Development 139, 3326-3331 (2012) doi:10.1242/dev.079418

Slit2 and Robo3 modulate the migration of GnRH-secreting neurons

Anna Cariboni[1,2,*], William D. Andrews[1], Fani Memi[1], Athena R. Ypsilanti[3], Pavol Zelina[3], Alain Chedotal[3] and John G. Parnavelas[1]

SUMMARY

Gonadotropin-releasing hormone (GnRH) neurons are born in the nasal placode and migrate along olfactory and vomeronasal axons to reach the forebrain and settle in the hypothalamus, where they control reproduction. The molecular cues that guide their migration have not been fully identified, but are thought to control either cell movement directly or the patterning of their axonal substrates. Using genetically altered mouse models we show that the migration of GnRH neurons is directly modulated by Slit2 and Robo3, members of the axon guidance Slit ligand and Robo receptor families. Mice lacking Slit2 or Robo3 have a reduced number of GnRH neurons in the forebrain, but a normal complement of their supporting axons, pointing to a direct role for these molecules in GnRH neuron migration.

KEY WORDS: GnRH neuron, Robo3, Slit2, Neuronal migration, Reproduction

INTRODUCTION

Reproduction in mammals is regulated by hypothalamic gonadotropin-releasing hormone (GnRH) neurons. In the adult rodent brain, these cells are dispersed between the olfactory bulbs and the medial preoptic area (MPOA) and project to the median eminence (ME), where they secrete GnRH into the portal vessels of the pituitary to induce the release of gonadotropins into the circulation (Merchenthaler et al., 1984). Deficiency in GnRH is the cause of hypogonadotropic hypogonadism and Kallmann syndrome, which are characterised by delayed or absent puberty and infertility (Hardelin, 2001).

In rodents, GnRH neurons originate in the nasal placode, which gives rise to the olfactory epithelium (OE) and the vomeronasal organ (VNO), and migrate towards the forebrain apposed to olfactory (OLF) and vomeronasal (VN) axons. Once in the forebrain, they make a turn towards the developing hypothalamus, guided by the caudal branch of the VN nerve. Throughout their migratory journey, which in mice starts at embryonic day (E) 10.5 and is complete by birth, many factors function as guidance cues. Thus, we found that Sema3A is important for the guidance of VN axons and, therefore, the migration of GnRH neurons into the brain, whereas reelin, present in the extracellular matrix of the olfactory bulbs deflects the course of migrating GnRH neurons into the ventral forebrain (Cariboni et al., 2011; Cariboni et al., 2005). Other authors have reported the importance of guidance molecules such as Sema4D, Sema7A, Hgf, netrin and SDF-1 (Cxcl12) in this process (Giacobini et al., 2007; Giacobini et al., 2008; Messina et al., 2011; Schwarting et al., 2004; Schwarting et al., 2006) by acting predominantly as directional cues in the nasal compartment (NC). By contrast, little is known about the factors that act at the final stages of migration in the MPOA (for reviews, see Cariboni et al., 2007a; Wray, 2010).

The secreted Slit glycoproteins (Slit1, Slit2 and Slit3) and their Roundabout receptors (Robo1, Robo2, Robo3) were originally identified as important axon guidance molecules (Ypsilanti et al., 2010; Kidd et al., 1998; Sundaresan et al., 1998; Yuan et al., 1999). During assembly of the nervous system, Slits function mainly as repulsive cues by preventing axons from growing to inappropriate locations. In addition, Slit-Robo interactions modulate neuronal migration, cell death and angiogenesis (Chédotal, 2007). Previous studies have established roles for Slit-Robo in the development of the olfactory system, but no function in the migration of GnRH neurons has been proposed, despite their intimate association with the OLF and VN tracts. Accordingly, in situ hybridisation studies in rat embryos have shown that olfactory receptor neurons in the OE and migrating cells resembling GnRH neurons express high levels of *Robo2* at E12-13 and E13-15, respectively (Marillat et al., 2002). Furthermore, genetic studies in mice have implicated Slits and Robos in the targeting of OLF (Cho et al., 2007; Nguyen-Ba-Charvet et al., 2008) and VN (Cloutier et al., 2004; Prince et al., 2009) axons throughout development.

Using genetically altered mouse models, we provide evidence for a direct role for Slit2 and Robo3 in the migration of GnRH neurons. Specifically, we found that mice lacking Slit2 contained fewer GnRH neurons in the forebrain compared with wild-type controls, resulting in much reduced innervation of the ME. However, these animals did not show defects in the fasciculation/targeting of their OLF/VN axons. Unexpectedly, we found that this defect is independent of Robo1 and Robo2, but that it closely phenocopies *Robo3* null mice, suggesting that Slit2 might function through Robo3 to modulate the migration of GnRH neurons.

MATERIALS AND METHODS

Animals

All animal procedures were performed in accordance with institutional and UK Home Office guidelines. Slit- and Robo-deficient mice (all in C57BL/6 inbred strain) were described previously (Plump et al., 2002; Grieshammer et al., 2004; Long et al., 2004; Sabatier et al., 2004).

[1]Department of Cell and Developmental Biology, University College London, London WC1E 6BT UK. [2]University of Milan, Department of Pharmalogical and Biomolecular Sciences, 20133 Milan, Italy. [3]Institut de la Vision, INSERM UMRS 968, F75012 Paris, France.

