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Oculomotor Nerve Development: Slit/Robo Repulsion Regulates Midline Crossing

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Abstract

The oculomotor nerve has a significant role in innervating the extraocular muscles of the eye that are necessary for the proper positioning and movement of the eye. Defects in the development of the oculomotor system can result in strabismus, a misalignment of the eyes, which left uncorrected can lead to partial blindness. Surprisingly, little is known about the embryonic development of the oculomotor nerve. Oculomotor neurons (OMNs) innervating the dorsal rectus undergo a unique migration through the midline to achieve a contralateral innervation pattern. *Slit/Robo* signaling has been shown to have an important role in the guidance of midline crossing axons, but less is known about midline crossing neurons. Here, we characterize normal migration of oculomotor neurons and analyze the oculomotor phenotypes of *Slit1/2* double mutants and *Robo 1/2* double mutants. We find that Slit/Robo repulsion (*Slit2* predominant) is important for the maintenance of the ipsilateral position of OMNs seen in early development. In addition, *Robo2* seems to be the main receptor governing this restriction. Therefore, the modulation of *Slit/Robo* signaling serves as an important mechanism governing the migration of dorsal rectus innervating OMNs.

Keywords: oculomotor, midline, floor plate, Slit, Robo, development,

Acknowledgements

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Introduction

The oculomotor system functions to coordinate the movements of the eye and to stabilize the position of the eye, allowing one to track visual stimuli and form a stable image on the back of the retina. The six extraocular muscles of the oculomotor system function in 3 antagonistic pairs; the dorsal and ventral recti controlling vertical movement, lateral and medial recti directing horizontal movement, while the dorsal and ventral oblique muscles provide rotational movement (Chilton & Guthrie, 2004). The extraocular muscles receive innervation from three separate cranial nerves, the abducent, trochlear, and the oculomotor. Defects in the development of the oculomotor system can lead to strabismus, misalignment of the eyes, which if left untreated can result in partial blindness.

The oculomotor nerve provides innervation to four of the six extraocular muscles. The oculomotor nerve arises from neurons of the ventral midbrain located next to the floor plate. Early in development oculomotor neurons (OMNs) form ipsilateral axonal projections to their extraocular targets. Most of these neurons maintain their ipsilateral connections throughout development. However, there is a subset of OMNs which innervate the dorsal rectus muscle that are known undergo a unique migratory pattern later in development to establish a contralateral innervation pattern. These dorsal rectus innervating OMNs migrate through the floorplate and integrate into the contralateral nucleus. This migration is evolutionarily conserved and has been described in various species to include chick, mouse, rat and human (Biondi, 1910; Chilton & Guthrie, 2004; Fritzsch et al, 1995; Hogg, 1966).

Midline crossing has been well described in commissural axons, where axons are thought to be guided across the midline by attractive and repellent cues originating at the floorplate. Two ligand/receptor couples play a significant role in this navigation: the attractive interaction of DCC/Netrin and the repulsive interaction of Slit/Robo (review by Dickson & Gilestro, 2006). More recently, Slit/Robo signaling has been shown to have a role in cell positioning and migration. In the hindbrain, Slit/Robo repulsion is required to keep neuronal cell bodies from entering the floor plate during development, allowing for proper positioning of neuron pools (Kim et al, 2011). In several other cases, Slit/Robo signaling regulates neuronal migration. For example, the migration of sensory neurons in drosophila is regulated by *Robo2* (Kraut & Zinn, 2004), while *Robo1* regulates the migration of interneurons in the forebrain (Andrews et al, 2006). Interestingly, a group of precerebellar neurons display a similar migratory pattern across the midline that is also regulated by Slit/Robo signaling (Marillat et al, 2004; Di Meglio et al, 2008). Therefore, it is feasible that Slit/Robo repulsion originating from the floorplate maintains the ipsilateral position of dorsal rectus innervating neurons seen early in development. Suppression of Slit/Robo signaling may mediate the contralateral migration of this subset of OMNs that is observed later in development.

A number of oculomotility disorders are thought to arise from developmental defects in axonal targeting systems, which may be a consequence of erroneous positioning of neuronal populations in the developing brain. One such syndrome, Horizontal Gaze Palsy with Progressive Scoliosis, has been attributed to a mutation in the *Robo3* gene, a negative regulator of Slit responsiveness (Engle, 2002; Engle, 2007; Sabatier et al, 2004). While congenital disorders of oculomotor development have been

well studied from a clinical perspective, little is known about the mechanisms regulating the neuronal midline crossing seen during oculomotor nerve development. As the neurons innervating the dorsal rectus are the only OMNs to display this contralateral migratory pattern, they provide a unique system to understanding the mechanisms governing neuronal midline crossing. Here we, show that modulation of Slit/Robo signaling governs the contralateral migration of OMNs spatially and temporally.

