The Amniote Oculomotor Complex

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ABSTRACT

The oculomotor (OM) complex is a combination of somatic and parasympatethic neurons. The correct development and wiring of this cranial pair is essential to perform basic functions: eyeball and eyelid movements, pupillary constriction, and lens accommodation. The improper formation or function of this nucleus leads pathologies such as strabismus. We describe the OM organization and function in different vertebrate brains, including chick, mouse, and human. The morphological localization is detailed, as well as the spatial relation with the trochlear nucleus in order to adjust some misleading anatomical topographic descriptions. We detailed the signaling processes needed for the specification of the OM neurons. The transcriptional programs driven the specification and differentiation of these neurons are partially determined. We summarized recent genetic studies that have led to the identification of guidance mechanisms involved in the migration, axon pathfinding, and targeting of the OM neurons. Finally, we overviewed the pathology associated to genetic malformations in the OM development and related clinical alterations. Anat Rec, 00:000–000, 2018. © 2018 Wiley Periodicals, Inc.

Key words: oculomotor nucleus; Peripheral nervous system; clinical anatomy; segmental organization; cranial nerve nuclei

THE OCULOMOTOR COMPLEX

The third cranial nerve neuronal nuclei are a compound of somatic motor neurons and preganglionar parasympatethic neurons. The somatic oculomotor (OM) neuronal populations are organized in five subnuclei in humans (Table 1), which, together with the trochlear and abducens nuclei, innervate the extraocular muscles. The five subnuclei are organized in a central and a lateral group (Wilson-Pauwels and Akesson, 2001). The

Abbreviations: III = Oculomotor nucleus; IV = trochlear nucleus; VII = facial nucleus; ANR = anterior neural ridge; BDNF = brain-derived neurotrophic factor; bp = basal plate; C = central subnucleus; ChAT = Choline acetyltransferase; DL = dorsal lateral subnucleus; DM = dorsal medial subnucleus; EW = Edinger-Westphal nucleus; GDNF = glial cell-line-derived neurotrophic factor; H = hindbrain; IL = Intermediate lateral subnucleus; IsO = isthmic organizer; M = medial subnucleus; Mb = midbrain; NGF = nerve growth factor; OM = oculomotor; p1 = prosomere 1; p2 = prosomere 2; p3 = prosomere 3; pre-EW = pre-Edinger-Westphal nucleus; VL = ventral lateral subnucleus; Vm = mesencephalic trigeminal nucleus; VM = ventral medial subnucleus; Zli = zona limitans intrathalamica

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TABLE 1. Components of the human oculomotor complex

Human nucleus	Chick nucleus	Innervation	Muscle	Action
Medial Subnucleus	Ventromedial	Contralateral	Superior rectus muscle	To roll the eye upwards and intort it
Central subnucleus	n/a	Bilateral	Levator palpebrae superioris	To raise the eyelid during upwards gaze
Lateral subnuclei				
Dorsal	Dorsolateral	Ipsilateral	Inferior rectus muscle	To turn the eye downwards and extort it
Intermediate	Ventromedial	Ipsilateral	Inferior oblique muscle	To roll the eye upwards and abduct and extort it
Ventral	Dorsomedial	Ipsilateral	Medial rectus muscle	To adduct the eye toward the nose

