Histol Histopathol (2012) 27: 1529-1538
DOI: 10.14670/HH-27.1529

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Mesencephalic neuronal populations. New insights on the ventral differentiation programs

Juan A. Moreno-Bravo*, Jesus E. Martinez-Lopez* and Eduardo Puelles
Institute of Neuroscience of Alicante, CSIC and University of Miguel Hernandez, Sant Joan d'Alacant, Spain
*Equal contribution

Summary. The midbrain is a complex structure where different functions are located. This formation is mainly involved in the visual and auditory information process (tectum) and visual movements and motor coordination (tegmentum). Here we display a complete description of midbrain anatomy based on the prosomeric model and of the developmental events that take place to generate this structure. We also summarize the new data about the differentiation and specification of the basal populations of the midbrain. The neural tube suffers the influence of several secondary organizers. These signaling centers confer exact positional information to the neuroblasts. In the midbrain these centers are the Isthmic organizer for the antero-posterior axis and the floor and roof plates for the dorso-ventral axis. This segment of the brain contains, in the dorsal part, structures such as the collicula (superior and inferior), tectal grey and the preisthmic segment, and in the basal plate, neuronal populations such as the oculomotor complex, the dopaminergic substantia nigra and the ventral tegmental area, the reticular formation and the periacueductal grey. Knowledge of the genetic cascades involved in the differentiation programs of the diverse populations will be extremely important to understand not only how the midbrain develops, but how degenerative pathologies, such as Parkinson's disease, occurs. These cascades are triggered by signaling molecules such as Shh, Fgf8 or Wnt1 and are integrated by receptor complexes and transcription factors. These are directly responsible for the induction or repression of the differentiation programs that will produce a specific neuronal phenotype.

Offprint requests to: Eduardo Puelles, PhD, Instituto de Neurociencias de Alicante, CSIC and Universidad Miguel Hernandez, 03550 Sant Joan d'Alacant, Spain. e-mail: epuelles@umh.es

Key words: Midbrain, Mesomere, Tectal grey, Superior colliculus, Inferior colliculus, Preisthmic region, Mesomere 1, Mesomere 2, Oculomotor complex, Substantia nigra, Ventral tegmental area, Red nucleus, Reticular formation, Periacueductal grey, Genetic mechanism

Introduction.

The neural tube is segmented during embryonic development. The rostral part is initially divided, along the antero-posterior axis (AP), into three main vesicles: the forebrain, midbrain, and hindbrain (Puelles and Rubenstein, 2003). This segmentation progresses further and results in the generation of the neuromeres. The most rostral vesicle, the forebrain, is subdivided into the diencephalon (prosomeres 1-3) and the secondary prosencephalon that gives rise to the telencephalic vesicles and hypothalamus (peduncular and terminal portions, Morales-Delgado et al., 2011). The hindbrain is the most caudal vesicle, and is subdivided into isthmus, 7 rhombomeres (r1-r7) and 4 pseudorhombomeres (r8-r11) (Marin and Puelles, 1995; Cambronero and Puelles, 2000; Puelles and Rubenstein, 2003). The midbrain is located between these two vesicles, and it is subdivided into two neuromeres (Hidalgo-Sanchez et al., 2005; Allen Developing Mouse Brain Atlas, 2009; Puelles et al., 2012). The rostral segment is larger and is named mesomere 1 (m1). It includes the tectal gray and the two colliculi among other populations. The mesomere 2 (m2) is a rather small segment localized between the inferior colliculus and the isthmus. It corresponds to the preisthmic region (Puelles et al., 2012). In addition to these transversal segments, in the neural tube four longitudinal domains along the dorso-ventral axis are present (DV). These domains are, from ventral to dorsal, the floor, basal, alar and roof plates (Puelles and

Rubenstein, 2003).

The DV patterning is due to two confronted structures that are sources of signaling molecules. On the one hand, the axial mesoderm (notochord and prechordal plate) and the floor plate express Sonic hedgehog (Shh), which is the most important morphogen with inducing capability of the ventral structures in the neural tube. (Patten and Placzek, 2000; Placzek and Briscoe, 2005; Ingham and Placzek, 2006) On the other hand, the roof plate expresses the Bone morphogenetic factor family, BMPs, among other molecules, which are involved in the specification of the dorsal regions (Lee and Jessell, 1999; Chizhikov and Millen, 2005).

The AP patterning is the result of the activity of the secondary organizers. These are groups of cells located in key regions of the neuroepithelium that emit signals with the capability of both inducing and patterning the surrounding tissue. So far, three of these structures have been identified; the anterior neural ridge (ANR) located at the rostral tip of the neural plate, the zona limitans intrathalamica (zli) at the thalamus-prethalamus boundary, and the isthmus (IsO) situated at the boundary between midbrain and hindbrain (Vieira et al., 2010; Martinez et al., 2012). The signals emitted by these organizers have been identified. One of these is Fibroblast growth factor 8 (Fgf8; Crossley and Martin, 1995), which is the main signaling molecule present in the ANR and the IsO. The zli combines the expression of Shh with the expression of Fgf8 at the dorsal tip.

Therefore, the specification of the mesencephalic territory is highly dependent on isthmic activity. The IsO is capable of inducing, when grafted in a more rostral region, the differentiation of an ectopic midbrain and cerebellum in the surrounding areas of the host (Martinez et al., 1991). This induction is due to the release of the Fgf8 protein from de IsO, generating a concentration gradient (Crossley et al., 1996). If this protein is placed directly in the diencephalon it has the capability to reproduce the effect of the isthmic transplant experiments (Martinez et al., 1999). The specification of the midbrain is heavily affected when the levels of Fgf8 are altered. The reduction of Fgf8 activity in the isthmus produces the absence of the anterior part of the midbrain by an apoptotic process. The Fgf8 deficient posterior midbrain suffers a change in its fate and is specified as anterior midbrain (Basson et al., 2008). Then Fgf8 in a low concentration is necessary and sufficient to specify the anterior midbrain, saving it from apoptosis, and a higher dose of this protein is needed for the specification of the posterior midbrain.

In order to localize the expression of Fgf8 in the isthmus the confrontation of two transcription factors is needed. Otx2 is expressed in the midbrain and Gbx2 in the hindbrain. These factors are not needed to induce but to restrain the expression of Fgf8 (Martinez-Barbera et al., 2001). The expression of Otx2 confers to the mesencephalic neuronal progenitors the capability of processing the positional signals received from the different organizers and of triggering the adequate differentiation program.

Antero-posterior and dorso-ventral organization of the midbrain

The midbrain is divided in two neuromeres along the AP axis: The m1, containing the tectal gray, the superior colliculus and the inferior colliculus, and the m2, comprising the preisthmic region. Complementary to this AP patterning, the confrontation of Shh from the floor plate with the BMPs and Wnts families from the roof plate generates the subdivision of the midbrain in the DV axis.

