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Development/Plasticity/Repair

Soluble Guanylate Cyclase Generation of cGMP Regulates Migration of MGE Neurons

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Here we have provided evidence that nitric oxide-cyclic GMP (NO-cGMP) signaling regulates neurite length and migration of immature neurons derived from the medial ganglionic eminence (MGE). Dlx1/2−/− and Lhx6−/− mouse mutants, which exhibit MGE interneuron migration defects, have reduced expression of the gene encoding the subunit of a soluble guanylate cyclase (Gucy1A3). Furthermore, Dlx1/2−/− mouse mutants have reduced expression of NO synthase 1 (NOS1). Gucy1A3−/− mice have a transient reduction in cortical interneuron number. Pharmacological inhibition of soluble guanylate cyclase and NOS activity rapidly induces neurite retraction of MGE cells in vitro and in slice culture and robustly inhibits cell migration from the MGE and caudal ganglionic eminence. We provide evidence that these cellular phenotypes are mediated by activation of the Rho signaling pathway and inhibition of myosin light chain phosphatase activity.

Introduction

Development of the ganglionic eminences (GEs), which produce basal ganglia projection neurons and interneurons that migrate tangentially to distant targets including the cerebral cortex, is regulated by the Dlx transcription factors. Mice lacking Dlx1 and Dlx2 (Dlx1/2−/− mutants) have a severe defect in interneuron migration (Anderson et al., 1997a; Cobos et al., 2007). Likewise, mice lacking either Lhx6 or Lhx6 and Lhx8 have a partial block in interneuron migration from one GE, the medial ganglionic eminence (MGE), where many neurons remain as a periventricular ectopia (Zhao et al., 2008; Flandin et al., 2011).

Analysis of changes in gene expression in the Dlx1/2−/− and Lhx6−/− mutants has identified candidate regulators of interneuron migration. For instance, the Pak3 kinase is overexpressed in mice lacking either Dlx1/2 (Mandal et al., 2013). NOS1 and CXCR7 receptor expression is reduced in the Dlx1/2−/− and Lhx6−/− mutants; these receptors guiding interneuron migration in the cortex (Fogarty et al., 2007; Sánchez-Alcañíz et al., 2011; Wang et al., 2011). ErbB4 expression is reduced in the Lhx6−/− mutants; this receptor tyrosine transduces the chemotactic signal from neuregulin in promoting migration (Flames et al., 2004). Finally, Dlx1/2−/− represses neuropilin2 expression (Le et al., 2007; Long et al., 2009a); neuropilins are coreceptors for semaphorins. Neuropilin/semaphorin signaling, particularly through Sema3A, is implicated in sorting MGE-derived cortical and striatal interneurons (Marin et al., 2001; Nóbrega-Pereira et al., 2008), and regulating their migration in the cortex (Tama-maki et al., 2003).

Semaphorins regulate axon guidance and axon/dendrite specification; Sema3A signaling may mediate these processes at least in part by increasing cyclic GMP (cGMP) concentration through “soluble” guanylyl cyclases (sGCs) (Polleux et al., 1998, 2000; Schmidt et al., 2002; Nishiyama et al., 2003, 2008, 2011; Togashi et al., 2008; Shelly et al., 2011). In many tissues, including striatal medium spiny neurons, sGC are activated by nitric oxide (NO) (Denning and Marletta, 1999; Lin et al., 2010). cGMP promotes slime mold cell migration (Sato et al., 2009).

