional questions and concerns. For example, do the caudoventral MGE and the commissural preoptic subdivisions produce different parts of the medial amygdala? Is there any embryological support for continuum of the medial extended amygdala and/or for the central extended amygdala? The results collected for this Thesis provide an answer to these questions.

Regarding the medial amygdala (Chapter 2), the results include experimental fate mapping data showing that it includes cell subpopulations from multiple origins, including the ventral pallium, the caudoventral MGE (MGEcv), the commissural preoptic area (POC) and the SPV hypothalamic domain (Bupesh et al., 2011a). It appears that the medial BST also includes distinct subpopulations of cells from at least MGEcv, POC and SPV (Bupesh et al., 2011a). Moreover, some data indicate that there is a trend for cells of the medial amygdala and medial BST with the same origin to be interconnected and to project to similar hypothalamic targets (for example, Lhx6-expressing cells of both the posterodorsal medial amygdala and the posteromedial part of BSTM, which derive from MGEcv; see discussion below). This suggests that the so-called medial extended amygdala may consist of several cell corridors (or subcorridors), each related to a distinct embryonic origin (see Chapter 2 and discussion below).

Regarding the central extended amygdala (Chapter 3), the results provide experimental fate mapping support for at least a triple origin of the neurons of the central amygdala and lateral BST, which derive from dorsal LGE, ventral LGE and MGE (Bupesh et al., 2011b). Moreover, it appears that cells of each specific origin of the central amygdala show a different phenotype and show a trend to be interconnected with cells of the same phenotype (and perhaps the same origin) in the lateral BST. Thus, this suggests that the central extended amygdala may consist of several cell corridors (or subcorridors), each related to a distinct embryonic origin. This is discussed in more detail below (see also Chapter 3).

1. Pallial, subpallial and hypothalamic contribution of neurons to the medial extended amygdala

As explained in the second Chapter of this Thesis (also Bupesh et al., 2011a), our results provide experimental support for a multiple origin of the cells of the medial extended amygdala, which derive at least from the following four embryonic subdivisions: the ventral pallium (VP), two subdivisions of the subpallium called the caudoventral MGE (MGEcv) and the commissural preoptic area (POC), and the supraopto-paraventricular domain of the hypothalamus (SPV). Each distinct contribution will be discussed in a separate section below. Before that, it is worth noting

that these different embryonic domains that contribute cells to the medial extended amygdala appear to produce different subpopulations of projection neurons, which reach the nuclear complex by either radial or tangential migration (based on comparison with radial glial fibers). In contrast to previous thoughts, this appears to be a common situation at least in the basal telencephalon, since it has also been observed in the globus pallidus (Nóbrega-Pereira et al., 2010) and the central amygdala (present Thesis). In the chicken and the frog *Xenopus*, the medial extended amygdala also appears to include subpopulations derived from VP, MGE and SPV (Brox et al., 2004; Moreno and González, 2006; Bardet et al., 2008; Abellán and Medina, 2008, 2009; Abellán et al., 2009, 2010; Moreno et al., 2010). The chicken medial extended amygdala (medial amygdalar nucleus and BSTM) also includes POC derived cells (Abellán and Medina, 2008, 2009). However, it is unclear whether in *Xenopus* the medial nucleus of the amygdala includes a POC-derived subpopulation (Domínguez et al., 2010; Medina et al., 2011).

1.1. Contribution of neurons from MGEcv to the medial extended amygdala.

In this study, we have experimentally shown that the caudoventral subdivision of the medial ganglionic eminence (MGEcv), previously called or included as part of the anterior peduncular area, or as part of the fifth MGE subdivision (Flames et al., 2007; García-López et al., 2008; this has also been referred as diagonal domain by Bardet et al., 2010), is a major source of neurons for the medial extended amygdala, including the medial bed nucleus of the stria terminalis (BSTM) and the anterior (MeA) and posterodorsal (MePD) parts of the medial amygdala. This agrees with a previous proposal based on expression of Lhx6 and calbindin, which are enriched in MGEcv and its derivatives (García-López et al., 2008). This also agrees with a previous study based on the genetic fate mapping of Nkx2.1 lineage cells, which has shown that the subpallial proliferative zone expressing the transcription factor Nkx2.1 during development (including MGE and the preoptic area) produce numerous cells for the medial amygdala (Xu et al 2008). However, this genetic fate mapping did not provide details on the exact subdivisions of the medial amygdala that receive Nkx2.1 lineage cells, and could not distinguished the exact origin of the cells within the large Nkx2.1 expressing subpallial domain. Some data based on combinatorial gene expression patterns during

development indicate that the Nkx2.1-expressing domain of the subpallium include at least five distinct MGE (pallidal) progenitor subdomains (pMGE 1-5) and two preoptic progenitor subdomains (PO1 or POC; and PO2 or POB) (Flames et al., 2007; García-López et al., 2008). Thus, our *in vitro* fate mapping study provides novel details on the exact origin and exact destiny of cells in the medial extended amygdala. Comparison with Lhx6 indicates that the MGEcv domain (roughly corresponding to caudal pMGE5 of Flames et al., 2007) is the source of most neurons of the MeA and MePD, and the principal subnucleus of BSTM (BSTMpm). Additionally, double labeling data indicate that most of these neurons contain calbindin. These cells appear to constitute a corridor of neurons that originate in MGEcv, express Lhx6 and calbindin, and extend from the MePD and MeA (caudolaterally) to the BSTM (rostromedially), as proposed by García-López et al. (2008). This cell corridor would constitute only one part of the medial extended amygdala of Alheid, Heimer, de Olmos and collaborators (Alheid and Heimer, 1988; Alheid et al., 1995; de Olmos et al., 2004). Interestingly, it appears that Lhx6-expressing cells of the MePD are GABAergic and are preferentially connected with the BSTM subdivision of the same origin (BSTMpm, which also derives from MGEcv and is rich in Lhx6 expression), and both project to the same hypothalamic targets that are involved in reproduction control (Choi et al., 2005; see further discussion in García-López et al., 2008, and in Chapter 2 of this Thesis). Therefore, this MGEcv-related (Lhx6-expressing) cell corridor of the medial extended amygdala appears to specifically control sexual behavior. As noted below, the medial extended amygdala also contains other neuron subpopulations with different origins (POC, VP, SPV), and future studies need to evaluate the existence of additional cell corridors related to these different embryonic origins.