The midbrain-hindbrain boundary was determined by fate map experiments (Echevarria et al., 2003). This limit coincides with the caudal limit of Wnt1 and Otx2 expression domains and with the rostral limit of Fgf8 and Gbx2 expression in the isthmus (Fig. 1A,B; Echevarria et al., 2003). The rostral boundary of the midbrain with the diencephalon coincides with the caudal limit of the alar pretectal domain, which expresses Pax6, and the rostral limit of the alar midbrain that expresses Meis2 (Fig. 1A,B; Ferrán et al., 2009; Agoston et al., 2012).

Along the AP axis, the m1 contains a homogeneous basal plate (midbrain tegmentum), which includes the substantia nigra, the oculomotor complex and the red nucleus, among other populations. The m1 alar plate contains three main regions: the tectal gray (TG; Fig. 1A,B), the superior colliculus (SC; Fig. 1A,B) and the inferior colliculus (IC; Fig. 1A,B).

The TG is a retinorecipient-layered formation and is selectively marked molecularly by the restricted strong expression of Ntng2 (Fig. 1A,B). The SC is also a retinorecipient structure and is involved in the integration of visual inputs. The TG and the SC receive the visual information following a precise retinotopic map (Puelles et al., 2012). During embryonic development different genes are expressed in gradients along the different axes of the SC. Among them, EphAs and ephrin-As are important players in these signaling mechanisms that produce the SC topographic map (Triplett and Feldheim, 2012). Other genes such as Meis2, Ntng2, Sox14, Pax3 or Tal2 are also expressed in gradients in the SC (Fig. 1A,B). All these gradients confer positional information to the navigating retinal axons allowing them to reach their final target. The IC is a massive globular formation, composed by a core (principal nucleus) and a shell, forming an important relay within the ascending auditory system. The expression of Ntng2 and Sox14 identifies the location of the IC, both being positive in the SC and negative in the IC (Fig. 1A,B; Puelles et al., 2012).

The m2 is a thin preisthmic transverse territory, whose basal plate is between the oculomotor nucleus (m1) and the trochlear nucleus (isthmus). This basal domain contains the retrorubral dopaminergic cell population (A8) and the dopaminergic interfascicular nucleus. The caudal limit of the basal m2 coincides with

the caudal end of the interfascicular nucleus and the rostral end of the trochlear nucleus (isthmus, Puelles et al., 2012). The alar plate of m2 contains, among less known structures, the cuneiform and the sagulum nuclei (Puelles et al., 2012), and it is identified by the expression of *Tal2* (Fig. 1A,B).

The mesencephalon has a clear DV organization, the dorsal midbrain, known generically as tectum, and the ventral midbrain, known as tegmentum (Puelles, 2007). Early on, roof (RP) and floor plate (FP) act as DV organizers conferring to the alar and basal plate their respective characteristics, and both of them are subdivided into smaller domains based on various gene expressions (Puelles et al., 2012). Each region shows three subdivisions, in the alar plate we can describe alar dorsal (AD), alar lateral (AL) and alar ventrolateral (AVL), and in the basal plate the basal lateral (BL), basal intermediate (BI) and basal medial (BM) (Fig. 1C; Puelles et al., 2012; Allen developing mouse brain atlas). From dorsal to ventral, the different regions can be revealed by the detection of specific molecules. The roof plate expresses signaling molecules such as BMPs or Wnts (Chizhikov and Millen, 2005), Wnt1 (Fig. 1C) among them. The whole alar plate is identified by the expression of Pou4f1. It is restricted to the mantle zone (Fig. 1C). AD and AL regions are detected by the ventricular expression of Dbx1 (Fig. 1C). The mantle layer of the AVL is positive medially for Nkx2.2 and laterally for Pou4f1 (Fig. 1C). With respect to the basal plate, BL express Olig2, BI express Pax6 and both share the expression of Nkx2.2 (Fig. 1C). BM expresses in the ventricular layer Shh while in the mantle layer Isl1