*Author for correspondence (a.cariboni@ucl.ac.uk)

Accepted 18 June 2012

Immunohistochemistry

Immunofluorescence on paraformaldehyde-fixed cryosections was as described previously (Cariboni et al., 2007b). Primary antibodies used were: rabbit anti-peripherin (1:500; Merck Millipore), anti-GnRH (1:400; Immunostar), anti-Robo1 (1:150; gift from Prof. F. Murakami, Osaka University, Japan), anti-Robo3 (R&D Systems) and chicken anti-GFP (1:500; Aves Laboratories), followed by Alexa Fluor-conjugated secondary antibodies (Invitrogen). For immunoperoxidase staining, sections were processed as described previously (Cariboni et al., 2011). The total number of GnRH neurons per head or forebrain was determined by counting all GnRH-positive cells in all sections through each embryo head/brain. We analysed at least three samples for each genotype. The density of GnRH neuron projections to the ME was calculated with ImageJ (NIH).

RT-PCR

Single-stranded cDNA was synthesized with AMV reverse transcriptase and random hexamers (Promega) and subjected to PCR with the following primers (5′-3′): *Slit2*, GCGCGTCTGGTGTGAATGAA and CACAGTGGCACCAGGAGCAT (product size 210 bp); *Robo1*, TGAAGCTTCCGCTACTTTGAC and GTTACTGATGTGATTGCAGACC (338 bp); *Robo2*, CAGGGCCGGACAGTGACATTC and CTTGGGGGTTGATCGCTCTGA (823 bp); *Robo3*, GTGTGGCTCGCAACTACCTG and AGGATCACCATACGTGGAGG (229 bp); *Gapdh*, TGGCATTGTGGAAGGGCTCATGAC and ATGCCAGTGAGCTTCCCGTTCAGC (188 bp).

Cell lines and chemomigration assays

GN11 and COS-7 cells were cultured as previously described (Cariboni et al., 2007b). Preparation of conditioned media from Slit2-expressing COS-7 cells and chemotactic assays were performed as described (Hernández-Miranda et al., 2011; Cariboni et al., 2007b). Rabbit anti-Robo antibodies (R&D Systems) were added to the GN11 cell suspension before the assay (1:100, for 1 hour at 37°C).

Statistical analysis

In all studies, we calculated the mean of at least three independent experiments; data are expressed as mean ± s.e.m. To determine statistical significance, we used a paired *t*-test or, for multiple comparisons, one-way ANOVA and Bonferroni post-hoc test. Statistical analysis was performed using Prism4 (GraphPad Software).

RESULTS AND DISCUSSION

Slit1 and Slit2 are expressed along the migratory route of GnRH neurons

Using transgenic mice carrying *GFP* in the deleted *Slit1* or *Slit2* allele (Plump et al., 2002), we studied Slit expression during GnRH neuron development. Sagittal sections of heterozygous *Slit1* and *Slit2* mouse embryos were stained with GFP, Tuj1 (Tubb3), GnRH (Gnrh1) and peripherin antibodies to reveal the expression of Slit proteins in relation to GnRH neurons and OLF/VN axons. We chose embryos at E14.5, as GnRH neuron migration is at its peak at this stage, and these cells and their associated axons can be visualised in the NC, nasal-forebrain junction and forebrain (FB). Double immunofluorescence revealed that Slit2 is co-expressed in nasal axons and FB neurons with Tuj1, a marker for newborn neurons and axons (Fig. 1A-C). To better characterise these cells, we performed double immunofluorescence for Slit2/GnRH and Slit2/peripherin, which are markers of GnRH neurons and OLF/VN axons, respectively, and found that Slit2 colabels with GnRH in neurons in the NC (Fig. 1D-F′) and with peripherin in OLF/VN axons (Fig. 1J-L), but not with GnRH in FB neurons (Fig. 1G-I) or with peripherin in the caudal branch of the VN nerve (Fig. 1M-O). Slit1 presents a similar pattern of expression, but is absent from GnRH neurons at E14.5 (supplementary material Fig. S1A-I).