1.2. Contribution of neurons from the commisural preoptic area to the medial extended amygdala.

The preoptic area (and in particular its commissural subdivision or POC) can be distinguished from MGE by expression of the signaling protein Shh in the ventricular zone and some of its derived cells, and based on Shh expression (in combination with other regulatory genes) it was proposed that POC produced part of the medial amygdala (MePV, and some cells of MeA) and part of BSTM (García-López et al., 2008). The results from this Thesis, obtained using in *vitro* fate mapping experiments, demonstrate

that POC is truly a source of neurons for part of BSTM and part of the medial amygdala. These results agree with recent data on Dbx1-Cre based genetic fate mapping, which show that the medial amygdala contain subpopulations of Dbx1-lineage cells, part of which (those that are GABAergic) originate in Dbx1-expressing progenitor cells of the preoptic area (Hirata et al., 2009). Our results also agree with another genetic fatemap of Shh-lineage cells in the medial amygdala, showing that these cells preferentialy populate MePV, but not MePD which is mostly populated by Nkx2.1 lineage cells (Carney et al., 2010). This complementary pattern of Nkx2.1-lineage versus Shh-lineage cells in the dorsal and ventral subdivisions of the posterior medial amygdala (Carney et al., 2010) completely agrees with the proposal of Garcia-López et al. (2008) and our data. However, our data are more specific on the exact origin of the neurons, since indicate that Nkx2.1-lineage cells of MePD specifically originate in MGEcv, while Shh-lineage cells originate from POC (POB does not appear to contribute cells to the medial amygdala). Moreover, while MGEcv (Nkx2.1/Lhx6 lineage) and POC (Shh-lineage) cells are segregated in the posterior amygdala, our fate mapping data indicate that they are intermingled in the anterior amygdala. This also agrees with the suggestion of García-López et al. (2008). Interestingly, this is correlated with the type of olfactory input, from the main olfactory bulb or the accessory olfactory bulb, that these different subdivisions of the medial amygdala receive. Both types of olfactory information segregate in the posterior amygdala (vomeronasal input to MePD, main olfactory input to MePV), while they intermingle in the anterior amygdala (Choi et al., 2005; Pro-Sistiaga et al., 2007).

Furthermore, our result showed that the POC also produces cells for other parts of the medial extended amygdala, such as the BSTM, and for other parts of the basal telencephalon (see details in Chapter 2). However, it is unclear whether Shh-lineage cells (POC origin) of the medial extended amygdala constitute a distinct cell corridor in which cells are interconnected and project to similar targets. Surely, this deserves further investigation. Some data of double labeling of cells from genetic fate mapping of the preoptic area indicate that many preoptic cells of the medial amygdala are GABAergic and nitrergic (Hirata et al., 2009; Carney et al., 2010). Altogether, this is a new starting point for future studies on the exact connections of these cells and their function.

1.3. Ventral pallial contribution of neurons to the medial amygdala.

In addition to subpallial marker genes, the medial amygdala was observed to express pallial marker genes such as Tbr1 and Lhx9 (Medina et al., 2004; García-López et al., 2008). Based on the expression pattern of Lhx9 during development, it was suggested that the ventral pallium is a specific source of cells for some subnuclei of the medial amygdala, including the MeA (where cells appeared to mix with those derived from MGEcv and POC) and the superficial part of MePV (where cells primarily segregated from those from POC and MGEcv) (García-López et al., 2008). However, experimental proof for this proposal was lacking. Here, we have shown experimentally by migration assays that VP truly contributes cells not only to typical ventral pallial derivatives (namely piriform cortex, basal amygdalar complex and cortical amygdalar areas), but also produces some cells that populate the anterior and posterior subdivisions of the medial amygdala. The VP derived cells tend to settle at a superficial band of the MePV, overlapping a similar superficial band of Lhx9 and Tbr1 expression. In contrast to the subpallial neurons of the medial amygdala (derived from MGEcv or POC), which are GABAergic (as noted above), cells of the medial amygdala derived from the ventral pallium are likely glutamatergic. However, the exact connections of this subpopulation of neurons are unknown and deserve investigation. Some studies have shown that some of the descending projections from the medial amygdala to the hypothalamus (such as those related to defense/aggressive behavior) are excitatory (Choi et al., 2005). These projections may originate in VP-derived cells of the medial amygdala, but – as explained below – may also relate to SPV-derived cells of this nucleus.

1.4. Contribution of neurons from the supraopto-paraventricular domain of the alar hypothalamus (SPV) to the medial extended amygdala.

Using the gene expression data on the transcription factors Otp and Lhx5, it was proposed that the supraopto-paraventricular domain of the alar hypothalamus (SPV) produces a subpopulation of cells for the medial extended amygdala, including the medial bed nucleus of the stria terminalis and the anterior medial amygdala (Otp: Bardet et al., 2008; Moreno et al., 2010; Lhx5: Abellán et al., 2010). This has been corroborated recently using genetic and *in vivo* experimental fatemap in the mouse

(García-Moreno et al., 2010). Our results on *in vitro* migration assays agree with these studies and provide further details on the route and destiny of the migrated cells, as well as on their phenotype. Based on correlation with the glutamatergic marker VGLUT2, SPV-derived cells are likely glutamatergic (Abellán et al., 2010; García-Moreno et al., 2010). Our data indicate that many SPV-derived neurons in the BSTM and medial amygdala also are immunoreactive for calbindin. In the BSTM, these cells appear to form a medial and caudal subdivision of the nucleus, which appears to correspond to the extratelencephalic, posterior part of the BST proposed by Puelles et al. (2000). Since the SPV domain is a source of vasopressin and oxytocin containing neurons of the paraventricular and supraoptic hypothalamic nuclei (Michaud et al., 1998; Wang and Lufkin, 2000), it is possible that it also contributes this type of cells to the medial extended amygdala. The presence of this type of neurons in the medial extended amygdala is highly variable between different mammals, and they have been involved in several aspects of social behavior (Cushing and Kramer, 2005). Neurons of this system are responsive to gonadal steroids and appear to be related to sex differencies in some social behaviors and functions (de Vries and Miller, 1998). It is therefore highly relevant to investigate their putative embryonic origin in SPV, analyze the molecular mechanisms behind their diverse invasion of the medial extended amygdala in different species, and the relation of this to species differences in some social behaviors (such as parental care or monogamous behavior). In addition to this, it would be important to analyze whether SPV-derived neurons of the medial extended amygdala show a trend to be connected with cells of the same origin within the corridor and project to the same targets. This trend has been reported for MGEcv-derived (Lhx6) cells (see above), and if also confirmed for SPV-, POC- and VP-derived cells it would indicate the existence of at least four distinct cells corridors in the medial extended amygdala, each related to a distinct embryonic origin, which cells are partially intermingled, but show distinct phenotype and connections.

2. Multiple origins of the neurons of the central extended amygdala.

As explained in the third Chapter of the Thesis (also Bupesh et al., 2011b), our results provide experimental evidence for at least a triple origin of the principal neurons of the central extended amygdala. Using *in vitro* fatemap analysis, combined with

immunohistochemistry and immunofluorescence, and compared with gene expression data from our laboratory, we have shown that the major components of the central extended amygdala, central amygdala and lateral BST, are mosaics formed by different proportions of dorsal LGE-, ventral LGE- and MGE-derived neurons. This will be discussed in separate sections below.

Based on similarity of neurochemistry and connections, it was proposed that the central extended amygdala constitutes a cell corridor that extends from the central amygdala and other striatal-like amygdalar cell groups to the BSTL (Alheid and Heimer, 1988; Alheid et al., 1995; de Olmos, 1999; Heimer 2003). Our results indicate that this continuum, from the central amygdala to the BSTL, is formed by different proportions of neurons from dorsal LGE, ventral LGE and MGE, which may explain the similarity of these cells in terms of neurochemistry and connections along the corridor. In the chicken and the frog *Xenopus*, the central extended amygdala includes LGE and MGE cell subpopulations (Abellán and Medina, 2009; Moreno and González, 2006), but more investigation is required to know the degree of similarity with the development of the central extended amygdala in the mouse. For example, the dorsal LGE contributes Pax6-expressing cells to the central extended amygdala in the mouse (present results) the chicken (Abellán and Medina, 2009) and the turtle (Moreno et al., 2010), but a similar contribution may be absent in *Xenopus* (Moreno et al., 2008b; Moreno et al., 2009). On the other hand, the ventral LGE produces Islet1-expressing cells for the central amygdala in the mouse (Waclaw et al., 2010; present results) and *Xenopus* (Moreno et al., 2008a; Moreno et al., 2009), but more studies are needed to know whether this is so in sauropsids (birds and reptiles).