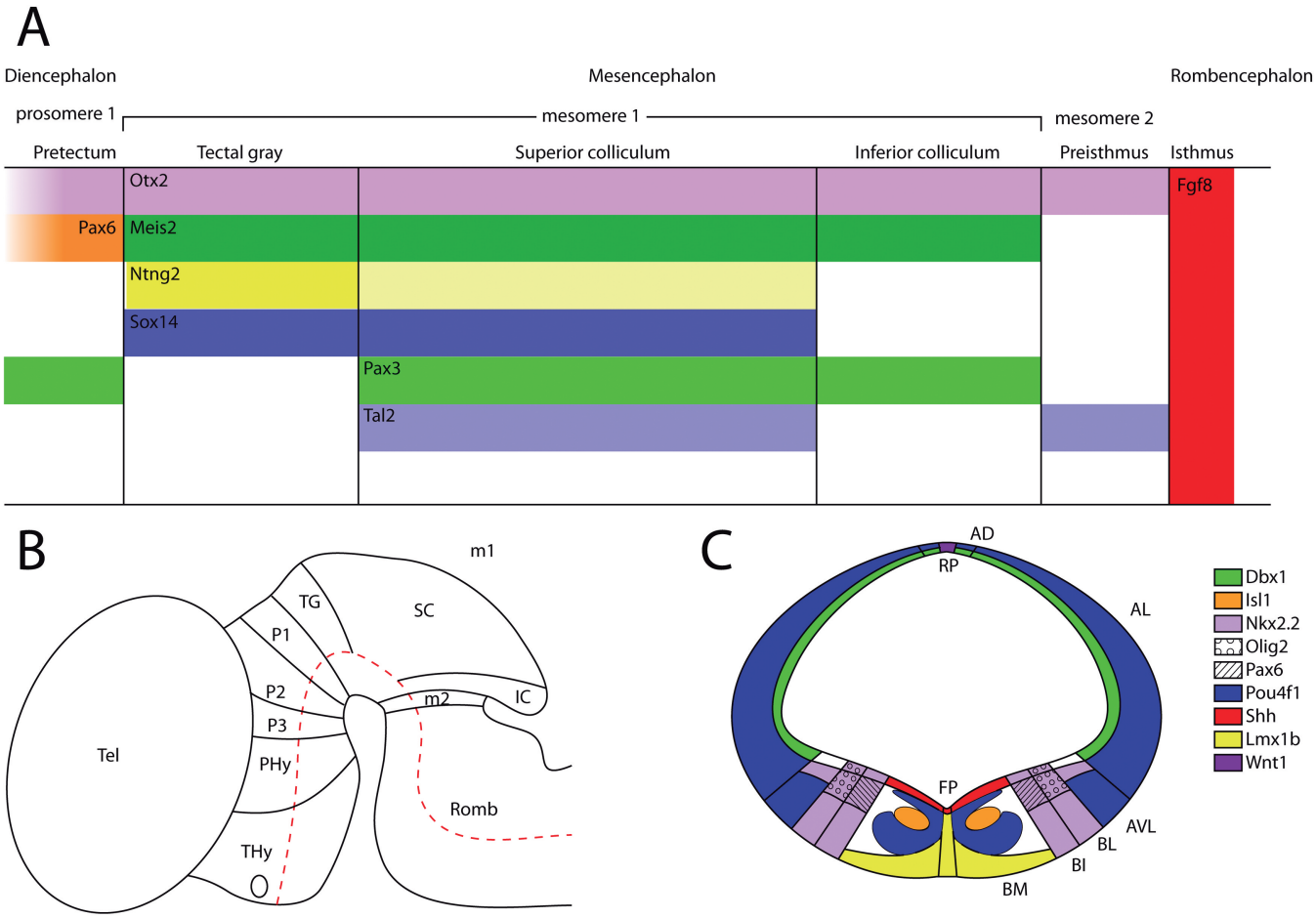

Fig. 1. Schematic representation of the antero-posterior and dorso-ventral organization of the midbrain. **A.** Diagram of the mesencephalic alar plate and surrounding territories displaying the expression pattern of several genes. The combination of these patterns helps us to identify the limits between the different domains. **B.** Schema of the neuromeres that compound the developing brain with special emphasis in the domains of the midbrain. The dashed red line identifies the antero-posterior axis of the neural tube and the alar-ventral limit. **C.** Schema of a transversal section through the midbrain. The different domains are represented and identified by the combination of expression patterns. AD, alar dorsal; AL, alar lateral; AVL, alar ventrolateral; BI, basal intermedial; BL, basal lateral; BM, basal medial; FP, floor plate; IC, inferior colliculus; m1, mesomere 1; m2, mesomere 2; p1, prosomere 1; p2, prosomere 2; p3, prosomere 3; PHy, peduncular hypothalamus; SC, superior colliculus; Romb, rombencephalon; RP, roof plate; Tel, telencephalon; Thy, terminal hypothalamus; TG, tectal grey.

identifies the oculomotor nucleus and Pou4f1 the magnocellular red nucleus (Fig. 1C). Finally, the floor plate and the BM share the expression of Shh in the ventricular layer. Lmx1b is specific of the floor mantle layer and participates in the determination of the dopaminergic populations. These dopaminergic neurons originated in the floor plate gives rise to the ventral tegmental area by radial migration and to the substantia nigra, located in the BL region, by tangential migration (Puelles et al., 2012).

Morphological structures of the midbrain.

Historically, there has been confusion in the delimitation of the mesencephalic territory. The extreme bend of the neural tube in the cephalic flexure produces in mammals a wedge-shape midbrain with a thin basal domain and a broader alar plate. In cross-section, past authors have attributed diencephalic and rombencephalic territories to the mesencephalon. Recent fate map and genetic expression patterns have confirmed the populations that belong to the midbrain (Puelles et al., 2012).

In order to describe the mesencephalic neural populations, we will follow the AP and the DV axis. Thus, we begin rostrally in m1, in the RP, a thin astroglial palisade in which there is a poor-cell mantle layer, where we can find the beds of tectal gray, tectal and intercollicular commissures. Lateral to the RP, it is located the AD. This domain shows a periventricular stratum that contains part of the periaqueductal gray (PAG). The PAG is a dense group of packed neurons, mainly glutamatergic, surrounding the aqueduct of the midbrain. The central and intermediate strata of the AD are occupied by the tectal paracomisural nuclei and the rostral territory contains a separated tectal gray paracommissural nucleus (Puelles et al., 2012). The next domain is AL, ventral to the AD. Its periventricular stratum is also part of the PAG. This domain presents several populations along the rostrocaudal axis of m1. From rostral to caudal, the tectal gray, the superior colliculus (SC; Fig. 2A,D) and the inferior colliculus (IC). The SC is a pluri-laminated retinorecipient structure divided in deep (white and gray layers), intermediate (white and gray layers) and superficial stratum, which receives the massive projections from the collateral retina. This last stratum is divided in the optical, superficial gray and zonal layer (Dräger and

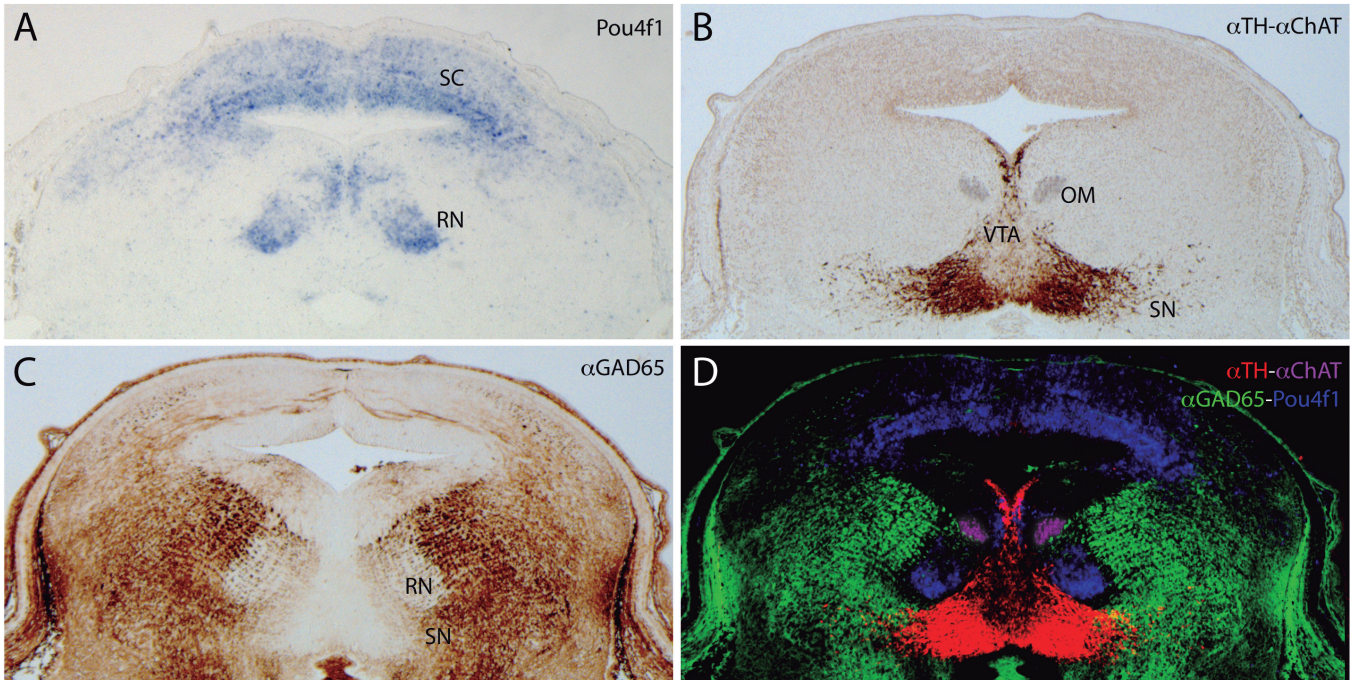

Fig. 2. Identification of the morphological structures of the midbrain. **A-D**, transversal section of the midbrain (mesomere 1) of an E15.5 mouse brain. **A.** *In situ* hybridization with a specific RNA probe against Pou4f1 displaying the localization of the red nucleus in the basal medial domain of the mesencephalon and the superior colliculus in the alar domain. **B.** Double immunohistochemistry with specific antibodies for TH (Institute Jacques Boy Cat. Nº 268020234) and ChAT (Chemicon Cat. Nº AB144P), identifying the dopaminergic substantia nigra and ventral tegmental area and the motorneurons of the oculomotor complex respectively. **C.** Immunohistochemistry with a specific antibody against Gad67 (Millipore Cat. Nº MAB5406) showing the gabaergic populations of the midbrain. **D.** Color-coded overlays of hybridization and immunohistochemistry for TH (red), ChAT (purple), Gad65 (green) and Pou4f1 (blue). The result displayed in the photomicrograph is the result of the superposition of adjacent sections **(B, C)** and a non-adjacent section **(A)**. This overlay allows us to display in the same image the combination of markers that identify the different mesencephalic populations. Abbreviations: OM, oculomotor nucleus; RN, red nucleus; SN, substantia nigra. SC, superior colliculus; VTA, ventral tegmental area.