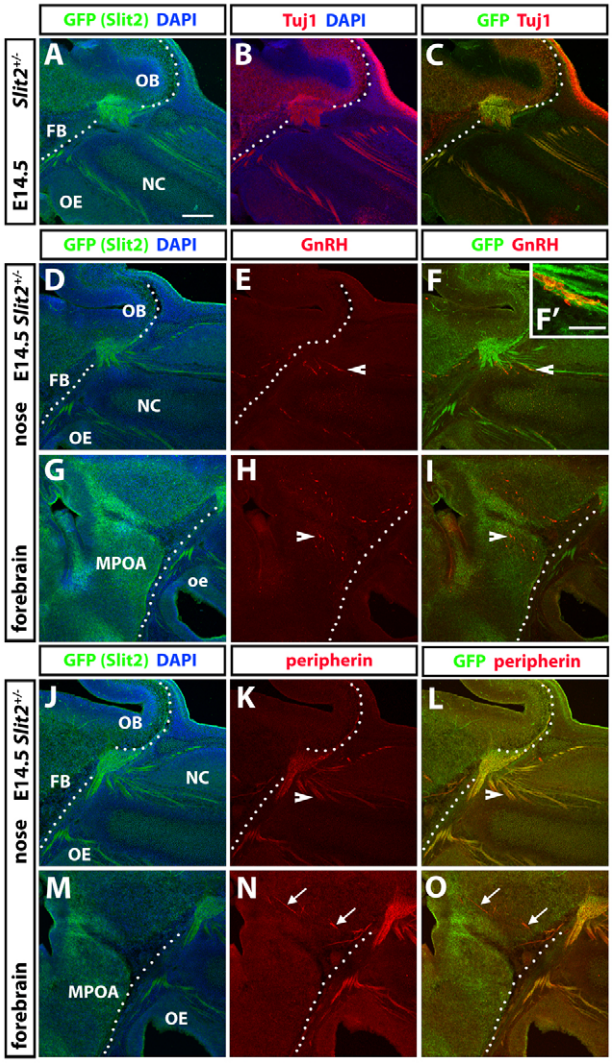

Fig. 1. Slit2 expression during GnRH neuron migration. Expression of Slit2 in the NC and FB of embryonic mouse heads. Contiguous sagittal sections from E14.5 *Slit2*$^{+/-}$ animals were colabelled with GFP and TuJ1/GnRH/peripherin antibodies to visualise co-expression (arrows and arrowheads) of Slit2 and Tuj1 in nasal axons and FB neurons (**A-C**), migrating GnRH neurons (**D-I**), and OLF/VN axons (**J-O**), respectively. The boundary between the NC and FB is indicated by a dotted line. NC, nasal compartment; OE, olfactory epithelium; OB, olfactory bulb; FB, forebrain; MPOA, medial preoptic area. Scale bars: 150 μm, except 50 μm in F′.

Slit2 controls GnRH neuron migration independently of OLF/VN axons

To study the role of Slit1 and Slit2 in the development of GnRH neurons, we analysed *Slit1* and *Slit2* single mutants and compound null mice at different embryonic stages. Sagittal sections taken from heads of E14.5 *Slit1*, *Slit2*, *Slit1/Slit2* null and wild-type littermates were stained for GnRH. No statistically significant differences in the total number of GnRH neurons were observed between the groups, thus excluding a role for Slit1 and Slit2 in the proliferation/survival of these cells (wild type, 1203±49.15; *Slit1*$^{-/-}$, 1120±18.34; *Slit2*$^{-/-}$, 1255±47.79; *Slit1*$^{-/-}$ *Slit2*$^{-/-}$, 1250±32.69; n=3; P>0.05, one-way ANOVA). A similar result was obtained following analysis of mice at E12.5 (wild type, 1042±25.41; *Slit1*$^{-/-}$, 1023±26.39; *Slit2*$^{-/-}$, 1001±9.866; P>0.05, one-way

ANOVA). Because of high expression of Slit1 and Slit2 on the axons that guide migrating GnRH neurons to the FB (Fig. 1; supplementary material Fig. S1), we also analysed the appearance of OLF/VN axons. Examination of peripherin-stained sagittal or coronal sections revealed no differences between genotypes in their patterning/fasciculation at either E12.5 or E14.5 (supplementary material Fig. S2).

We then counted GnRH neurons separately in the NC and FB, and observed differences in their distribution in *Slit2*$^{-/-}$ mice. Specifically, we found an accumulation of cells in the NC compared with wild-type animals at E12.5 (wild type, 733.0±38.19; *Slit2*$^{-/-}$, 910.0±9.713; n=3; P<0.05, t-test), resulting in a significantly reduced number of cells in the FB (wild type, 237.0±27.43; *Slit2*$^{-/-}$, 94.00±3.786; n=3; P<0.01, t-test) (Fig. 2A-D). Slit2 expression in GnRH neurons was also found at E12.5 (Fig. 3A-C), whereas Slit1 was expressed only by a small number of cells at this stage (Fig. 3D-F), suggesting a possible autocrine role for Slit2. GnRH neuron accumulation in the NC and reduction in the FB of *Slit2*$^{-/-}$ mice were also observed at E14.5 (NC: wild type, 578.0±14.22; *Slit2*$^{-/-}$, 3912.3±10.11; n=3; P<0.01, t-test; FB: wild type, 675.0±90; *Slit2*$^{-/-}$, 317.7±18.81; n=3; P<0.05, t-test) (Fig. 2E-K). No differences were found in *Slit1*$^{-/-}$ null mice at E14.5 (supplementary material Fig. S3A,B), suggesting that Slit1 does not play a role in GnRH neuron migration.

A significantly reduced number of GnRH neurons was also observed in the MPOA of *Slit2*$^{-/-}$ mice at E18.5 (wild type, 855.3±25.86; *Slit2*$^{-/-}$, 398.3±36.06; n=3; P<0.05, t-test) (Fig. 2L-O) accompanied by markedly reduced GnRH innervation of the