2.1. Contribution of neurons from dorsal LGE, ventral LGE and MGE to the lateral parts of the central extended amygdala.

According to Alheid, Heimer, de Olmos and collaborators, the central extended amygdala includes the intercalated amygdalar cells (ITC), the central amygdala, the interstitial nucleus of the posterior limb of the anterior commissure (IPAC), part of the substantia innominata, and the lateral BST, and is essential for control of ingestion and for fear, stress and anxiety responses (Alheid et al., 1995; de Olmos et al., 2004; Heimer 2003; Walker and Davis 2008; for function see also Swanson, 2000). Developmental

data of the nuclei conforming the central extended amygdala were scarce until recently. Based on expression of Pax6 and Dlx5, it was proposed that the central amygdala and at least part of the ITC are LGE derivatives (Puelles et al., 2000; Medina et al., 2004). More recently, based on expression of Pax6 and Foxp2, which are distinctly expressed in dorsal LGE derivatives, the ITC was proposed to specifically derive from the dorsal LGE subdivision (Kaoru et al., 2010), while based on expression of Islet1 and genetic fate mapping of Isletl-lineage cells, many neurons of the central amygdala, including projection neurons, were shown to derive specifically from ventral LGE (Waclaw et al., 2010). However, as noted above, the central amygdala also includes Pax6-expressing cells in mouse and chicken, suggesting that at least some neurons of this nucleus derive from dorsal LGE (Puelles et al., 2000; Abellán and Medina, 2009). Our results help to clarify what specific parts or cell subpopulations of the central amygdala derive from either dorsal LGE or ventral LGE. Our data on *in vitro* fate mapping combined with immunofluorescence for Pax6 show that dorsal LGE produces the majority of Pax6-immunoreactive (Pax6+) neurons of the capsular central amygdala (CeC) and many neurons (Pax6+) of the lateral central amygdala (CeL). On the other hand, our data on *in vitro* fate mapping combined with immunofluorescence for Islet1 show that ventral LGE primarily produces Islet1-immunoreactive (Islet1+) neurons for the medial and part of the lateral subnuclei of the central amygdala.

Based on comparison of these fate mapping and Pax6 data with the mRNA expression of Pax6 and pre-proenkephalin (see Chapter 3 for mouse, and Poulin et al., 2006, 2008, for rat), it appears that many cells derived from dorsal LGE are enkephalinergic neurons, which constitute a subpopulation of projection neurons of the central amygdala (Rao et al., 1987). It also appears that these cells primarily populate the ITC, the capsular central amygdala, the lateral third of the lateral central amygdala, and the IPAC. On the other hand, based on comparison of our fate mapping and Islet1 data with the distribution of cells containing corticotropin-releasing factor (CRF+) (Marchant et al., 2007), it appears that cells derived from ventral LGE may include CRF+ projection neurons (often co-containing neurotensin and/or dynorphin), which primarily locate in the central part of the lateral central amygdala (Moga and Gray, 1985; Gray and Magnuson, 1992; Gray, 1993; Marchant et al., 2007). Thus, it appears that dorsal LGE and ventral LGE produce two distinct projection neurons of the central amygdala.

In addition, previous data also suggested that the medial part of the central amygdala includes another subpopulation of projection neurons, containing the neuropeptide somatostatin (SOM+) (Shimada et al., 1989), which immigrate from MGEcv (García-López et al., 2008; Real et al., 2009). Our data on *in vitro* migration assays combined with immunofluorescence for somatostatin show that this is so. The cells derived from MGEvc that invade the medial part of the central amygdala (medial subnucleus and the medialmost part of the lateral subnucleus) are SOM+. These cells appear to represent the SOM+ projection neurons (co-containing substance P) of the central amygdala described in some studies (see Shimada et al., 1989; Gray and Magnuson, 1992, and more references in Chapter 3).

Therefore, it appears that different projection neurons of the central amygdala originate in three different embryonic subdivisions. Moreover, as explained in the next section, these different neurons of the central amygdala appear to project to cells of the BSTL having a similar phenotype (Gray et al., 1987; Moga et al., 1989).

2.2. Contribution of neurons from dorsal LGE, ventral LGE and MGE to the BSTL and general remarks on the central extended amygdala continuum.

While the central amygdala was once thought to only derive from LGE, based on expression of Nkx2.1 and other transcription factors, the lateral BSTL was though to be a pallidal (MGE) (Puelles et al., 2000; Xu et al., 2008; García-López et al., 2008). Therefore, these data did not support a uniformity along the cell corridor of the central extended amygdala in terms of embryonic origin, and could not explain the similarity of their cells regarding neurochemistry and connectivity.

However, our *in vitro* fate mapping data combined with immunofluorescence indicate that the BSTL does receive neurons from the same three progenitor domains that produce the neurons of the central amygdala, dorsal LGE, ventral LGE, and MGE. Nevertheless, these three domains contribute cells to the BSTL in different proportions that they do to the central amygdala. Most neurons of BSTL appear to derive from MGE, including cells from dorsal MGE and from MGEcv (results of Chapter 2 and 3 of this Thesis), thus supporting the general "pallidal" nature of this nucleus (Puelles et al., 2000; Xu et al., 2008; García-López et al., 2008). In addition, here we show that many

Islet1+ neurons of BSTL immigrate from the ventral LGE, while a few Pax6+ neurons do so from dorsal LGE. Since the BSTL contains the same three types of projection neurons described in the central amygdala, ENK+, CRF+, and SOM+ (Moga et al., 1989; Poulin et al., 2008), it is tempting to suggest that at least some of them may have a similar embryonic origin to those of the central amygdala. However, this requires further investigation. Interestingly, there appears to be a trend for cells of the same neurochemical profile to be connected with "homologous" cells (cells with the same phenotype) along the corridor, and for homologous cells to project to similar targets in the hypothalamus/brainstem (Moga et al., 1989; Poulin et al., 2008, and other references in Chapter 3).

In conclusion, our data indicate that the nuclei that conform the central extended amygdala, particularly the central amygdala and the lateral BST, are formed by different proportions of neurons derived from either dorsal LGE, ventral LGE or MGE. Thus, our data provide embryonic support for the existence of the central extended amygdala corridor and for their similarity in terms of neurochemistry and connections. Our data also indicate that distinct projection neurons of the corridor originate in separate progenitor domains, and open new venues for further investigation on the existence of separate subcorridors within the general continuum of the central extended amygdala.

CHAPTER 5: CONCLUSIONS

Conclusions

1. The results obtained by *in vitro* fate mapping assays provide experimental evidence for the multiple embryonic origins of the neurons of the medial extended amygdala, which are produced in at least three telencephalic and one extratelencephalic domains. Correlations with other studies and with radial glia indicate that many of these cells are projection neurons, which reach the medial extended amygdala by either radial or tangential migrations.