Materials and methods

Mouse embryos

Wild type CD-1 mice (6-8 weeks old) were purchased from Charles River Laboratories (Wilmington, MA USA). Mouse maintenance and experimentation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All protocols were approved by the University of Nevada, Reno Institutional Animal Care and Use Committee. The animals were anesthetized by CO_2 inhalation and killed by decapitation. Embryonic day 11 (E11) and E13 embryos were obtained via uterine dissection. The Slit and Robo mutant mouse strains were a gift from Marc Tessier-Lavigne, Genetech. PCR genotyping was performed as previously described (Grieshammer, et al. 2004; Plump, et al. 2002).

Immunohisotochemistry

For cryostat section immunofluorescent labeling of the ventral midbrain embryos were fixed in 4% PFA. Embryos were embedded in sucrose/gelatin. 20 μm sections were taken transverse to the cephalic flexure using a cryostat. Gelatin was removed from specimens by immersing slides in warm (37-45˚C) 0.1 M phosphate buffer for 1 minute. For Robo1 and Robo 2 labeling, sections were washed for 1 hour with PBS (10% FBS 0.1% Trition X-100). For Islet-1 and β-galactosidase labeling, sections were washed for 1 hour in PBS (1% NGS 0.1% Triton X-100). Primary antibodies rabbit anti-Robo1(1:10,000 dilution, E.Stein) and rabbit anti-Robo2 (1:10,000 dilution, E.Stein), mouse anti-Islet-1(1:100 dilution, Jackson) and rabbit anti-β galactosidase (1:100 dilution, Jackson) were applied and were incubated in a humidified chamber overnight.

Secondary antibody, anti-rabbit Alexa (1:200 dilution, Jackson), was applied to sections and incubated in a humidified chamber for 1 hour. A tertiary system was used for Islet-1 immunolabeling using secondary antibody donkey anti-mouse Biotin (1:100 dilution, Jackson) and tertiary antibody Avidin Cy3 (1:200 dilution, Jackson). Slides were mounted with floursafe solution and covered with a glass cover slip. Labels were visualized under a fluorescence microscope.

DiI labeling

To trace projection fibers of OMNs, embryos were fixed in 4% PFA overnight. Embryos were dissected to expose the oculomotor nerve. Small crystals of lipophilic dye (DiI) were applied to the oculomotor nerve. The embryos were incubated for 2 days in 4% PFA at 37° to allow the dye to diffuse to the oculomotor nucleus. Dyed embryos were embedded in 2% agrose/PBS. 200 µm sections were taken parallel to the oculomotor nerve and visualized under a fluorescence microscope.

Results

Oculomotor neurons begin contralateral migration at Embryonic Day 13.

A subset of OMNs that innervate the dorsal rectus muscle are known to migrate contralaterally during normal oculomotor development (Fritzsch et al, 1995) To characterize this migration through the course of development, we analyzed two critical time points. We began with E11, when all OMNs are ispilateral to their muscle targets. At this time point the oculomotor nerve has reached primordial ventral oblique, ventral rectus and medial rectus muscles. The second time point we examined was E13, at which time innervation has reached the dorsal rectus muscle and OMNs are seen migrating across the midline (Fritztsch et al, 1995). The oculomotor nuclei were visualized by immunofluorescence labeling of 20 μm sections of the mid-brain taken transverse to the cephalic flexure for Islet-1, a motor neuron specific transcription factor. Prior to E13, the OMNs are restricted to nuclear positions adjacent to the floor plate (Fig 1A). The neurons appear densely packed along the entire anterior- posterior length of the nuclei (data not shown). At E13, the oculomotor nuclei borders are less distinct and the cells are less densely packed. An abundance of Islet-1 positive cells are now present in the floor plate (Fig 1B).

Oculomotor neurons of Slit mutants migrate prematurely.

As a first step to determine if the contralateral migration of OMNs is regulated by Slit signaling, the oculomotor nuclei of Slit mutants was examined at several timepoints using a lipophilic dye introduced at the oculomotor nerve. At E11, OMNs should maintain ipsilateral positioning (Fig 1A). Surprisingly, in *Slit1/Slit 2* double mutants at this same

time period dendritic projections into the midline can been seen. Many fibers were observed at the midline, while some had already reached the contralateral nucleus. Neuronal cell bodies were observed to be at various stages of migration along the dendritic projection. Many cells were observed in the floor plate, while some neurons had already begun to integrate into the contralateral nucleus (Fig 2D). We found that a single copy of *Slit2* was sufficient to rescue the ipsilateral phenotype. Dendritic projections and neuronal cell bodies were absent from the floor plate of *Slit 1 (-/-) Slit 2 (+/-)* mutants at E11 (Fig 2C).