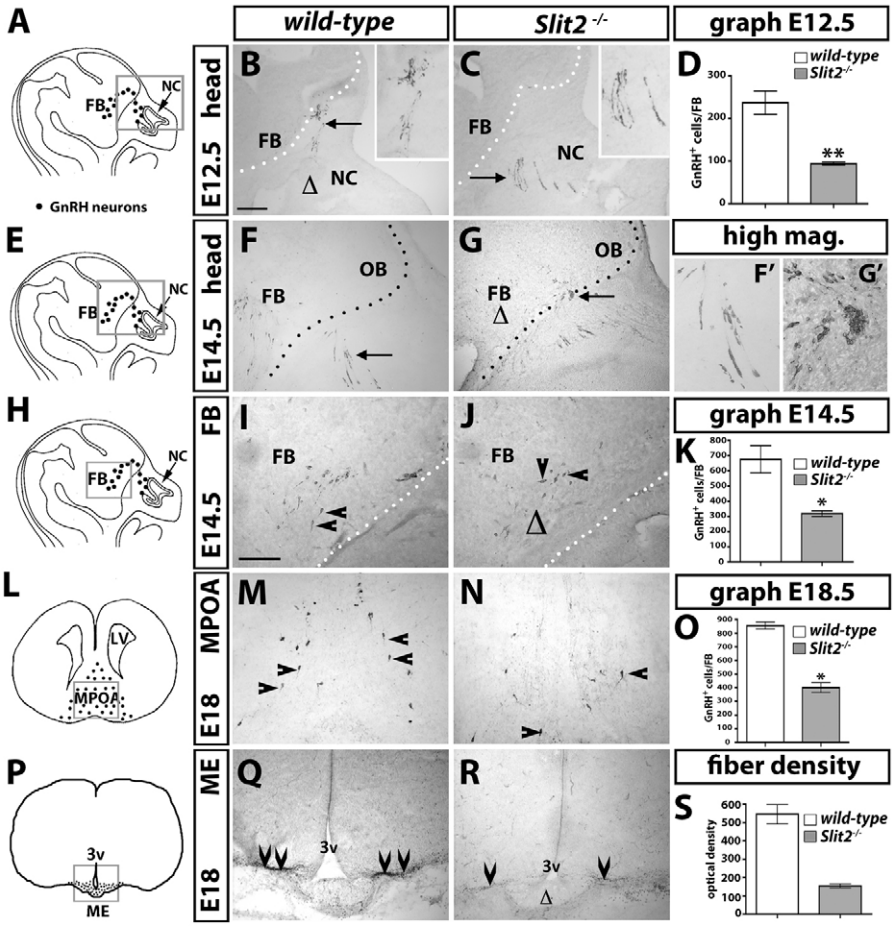

Fig. 2. Reduced number of GnRH neurons in the forebrain of *Slit2*$^{-/-}$ mice. (**A**) Schematic of the mouse head area displayed in B,C. (**B**,**C**) Sagittal sections of E12.5 mouse heads were immunolabelled for GnRH to reveal neurons migrating in the NC and FB. The FB boundary is delineated by a dotted line. Arrows (B,C) indicate areas displayed at higher magnification in insets. Arrow in C also points to abnormal accumulation of cells in the NC of *Slit2*$^{-/-}$ mice as compared with wild type (Δ in B). (**D**) Counts of GnRH neurons in the FB of E12.5 wild-type and *Slit2*$^{-/-}$ mice revealed a significant reduction in the mutant. (**E-G′**) Schematic (E) and sagittal sections (F,G) of E14.5 mouse heads immunolabelled for GnRH to reveal neurons migrating in the NC and FB. Arrows indicate areas displayed at high magnification in F′,G′. (**H-J**) Schematic (H) and representative images of sagittal sections (I,J) showing migrating GnRH neurons in the basal FB of E14.5 wild-type and *Slit2*$^{-/-}$ mice. There is a reduction in cells in the FB of the mutant (Δ in J). Arrowheads indicate examples of migrating GnRH neurons. (**K**) Counts of GnRH neurons in the FB of E14.5 wild-type and *Slit2*$^{-/-}$ mice revealed a statistically significant reduction in the mutant. (**L-N**,**P-R**) Schematic drawings (L,P) and coronal sections (M,N,Q,R) of E18.5 mouse brains immunolabelled for GnRH to reveal neurons projecting to the MPOA (M,N) and extending their axons to the ME (Q,R). Arrowheads indicate examples of migrating GnRH neurons (M,N) or GnRH^{+} fibres projecting to the ME (Q,R). The paucity of such projections in the *Slit2*$^{-/-}$ mice is indicated by Δ. (**O**) Counts of GnRH neurons in the brain of E18.5 wild-type and *Slit2*$^{-/-}$ mice revealed a statistically significant reduction in the mutant. (**S**) Optical density measurement of GnRH neuron fibres projecting to the ME of E18.5 wild-type and *Slit2*$^{-/-}$ mice revealed a statistically significant reduction in the mutant as compared with wild-type littermates. Mean ± s.e.m. *P<0.05, **P<0.01. ME, median eminence; LV, lateral ventricle; 3v, third ventricle. Scale bars: 100 μm in B,G; 75 μm in I,J,M,N,Q,R.