2. Two distinct subdivisions of the telencephalic subpallium contribute neurons to the medial extended amygdala: the caudoventral part of the medial ganglionic eminence (MGEcv) and the commissural preoptic area (POC). Cells from both domains populate the medial amygdala and the medial bed nucleus of the stria terminalis (BSTM). The distribution of migrated cells derived from MGEcv correlate well with expression Lhx6 along the radial dimension of this subdivision, while those derived from POC correlate well with expression of Shh. While MGEcv- and POC-derived cells intermingle in the anterior part of the medial amygdala, they show a trend to segregate to dorsal or ventral parts, respectively, of the posterior part of the medial amygdala.

3. The ventral pallium (VP) of the telencephalon produces some cells that invade the anterior and posterior parts of the medial amygdala by tangential migration. In the posterior medial amygdala, VP-derived cells show a trend to occupy a superficial position, overlapping with a band of Lhx9 expression that appears to extend from VP.

4. The supraopto-paraventricular domain (SPV) of the alar hypothalamus also produces cells for the medial extended amygdala, including the BSTM and the medial amygdala. The SPV-derived cells invade the medial amygdala by tangential migration, and correlate well with expression of Lhx5 that appears to extend from SPV. The SPV also produces cells that migrate tangentialy into the subparaventricular domain of the alar hypothalamus (Spa).

5. Double labeling with calbindin shows that many MGEcv-derived cells of the medial extended amygdala are calbindin immunoreactive, and likely GABAergic (as expected from the results of other studies). However, our data also indicate that calbindin

immunoreactive cells of the amygdala are also produced in the ventral pallium and the hypothalamic SPV, and in these two cases calbindin cells are likely glutamatergic. Therefore, calbindin cells of the medial extended amygdala appear to include both GABAergic and glutamatergic neurons.

6. Our *in vitro* migration assays show that the central extended amygdala, including the central amygdala and the lateral bed nucleus of the stria terminalis (BSTL), are mosaics formed by different proportions of dorsal LGE-, ventral LGE- and MGE-derived neurons.

7. The dorsal LGE produces Pax6-expressing neurons that primarily populate lateral parts of the central extended amygdala, including the capsular and part of the lateral central amygdala, but also produces a few cells for the lateral BST. Correlation between Pax6 and the mRNA of pre-proenkephalin suggests that dorsal LGE-derived cells of the central amygdala are enkephalinergic.

8. The ventral LGE produces Islet1-expressing neurons that primarily populate the central and medial parts of the central amygdala, but also produces numerous neurons for the lateral BST.

9. Double-labeling with calbindin indicate that both dorsal LGE and ventral LGE produce calbindin-containing neurons for the central amygdala.

10. The MGE produces the majority of neurons of the lateral BST and many interneurons for the striatal (central) and pallial amygdala, but our migration assays combined with immunofluorescence show that its ventrocaudal subdivision (MGEcv) also produces an important subpopulation of projection neurons containing somatostatin for medial aspects of the central amygdala.

11. Regarding other nuclei of the central extended amygdala, most neurons of the intercalated cells (ITC), the dorsal anterior amygdala (AAd) and the interstitial nucleus of the anterior commissure (IPAC) express Pax6 and pre-proenkephalin, and appear to derive from dorsal LGE. However, ITC and IPAC also include a subpopulation of

somatostatin immunoreactive neurons that derive from MGEcv. It is not clear if these MGEcv-derived cells are only interneurons or also include projection neurons.

12. The medial extended amygdala, including BSTM and medial amygdala, also include a subpopulation of cells that express Pax6. Our migration data indicate that these cells do not originate in LGE, and may derive from the prethalamic eminence.

LIST OF REFERENCES

A

Abellán A, Legaz I, Vernier B, Rétaux S, Medina L. 2009. Olfactory and amygdalar structures of the chicken ventral pallium based on the combinatorial expression patterns of LIM and other developmental regulatory genes. J Comp Neurol 20:166–186.

Abellán A, Medina L. 2009. Subdivisions and derivatives of the chicken subpallium based on expression of LIM and other regulatory genes and markers of neuron subpopulations during development. J Comp Neurol 515:465–501.

Abellán A, Vernier B, Rétaux S, Medina L. 2010. Similarities and differences in the forebrain expression of Lhx1 and Lhx5 between chicken and mouse: insights for understanding telencephalic development and evolution. J Comp Neurol 518:3512–3528.

Adolphs R, Tranel D, Damasio H, Damasio A.1994. Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. Nature 372:669-672.

Aggleton JP. 2000. The amygdala. A functional analysis. Second Ed. Oxford: Oxford University press.

Alheid GF, de Olmos J, Beltramino CA.1995. Amygdala and extended amygdala. In: Paxinos G, editor. The rat nervous system. San Diego: Academic Press. p 495–578.

Alheid GF, Heimer L. 1988. New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience 27:1–39.

Amaral DG, Schumann CM, Nordahl CW. 2008. Neuroanatomy of autism.Trends Neurosci 31:137–145.

Amaral DG, Price JL, Pitkänen A, Carmichael ST. 1992. Anatomical organization of the primate amygdaloid complex. In: Aggleton J, editor.

Ariëns Kappers CU, Huber GC, Crosby EC. 1936. The comparative anatomy of the nervous system of vertebrates, including man. 2 volumes.New York: Macmillan.

B

Bachy I, Vernier P, Retaux S. 2001. The LIM homeodomain gene family in the developing *Xenopus* brain: conservation and divergences with the mouse related to the evolution of the forebrain. J Neurosci 21:7620–7629.

Backman M, Machon O, Mygland L, van den Bout CJ, Zhong W, Taketo MM, Krauss S. 2005. Effects of canonical Wnt signaling on dorso-ventral specification of the mouse telencephalon. Dev Biol 279:155-68.

Bardet SM, Martinez-de-la-Torre M, Northcutt RG, Rubenstein JL, Puelles L. 2008. Conserved pattern of OTP-positive cells in the paraventricular nucleus and other hypothalamic sites of tetrapods. Brain Res Bull 75:231–235.

Bardet SM, Ferran JL, Sanchez-Arrones L, Puelles L. 2010. Ontogenetic expression of sonic hedgehog in the chicken subpallium. Front Neuroanat 4. pii: 28 (p. 1-16).

Berdel B, Moryś J. 2000. Expression of calbindin-D28k and parvalbumin during development of rat's basolateral amygdaloid complex. Int J Dev Neurosci 18:501–513.

Bielsky IF, Hu SB, Ren X, Terwilliger EF, Young LJ. 2005. The V1a vasopressin receptor is necessary and sufficient for normal social recognition: a gene replacement study. Neuron 47:503-13.

Bishop KM, Goudreau G, O'Leary DD. 2000. Regulation of area identity in the mammalian neocortex by Emx2 and Pax6. Science 288:344-349.

Bishop KM, Garel S, Nakagawa Y, Rubenstein JL, O'Leary DD. 2003. Emx1 and Emx2 cooperate to regulate cortical size, lamination, neuronal differentiation, development of cortical efferents, and thalamocortical pathfinding. J Comp Neurol 457:345-60.