Robo-2 but not Robo-1 is transcribed by oculomotor neurons prior to migration.

To determine if OMNs transcribe the Slit receptor proteins Robo1 and Robo 2, we utilized a reporter gene, *β-galactosidase,* in the place of *Robo1* or *Robo2* indicating *Robo* transcription. Prior to migration at E11, the OMNs of *Robo 1* $^{-/-}$ *;Robo 2* $^{+/+}$ embryos do not label for *β-galactosidase* indicating that *Robo1* is transcriptionally inactive at this time point (Fig 3A-C). However, in *Robo1* $^{+/+}$ *;Robo 2* $^{-/-}$ embryos β-*galactosidase* labeling co-localized with *Islet-1* labeling in the oculomotor nucleus, showing that *Robo2* is transcriptionally active in OMNs prior to migration at E11 (Fig 3D-F).

Robo-1 and Robo-2 are not highly expressed on the cell surface of oculomotor neurons.

Repulsive Slit/Robo interactions have a significant role in guiding axons. More recently, Slit/Robo signaling has also been shown to have a role in the positioning and migration of neurons. *Robo2* was shown to prevent motor neurons in the hindbrain from entering the floor plate (Kim, unpublished). Additionally, the modulation of Slit/Robo signaling

by *Robo3* was shown to regulate the midline crossing of precerebellar neurons (Di Meglio et al, 2008). We previously showed that prior to migration OMNs extend a dendritic projection into the floor plate (Fig 2D). We next wanted to determine if direct Slit/Robo signaling with the cell body was important regulator for the restriction of OMNs to the ipsilateral nucleus. Cryostat sections of wild-type CD1 strain embryos at E11 were antibody labeled for *Islet-1* and *Robo1* or *Robo2* and examined for co-localization. While Islet-1 positive cells of the OM nucleus are restricted to the ipsilateral position (Fig 4A, Fig 4D), with this technique we did not observe high levels of *Robo1* or *Robo2* expression in the oculomotor nucleus . *Robo2* expression was observed at very low levels in the oculomotor nucleus (Fig 4E, 4F). Anticipated *Robo2* expression was seen in the area of pre and post-crossing commissural axons (Fig 4E, 4F; review by Dickson & Gilestro, 2006) *Robo1* expression was seen in the area of the commissural axons in the ventral commissure and the ventral motor exit points, as expected (Fig 4B, 4C; Mambetisaeva et al , 2005). *Robo1* expression did not co-localize with *Islet-1* region containing the oculomotor nucleus (Fig 4B, 4C)

Robo1/2 mutants have ectopic motor neurons in the floor plate

Next, we wanted to determine if Slit interaction with Robo was responsible for the maintenance of the ipsilateral position of OMNs seen early in development of the oculomotor nerve. We analazyed wild type embryos and *Robo1/2* mutant embryos at E11, a time in which oculomotor neurons should be restricted to the ipsilateral position and not migrating. OMNs were visualized by antibody labeling transverse sections of the midbrain for *Islet-1*. As expected at E11, in $Robo1^{+/+};Robo2^{+/+}$embryos OMNs are

located in two distinct nuclei on either side of the floorplate and no motor neurons are observed in the floorplate (Fig 5A). In contrast, several neuronal cell bodies are seen in the floorplate of $Robo1^{-/-}; Robo2^{-/-}$ embryos at E11 (Fig 5B).

Discussion

The purpose of this study was to examine the role of Slit/Robo signaling in the regulation of midline crossing of oculomotor neurons. Our findings suggest that the modulation of Slit/Robo signaling regulates the midline crossing of OMNs. Despite, overlapping expression of the various isoforms of the Slit signaling molecule (Yuan et al, 1999), it appears that the midline attractive cue Slit2 has a predominant role in the maintenance of the ipsilateral position of OMNs. In addition, co-localization of the Slit receptor Robo2 with OM cells prior to migration at E11, suggests that *Robo2* may govern the ipsilateral restriction. Furthermore, in *Robo1* and *Robo2* mutant mice, ectopic neurons are seen in the floor plate, suggesting that *Robo* is necessary to maintain the ipsilateral restriction seen in early oculomotor development. In addition, we show that OM migration into the floorplate in $Slit1^{-/-}; Slit2^{-/-}$ mutant mice is premature. This suggests that migrating OMNs modulate their ability to respond to midline repulsive cues during development. Lastly, our results suggest that Slit signaling plays a role in inhibiting the initiation of axon outgrowth from OM nucleus into and across the midline— an initial process necessary for OMN migration.