Hubel, 1975a,b). The IC is constituted by a periventricular central nucleus (CIC) that contains densely packed neurons that are more strongly acetycholinesterase positive than the cortical regions (Franklin and Paxinos, 2008). It receives inputs from all auditory centers in the hindbrain: the cochlear nuclei, the superior olivary complex and the nuclei of the lateral lemniscus, and it projects to the ventral division of the medial geniculate nucleus. Dorsomedially to CIC, the dorsal cortex is located (DCIC) which receives similar inputs from the hindbrain auditory centers and from the nucleus sagulum, and projects to the dorsal division of medial geniculate nucleus. Dorsocentrally to CIC, we can localize the external cortex (ECIC), defined by four layers, two layers contain neurons rich in Acetylcholinesterase and GABA, and the other two link up with the non-stratified rostral part of IC and receive auditory and somatosensory inputs from the hindbrain and spinal cord nuclei. The ECIC also projects to the dorsal division of the medial geniculate and to the magnocellular nucleus (Puelles et al., 2012). Finally, the last subdivision in the dorsal domain, the AVL, contains the liminar alar structures (from *limen alaris*, rim of the alar plate), composed in its periventricular zone by the liminar part of the PAG and in its intermediate stratum by the subtectal portion of the midbrain reticular formation. The superficial stratum is traversed longitudinally by the brachium of SC and IC.

Continuing with the basal plate, the most dorsal parts, BL and BI, are composed in their periventricular stratum by the PAG, possibly containing specialized cells in vocalization, and in their mantle layer by the reticular formation, the largest component of the midbrain tegmentum with non-specific nuclei characterized so far (Puelles et al., 2012). The best-known domain of the basal plate, the BM, contains well-defined neuronal populations. Lateral to the median floor plate and at the border of PAG is the oculomotor nucleus (OM; Fig. 2B,D), a population of motoneurons which innervates several eye muscles and, together with Edinger-Westphal nucleus (EW), preganglionic efferent neurons which project to the ciliary parasympathetic ganglion in the orbit, compromising the most rostral group of motoneurons in the neural tube (Puelles, 2007). Some studies reporting descendent projections from EW to the spinal cord, referred to a pre-EW population, distinct from the proper EW by not being cholinergic and expressing early Nkx6.1 and Nkx6.2 (Puelles et al., 2012). It is also hypothesized that this population correspond to the mesencephalic interstitial nucleus (Moreno-Bravo et al., 2010). Ventral to the OM, in the outer intermediate stratum, is a glutamatergic reticular formation, the red nucleus (RN; Fig. 2A,D) whose magnocellular part, specifically of the midbrain, integrates inputs from the motor cortex and the spinocerebellum and it is part of the rubrospinal tract that controls movement of distal parts of the limbs (Puelles, 2007). Lateral and dorsal to the RN, there is a large reticular area of GABAergic cells, the pararubral area (Paxinos and Watson, 1998; Paxinos, 2009) that expresses Gad67 and parvalbumin (Fig. 2C,D). Projections from somatosensory and motor cortex to this area and to the rostral part of RN are suggested as the basis of an inhibitory pathway to modulate the rubroespinal control over flexor movements of the limbs (Puelles et al., 2012). Lateroventrally in this stratum there is the substantia nigra (SN; Fig. 2B,D), divided into superficial (pars reticularis, SNr) and deep portions (pars compacta, SNc) (Puelles, 2007) and its main input is inhibitory from the striatum, receiving also projections from a range of other structures (Fallon and Loughlin, 1995; Puelles et al., 2012). Neurons of SNr are GABAergic and project to the superior colliculus, thalamus and some midbrain and hindbrain nuclei involved in motor control (Fig. 2C,D; Faull and Mehler, 1978; Puelles et al., 2012). Neurons of SNc project to the striatum, via the nigrostriatal tract, which is affected in Parkinson's disease (Lang and Lozano, 1998a,b) and express tyrosine hydroxylase (TH) early in development (Marin et al., 2005) like the group of neurons located near the midline known as the ventral tegmental area (VTA; Fig. 2B,D). These neurons, derived from developing cells of the floor plate, project to the nucleus accumbens and the limbic cerebral cortex modulating cognitive process (Prakash and Wurst, 2006) and its deregulation is related to the development of addiction (Kelley and Berridge, 2002) and depression (Dailly et al., 2004). Finally, the last domain in the m1 is the floor plate, crossed by two distinct bundles of fibers, the dorsal tegmental decussation (tectobulbar and tectospinal tracts) and the dorsal tegmental decussation (rubrospinal tract). In its intermediate stratum there is the rostral linear nucleus located along the midline and medial to the parabrachial pigmented nuclei, which together with the paranigral and parainterfascicular nuclei form the different subnuclei recognized in the VTA (Puelles et al., 2012).

Continuing in the caudal part of the midbrain, the m2 is a thin transverse ring domain known as preisthmus (Hidalgo-Sanchez et al., 2005) and owing to its different molecular profile it has been recently recognized as a distinct domain (Puelles et al., 2012 and Allen developing mouse brain atlas). Like a complete neuromeric ring of the neural tube, it has roof, alar, basal and floor components (Puelles et al., 2012). The RF, in front of the decussation of the trochlear nerve, is very thin and unremarkable (Puelles et al., 2012). The AD, at both sides of the roof plate, contains the nucleus sagulum (Sag), which is linked to a variety of auditory areas in the hindbrain, midbrain and forebrain. The AL, caudal to the IC, contains the stratified cuneiform gray (CnG) that it is adjacent to the isthmo-mesencephalic border and its periventricular stratum belongs to the PAG. In the AVL, ventral to the cuneiform gray there is the subcuneiform nucleus, which projects to the contralateral spinal cord (Puelles et al., 2012).

The more dorsal domain of the basal plate, the BL and BI, contribute with their periventricular stratum to

the PAG and with their intermediate stratum to the mesencephalic reticular formation in the mantle layer (Puelles et al., 2012) with a large derivate of GABAergic neurons due to the expression of the transcription factor Helt (Nakatani et al., 2007; Guimera et al., 2006). The BM of m2 contributes to the same populations described in the BM of m1. It is specifically located the dopaminergic retrorubral area in the intermediate stratum of this domain. The periventricular PAG also shows the rostral tip of the dorsal raphe nucleus, composed mainly by neurons that express serotonin and by others that express somatostatin (Puelles et al., 2012). The last basal domain of m2, the FP, has only one derivate, the interfascicular nucleus (Puelles et al., 2012), included in the caudal VTA (Phillipson, 1979a-c; Ferreira et al., 2008) and projects opportunistically to the habenula via the retroflex tract (Puelles et al., 2012).