Briscoe J, Ericson J. 2001. Specification of neuronal fates in the ventral neural tube. Curr Opin Neurobiol 11:43-49.

Broglio C, Gómez A, Durán E, Ocaña FM, Jiménez-Moya F, Rodríguez F, Salas C. 2005. Hallmarks of a common forebrain vertebrate plan: specialized pallial areas for spatial, temporal and emotional memory in actinopterygian fish. Brain Res Bull 66:277-81.

Bronner-Fraser, M.E. and Fraser S.E.1997. Differentiation of the vertebrate neural tube. Current Opinion in Cell Biology 9:885-891.

Brox A, Puelles L, Ferreiro B, Medina L. 2003. Expression of the genes GAD67 and Distalless- 4 in the forebrain of *Xenopus* laevis confirms a common pattern in tetrapods. J Comp Neurol 461:370–393.

Brox A, Puelles L, Ferreiro B, Medina L 2004. Expression of the genes Emx1, Tbr1, and Eomes (Tbr2) in the telencephalon of *Xenopus laevis* confirms the existence of a ventral pallial division in all tetrapods. J Comp Neurol 474:562–577.

Brunelli S, Faiella A, Capra V, Nigro V, Simeone A, Cama A, Boncinelli E. 1996. Germline mutations in the homeobox gene EMX2 in patients with severe schizencephaly. Nat Genet 12:94–96.

Bulchand S, Grove EA, Porter FD, Tole S. 2001. LIM-homeodomain gene Lhx2 regulates the formation of the cortical hem. Mech Dev 100:165-175.

Bulfone A, Martinez S, Marigo V, Campanella M, Basile A, Quaderi N, Gattuso C, Rubenstein JL, Ballabio A.1999. Expression pattern of the Tbr2 (Eomesodermin) gene during mouse and chick brain development. Mech Dev 84:133–138.

Bulfone A, Smiga SM, Shimamura K, Peterson A, Puelles L, Rubenstein JL 1995. T-brain-1: a homolog of Brachyury whose expression defines molecularly distinct domains within the cerebral cortex. Neuron 15:63-78.

Butler AB, Hodos W. 2005. Comparative Vertebrate Neuroanatomy: John Wiley and sons.

C

Campbell K. 2003. Dorsal-ventral patterning in the mammalian telencephalon. Curr Opin Neurobiol.13:50-56.

Carney RS, Mangin JM, Hayes L, Mansfield K, Sousa VH, Fishell G, Machold RP, Ahn S, Gallo V, Corbin JG. 2010. Sonic hedgehog expressing and responding cells generate neuronal diversity in the medial amygdala. Neural Dev 14:1-17.

Choi GB, Dong HW, Murphy AJ, Valenzuela DM, Yancopoulos GD, Swanson LW, Anderson DJ. 2005. Lhx6 delineates a pathway mediating innate reproductive behaviors from the amygdala to the hypothalamus. Neuron 46:647–660.

Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, Westphal H, Beachy PA. 1996. Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature 383:407-413.

Cobos I, Shimamura K, Rubenstein JL, Martínez S, Puelles L. 2001. Fate map of the avian anterior forebrain at the four-somite stage, based on the analysis of quail-chick chimeras. Dev Biol 239:46–67.

Crosby G, Ito M, Kaufman E, Nelson T, Sokoloff L.1983. Naloxone pretreatment alters the local cerebral metabolic effect of gamma-hydroxybutyrate in rats. Brain Res 75:194-197.

D

Davis M. 2000. The role of the amygdala in conditioned and unconditioned fear and anxiety. In: Aggleton JP, editor. The Amygdala: A functional analysis. Second Ed. Oxford: Oxford University Press. p. 213-287.

de Olmos. JS. 1969. A cupric-silver method for impregnationof terminal axon degeneration and its further use in staining granular argyrophilic neurons. Brain Behav.Evol 2:213-237.

de Olmos. JS.1972. The amygdaloid projection field in the rat as studied with the cupric-silver method. In: The Neurobiology of the amygdala. B.E. Eleftheriou, ed. Plenum Press, New York. p.145-204.

de Olmos JS, Alheid GF, Beltramino CA. 1985. Amygdala. In: Paxinos G, editor. The rat nervous system. Sydney: Academic Press. p 223-334.

de Olmos JS, Beltramino CA, Alheid G. 2004. Amygdala and extended amygdala of the rat: a cytoarchitectonical, fibroarchitectonical, and chemoarchitectonical survey. In: Paxinos G, editor. The rat nervous system, 3rd ed. Elsevier Academic press. Sandiago. p510-601.

de Olmos JS, Heimer L. 1999. The concepts of the ventral striatopallidal system and extended amygdala. Ann N Y Acad Sci 877:1-32.

Domínguez L, Gonzalez A, Moreno N. 2010. Sonic hedgehog expression during Xenopus laevis forebrain development. Brain Res 1347:19-32.

Dong HW, Petrovich GD, Swanson LW. 2001a. Topography of projections from amygdala to bed nuclei of the stria terminalis. Brain Res Rev 38:192–246.

E

Eagleson G, Ferreiro B, Harris WA.1995. Fate of the anterior neural ridge and the morphogenesis of the Xenopus forebrain. J Neurobiol 2:146-158.

Ericson J, Muhr J, Jessell TM, Edlund T. 1995. Sonic hedgehog: a common signal for ventral patterning along the rostrocaudal axis of the neural tube. Int J Dev Biol. 5:809-816.

F

Ferran JL, de Oliveira ED, Merchán P, Sandoval JE, Sánchez-Arrones L, Martínez-de-la- Torre M, Puelles L. 2009. Genoarchitectonic profile of developing nuclear groups in the chicken pretectum. J Comp Neurol 517:405–451.

Ferran JL, Sánchez-Arrones L, Sandoval JE, Puelles L. 2007. A model of early molecular regionalization in the chicken embryonic pretectum. J Comp Neurol 505: 379–403.

Ferran JL, de Oliveira ED, Merchán P, Sandoval JE, Sánchez-Arrones L, Martínez-de-la- Torre M, Puelles L. 2009. Genoarchitectonic profile of developing nuclear groups in the chicken pretectum. J Comp Neurol 517:405– 451.

Ferrante RJ. 2009. Mouse models of Huntington's disease and methodological considerations for therapeutic trials. Biochim Biophys Acta 1792:506-20.

Flames N, Long JE, Garratt AN, Fischer TM, Gassmann M, Birchmeier C, Lai C, Rubenstein JL, Marín O. 2004. Short- and long-range attraction of cortical GABAergic interneurons by neuregulin-1. Neuron 44(2):251-261.

Flames N, Pla R, Gelman DM, Rubenstein JL,Puelles L, Marín O. 2007. Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. J Neurosci 27:9682–9695.

Fernandez AS, Pieau C, Repérant J, Boncinelli E, Wassef M.1998. Expression of the Emx-1 and Dlx-1 homeobox genes define three molecularly distinct domains in the telencephalon of mouse, chick, turtle and frog embryos: implications for the evolution of telencephalic subdivisions in amniotes. Development 125:2099–2111.

Fernández-Garre P, Rodríguez-Gallardo L, Gallego-Díaz V, Alvarez IS, Puelles L. 2002. Fate map of the chicken neural plate at stage 4. Development 129:807-822.