Slit signaling maintains the ipsilateral position of OMNs.

Prior to migration at E11, the oculomotor nucleus consists of densely packed neurons located next to the floor plate (Fig 1). The repellent cue Slit is expressed by glial cells in the floorplate at E11 (Brose 1999; Kidd 1999; Yuan 1999) and has previously been shown to repel motor neurons from the floorplate in hindbrain (Kim , unpublished). We find that OMNs are responsive to midline derived Slit. Slit acts to maintain the ipsilateral

position of OMNs to their muscle target, and prevents axonal outgrowth towards the midline (Fig 1, Fig2). However, in the absence of *Slit* expression, OMNs no longer receive this repulsive cue from the floor plate, resulting in outgrowth and neuronal wandering into the midline (Fig 2B). Remarkably, we found that a single copy of Slit2 is sufficient to rescue the ipsilateral phenotype, whereas Slit1 had no effect (Fig 2A, 2B). Thus, midline expression of *Slit2* has a more significant role in restricting OMNs to the ipsilateral nucleus.

Slit signaling regulates the organization of OMN migration temporally.

In agreement with previous studies, we found that contralateral migration of OMNs begins around E13 (Fritzsch et al, 1995; Chilton & Guthrie, 2004). Surprisingly, full slit mutants *Slit1*$^{-/-}$*; Slit2* $^{-/-}$ display an early migratory pattern at E11 (Fig 2B). This suggests that regulation of responsiveness to midline slit in OMNs has a distinct temporal organization. At E13, a select group of OMNs are no longer responsive to Slit, allowing for migration into high Slit concentration at the midline (Fig 1). However, the manner in which OMNs proceed through this migration is atypical. Prior to migration, OMNs have been observed to extend a leading process into the midline. The cell body then migrates along the process through the floor plate, ultimately integrating into the contralateral nucleus (Fig 2D) Thus, OMNs responsiveness to Slit varies temporally. There are several other cases in which cellular response to Slit varies with time or position. For example, commissural axons become less responsive to slit as they navigate toward the floor plate by post-transcriptional regulation of *Robo1/Robo2* receptors in drosophila and mouse (Kidd et al, 1998; Long, et al 2004) . Slit responsiveness is further decreased by an

additional negative regulatory interaction with *Robo3* to facilitate midline crossing (Sabatier et al, 2004). Interestingly, the appearance of midline cells coincides with the innervation of the dorsal rectus muscle (Chilton & Guthrie, 2004). This suggests that an outside signal, perhaps originating from the muscle, may regulate the responsiveness to Slit.

Robo1/2 expression is sufficient to repel neurons from the floorplate

β-*galactosidase* labeling indicates that *Robo2* is actively transcribed prior to migration, while *Robo1* transcription appears to be relatively inactive. We expected to see increased expression of Robo proteins on the cell bodies of OMNs prior to migration. Surprisingly, this was not the case. Instead, we found that cell bodies of OMNs express *Robo2,* but only at low levels. However, it seems that even low levels of *Robo2* expression is sufficient to repel neurons from the floor plate. Our preliminary data shows that in *Robo1/2* mutant mice some neurons collapse into the floorplate at E11, a time in which neurons should be absent from the floorplate (Fig 5B). In addition, previous studies have shown similar results in other brain regions. For instance, in the hindbrain ectopic neurons have been observed in the floor plate of embryos lacking functional *Robo2* (Kim et al, 2011). Together these findings suggest that Robo2 may be receptor that signals Slit dependent repulsion which restricts OMNs to the ipsilateral nucleus by preventing dendritic extension into the midline, thereby stalling migration. While it seems that Slit signaling with Robo2 is a good candidate mechanism for the regulation of this migratory pattern, further functional study of the Robo receptors are needed to make definitive conclusions about the signaling pathway regulating oculomotor neuron migration.