Genetic specification of the basal midbrain

Substantia nigra and ventral tegmental area

The mesencepahlic dopaminergic neurons (mDN) are the most studied population owing to their neurodegenerative alteration in several motor syndromes like Parkinson's disease or schizophrenia. The genetic cascade responsible for their differentiation has been amply elucidated (Simeone, 2005; Prakash and Wurst, 2006). There are three main groups of factors composed by extracellular, intracellular and other signaling factors that act on the specification of the mitotic precursors, post-mitotic precursors and on early dopaminergic specified cells.

During the mitotic progenitor stage, the Shh secreted by the floor plate organizer is necessary and sufficient to instruct the ventral cell fate and defines a multiple progenitor pool of early mDNs (Hynes et al., 1995; Abeliovich and Hammond, 2007; Joksimovic et al., 2009). Progenitor cells expressing Patched receptor complex transduce the Shh signal to the transmembrane protein Smoothened. Then, this complex induces the intracellular transcriptional activation form of Gli2 which upregulates Gli1, sufficient for the induction of floor plate markers as Foxa2 (Fuccillo et al., 2006; Blaess et al., 2006, Abeliovich and Hammond, 2007). Fate-map and birth-dating analysis has proven that progenitors in the ventral midline contribute to mDNs in response to Shh before E10.5 (Joksimovic et al., 2009; Blaess et al., 2011). The inhibition of the ventral organizer alters the function of the IsO through the interaction between Shh-Gli3-Fgf8 (Blaess et al., 2006). The two main organizer centers involved in the production of the positional information needed for the specification of the mDNs are closely interrelated (Ye et al., 1998).

The Otx genes, Otx1 and Otx2, participate in the control of the proper position of the IsO (Fgf8 and Wnt1 expression domains). They also instruct the mesencephalic precursors to generate the correct response to the positional information received (Puelles et al., 2003; Simeone, 2005; Simeone et al., 2011). One of Otx2 functions is to repress the expression of the transcription factor Nkx2.2 in the basal medial area in order to prevent the specification of serotoninergic neurons instead of mDN (Puelles et al., 2004) and is intrinsically required to control the mDN neurogenesis through a graded AP effect (Omodei et al., 2008) possibly through the regulation of Wnt1 (Alves dos Santos and Smidt, 2011; Simeone et al., 2011).

The mesencephalic precursors with the correct positional information and capabilities trigger the dopaminergic differentiation program, expressing transcription factors such as Lmx1a and Lmx1b (Andersson et al., 2006; Smidt and Burbach, 2007; Deng et al., 2011). Lmx1a is selectively required for the specification of mDNs in the medial area, whereas Lmx1b is necessary for the mDN progenitors located laterally (Deng et al., 2011). Therefore, the Lmx1a/Lmx1b combination has proven essential to initiate the process of all mDNs generation. Lmx1a induces Msx1, which inhibits the expression of negative regulators such as Nkx6.1 and induces the expression of Ngn2, which in turn will induce the expression of Sox2. This is a required factor for the differentiation of the early progenitors into postmitotic mDN that will express Nurr1 (Simeone et al., 2011; Abeliovich and Hammond, 2007). Lmx1b is also suggested to be involved in the activation of Pitx3, which is required for the correct formation, specification and/or survival of mDN in the SN but not in VTA (Smidt et al., 2004; Smidt and Burbach, 2007).

The Nurr1 positive early neurons initiate their postmitotic development. Nurr1 is an important intrinsic factor for the generation of mature mDN (Saucedo-Cardenas et al., 1998). It regulates the expression of several genes involved in the generation and regulation of dopamine (Smidt and Burbach, 2007), such as tyrosine hydroxylase, a limiting enzyme involved in the synthesis of this neurotransmitter. The lack of Nurr1 function produces a complete absence of mDN (Abeliovich and Hammond, 2007).

There is a group of transcription factors involved not only in the determination of the mDN, but also in their late survival. Engrailed-1 (En1) is a crucial factor in early mesencephalic development (Alves dos Santos and Smidt, 2011). Adult mice heterozygous for En1 suffer a progressive loss of mDN (Sonnier et al., 2007). Recently it has been proven that En1 protects dopaminergic neurons against mitochondrial insults (Alvarez-Fischer et al., 2011). Another transcription factor involved in the survival of the mDN is Foxa2. Adult heterozygous mice for Foxa2 suffer a spontaneous and progressive loss of these cells (Kittappa et al., 2007). The activation of Otx2 in the VTA mDN modifies their cellular biology and confers them resistance to MPTP mediated degeneration

(Di Salvio et al., 2010).

Red nucleus

The RN is organized in two subpopulations, the parvocellular part located in the posterior diencephalon and the magnocellular part located in the mesencephalon.

The mesencephalic RN population appears to be related to the Shh-positive basal domain. Its ventricular region is also positive for Nkx.6.1 and gives rise to the oculomotor nucleus as well (Agarwala and Ragsdale, 2002). The progenitors committed to form the RN switch off the expression of Nkx6.1 as soon as they leave the proliferating ventricular layer and switch on the expression of the transcription factor Pou4f1. It has been demonstrated that a Shh overexpression in the developing midbrain produces inactivation of this ventricular Nkx6.1 domain, resulting in a complete lack of RN (Puelles et al., 2003, 2004). Recently, it has been confirmed that the lack of Nkx6.1 generates an abnormal RN with a reduced number of Pou4f1 positive neurons (Prakash et al., 2009).

We demonstrated that this nucleus is maintained in the absence of Shh in the midbrain. Even so this population displayed some disorganization with some ectopic Pou4f1 positive neurons crossing the ventral midline and colonizing the putative location of the lost OM and SN neurons. The number of Pou4f1 positive cells did not increase in this conditional mutant mouse. We hypothesized that the maintenance of the red nucleus could be due to the role of a Foxa2 mechanism in a Shh-independent manner (Perez-Balaguer et al., 2009).

In addition, it has been demonstrated that Foxa2 and Foxa1 are necessary and sufficient to induce the development of this nucleus. The suppression of both genes is required to produce a complete absence of the red nucleus (Ferri et al., 2007). Recently, it has been described that Foxa1 and Foxa2 participate in the specification of the ventral midbrain progenitors interacting with the Shh genetic cascade (Mavromatakis et al., 2011).

OM and RN neurons are derived from the same progenitor domain but at different time points in development. Lmx1b is necessary for the early inhibition of the specification of the red nucleus differentiation program at the beginning of the midbrain basal plate development (Deng et al., 2011). In Lmx1b mutants, there is an increase of the Pou4f1 positive cells and a decrease in the expression of Phox2a (marker of OM neurons). The lack of Lmx1b function generates a change in the progenitors fate from OM to RN neurons, giving rise to an overproduction of Pou4f1 positive cells (Deng et al., 2011).