Fazzari P, Paternain AV, Valiente M, Pla R, Luja´n R, Lloyd K, Lerma J, Marín O, Rico B. 2010. Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. Nature 464:1376 –1380.

G

Gallagher M. 2000. The amygdala and associative learning. In: Aggleton JP, editor. The Amygdala: A functional analysis. Second Ed. Oxford: Oxford University Press. p 311-329.

Galceran J, Miyashita-Lin EM, Devaney E, Rubenstein JL, Grosschedl R. 2000. Hippocampus development and generation of dentate gyrus granule cells is regulated by LEF1. Development 127:469-482.

García-López M, Abellán A, Legaz I, Rubenstein JL, Puelles L, Medina L. 2008. Histogenetic compartments of the mouse centromedial and extended amygdala based on gene expression patterns during development. J Comp Neurol 506:46-74.

García-Moreno F, Pedraza M, Di Giovannantonio LG, Di Salvio M, López-ascaraque L, Simeone A, De Carlos JA. 2010. A neuronal migratory pathway crossing from diencephalon to telencephalon populates amygdala nuclei. Nat Neurosci 13:680–689.

Geng X, Oliver G. 2009. Pathogenesis of holoprosencephaly. J Clin Invest.19:1403-1413.

Gorski JA, Talley T, Qiu M, Puelles L, Rubenstein JLR, Jones KR (2002): Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. J Neurosci 22:6309–6314.

Grove EA, Fukuchi-Shimogori T. 2003. Generating the cerebral cortical area map. Annu Rev Neurosci 26:355-380.

Gunhaga L, Marklund M, Sjödal M, Hsieh JC, Jessell TM, Edlund T. 2003. Specification of dorsal telencephalic character by sequential Wnt and FGF signaling. Nat Neurosci 7:701-707.

H

Heimer L. 2003. A new anatomical framework for neuropsychiatric disorders and drug abuse. Am J Psychiatry 160:1726–1739.

Hevner RF, Shi L, Justice N, Hsueh Y, Sheng M, Smiga S, Bulfone A, Goffinet AM, Campagnoni AT, Rubenstein JLR. 2001. Tbr1 regulates differentiation of the preplate and layer 6. Neuron 29:353–366.

Hirata T, Li P, Lanuza GM, Cocas LA, Huntsman MM, Corbin JG. 2009. Identification of distinct telencephalic progenitor pools for neuronal diversity in the amygdala. Nat Neurosci 12:141–149.

Holmgren N. 1925. Points of view concerning forebrain morphology in higher vertebrates. Acta Zool 6:413–477.

Houart C, Caneparo L, Heisenberg C, Barth K, Take-Uchi M, Wilson S. 2002. Establishment of the telencephalon during gastrulation by local antagonism of Wnt signaling. Neuron 35(2):255-265.

J

Johnston JB. 1923. Further contributions to the study of the evolution of the forebrain. J Comp Neurol 35:337–481.

K

Källén B. 1951. The nuclear development in the mammalian forebrain with special regard to the subpallium. Kungl Fysiogr Sällsk Handl 61,9:1-40.

Kaas JH, Bullock TH. 2007. Evolution of Nervous Systems: A Comprehensive Reference. Amsterdam, Academic Press-Elsevier, vol 2.

Kaoru T, Liu FC, Ishida M, Oishi T, Hayashi M, Kitagawa M, Shimoda K, Takahashi H. 2010. Molecular characterization of the intercalated cell masses of the amygdala: implications for the relationship with the striatum. Neuroscience 166:220–230.

Kato M, Dobyns WB. 2005. X-linked lissencephaly with abnormal genitalia as a tangential migration disorder causing intractable epilepsy: proposal for a new term, "interneuronopathy". J Child Neurol. 20(4):392-397.

Kemppainen S, Pitkänen A. 2000. Distribution of parvalbumin, calretinin, and calbindin-D(28k) immunoreactivity in the rat amygdaloid complex and colocalization with gamma-aminobutyric acid. J Comp Neurol 426:441–467.

Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M, Omichi K, Suzuki R, Kato-Fukui Y, Kamiirisa K, Matsuo M, Kamijo S, Kasahara M, Yoshioka H, Ogata T, Fukuda T, Kondo I, Kato M, Dobyns WB, Yokoyama M, Morohashi K. 2002. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. Nat Genet 32(3):359-369.

Kuschel S, Rüther U, Theil T. 2003. A disrupted balance between Bmp/Wnt and Fgf signaling underlies the ventralization of the Gli3 mutant telencephalon.
Dev Biol 260(2):484-495.

Kaoru T, Liu FC, Ishida M, Oishi T, Hayashi M, Kitagawa M, Shimoda K, Takahashi H. 2010. Molecular characterization of the intercalated cell masses of the amygdala: implications for the relationship with the striatum. Neuroscience 166:220–230.

L

Lagutin OV, Zhu CC, Kobayashi D, Topczewski J, Shimamura K, Puelles L, Russell HR, McKinnon PJ, Solnica-Krezel L, Oliver G. 2003. Six3 repression of Wnt signaling in the anterior neuroectoderm is essential for vertebrate forebrain development.
Genes Dev 17:368–379.

LeDoux JE. 2000. Emotion circuits in the brain. Annu Rev Neurosci 23:155-184.

LeDoux JE. 1992. Brain mechanisms of emotion and emotional learning. Curr Opin Neurobiol 2:191-197.

LeDoux JE, Farb C, Ruggiero DA. 1990. Topographic organization of neurons in the acoustic thalamus that project to the amygdala. J Neurosci 4:1043-1054.

Lee SM, Tole S, Grove E, McMahon AP. 2000. A local Wnt-3a signal is required for development of the mammalian hippocampus. Development 127(3):457-467.

Linke R, De Lima AD, Schwegler H, Pape HC. 1999. Direct synaptic connections of axons from superior colliculus with identified thalamo-amygdaloid projection neurons in the rat: possible substrates of a subcortical visual pathway to the amygdala. J Comp Neurol 403(2):158-170.

Lupo G, Harris WA, Lewis KE. 2006. Mechanisms of ventral patterning in the vertebrate nervous system. Nat Rev Neurosci 7:103–114.

M

Macdonald R, Barth KA, Xu Q, Holder N, Mikkola I, Wilson SW. 1995. Midline signalling is required for Pax gene regulation and patterning of the eyes. Development 121(10):3267-3278.

MacLean P.1990. The Triune Brain in Evolution, Plenum Press: New York. p. 543.

Marchant NJ, Densmore VS, Osborne PB. 2007. Coexpression of prodynorphin and corticotrophin-releasing hormone in the rat central amygdala: evidence of two distinct endogenous opioid systems in the lateral division. J Comp Neurol 504:702–715.

Marín O, Smeets WJ, González A. 1998. Evolution of the basal ganglia in tetrapods: a new perspective based on recent studies in amphibians Trends Neurosci 11:487-494.

Marín O, Rubenstein JLR. 2001. A long, remarkable journey: tangential migration in the telencephalon. Nature Reviews Neuroscience 2:780-790.

Marín O, Rubenstein JL. 2003. Cell migration in the forebrain. Annu Rev Neurosci 26:441-483.