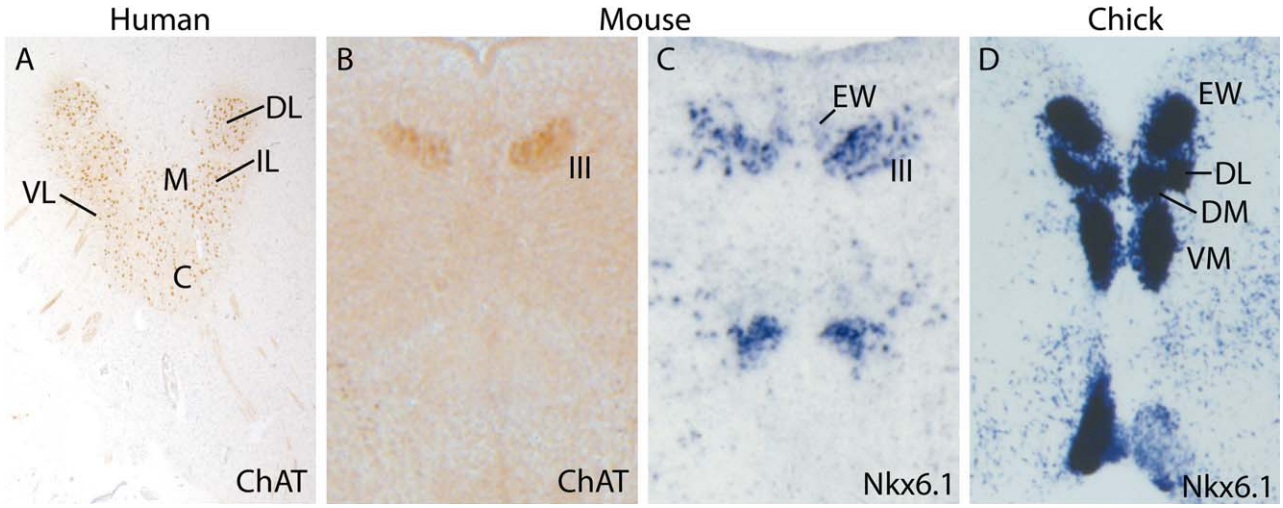

Fig. 1. Description of the OM components in different vertebrates. We show the components of the OM complex in human, mouse, and chick samples. (**A**) Transverse section of a 54 days old human midbrain immunohistochemically labeled for ChAT. (**B**) Transverse section of a E15.5 mouse embryo labeled with immunohistochemistry against ChAT. (**C**) Transverse section of a E15.5 mouse embryo labeled with *in situ* hybridization against Nkx6.1. (**D**) Transverse section of a HH29 chick embryo labeled with *in situ* hybridization against Nkx6.1. Abbreviations: C, central subnucleus; DL, dorsal lateral subnucleus; DM, dorsal medial subnucleus; EW; Edinger-Westphal nucleus; IL, intermediate lateral subnucleus; M, medial subnucleus; VM, ventral medial subnucleus; VL, ventral lateral subnucleus; III, oculomotor nucleus.

central group contains the medial subnucleus, which contralaterally innervates the superior rectus muscle, and the central subnucleus that innervates the levator palpebrae superioris, bilaterally. The lateral group contains the dorsal lateral subnucleus that sends projections ipsilaterally to the inferior rectus muscle, the intermediate subnucleus, which innervates the inferior oblique muscle, and the ventral lateral subnucleus that projects to the medial rectus muscle (Wilson-Pauwels and Akesson, 2001; Table 1 and Fig. 1A).

This organization varies in amniotes, depending on the species reviewed. In rodents, such as mice or rats, the somatic neurons are organized in a unique mass that contains all the different subnuclei described (compare ChAT labeling between Fig. 1A,B). This organization was proved by neuronal tracers in rats (Glicksman, 1980; Fernandez et al., 1987). While in chicken, Nkx6.1 labeling, a transcription factor that labels all the three different components of the OM complex (Moreno-Bravo et al., 2010; Fig. 1C) allowed us to nicely identify three somatic populations: a dorsolateral subnucleus equivalent to the human dorsal lateral subnucleus, a dorsomedial subnucleus that corresponds with the human ventral lateral subnucleus and a ventromedial nucleus equivalent to the human intermediate lateral subnucleus and medially to the medial subnucleus (Table 1 and Fig. 1D). There is no equivalent to the human central subnucleus as the avian do not have the levator palpebrae superioris muscle (Dubbeldam, 1998).

The preganglionar parasympathetic neurons are organized in the Edinger-Westphal nucleus, also known as accessory OM nucleus (Fig. 1C,D). They innervate the ciliary parasympathetic ganglia that controls the constrictor pupillae (pupil reflex) and ciliary muscles (lens accommodation).