The proportion of RN and OM population seems to be related to isthmic signaling. Near the Fgf8 source the number of OM neurons are predominant over the RN. As we move rostrally the relation is inverted. An ectopic source of Fgf8 in the anterior midbrain alters this proportion. It produces the differentiation of ectopic Islet1-positive cells (OM) in the new location in detriment of the Pou4f1-positive neurons (RN; Fedtsova and Turner, 2001).

Oculomotor nucleus

It is well known that the morphogen Shh is essential for the determination of the motor neurons in all the neural tube, and the lack of this gene produces the absence of ventral structures and, consequently, the absence of motorneurons (Litingtung and Chiang, 2000). On the one hand Shh is able, by itself, to induce this neural type in in vitro assays (Tanabe et al., 1995). On the other hand Fgf8 signaling is implicated in the specification of this nucleus (see above).

The OM is specified early on in embryonic development. Overexpression of Shh in 9.5 dpc embryos does not produce changes in this population, due to the fact that it is already specified at this time (Puelles et al., 2003, 2004).

In the Shh conditional mutant mouse, where the lack of this gene (form 8.5dpc onwards) is driven by the expression of Engrailed1, we could only detect some Nkx6.1 and Islet1 co-expressing positive cells belonging to the OM at E10.5, but at 12.5 they were completely absent. For the first time is was proved that Shh is not only necessary to generate the OM neurons, but also to maintain this population (Perez-Balaguer et al., 2009).

In cooperation with Shh, Nkx6.1 is involved in the differentiation of this nucleus. It is expressed in two ventricular domains in the midbrain, one ventral related to the Shh expression domain, and the other dorsal separated from the first by the Nkx2.2-positive band. The OM originates from this Nkx6.1-positive ventricular ventral domain. It was previously described that there is a complete lack of these somatic motor neurons in the loss of function mouse for this gene (Sander et al., 2000), although recently it was proved that there are some Islet1-positive neurons remaining. These neurons have an abnormal migration in the mesencephalic neural tube and project their axons in erroneous directions (Prakash et al., 2009).

There is also evidence, that Lmx1b is involved in the specification of this complex, through the activity of Phox2a. It controls the switch in time in the ventricular progenitors from the OM to the RN differentiation program (Deng et al., 2011).

Mesencephalic interstitial nucleus

Recently, a population positive for Nkx6.1 and Nkx6.2 with a longitudinal distribution along the basal midbrain and diencephalon was described. This population may correspond from rostral to caudal to a functional column integrated in the reticular formation (Moreno-Bravo et al., 2010). Due to its location, the medial part of this positive column for Nkx6.2 corresponds to the pre-Edinger-Westphal nucleus

(Puelles, 2007; Franklin and Paxinos, 2008).

The lateral part of this column would contribute to the rostral interstitial nucleus in the thalamic segment (prosomere 2, Puelles and Rubenstein, 2003), to the interstitial nucleus of Cajal in the pretectal segment (prosomere 1, Puelles and Rubenstein, 2003) and to the interstitial mesencephalic nucleus in the midbrain (Moreno-Bravo et al., 2010). All these populations would constitute a functional column of pre-motor nuclei involved in the saccadic movements of the eyes.

This Nkx6.2-positive population is not only maintained in the absence of Shh in the basal midbrain, but also increased. Therefore, the specification of these Nkx6.2 positive cells is independent of this morphogen. We hypothesized that Foxa2 could be the candidate to induce this differentiation program (Perez-Balaguer et al 2009).

Periacueductal gray and diffuse reticular formation

The mesencephalic populations with a less characterized genetic differentiation program are the periaqueductal gray (PAG) and the diffuse reticular formation (Puelles, 2007). The PAG is a dense group of neurons located around the mesencephalic ventricle. It is divided into longitudinal domains parallel to the AP axis corresponding to the DV territories described previously. Each domain has different functional roles; the dorsal area is involved in active defensive/coping strategies while the ventral area has been related with passive coping strategies (Puelles et al., 2012). The diffuse reticular formation is a dispersed group of neurons occupying the remaining mantle layer in the midbrain. In this region no specific areas have been described as specific nuclei or functional centers so far (Puelles et al., 2012).

Conclusions

The development of molecular biology techniques and the increasing number of available specific antibodies allows us to increase the morphological knowledge of the different regions of the developing brain. Simultaneously, the generation of different mouse models through genetic engineering permits us to elucidate the genetic cascades that control the differentiation program of the neuronal nuclei that populate the nervous system. In this review we have joined the explanation of developing embryonic events that give rise to the mesencephalon with the latest updates of the morphological structure of the midbrain, and with the known genetic cascades that control the development of the mesencephalic basal plate.

Acknowledgements. MICINN (BFU2010-16548, FEDER Fonds), MICINN Consolider Grant (CSD2007-00023) and European commission (EUCOMMTOOLS, contract 261492) supported this work. The CSIC-JAE-Predoc program co-financed by FSE supports JA Moreno-Bravo.

References

Abeliovich A. and Hammond R. (2007). Midbrain dopamine neuron differentiation: factors and fates Dev. Biol. 304, 447-454.

Allen Developing Mouse Brain Atlas (2009). Allen Institute for Brain Science. Seattle. Available from: http://developingmouse.brain-map.org.

Agarwala S. and Ragsdale C.E. (2002). A role for midbrain arcs in nucleogenesis. Develpment 129, 5779-5788.

Agoston Z., Li N., Haslinger A., Wizenmann A. and Schulte D. (2012). Genetic and physical interaction of Meis2, Pax3 and Pax7 during dorsal midbrain development BMC Dev. Biol. 12, p10.

Alvarez-Fischer D., Fuchs J., Castagner F., Stettler O., Massiani-Beaudoin O., Moya K.L., Bouillot C., Oertel W., Lombès A., Faigle W., Joshi R., Hartmann A. and Prochiantz A. (2011). Engrailed protects mouse midbrain dopaminergic neurons against mitochondrial complex I insults. Nat. Neurosci. 14, 1260-1266.

Alves dos Santos M.T.M. and Smidt M.P. (2011). En1 and Wnt signaling in midbrain dopaminergic neuronal development. Neural Dev. 6, 23.

Andersson E., Tryggvason U., Deng Q., Friling S., Alekseenko Z., Robert B., Perlmann T. and Ericson J. (2006). Identification of intrinsic determinants of midbrain dopamine neurons. Cell 124, 393-405.

Basson M.A., Echevarria D., Ahn C.P., Sudarov A., Joyner A.L., Mason I.J., Martinez S. and Martin G.R.(2008). Specific regions within the embryonic midbrain and cerebellum require different levels of FGF signaling during development. Development 135, 889-898.

Blaess S., Corrales J.D. and Joyner A.L. (2006). Sonic hedgehog regulates Gli activator and repressor functions with spatial and temporal precision in the mid/hindbrain region. Development 133, 1799-1809.