Matsuo I, Kuratani S, Kimura C, Takeda N, Aizawa S. 1995. Mouse Otx2 functions in the formation and patterning of rostral head. Genes Dev 21:2646-2658.

McDonald AJ. 1998. Cortical pathways to the mammalian amygdala. Prog Neurobiol 55(3):257-332.

McDonald AJ, Mascagni F. 2001. Colocalization of calcium-binding proteins and GABA in neurons of the rat basolateral amygdala. Neuroscience 105:681–693.

Medina L. 2007. Field homologies; in Kaas JH, Striedter GF, Rubenstein JL. eds: Evolution of Nervous Systems: A Comprehensive Reference. Amsterdam, Academic Press-Elsevier, vol 1, p 73–87.

Medina L. 2008a. Evolution and embryological development of forebrain; in Binder MD, Hirokawa N, Windhorst U (eds): Encyclopedia of Neuroscience. Berlin, Springer, p 1172–1192.

Medina L. 2008b. Basal ganglia: evolution; in Squie LR (ed): Encyclopedia of Neuroscience.Amsterdam, Elsevier, p 67–85.

Medina L, Abellán A. 2009. Development and evolution of the pallium. Semin Cell Dev Biol 20:698–711.

Medina L, Brox A, Legaz I, García-López M, Puelles L. 2005. Expression patterns of developmental regulatory genes show comparable divisions in the telencephalon of *Xenopus* and mouse: insights into the evolution of the forebrain. Brain Res Bull 66:297-302.

Medina L, Bupesh M, Abellán A. 2011. Contribution of Genoarchitecture to Understanding Forebrain Evolution and Development, with Particular Emphasis on the Amygdala. Brain Behav Evol. [Epub ahead of print].

Medina L, Legaz I, González G, de Castro F, Rubenstein JLR, Puelles L. 2004. Expression of Dbx1, neurogenin 2, semaphoring 5A, cCadherin 8, and Emx1 distinguish ventral and lateral pallial histogenetic divisions in the developing claustroamygdaloid complex. J Comp Neurol 474:504–523.

Moga MM, Saper CB, Gray TS. 1989. Bed nucleus of the stria terminalis: cytoarchitecture, immunohistochemistry, and projection to the parabrachial nucleus in the rat. J Comp Neurol 283:315–332.

Moreno N, Bachy I, Rétaux S, González A. 2004. LIM-homeodomain genes as developmental and adult genetic markers of *Xenopus* forebrain functional subdivisions. J Comp Neurol 472:52–72.

Moreno N, González A. 2006. The common organization of the amygdaloid complex in tetrapods: new concepts based on developmental, hodological and neurochemical data in anuran amphibians. Prog Neurobiol 78:61–90.

Moreno N, Domínguez L, Rétaux S, González A. 2008a. Islet1 as a marker of subdivisions and cell types in the developing forebrain of *Xenopus*. Neuroscience 154:1423-39.

Moreno N, Rétaux S, González A. 2008b. Spatio-temporal expression of Pax6 in *Xenopus* forebrain. Brain Res 1239:92-9.

Moreno N, González A, Rétaux S. 2009. Development and evolution of the subpallium. Semin Cell Dev Biol 20:735–743.

Moreno N, Morona R, López JM, González A. 2010. Subdivisions of the turtle *Pseudemys scripta* subpallium based on the expression of regulatory genes and neuronal markers. J Comp Neurol 518:4877–4902.

Mueller T, Wullimann MF. 2009. An evolutionary interpretation of teleostean forebrain anatomy. Brain Behav Evol 74:30–42.

Mukhopadhyay M, Shtrom S, Rodriguez-Esteban C, Chen L, Tsukui T, Gomer L, Dorward DW, Glinka A, Grinberg A, Huang SP, Niehrs C, Izpisúa Belmonte JC, Westphal H. 2001 Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. Dev Cell 3:423-434.

Muller JF, Mascagni F, McDonald AJ. 2003. Synaptic connections of distinct interneuronal subpopulations in the rat basolateral amygdalar nucleus. J Comp Neurol 456: 217–236.

Murakami Y, Ogasawara M, Sugahara F, Hirano S, Satoh N, Kuratani S. 2001. Identification and expression of the lamprey Pax6 gene:Evolutionary origin of the segmented brain of vertebrates. Development 128:3521–3531.

N

Nieuwenhuys R, ten Donkelaar HJ, Nicholson C. 1998. The Central Nervous System of Vertebrates.Berlin, Springer.

Nóbrega-Pereira S, Gelman D, Bartolini G, Pla R, Pierani A, Marı ´n O. 2010. Origin and molecular specification of globus pallidus neurons. J Neurosci 30:2824–2834.

O

Osório J, Mueller T, Rétaux S, Vernier P, Wullimann MF. 2010. Phylotypic expression of the bHLH genes Neurogenin2, Neurod, and Mash1 in the mouse embryonic forebrain. J Comp Neurol 518:851–871

P

Petrovich GD, Canteras NS, Swanson LW. 2001. Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. Brain Res Brain Res Rev 38: 247–289.

Pellegrini M, Mansouri A, Simeone A, Boncinelli E, Gruss P. 1996. Dentate gyrus formation requires Emx2. Development 122(12):3893-3898.

Pitkänen A. 2000. Connectivity of the rat amygdaloid complex. In: Aggleton JP, editor.The amygdala: A functional analysis. Second ed. Oxford: Oxford University Press. p31-115.

Pitkänen A, Tuunanen J, Ka¨lvia¨inen R, Partanen K, Salmenpera¨ T. 1998. Amygdala damage in experimental and human temporal lobe epilepsy. Epilepsy Res 32:233–253.

Poulin JF, Castonguay-Lebel Z, Laforest S, Drolet G. 2008. Enkephalin co-expression with classic neurotransmitters in the amygdaloid complex of the rat. J Comp Neurol 506: 943–959.

Price JL, Russchen FT, Amaral DG. 1987. The Limbic region. II. The amygdaloid complex. In: Bjorkland A, Hokfelt T, Swanson L, editors. Handbook of Chemical Neuroanatomy. Amsterdam: Elsevier. p279-381.

Pro-Sistiaga P, Mohedano-Moriano A, Ubeda-Bañon I, Del Mar Arroyo-Jimenez M, Marcos P, Artacho-Pérula E, Crespo C, Insausti R, Martinez-Marcos A. 2007. Convergence of olfactory and vomeronasal projections in the rat basal telencephalon. J Comp Neurol 504:346-362.

Puelles L. 2001a. Brain segmentation and forebrain development in amniotes. Brain Res Bull 55:695–710.

Puelles L. 2001b. Thoughts on the development, structure and evolution of the mammalian and avian telencephalic pallium. Philos Trans R Soc Lond B Biol Sci 356: 1583–1598.

Puelles L, Amat JA, Martinez-de-la-Torre M. 1987. Segment-related, mosaic neurogenetic pattern in the forebrain and mesencephalon of early chick embryos. 1.

Topography of AChE-positive neuroblasts up to stage HH18. J Comp Neurol 8:247–268.

Puelles L., Kuwana E., Puelles E., Bulfone A., Shimamura K., Keleher J., Smiga S. and Rubenstein J.L.R. 2000. Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. J Comp Neurol 424:409- 438.