Figures

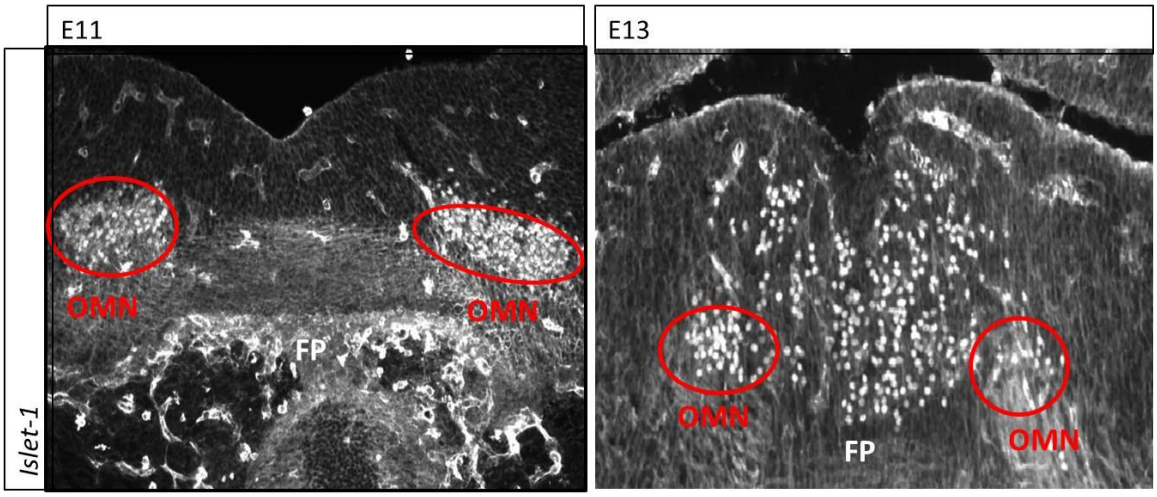

Figure 1. Oculomotor neurons migrate into the floor plate at embryonic day 13. Transverse sections of the mid-brain containing the oculomotor nuclei labeled for Islet-1, a motor neuron specific transcription factor, at E11(A) and E13(B). A. At E11, OMNs are restricted to positions adjacent to the floorplate. B. At E13, an abundance of OMNs are seen in the floor plate in addition to their lateral positions.