Finally, rostral to the Edinger-Westphal nucleus, in the diencephalon of vertebrates, a group of cells have been identified, positive to urocortin and Nkx6.1 (Fig. 2; Ryabinin et al., 2005; Moreno-Bravo et al., 2010) that project to the brainstem, the spinal cord and prosencephalic regions. Its function is related with feeding behavior, stress responses, addiction and pain (Dos Santos Júnior et al., 2015). Due to its location, this population has been named as periventricular tegmental area in chick (Puelles et al., 2001), pre-Edinger Westphal

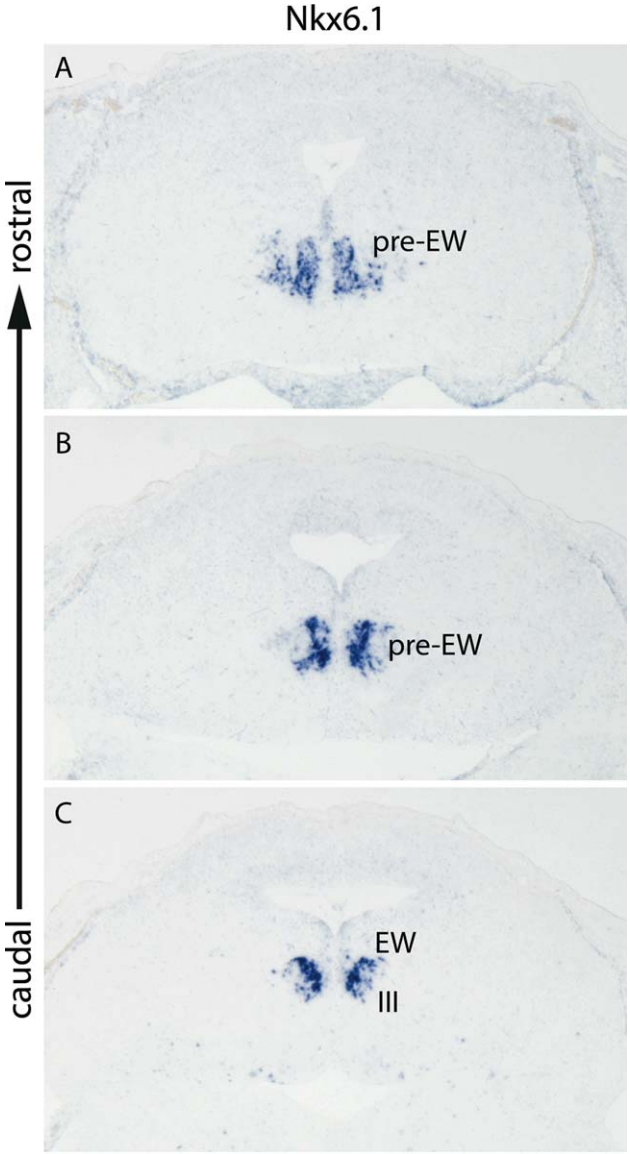

Fig. 2. Location of the pre-Edinger Westphal nucleus. (**A**–**C**) Transverse section of a E15.5 mouse embryo labeled with *in situ* hybridization against Nkx6.1. Abbreviations: EW, Edinger-Westphal nucleus; pre-EW: pre-Edinger-Westphal nucleus; III, oculomotor nucleus.

nucleus (Puelles et al., 2012) or as centrally projecting Edinger Westphal nucleus in mouse (Da Silva et al., 2013; Che Ngwa et al., 2014; Dos Santos Júnior et al., 2015). In our opinion, the term pre-Edinger Westphal is more appropriated as it describes the rostral location of this neural population with respect to the Edinger Westphal nucleus. Due to its function and location this nucleus should not be considered as part of the OM complex.