Blaess S., Bodea G.O., Kabanova A., Chanet S., Mugniery E., Derouiche A., Stephen D. and Joyner A.L. (2011). Temporal-spatial changes in Sonic Hedgehog expression and signaling reveal different potentials of ventral mesencephalic progenitors to populate distinct ventral midbrain nuclei. Neural Dev. 6, 29.

Cambronero F. and Puelles L. (2000). Rostrocaudal nuclear relationships in the avian medulla oblongata: a fate map with quail chick chimeras J. Comp. Neurol. 427, 522-545.

Chizhikov V.V. and Millen K.J. (2005). Roof plate-dependent patterning of the vertebrate dorsal central nervous system. Dev. Biol. 277, 287-295.

Crossley P.H. and Martin G.R. (1995). The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. Development 121, 439-451.

Crossley P.H., Martínez S. and Martin G.R. (1996). Midbrain development induced by FGF8 in the chick embryo. Nature 380, 66-68.

Dailly E., Chenu F., Renard C.E. and Bourin M. (2004). Dopamine, depression and antidepressants. Fundam. Clin. Pharmacol. 18, 601-607.

Deng Q., Andersson E., Hedlund E., Alekseenko Z., Coppola E., Panman L., Millonig J.H., Brunet J.F., Ericson J. and Perlmann T. (2011). Specific and integrated roles of Lmx1a, Lmx1b and Phox2a in ventral midbrain development. Development 138, 3399-3408.

Di Salvio M., Di Giovannantonio L.G., Acampora D., Prosperi R., Omodei D., Prakash N., Wurst W. and Simeone A. (2010). Otx2 controls neuron subtype identity in ventral tegmental area and

antagonizes vulnerability to MPTP. Nat. Neurosci. 13, 1481-1488.

Drager U.C. and Hubel D.H. (1975a). Physiology of visual cells in mouse superior colliculus and correlation with somatosensory and auditory input. Nature 253, 203-204.

Drager U.C. and Hubel D.H. (1975b). Responses to visual stimulation and relationship between visual, auditory, and somatosensory inputs in mouse superior colliculus. J. Neurophysiol. 38, 690-713.

Echevarria D., Vieira C., Gimeno L. and Martinez, S. (2003). Neuroepithelial secondary organizers and cell fate specification in the developing brain. Brain Res. Brain Res. Rev. 43, 179-191.

Fallon J.H. and Louglin S.E. (1995). Substantia nigra. In: The rat nervous system. Paxinos G (ed). Academic Press. San Diego. pp 215-237.

Faull R.L. and Mehler W.R. (1978). The cells of origin of nigrotectal, nigrothalamic and nigrostriatal projections in the rat. Neuroscience 3, 989-1002.

Fedtsova N. and Turner E.E. (2001). Signals from the ventral midline and isthmus regulate the development of Brn3.0-expressing neurons in the midbrain. Mech. Dev. 105, 129-144.

Ferran J.L., de Oliveira E.D., Merchán P., Sandoval J.E., Sánchez-Arrones L., Martínez-De-La-Torre M. and Puelles L. (2009). Genoarchitectonic profile of developing nuclear groups in the chicken pretectum. J. Comp. Neurol. 1, 405-451.

Ferreira J.G.P., Del-Fava F., Hasue R.H. and Shammah-Lagnado S.J. (2008). Organization of ventral tegmental area projections to the ventral tegmental area-nigral complex in the rat. Neuroscience 153, 196-213.

Ferri A.L.M., Lin W., Mavromatakis Y.E., Wang J.C., Sasaki H., Whitsett J.A. and Ang S. (2007). Foxa1 and Foxa2 regulate multiple phases of midbrain dopaminergic neuron development in a dosage-dependent manner. Development 134, 2761-2769.

Franklin K.B.J. and Paxinos G. (2008). The mouse brain in stereotaxic coordinates. 3rd Edition. Elsevier Academic Press. San Diego.

Fuccillo M., Joyner A.L. and Fishell G. (2006). Morphogen to mitogen: the multiple roles of hedgehog signalling in vertebrate neural development. Nat. Rev. Neurosci. 7, 772-783.

Guimera J., Weisenhorn D.V. and Wurst W. (2006). Megane/Heslike is required for normal GABAergic differentiation in the mouse superior colliculus. Development 133, 3847-3857.

Hidalgo-Sánchez M., Martínez-de-la-Torre M., Alvarado-Mallart R. and Puelles L. (2005). A distinct preisthmic histogenetic domain is defined by overlap of Otx2 and Pax2 gene expression in the avian caudal midbrain. J. Comp. Neurol. 483, 17-29.

Hynes M., Porter J.A., Chiang C., Chang D., Tessier-Lavigne M., Beachy P.A. and Rosenthal A. (1995). Induction of midbrain dopaminergic neurons by Sonic hedgehog. Neuron 15, 35-44.

Ingham P.W. and Placzek M. (2006). Orchestrating ontogenesis: variations on a theme by sonic hedgehog. Nat. Rev. Genet. 7, 841-850.

Joksimovic M., Anderegg A., Roy A., Campochiaro L., Yun B., Kittappa R., McKay R. and Awatramani R.(2009). Spatiotemporally separable Shh domains in the midbrain define distinct dopaminergic progenitor pools. Proc. Natl. Acad. Sci. USA 106, 19185-19190.

Kelley A.E. and Berridge K.C. (2002). The neuroscience of natural rewards: relevance to addictive drugs. J. Neurosci. 22, 3306-3311.

Kittappa R., Chang W.W., Awatramani R.B. and McKay R.D.G. (2007). The foxa2 gene controls the birth and spontaneous degeneration of dopamine neurons in old age. PLoS Biol. 5, e325.

Lang A.E. and Lozano A.M. (1998a). Parkinson's disease. First of two parts. N. Engl. J. Med. 339, 1044-1053.

Lang A.E. and Lozano A.M. (1998b). Parkinson's disease. Second of two parts. N. Engl. J. Med. 339, 1130-1143.

Lee K.J. and Jessell T.M. (1999). The specification of dorsal cell fates in the vertebrate central nervous system. Annu. Rev. Neurosci. 22, 261-294.

Litingtung Y. and Chiang C. (2000). Specification of ventral neuron types is mediated by an antagonistic interaction between Shh and Gli3. Nat. Neurosci. 3, 979-985.

Marín F. and Puelles L. (1995). Morphological fate of rhombomeres in quail/chick chimeras: a segmental analysis of hindbrain nuclei. Eur. J. Neurosci. 7, 1714-1738.

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

Martínez S., Wassef M. and Alvarado-Mallart R.M. (1991). Induction of a mesencephalic phenotype in the 2-day-old chick prosencephalon is preceded by the early expression of the homeobox gene en. Neuron 6, 971-981.

Martínez S., Crossley P.H., Cobos I., Rubenstein J.L. and Martin G.R. (1999). FGF8 induces formation of an ectopic isthmic organizer and isthmocerebellar development via a repressive effect on Otx2 expression. Development 126, 1189-1200.