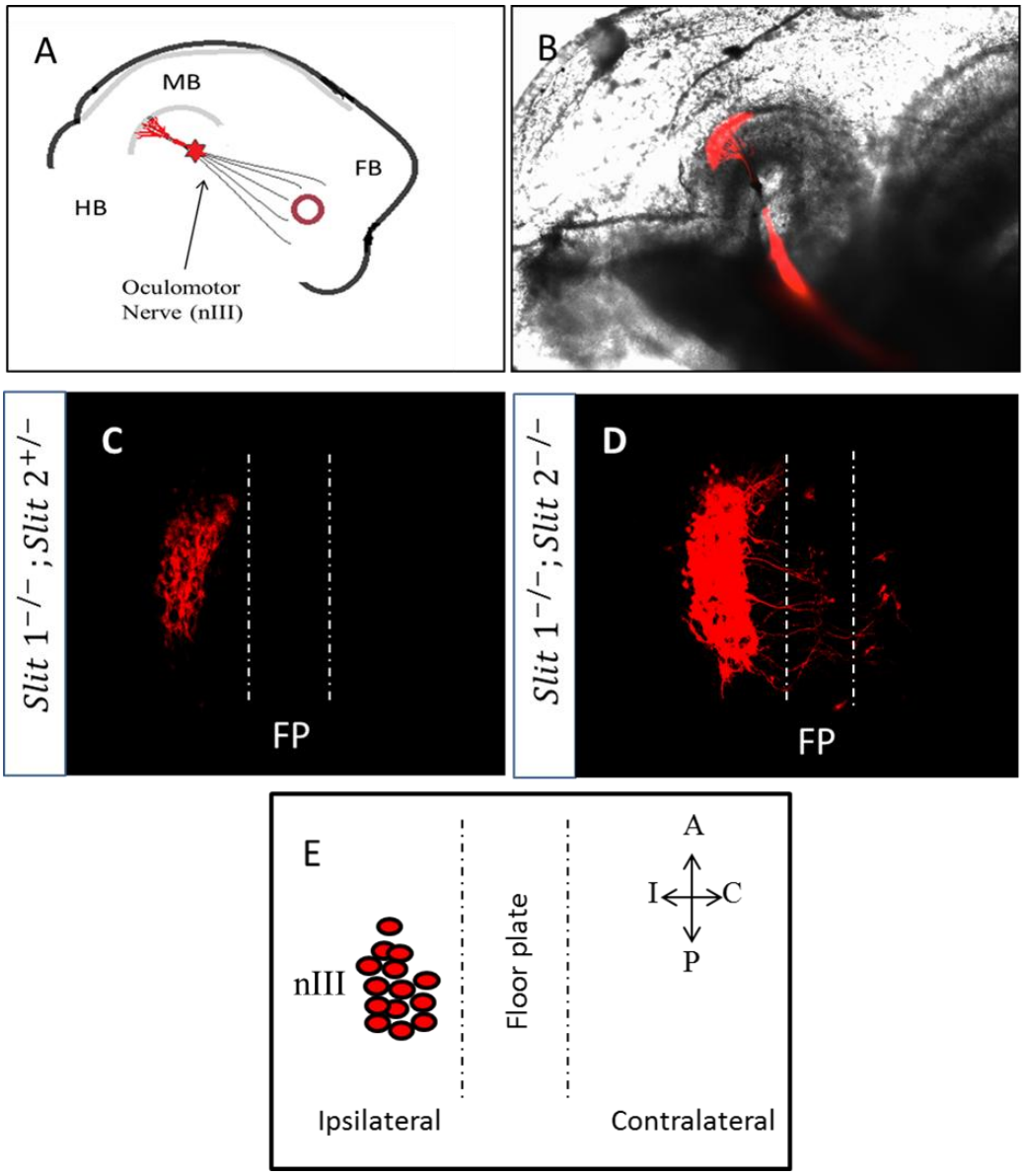

Figure 2. Oculomotor neurons migrate through the floor plate to the contralateral nucleus at E11 in Slit1/2 mutants. A. Schematic of DiI labeling of oculomotor nucleus (nIII). DiI crystals were placed on the exposed oculomotor nerve at embryonic day 11 in Slit1/2 mutants and allowed to travel up the nerve to the oculomotor nucleus. B. DiI labeled oculomotor nucleus and oculomotor nerve. C. OMNs maintain their ipsilateral position with a single copy of *Slit2*. D. Full *Slit1/2* mutants have dendritic projections that extend from the oculomotor nucleus through the floor plate to the contralateral nucleusa 2 days early. Cell bodies are seen at various stages of migration along midline projection fibers. E. Schematic of open book preparation of DiI labeled oculomotor nucleus. Anterior-posterior and ipsilateral-contralateral axes are labeled.

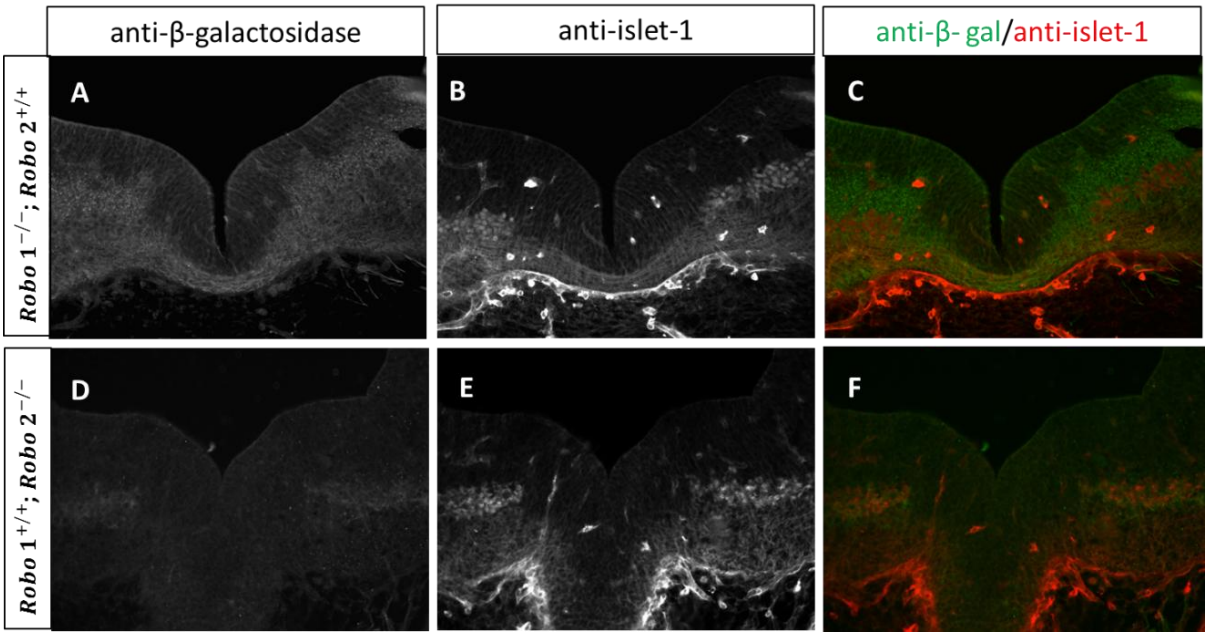

Figure 3. Slit receptor Robo-2 is transcribed by oculomotor neurons at E11, prior to contralateral migration. β-galactosidase antibody labeling indicating the transcription of *Robo1* (A-C) and *Robo2* (D-F) in cryostat sections of embryos at E11. A-C. A lack of .β-galactosidase labeling of the Islet-1 positive oculomotor nucleus in *Robo 1* $^{-/-}$; *Robo 2* $^{+/+}$ embryos indicates that *Robo1* is not transcribed in the nucleus prior to migration. D-F. β- galactosidase labeling co-localizes with Islet-1 positive cells in *Robo 1* $^{+/+}$ *; Robo 2* $^{-/-}$ embryos showing that Robo 2 is transcribed by OMNs prior to migration.