LOCALIZATION

The OM neurons occupy a periventricular position close to the mesencephalic ventricle in all vertebrates. The shape of the midbrain with a rather small tegmentum (basal plate) and a large tectum (alar plate) has misled in the past the description of the real location of the OM and trochlear nuclei. These two neural populations are almost continuous along the anteroposterior axis but they are separated by the isthmic constriction (mid-hindbrain boundary; Fig. 3A). Thus, the OM complex locates in the midbrain and the trochlear nucleus in the hindbrain. Therefore, due to its location the OM complex is the most rostral somatic motor neuron and parasympathetic population in the brain. The OM complex, like all somatic motor neurons, is located in the basal plate of the neural tube (Fig. 3B).

DEVELOPMENT

The neuroblasts that will give rise to the OM complex "need to know" their position along the anteroposterior and dorsoventral axes in order to trigger the correct differentiation genetic program. They receive this information from two secondary organizers, these are groups of cells located in key regions of the neural tube that emit signals to the surrounding tissue driving their specification and differentiation.

The isthmic organizer (anteroposterior information) locates at the mid-hindbrain boundary (Vieira et al., 2010; Fig. 4A). It is responsible for the induction of the mesencephalic vesicle, and therefore the OM complex, and, the rostral hindbrain. The key signal emitted by this organizer is the fibroblast growth factor 8 (Fgf8; Crossley and Martin, 1995). Genetic alterations of this morphogen, its receptors or responding intracellular cascades invariably produce the non-specification of the mid-rostral hindbrain the territory (Basson et al., 2008; Yu et al., 2011).

On the other hand, the floor plate and its morphogen Sonic Hedgehog (Shh; Tanabe et al., 1995; Ericson et al., 1995) are also responsible for the specification of the OM complex (Fig. 4A,B). Experiments of gain of function in chick demonstrated a direct relation between the overexpression of Shh and the induction of ectopic motor neurons (Patten and Placzek, 2000). Loss of function mutations generate a complete ablation of floor and basal plate neural structures (Chiang et al., 1996; Fogel et al., 2008). We also demonstrated that Shh is not only needed to induce the OM neurons but is also necessary for their maintenance (Perez-Balaguer et al., 2009).

The genetic cascade triggered by these signals is headed by the transcription factor Nkx6.1, the lack of function of this gene produces a strong effect in the generation of the OM complex (Vallstedt et al., 2001; Müller et al., 2003; Prakash et al., 2009). This factor is expressed in ventricular layer neuroblasts, which give rise to different neuronal basal populations as the OM and the red nucleus. The only mature neurons that retain the expression of Nkx6.1 are the ones in the OM complex. Moreover, the OM complex differentiation process requires the transient expression of Lmx1b. The lack of function of this gene produces a complete absence of OM complex neurons, their territory is then occupied by red nucleus neurons (Deng et al., 2011). The Lmx1b expression is followed by Phox2A and B (Hasan et al., 2010) and by Isl1 and 2. Failure to express any of these factors results in complete OM complex loss (Pfaff et al., 1996; Deng et al., 2011). All the mentioned genes are transcription factors integrated in the genetic cascade responsible for the differentiation of the OM complex motor neurons.