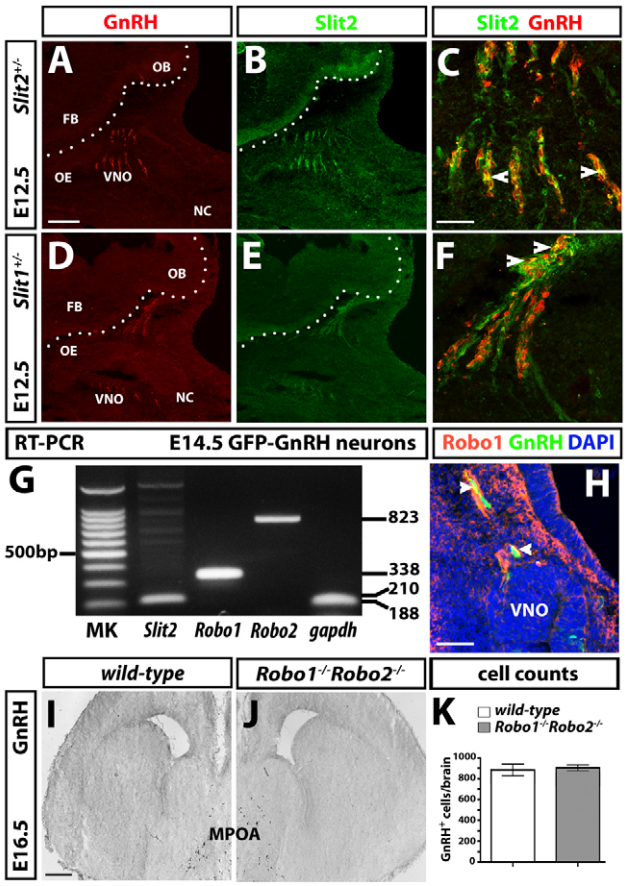

Fig. 3. Slit and Robo in the GnRH neuronal system. (**A-F**) Expression of Slit2 and Slit1 in GnRH neurons. Sagittal sections from E12.5 *Slit2*$^{+/-}$ (A-C) and *Slit1*$^{+/-}$ (D-F) mouse heads were immunostained for GnRH (A,D) and GFP (B,E); colocalisation between GnRH (red) and Slit2/Slit1 (green) is indicated with arrowheads in the corresponding higher magnification images (C,F). The boundary between NC and FB is indicated by a dotted line. (**G**) RT-PCR on FACS-sorted E14.5 GFP-GnRH neurons revealed expression of *Slit2* (210 bp), *Robo1* (338 bp) and *Robo2* (823 bp). *Gapdh*, positive control (188 bp). (**H**) Coronal section taken from E14.5 mouse head shows that Slit1 is expressed in GnRH neurons emerging from the VNO (yellow; arrowheads). (**I**,**J**) Coronal sections through the FB of E16.5 wild-type and *Robo1*$^{-/-}$ *Robo2*$^{-/-}$ littermates were immunolabelled to visualise GnRH neurons in the MPOA. (**K**) Counts of labelled cells did not show statistically significant differences between the wild-type and *Robo1*$^{-/-}$ *Robo2*$^{-/-}$ littermates. Mean ± s.e.m. VNO, vomeronasal organ. Scale bars: 150 μm in A,B,D,E; 50 μm in C,F,H; 100 μm in I,J.

ME (Fig. 2P-S). Analysis of *Slit1/Slit2* compound null mice revealed a similar reduction in the number of cells in the FB at E14.5 (wild type, 587.0±47.35; *Slit1*$^{-/-}$ *Slit2*$^{-/-}$, 311.7±22.42; n=3; P<0.01, t-test) and E18.5 (wild type, 682.0±45.71; *Slit1*$^{-/-}$ *Slit2*$^{-/-}$, 344±20.55; n=3; P<0.001, t-test), confirming that Slit1 is dispensable for the migration of GnRH neurons.

We also analysed mice lacking *Slit3* at E14.5 (n=3), although expression of this molecule in the rodent olfactory system has not been reported (Marillat et al., 2002). This analysis revealed no statistically significant differences in the number or position of GnRH neurons compared with wild-type controls (GnRH^{+} neurons/head: wild type, 1320±35.79; *Slit3*$^{-/-}$, 1290±75.21; n=3; GnRH^{+} neurons/FB: wild type, 699.0±34.22; *Slit3*$^{-/-}$, 696.0±81.09, n=3) (supplementary material Fig. S3A-C,F), excluding its involvement in this system. Accordingly, a similar distribution of GnRH neurons in the MPOA and innervation of the ME were noted in the two groups of mice at E18.5 (supplementary material Fig. S4D,E,G,H).

Robo expression and function in the GnRH neuronal system

Robo1 and Robo2 are the predominant Slit receptors in the CNS (Sundaresan et al., 2004), including the olfactory system (Marillat et al., 2002), but no binding preference has been described between the different Slits and these receptors (Mambetisaeva et al., 2005). We performed RT-PCR on RNA isolated from FACS-sorted mouse GFP-GnRH neurons at E14.5 as described previously (Cariboni et al., 2007b) and found expression of *Slit2*, as well as of *Robo1* and *Robo2*, in these cells (Fig. 3G). Expression of *Robo2* mRNA in neurons leaving the OE has been reported in E15 rats (Marillat et al., 2002), and here we confirmed the expression of Robo1 in GnRH neurons by immunostaining coronal sections from E15.5 GFP-GnRH rat embryos with GFP and Robo1 antibodies (Fig. 3H).

We subsequently assessed the number and distribution of GnRH neurons in *Robo1*$^{-/-}$ *Robo2*$^{-/-}$ mice at E16.5. Counts of labelled cells in coronal sections taken through the FB showed no difference between genotypes (wild type, 890±18.02; *Robo1*$^{-/-}$ *Robo2*$^{-/-}$, 921±36.47; n=3; P>0.05, t-test) (Fig. 3I-K), suggesting that Slit2 acts independently of these receptors.

A previous study reported that Robo3 is expressed by populations of tangentially migrating neurons, including GnRH cells (Marillat et al., 2004). We confirmed this finding both by RT-PCR of RNA from E14.5 GFP-GnRH neurons and by immunofluorescence (Fig. 4A,B). We then analysed *Robo3*$^{-/-}$ mice and found, similar to *Slit2*$^{-/-}$ animals, a significantly reduced number of GnRH neurons in the FB and a concomitant accumulation of cells in the NC compared with wild-type littermates at E14.5 (wild type, 658.0±31.90; *Robo3*$^{-/-}$, 343.7±46.36; n=3; P<0.001, t-test) (Fig. 4C-F). Also, as in *Slit2*$^{-/-}$ mice, there were no apparent defects in the fasciculation/patterning of the OLF/VN nerves (supplementary material Fig. S3C-D′).