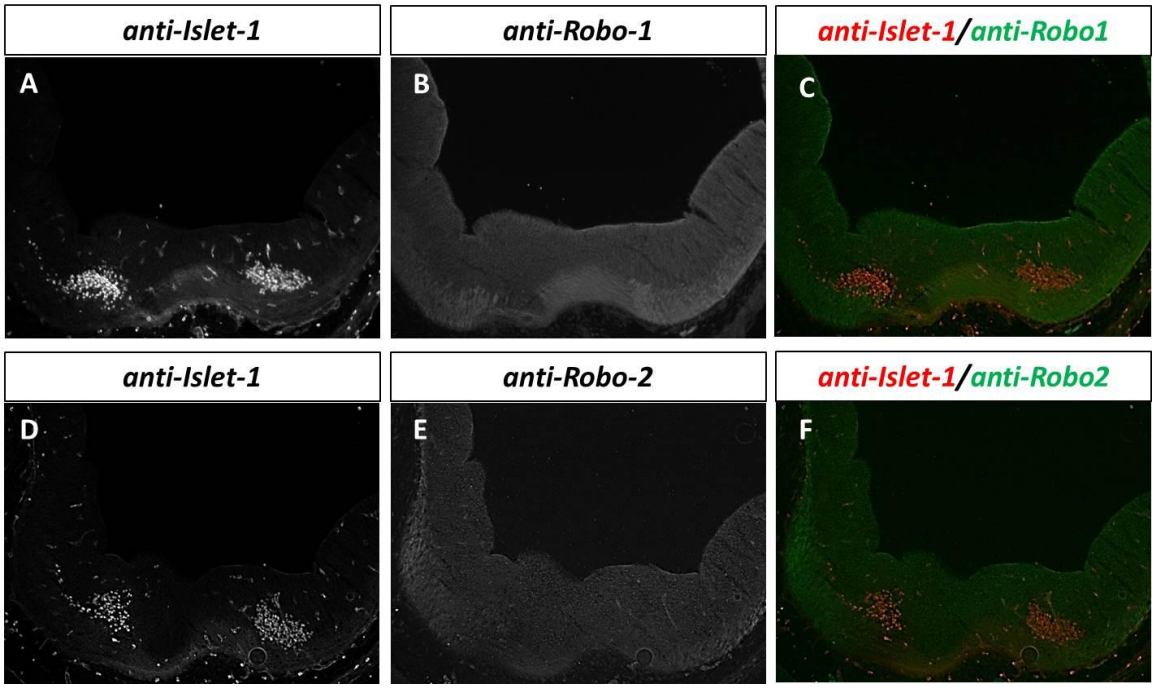

Figure 4. Robo1 and Robo2 are not highly expressed on the surface of oculomotor neuron cell bodies prior to migration. Antibody labelings for *Islet-1* and *Robo1 or Robo 2*, in wild type CD1 embryos at E11. A-C. *Robo1* is expressed highly at the ventral commissure and ventral motor exit points, but is not shown to be highly expressed in the oculomotor nucleus. D-F. *Robo2* is highly expressed on the pre and post crossing commissural axons and does appear to be expressed at low levels on the surface of oculomotor neuronal cell bodies.