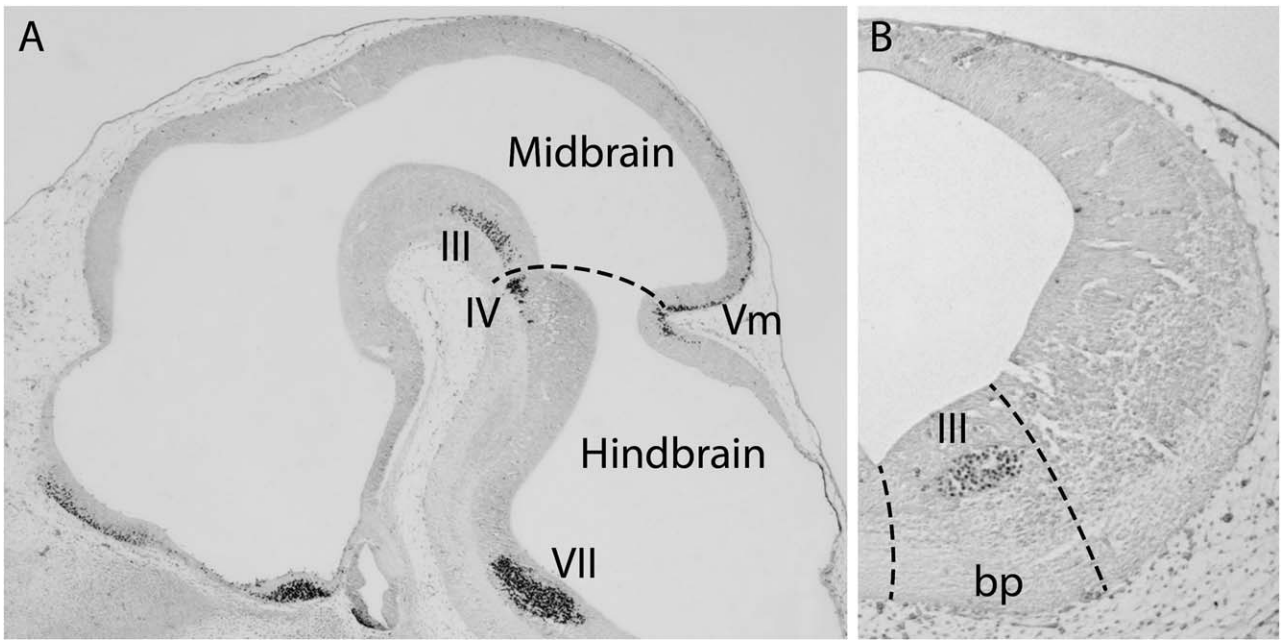

Fig. 3. Location of the OM complex in the mouse embryo. We display the location of the OM along the antero-posterior and dorsoventral axes. (**A**) Sagittal section of a E12.5 mouse embryo labeled with immunohistochemistry against Isl1. (**B**) Transverse section of a E12.5 mouse embryo labeled with immunohistochemistry against Isl1. Abbreviations: bp, basal plate; III, oculomotor nucleus; IV, trochlear nucleus; Vm, mesencephalic trigeminal nucleus; VII, facial nucleus.

The specified OM neurons suffer a short radial migration to occupy their final destination. Nevertheless, the neurons of the medial subnucleus suffer an important tangential migration of their cell bodies toward the contralateral after the innervation of the superior rectus muscle. This is an atypical process in the development of the brain to obtain a contralateral projection. It is habitual that neurons send their axonal process toward contralateral side of the brain but not that neurons project ipsilaterally and afterwards they migrate their soma to the contralateral side. The migration is independent of Netrin1 (present in the floor and basal plates) and dependent of the receptor family Robo and their ligand family Slits (Bjorke et al., 2016). This phenomenon has been described in several vertebrate species (Puelles and Privat, 1977; Puelles, 1978; Naujoks-Manteuffel et al., 1991).

Finally, the differentiated OM neurons must reach their targets in the ocular muscles. The molecular tools needed to direct the OM axons are common to other cranial and spinal nerves (Netrin1-Dcc, Robo-Slits, Ephrins-Eph, Semaphorins-Plexins, etc.; Beaubien et al., 2013; Chisholm and Feldheim, 2013; Falk and Castellani, 2013; Izzi and Charron, 2013). Nevertheless, alterations in their maturation can produce aberrant projections of the OM neurons, as in the case of Nkx6.1 lack of function (Prakash et al., 2009).

PATHOLOGY

Injury to the OM complex results in paralysis of the intraocular and extraocular muscles called OM palsy. It causes strabismus, which is defined as the condition of having eyes that look in different directions from each other. In addition, if the central subnucleus is damaged, there will be difficulty in keeping the eyelid open, known as ptosis.