To confirm that Slit2 acts through Robo3 to guide GnRH neurons in the NC, we performed in vitro chemotactic assays using immortalised GnRH neurons (GN11 cell line). We have previously reported that GN11 cells express Robo1-3 and respond to Slit1 (Hernández-Miranda et al., 2011), and may thus be used to test the effects of Slit2. As shown in Fig. 4, the chemomigration of GN11 cells is reduced in the presence of Slit2-conditioned media (CM) (Fig. 4H) compared with mock CM from COS-7 cells (Fig. 4G). In addition, this effect was blocked by pre-incubation with Robo3 antibody (Fig. 4J,K), but not by Robo1 and Robo2 antibodies (Fig. 4I), suggesting that Slit2 exerts its effects specifically through Robo3.

Taken together, our data suggest that Slit2 and Robo3 are important in the development of the GnRH neuronal system, as lack of these molecules alters the migration of GnRH cells, resulting in their abnormal accumulation in the NC. The similarity of the phenotypes observed in the *Slit2*$^{-/-}$ and *Robo3*$^{-/-}$ mice, together with the results of the chemotactic assays, suggest that this ligand-receptor pair modulates the migration of GnRH neurons, as reported for cancer cells (Bauer et al., 2011). However, the possibility of interaction with other signalling pathways cannot be excluded. For example, genetic data from *C. elegans* indicate that Slits might function through DCC (Deleted in colorectal cancer) (Yu et al., 2002), a molecule found to be important in the migration of many neuronal cell types, including GnRH neurons. Mice

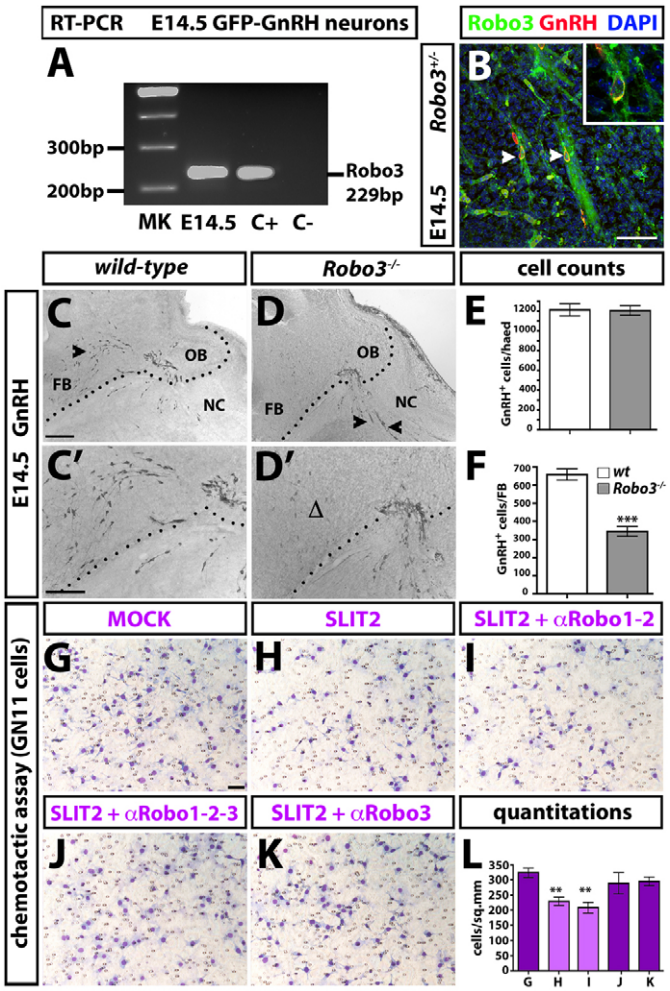

Fig. 4. Robo3 in the GnRH neuronal system. (**A**,**B**) Expression of Robo3 in GnRH neurons. (A) RT-PCR on FACS-sorted E14.5 GFP-GnRH neurons revealed the expression of *Robo3* (229 bp). C+, positive control E14.5 mouse head; C–, negative control water. (B) Sagittal sections from E14 *Robo3*$^{+/-}$ animals were immunostained for GnRH and GFP. Colocalisation of GnRH (red) and Robo3 (green) is indicated (arrowheads and inset). (**C-F**) *Robo3*$^{-/-}$ mice have a reduced number of GnRH neurons in the FB. (C-D′) Sagittal sections of E14.5 mouse heads were immunolabelled for GnRH to reveal migrating neurons (arrowheads) in NC and FB. Higher magnification images are shown in C′,D′. There is a reduction of GnRH neurons in the FB of *Robo3*$^{-/-}$ mice (Δ in D′). (E,F) Counts of GnRH^{+} neurons in the head (E) and FB (F) of E14.5 wild-type and *Robo3*$^{-/-}$ mice revealed no differences in total number (E), but a statistically significant reduction in the FB of *Robo3* mutants compared with wild-type littermates (F). (**G-K**) Chemomigratory response of GN11 cells towards Slit2. Representative images of migrated GN11 cells in a Boyden chamber assay with mock conditioned media of COS-7 cells (G) and Slit2-expressing COS-7 cells in the absence (H) or presence of Robo1/2 (I), Robo1-3 (J) or Robo3 (K) antibodies. (**L**) Counts of migrated GN11 cells/mm^2 towards the indicated stimuli. Mean ± s.e.m. $^{**}P<0.01$, $^{***}P<0.001$. Scale bars: 50 μm in B; 100 μm in C,D; 75 μm in C′,D′; 50 μm in G-K.