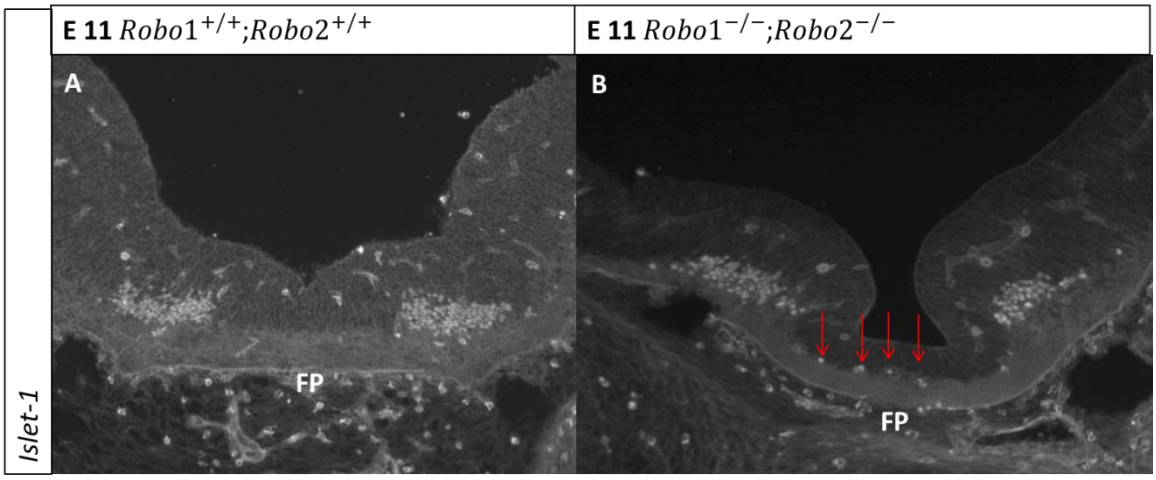

Figure 5. *Robo 1/2* mutants have ectopic motor neurons in the floorplate. *Islet-1* antibody labeling for motor neurons at E11 in wild type embryos (A) and full *Robo1/2* mutants embryos (B). A. In $Robo1^{+/+};Robo2^{+/+}$ embryos, OMNs are restricted to positions lateral to the floorplate B. In $Robo1^{-/-};Robo2^{-/-}$ embryos, several motor neuron cell bodies are seen in the floorplate at E11.

References

Andrews, W., et al. 2006. Robo1 regulates the development of major axon tracts and interneuron migration in the forebrain. Development. 133:2243-2252.

Biondi, G., et al. 1910. Osservzioni sullo sviluppo e sullus strutura dei nuclei d'origine dei nervi Oculomotore e trocleare nel pollo. Review of Italian Neuropathology 3:302-327.

Brose, K., et al., 1999. Slit proteins bind Robo receptors and have evolutionarily conserved roles in repulsive axon guidance. Cell. 96:785-794.

Chilton, J. & Guthrie, S. 2004. Development of oculomotor axon projections in the chick embryo. The Journal of Comparative Neurology. 472:308-317.

Dickson, B., & Gilestro, G. 2006. Regulation of commissural axon pathfinding by slit and its Robo receptors. Annual Review of Cell and Developmental Biology. 22:651-675.

Di Meglio, T., et al. 2008. Molecular mechanisms controlling crossing by precerebellar neurons. Journal of Neuroscience. 28: 6285-6294.

Engle, E. 2002. Applications of molecular genetics to the understanding of congenital ocular motility disorders. Annals of N.Y. Academy of Sciences. 956: 55-63

Engle, E. 2007. Oculomotility disorders arising from disruptions in brainstem motor neuron development. Archives of Neurology. 64:633-637.

Grieshammer, U., et al. 2004. Slit 2 mediated Robo 2 signaling restricts kidney induction to a single site. Dev. Cell. 6: 709-717.

Hogg, I. 1966. Observations on the development of the nucleus of Edinger-Westphal in man and the albino rat. Journal of Comparative Neurology. 126:567-585.

Fritzsch, B., et al., 1995. Development of midbrain and anterior hindbrain ocular motoneurons in Normal and Wnt-1 knockout mice. Journal of Neurobiology. 27(4):457-469.

Kidd, T., et al., 1998. Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. Cell 92:205-215.

Kidd, T., et al., 1999. Slit is the midline repellent for the Robo receptor in Drosophila. Cell. 96: 795-806.

Kim, M., et al. 2011. Robo 1 and Robo2 have distinct roles in pioneer axon guidance. Developmental Biology 358(1):181-188.

Kim, M., unpublished data. 2012. Robo2 repels motors neurons from the floorplate in he hindbrain. Verbal Communication.

Kraut, R. & Zinn, K. 2004. Roundabout 2 regulates migration of sensory neurons by signaling in trans. Current Biology. 14: 1319-1329.

Long, H., et al. 2004. Conserved roles for Slit and Robo proteins in midline commissural axon guidance. Neuron 42:213-223.

Marillat, V., et al. 2004. The Slit receptor rig-1/Robo3 controls midline crossing by hindbrain precerebellar neurons and axons. Neuron. 43:69-79.

Mambetisaeva, E., et al. 2005. Robo family of proteins exhibit differential expression in mouse spinal cord and Robo-Slit interaction is required for midline crossing in vertebrate spinal cord. Developmental Dynamics. 233(1):41-51.

Plump, A., et al. 2002. Slit 1 and Slit 2 cooperate to prevent premature midline crossing of retinal axons in the mouse visual system. Neuron. 33: 219-232.

Sabatier, C., et al. 2004. The divergent Robo family protein rig-1/Robo3 is a negative Regulator of slit responsiveness required for midline crossing by commissural axons.Cell. 117: 157-169.

Yuan, W., et al. 1999. The mouse Slit family: secreted ligand for Robo expressed in Patterns that suggest a role in morphogenesis and axon guidance. Dev. Biology 207:62-75.