Martinez S., Puelles E., Puelles L. and Echevarría D. (2012). Molecular regionalization of the developing neural tube. In: The mouse nervous system. First Edition. Watson C., Paxinos G. and Puelles L. (eds). Elsevier Academic Press. San Diego. pp 2-18.

Martinez-Barbera J.P., Signore M., Boyl P.P., Puelles E., Acampora D., Gogoi R., Schubert F., Lumsdem A. and Simeone A. (2001). Regionalisation of anterior neuroectoderm and its competence in responding to forebrain and midbrain inducing activities depend on mutual antagonism between OTX2 and GBX2. Development 128, 4789-4800.

Mavromatakis Y.E., Lin W., Metzakopian E., Ferri A.L.M., Yan C.H., Sasaki H., Whisett J. and Ang S. (2011). Foxa1 and Foxa2 positively and negatively regulate Shh signalling to specify ventral midbrain progenitor identity. Mech. Dev. 128, 90-103.

Morales-Delgado N., Merchan P., Bardet S.M., Ferrán J.L., Puelles L. and Díaz C. (2011). Topography of somatostatin gene expression relative to molecular progenitor domains during ontogeny of the mouse hypothalamus. Front. Neuroanat. 5, 10.

Moreno-Bravo J.A., Perez-Balaguer A., Martinez S. and Puelles E. (2010). Dynamic expression patterns of Nkx6.1 and Nkx6.2 in the developing mes-diencephalic basal plate. Dev. Dyn. 239, 2094-2101.

Nakatani T., Minaki Y., Kumai M. and Ono Y. (2007). Helt determines GABAergic over glutamatergic neuronal fate by repressing Ngn genes in the developing mesencephalon. Development 134, 2783-2793.

Omodei D., Acampora D., Mancuso P., Prakash N., Di Giovannantonio L.G., Wurst W. and Simeone, A. (2008). Anterior-posterior graded response to Otx2 controls proliferation and differentiation of dopaminergic progenitors in the ventral mesencephalon. Development 135, 3459-3470.

Patten I. and Placzek M. (2000). The role of Sonic hedgehog in neural tube patterning. Cell. Mol. Life Sci. 57, 1695-1708.

Paxinos G. and Watson C. (1998). The rat brain in stereotaxic coordinates. 4th ed. Academic Press. San Diego.

Paxinos G., Watson C., Carrive P., Kirkcaldie M. and Ashwell K. (2009). Chemoarchitectonic atlas of the rat brain. Second edition. Elsevier Academic Press. San Diego.

Perez-Balaguer A., Puelles E., Wurst W. and Martinez S. (2009). Shh dependent and independent maintenance of basal midbrain. Mech. Dev. 126, 301-313.

Phillipson O.T. (1979a). The cytoarchitecture of the interfascicular nucleus and ventral tegmental area of Tsai in the rat. J. Comp. Neurol. 187, 85-98.

Phillipson O.T. (1979b). A Golgi study of the ventral tegmental area of Tsai and interfascicular nucleus in the rat. J. Comp. Neurol. 187, 99-115.

Phillipson O.T. (1979c). Afferent projections to the ventral tegmental area of Tsai and interfascicular nucleus: a horseradish peroxidase study in the rat. J. Comp. Neurol. 187, 117-143.

Placzek M. and Briscoe J. (2005). The floor plate: multiple cells, multiple signals. Nat. Rev. Neurosci. 6, 230-240.

Prakash N. and Wurst W. (2006). Development of dopaminergic neurons in the mammalian brain. Cell. Mol. Life Sci. 63, 187-206.

Prakash N., Puelles E., Freude K., Trümbach D., Omodei D., Di Salvio M., Sussel L., Ericson J., Sander M., Simeone A. and Wurst W. (2009). Nkx6-1 controls the identity and fate of red nucleus and oculomotor neurons in the mouse midbrain. Development 136, 2545-2555.

Puelles E. (2007). Genetic control of basal midbrain development. J. Neurosci. Res. 85, 3530-3534.

Puelles L. and Rubenstein J.L.R. (2003). Forebrain gene expression domains and the evolving prosomeric model. Trends Neurosci. 26, 469-476.

Puelles E., Acampora D., Lacroix E., Signore M., Annino A., Tuorto F., Filosa S., Corte G., Wurst W., Ang S. and Simeone A. (2003). Otx dose-dependent integrated control of antero-posterior and dorso-ventral patterning of midbrain. Nat. Neurosci. 6, 453-460.

Puelles E., Annino A., Tuorto F., Usiello A., Acampora D., Czerny T., Brodski C., Ang S., Wurst S. and Simeone A. (2004). Otx2 regulates the extent, identity and fate of neuronal progenitor domains in the ventral midbrain. Development 131, 2037-2048.

Puelles E., Martinez-de-la-Torre M., Watson C. Puelles L. (2012). Midbrain. In: The mouse nervous system. First Edition. Watson C., Paxinos G and Puelles L. (eds). Elsevier Academic Press. San Diego. pp 337-359.

Sander M., Sussel L., Conners J., Scheel D., Kalamaras J., Cruz F., Schwitzgebel V., Hayes-Jordan A. and German M. (2000). Homeobox gene Nkx6.1 lies downstream of Nkx2.2 in the major pathway of beta-cell formation in the pancreas. Development 127, 5533-5540.

Saucedo-Cardenas O., Quintana-Hau J.D., Le W.D., Smidt M.P., Cox J.J., de Mayo F., Burbach J.P. and Conneely M.O. (1998). Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. Proc. Natl. Acad. Sci. USA 95, 4013-4018.

Simeone A. (2005). Genetic control of dopaminergic neuron differentiation Trends Neurosci. 28, 62-65.

Simeone A., Puelles E., Omodei D., Acampora D., Di Giovannantonio L.G., Di Salvio M., Mancuso P. and Tomasetti C.(2011). Otx genes in neurogenesis of mesencephalic dopaminergic neurons. Dev. Neurobiol. 71, 665-679.

Smidt M.P. and Burbach J.P.H. (2007). How to make a meso-diencephalic dopaminergic neuron. Nat. Rev. Neurosci. 8, 21 -32.

Sonnier L., Le Pen G., Hartmann A., Bizot J., Trovero F., Krebs M. and Prochiantz A. (2007). Progressive loss of dopaminergic neurons in the ventral midbrain of adult mice heterozygote for Engrailed1. J. Neurosci. 27, 1063-1071.

Tanabe Y., Roelink H. and Jessell T.M. (1995). Induction of motor neurons by Sonic hedgehog is independent of floor plate differentiation. Curr. Biol. 5, 651-658.

Triplett J.W. and Feldheim D.A. (2012). Eph and ephrin signaling in the formation of topographic maps. Semin. Cell Dev. Biol. 23, 7-15.

Vieira C., Pombero A., García-Lopez R., Gimeno L., Echevarria D. and Martinez S. (2010). Molecular mechanisms controlling brain development: an overview of neuroepithelial secondary organizers. Int. J. Dev. Biol. 54, 7-20.

Ye W., Shimamura K., Rubenstein J.L., Hynes M.A. and Rosenthal A. (1998). FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. Cell 93, 755-766.

Accepted August 16, 2012