lacking Dcc or its ligand netrin 1 show a defect in the migration of GnRH cells similar to that reported here (Schwarting et al., 2001; Schwarting et al., 2004), supporting the possibility of cross-talk between Slit2 and netrin 1 signalling in this system. In conclusion, our results highlight the importance of Slit2 and Robo3 in the migration of GnRH neurons in a cell-autonomous manner, and might shed light on the pathogenesis of idiopathic forms of hypogonadotropic hypogonadism and Kallmann syndrome.

Acknowledgements
We thank Drs M. Tessier-Lavigne and D. Ornitz for supplying Slit mutant mice; Roberta Noseda and Alessandro Fantin for technical assistance; and Drs Sonja Rakic and Nadia Liapi for discussion of results.

Funding
This work was funded by a Wellcome Trust Programme Grant [089775]. Deposited in PMC for immediate release.

Competing interests statement
The authors declare no competing financial interests.

Supplementary material
Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.079418/-/DC1

References

Bauer, K., Dowejko, A., Bosserhoff, A. K., Reichert, T. E. and Bauer, R. (2011). Slit-2 facilitates interaction of P-cadherin with Robo-3 and inhibits cell migration in an oral squamous cell carcinoma cell line. *Carcinogenesis* **32**, 935-943.
Cariboni, A., Rakic, S., Liapi, A., Maggi, R., Goffinet, A. and Parnavelas, J. G. (2005). Reelin provides an inhibitory signal in the migration of gonadotropin-releasing hormone neurons. *Development* **132**, 4709-4718.
Cariboni, A., Maggi, R. and Parnavelas, J. G. (2007a). From nose to fertility: the long migratory journey of gonadotropin-releasing hormone neurons. *Trends Neurosci.* **30**, 638-644.
Cariboni, A., Hickok, J., Rakic, S., Andrews, W., Maggi, R., Tischkau, S. and Parnavelas, J. G. (2007b). Neuropilins and their ligands are important in the migration of gonadotropin-releasing hormone neurons. *J. Neurosci.* **27**, 2387-2395.
Cariboni, A., Davidson, K., Rakic, S., Maggi, R., Parnavelas, J. G. and Ruhrberg, C. (2011). Defective gonadotropin-releasing hormone neuron migration in mice lacking SEMA3A signalling through NRP1 and NRP2: implications for the aetiology of hypogonadotropic hypogonadism. *Hum. Mol. Genet.* **20**, 336-344.
Chédotal, A. (2007). Slits and their receptors. *Adv. Exp. Med. Biol.* **621**, 65-80.
Cho, J. H., Lépine, M., Andrews, W., Parnavelas, J. and Cloutier, J. F. (2007). Requirement for Slit-1 and Robo-2 in zonal segregation of olfactory sensory neuron axons in the main olfactory bulb. *J. Neurosci.* **27**, 9094-9104.
Cloutier, J. F., Sahay, A., Chang, E. C., Tessier-Lavigne, M., Dulac, C., Kolodkin, A. L. and Ginty, D. D. (2004). Differential requirements for semaphorin 3F and Slit-1 in axonal targeting, fasciculation, and segregation of olfactory sensory neuron projections. *J. Neurosci.* **24**, 9087-9096.
Giacobini, P., Messina, A., Wray, S., Giampietro, C., Crepaldi, T., Carmeliet, P. and Fasolo, A. (2007). Hepatocyte growth factor acts as a motogen and guidance signal for gonadotropin hormone-releasing hormone-1 neuronal migration. *J. Neurosci.* **27**, 431-445.
Giacobini, P., Messina, A., Morello, F., Ferraris, N., Corso, S., Penachioni, J., Giordano, S., Tamagnone, L. and Fasolo, A. (2008). Semaphorin 4D regulates gonadotropin hormone-releasing hormone-1 neuronal migration through PlexinB1-Met complex. *J. Cell Biol.* **183**, 555-566.
Grieshammer, U., Ma, L., Plump, A. S., Wang, F., Tessier-Lavigne, M. and Martin, G. R. (2004). SLIT2-mediated ROBO2 signaling restricts kidney induction to a single site. *Dev. Cell* **6**, 709-717.
Hardelin, J. P. (2001). Kallmann syndrome: towards molecular pathogenesis. *Mol. Cell. Endocrinol.* **179**, 75-81.
Hernández-Miranda, L. R., Cariboni, A., Faux, C., Ruhrberg, C., Cho, J. H., Cloutier, J. F., Eickholt, B. J., Parnavelas, J. G. and Andrews, W. D. (2011). Robo1 regulates semaphorin signaling to guide the migration of cortical interneurons through the ventral forebrain. *J. Neurosci.* **31**, 6174-6187.
Kidd, T., Brose, K., Mitchell, K. J., Fetter, R. D., Tessier-Lavigne, M., Goodman, C. S. and Tear, G. (1998). Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. *Cell* **92**, 205-215.
Long, H., Sabatier, C., Ma, L., Plump, A., Yuan, W., Ornitz, D. M., Tamada, A., Murakami, F., Goodman, C. S. and Tessier-Lavigne, M. (2004). Conserved roles for Slit and Robo proteins in midline commissural axon guidance. *Neuron* **42**, 213-223.
Mambetisaeva, E. T., Andrews, W., Camurri, L., Annan, A. and Sundaresan, V. (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. *Dev. Dyn.* **233**, 41-51.