The causes of OM palsy can be divided into genetically determined (mutations in the components of the genetic cascade) and acquired causes (vascular lesions or secondary symptoms to other pathologies).

Within the genetic causes group, two different types of principal alterations have been described (Engle, 2006). First, the congenital fibrosis of the extraocular muscles, which can be caused by faulty development of the OM motor neuron. Three different types of phenotypes and their respective associated genotypes have been identified to confirm this hypothesis. Most individuals with type 1 phenotype have KIF21A mutations and most individuals with type 2 have PHOX2A mutations. The exception is type 3 phenotype, for which the mutated gene is not yet found (Engle, 2006). The second congenital cause consists on anomalies in axonal targeting. Most individuals with congenital type 1 phenotype also exhibit an absence of the superior division of the OM nerve and of motor neurons in the nuclei that innervate the levator palpebrae superioris and superior rectus muscles. This could indicate that KIF21A is important for the development of this branch of the OM nerve and axonal targeting of the extraocular muscles. Consequently, mutations in this developmental kinesin cause failures in the synapse of the developing neuromuscular junction of the extraocular muscle (Engle, 2006). Mutations in several genes, not directly related with OM neuron generation, have been identified in both the Duane retraction syndrome and the Moebius syndrome. These two are examples of other genetic syndromes related with alterations in the OM complex (Doherty et al., 2013).