Puelles L, Martinez de-la-Torre M, Paxinos G, Watson C, Martinez S. 2007. The Chick Brain in Stereotaxic Coordinates. New York, Academic Press.

Puelles L, Martínez S, Martínez-de-la-Torre M, Rubenstein JL. 2004. Gene maps and related histogenetic domains in the forebrain and midbrain; in Paxinos G (ed): The Rat Nervous System, ed 3. Amsterdam, Elsevier-Academic Press. p3–25.

Puelles L, Medina L. 2002. Field homology as a way to reconcile genetic and developmental variability with adult homology. Brain Res Bull 57:243–255.

Puelles L, Rubenstein JL. 1993. Expression patterns of homeobox and other putative regulatory genes in the embryonic mouse forebrain suggest a neuromeric organization. Trends Neurosci 16:472–479.

Puelles L, Rubenstein JLR. 2003. Forebrain gene expression domains and the evolving prosomeric model. Trends Neurosci 26:469–476.

Q

Qiu M, Anderson S, Chen S, Meneses JJ, Hevner R, Kuwana E, Pedersen RA, Rubenstein JL.1996. Mutation of the Emx-1 homeobox gene disrupts the corpus callosum. Dev Biol 178(1):174-178.

R

Real MA, Heredia R, Labrador MC, Da´vila JC, Guirado S. 2009. Expression of somatostatin and neuropeptide Y in the embryonic, postnatal, and adult mouse amygdalar complex. J Comp Neurol 513:335–348.

Remedios R, Subramanian L, Tole S. 2004. LIM genes parcellate the embryonic amygdala and regulate its development. J Neurosci 24:6986–6990.

Rauch SL, Shin LM, Phelps EA. 2006. Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research--past, present, and future.Biol Psychiatry 60(4):376-82.

Rubenstein JL, Shimamura K, Martinez S, Puelles L. 1998: Regionalization of the prosencephalic neural plate. Annu Rev Neurosci 21:445–477.

S

Sánchez-Arrones L, Ferrán JL, Rodríguez-Gallardo L, Puelles L. 2009. Incipient forebrain boundaries traced by differential gene expression and fate mapping in the chick neural plate. Dev Biol 335: 43–65.

Shanmugalingam S, Houart C, Picker A, Reifers F, Macdonald R, Barth A, Griffin K, Brand M, Wilson SW. 2000 Ace/Fgf8 is required for forebrain commissure formation and patterning of the telencephalon. Development 127(12):2549-2561.

Shimada S, Inagaki S, Kubota Y, Ogawa N, Shibasaki T, Takagi H. 1989. Coexistence of peptides (corticotropin releasing factor/neurotensin and substance P/somatostatin) in the bed nucleus of the stria terminalis and central amygdaloid nucleus of the rat. Neuroscience 30:377-383.

Shimamura K, Hartigan DJ, Martinez S, Puelles L, Rubenstein JL. 1995. Longitudinal organization of the anterior neural plate and neural tube. Development 121:3923–3933.

Shimamura K, Rubenstein JLR. 1997. Inductive interactions direct early regionalization of the mouse forebrain. Development 124:2709–2718.

Smidt MP, Burbach JP. 2007. How to make a mesodiencephalic dopaminergic neuron. Nat Rev Neurosci 8:21–32.

Soma M, Aizawa H, Ito Y, Maekawa M, Osumi N, Nakahira E, Okamoto H, Tanaka K, Yuasa S. 2009. Development of the mouse amygdala as revealed by enhanced green fluorescent protein gene transfer by means of in utero electroporation. J Comp Neurol 513:113–128.

Stoykova A, Fritsch R, Walther C, Gruss P. 1996. Forebrain patterning defects in Small eye mutant mice. Development 122(11):3453-3465.

Striedter GF. 2005. Principles of Brain Evolution. Sunderland, Sinauer Associates. Elsevier-Academic Press. p509–603.

Striedter GF. 1997. The telencephalon of tetrapods in evolution. Brain Behav Evol 49: 179–213.

Swanson LW. 2000. Cerebral hemisphere regulation of motivated behavior. Brain Res 886:113–164.Swanson LW, Petrovich GD. 1998. What is the amygdala? Trends Neurosci 21:323–331.

Swanson LW. 2005. Anatomy of the soul as reflected in the cerebral hemispheres: neural circuits underlying voluntary control of basic motivated behaviors. J Comp Neurol 493:122-131.

Swanson LW, Petrovich GD. 1998. What is the amygdala? Trends Neurosci 21:323–331.

T

Tole S, Remedios R, Saha B, Stoykova A. 2005. Selective requirement of Pax6, but not Emx2, in the specification and development of several nuclei of the amygdaloid complex. J Neurosci 25:2753–2760.

V

Vyas A, Saha B, Lai E, Tole S. 2003. Paleocortex is specified in mice in which dorsal telencephalic patterning is severely disrupted. J Comp Neurol 466:545-553.

W

Waclaw RR, Ehrman LA, Pierani A, Campbell K. 2010. Developmental origin of the neuronal subtypes that comprise the amygdalar fear circuit in the mouse. J Neurosci 30: 6944–6953.

Walker DL, Davis M. 2008. Role of the extended amygdala in short-duration vs. sustained fear: a tribute to Dr. Lennart Heimer. Brain Struct Funct 213:29–42.

Wilson SW, Houart C. 2004. Early steps in the development of the forebrain. Dev Cell 6:167–181.

Wilson L, Maden M. 2005. The mechanisms of dorsoventral patterning in the vertebrate neural tube. Dev Biol 282(1):1-13.

Wilson SW, Rubenstein JLR. 2000. Induction and dorsoventral patterning of the telencephalon.Neuron 28:641–651.

Wolterink G, Daenen LE, Dubbeldam S, Gerrits MA, van Rijn R, Kruse CG, Van Der Heijden JA, Van Ree JM. 2001. Early amygdala damage in the rat as a model for neurodevelopmental psychopathological disorders.Eur Neuropsychopharmacol 1:51-59.

Wullimann MF, Mueller T. 2004. Teleostean and mammalian forebrains contrasted: evidence from genes to behavior. J Comp Neurol 475:143–162.

X

Xu Q, Tam M, Anderson SA. 2008. Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. J Comp Neurol 506:16–29.

Xu Q, Cobos I, De la Cruz E, Rubenstein JLR, Anderson SA. 2004. Origins of cortical interneuron subtypes. J Neurosci 24:2612–2622

Y

Yamaguchi TP. 2001. Heads or tails: Wnts and anterior-posterior patterning. Curr Biol 11(17):713-724.

Yoshida M, Suda Y, Matsuo I, Miyamoto N, Takeda N, Kuratani S, Aizawa S. 1997. Emx1 and Emx2 functions in development of dorsal telencephalon. Development 124(1):101-111.

Yun K, Garel S, Fischman S, Rubenstein JL. 2003. Patterning of the lateral ganglionic eminence by the Gsh1 and Gsh2 homeobox genes regulates striatal and olfactory bulb histogenesis and the growth of axons through the basal ganglia.
J Comp Neurol 461:151-165.

Yun K, Potter S, Rubenstein JL. 2001. Gsh2 and Pax6 play complementary roles in dorsoventral patterning of the mammalian telencephalon. Development 128(2):193-205.