Marillat, V., Cases, O., Nguyen-Ba-Charvet, K. T., Tessier-Lavigne, M., Sotelo, C. and Chédotal, A. (2002). Spatiotemporal expression patterns of slit and robo genes in the rat brain. *J. Comp. Neurol.* **442**, 130-155.

Marillat, V., Sabatier, C., Failli, V., Matsunaga, E., Sotelo, C., Tessier-Lavigne, M. and Chédotal, A. (2004). The slit receptor Rig-1/Robo3 controls midline crossing by hindbrain precerebellar neurons and axons. *Neuron* **43**, 69-79.

Merchenthaler, I., Görcs, T., Sétáló, G., Petrusz, P. and Flerkó, B. (1984). Gonadotropin-releasing hormone (GnRH) neurons and pathways in the rat brain. *Cell Tissue Res.* **237**, 15-29.

Messina, A., Ferraris, N., Wray, S., Cagnoni, G., Donohue, D. E., Casoni, F., Kramer, P. R., Derijck, A. A., Adolfs, Y., Fasolo, A. et al. (2011). Dysregulation of Semaphorin7A/β1-integrin signaling leads to defective GnRH-1 cell migration, abnormal gonadal development and altered fertility. *Hum. Mol. Genet.* **20**, 4759-4774.

Nguyen-Ba-Charvet, K. T., Di Meglio, T., Fouquet, C. and Chédotal, A. (2008). Robos and slits control the pathfinding and targeting of mouse olfactory sensory axons. *J. Neurosci.* **28**, 4244-4249.

Plump, A. S., Erskine, L., Sabatier, C., Brose, K., Epstein, C. J., Goodman, C. S., Mason, C. A. and Tessier-Lavigne, M. (2002). Slit1 and Slit2 cooperate to prevent premature midline crossing of retinal axons in the mouse visual system. *Neuron* **33**, 219-232.

Prince, J. E., Cho, J. H., Dumontier, E., Andrews, W., Cutforth, T., Tessier-Lavigne, M., Parnavelas, J. and Cloutier, J. F. (2009). Robo-2 controls the segregation of a portion of basal vomeronasal sensory neuron axons to the posterior region of the accessory olfactory bulb. *J. Neurosci.* **29**, 14211-14222.

Sabatier, C., Plump, A. S., Ma, L., Brose, K., Tamada, A., Murakami, F., Lee, E. Y. and Tessier-Lavigne, M. (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.

Schwarting, G. A., Kostek, C., Bless, E. P., Ahmad, N. and Tobet, S. A. (2001). Deleted in colorectal cancer (DCC) regulates the migration of luteinizing hormone-releasing hormone neurons to the basal forebrain. *J. Neurosci.* **21**, 911-919.

Schwarting, G. A., Raitcheva, D., Bless, E. P., Ackerman, S. L. and Tobet, S. (2004). Netrin 1-mediated chemoattraction regulates the migratory pathway of LHRH neurons. *Eur. J. Neurosci.* **19**, 11-20.

Schwarting, G. A., Henion, T. R., Nugent, J. D., Caplan, B. and Tobet, S. (2006). Stromal cell-derived factor-1 (chemokine C-X-C motif ligand 12) and chemokine C-X-C motif receptor 4 are required for migration of gonadotropin-releasing hormone neurons to the forebrain. *J. Neurosci.* **26**, 6834-6840.

Sundaresan, V., Roberts, I., Bateman, A., Bankier, A., Sheppard, M., Hobbs, C., Xiong, J., Minna, J., Latif, F., Lerman, M. et al. (1998). The DUTT1 gene, a novel NCAM family member is expressed in developing murine neural tissues and has an unusually broad pattern of expression. *Mol. Cell. Neurosci.* **11**, 29-35.

Sundaresan, V., Mambetisaeva, E., Andrews, W., Annan, A., Knöll, B., Tear, G. and Bannister, L. (2004). Dynamic expression patterns of Robo (Robo1 and Robo2) in the developing murine central nervous system. *J. Comp. Neurol.* **468**, 467-481.

Wray, S. (2010). From nose to brain: development of gonadotrophin-releasing hormone-1 neurones. *J. Neuroendocrinol.* **22**, 743-753.

Ypsilanti, A. R., Zagar, Y. and Chédotal, A. (2010). Moving away from the midline: new developments for Slit and Robo. *Development* **137**, 1939-1952.

Yu, T. W., Hao, J. C., Lim, W., Tessier-Lavigne, M. and Bargmann, C. I. (2002). Shared receptors in axon guidance: SAX-3/Robo signals via UNC-34/Enabled and a Netrin-independent UNC-40/DCC function. *Nat. Neurosci.* **5**, 1147-1154.

Yuan, W., Zhou, L., Chen, J. H., Wu, J. Y., Rao, Y. and Ornitz, D. M. (1999). The mouse SLIT family: secreted ligands for ROBO expressed in patterns that suggest a role in morphogenesis and axon guidance. *Dev. Biol.* **212**, 290-306.