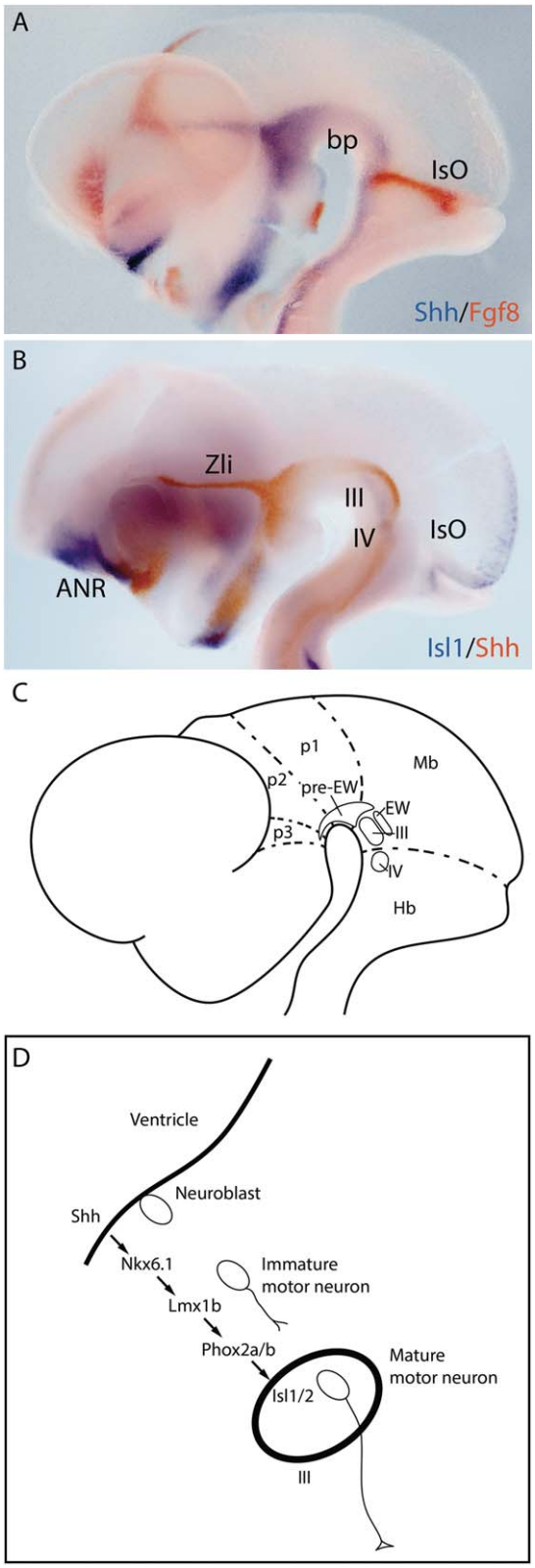

Fig. 4

The most important acquire causes are vascular lesion, tumoral outgrowth, infectious processes, or trauma. Lesions in origin, within the midbrain, may result in distinct syndromes. Infarction of the ventromedial midbrain causes Weber's syndrome, which results in an ipsilateral third nerve palsy and a contralateral weakness (Ruchalski and Hathout, 2012). Claude syndrome is produced by an injury to the dorsal tegmentum, including the OM nerve, and dentato-rubro fibers. It causes an ipsilateral OM nerve palsy with contralateral cerebellar ataxia (Ruchalski and Hathout, 2012). A lesion within the tegmentum of the midbrain produced by an infarction of the posterior cerebral artery causes Benedikt syndrome. Its symptoms include incoordination and OM nerve palsy and a contralateral hemiparesis (Ruchalski and Hathout, 2012).

The OM nerve is also susceptible to be injured along its course to the ocular orbit (Ruchalski and Hathout, 2012). An aneurysm in the posterior cerebral artery or superior cerebellar artery can produce the compression of the nerve. A cavernous sinus infection, venous thrombosis, carcinomatosis, trauma, immune disorders, or infectious processes (tuberculous meningitis), can also injure the OM nerve (Ruchalski and Hathout, 2012).

The treatment of this symptoms includes solving the primary cause of the OM palsy or the symptomatology by surgery (eye muscle resection or recession) or botulinum injections. Recently the OM axonal recovery after axotomy by the action of neurotrophins has been proved. Some of these neurothophins, specially GDNF and NGF, show a protective action against an induced axotomy, but only two, NGF and BDNF prevent the downregulation in ChAT (Morcuende et al.,2013; Benítez-Temiño et al., 2016).

MATERIALS AND METHODS

The immunohistochemistry was developed in wax embedded embryos sectioned in parallel series and incubated with the antibodies: anti-ChAT, Chemicon Cat. N° AB144P, 1:100 and anti-Isl1, Hybridoma Bank Cat. N° 39.4D5, 1:13. *In situ* hybridization was performed on whole-mount embryos while others were wax embedded sectioned. The *in situ* hybridization was developed after dewaxing and rehydration of the sections. RNA probes were prepared from plasmids (Fgf8, Isl1, Nkx6.1, and Shh). The techniques were applied to mouse and chick embryos (staged by Hamburger and Hamilton, 1992). The 54 days old human sample, was nicely supplied by Dr. Roig Quilis (Boix et al., 2010). All the work in this study has been conducted following the Spanish and European

Fig. 4. Development of the OM complex in mouse. Identification of the secondary organizers related with the OM specification, the isthmic organizer (Fgf8) and the basal plate (Shh). The position of the OM and trochlear nucleus in relation with the organizers is also shown. (**A**) Lateral view of a whole mount *in situ* hybridization in E12.5 mouse brain with probes against Fgf8 (red) and Shh (blue). (**B**) Medial view of a whole mount *in situ* hybridization in E12.5 brain with probes against Shh (red) and Isl1 (blue). (**C**) Scheme representing the location of the different OM complex components along the dorsoventral axis of the brain. (**D**) Scheme of the genetic cascade responsible for the differentiation of the somatic motor neurons of the OM complex. Abbreviations: ANR, anterior neural ridge; bp, basal plate; EW, Edinger-Westphal nucleus; Hb, Hindbrain; IsO, isthmic organizer; Mb, Midbrain; pre-EW, pre-Edinger-Westphal nucleus; p1–3, prosomere 1–3; III, oculomotor nucleus; IV, trochlear nucleus; Zli, zona limitans.

legislation. The experiments were performed according to protocols approved by the Universidad Miguel Hernandez "Oficina Evaluadora de Proyectos" committee.

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