Here we have explored whether the NO-cGMP signaling system regulates the migration of immature neurons in the developing telencephalon. Preliminary analyses of Dlx1/2−/− mutants showed that expression of the subunit of sGC (Gucy1A3) was decreased in the E15.5 GEs (Long et al., 2009a). Herein we demonstrate that Gucy1A3 is expressed in the dorsal MGE, the primordium where cortical interneurons are generated, where its expression is reduced in Dlx1/2−/− and Lhx6−/− mutants. We show that NOS1 (nNOS) expression in the MGE is lost in the Dlx1/2−/− mutants. We find that Gucy1A3−/− mice have a transient prenatal reduction in cortical interneuron number. Fur-
thermore, pharmacological inhibition of sGC and NOS activity robustly inhibits cell migration from the MGE and caudal ganglionic eminence (CGE) and rapidly induces neurite retraction of MGE cells in vitro and in slice culture. We provide evidence that these cellular phenotypes are mediated by activation of the Rho signaling pathway and inhibition of myosin light chain phosphatase (MLCP) activity (see Fig. 14).

Materials and Methods

Animals and tissue preparation. The following mouse strains (of either sex) were used and genotyped as described in the associated references: Dlx1/2<sup>+/−</sup> (Anderson et al., 1997a), Lhx6<sup>−/−</sup>PLAP (Choi et al., 2005), Lhx6-GFP (http://www.gensat.org/index.html), Gucy1A3<sup>−/−</sup> (Buys et al., 2008), and Nos1<sup>−/−</sup> (Packer et al., 2003). CD-1 females were obtained from Charles River Laboratories. For staging of embryos, midday of the vaginal plug was calculated as embryonic day 0.5 (E0.5). Littermate mice, either male or female (wild-type [WT] or, when not possible, heterozygous) were used as controls for all experiments. Mouse colonies were maintained in accordance with the guidelines set by the author’s institutions and the National Institutes of Health.

For embryonic brains (E13.5, E15.5), animals were deeply anesthetized, dissected, and fixed by immersion in 4% PFA in PBS overnight at 4°C. The following day, tissue was cryoprotected by immersion in 30% sucrose, embedded in OCT (Tissue-Tek), cryostat sectioned (20 μm), and used either for in situ hybridization or immunohistochemistry analysis.

In situ hybridization. Section in situ hybridization experiments were performed using digoxigenin riboprobes on 20 μm frozen sections (from either male or females) as described previously (Jeong et al., 2004). The probes used and their sources are as follows: Gucy1A3 (GenBank accession no. NM_021896), Nos1 (GenBank accession and KTs5823, protein kinase G [PKG] inhibitor) were all purchased from Enzo Life Sciences. NAME86 (N<sup>0</sup>-monomethyl-L-arginine, monoacetate salt) was purchased from Calbiochem. Y-27632 was purchased from Sigma. C3 transferase, calpeptin, and EGF were all purchased from Calpeptin, and KT5823 (protein kinase G [PKG] inhibitor) were all purchased from Enzo Life Sciences. NAME86 (N<sup>0</sup>-monomethyl-L-arginine, monoacetate salt) was purchased from Calbiochem. Y-27632 was purchased from Sigma. C3 transferase, calpeptin, and EGF were all purchased from Calbiochem.

Slice culture and electroporation. Slices (300 μm) were prepared from E13.5 mouse embryos (from either male or female) (Anderson et al., 1997a). Electroporations (pCAGGS-GFP) followed the methods of Stuhmer et al. (2002).

Matrigel explant assay. The 250 μm coronal vibromate sections were made from E13.5 WT, CD-1 telencephalons (from either sex), followed by microdissection of the MGE and CGE progenitor zones. The explants were embedded in Matrigel and placed into a 6-well insert (1 mm) were prepared from E13.5 mouse embryos (from either male or female) (Anderson et al., 1997a). Electroporations (pCAGGS-GFP) followed the methods of Stuhmer et al. (2002).

Figure 1. Dlx1/2 regulate the expression of Gucy1A3, Gucy1B3, and Nos1 (nNOS) in the developing basal ganglia. In situ RNA hybridization analysis of Gucy1A3 (A–D, I–J), Gucy1B3 (K–N, O–P), and Nos1 (S–V) expression in the telencephalon of WT (left panels) and Dlx1/2<sup>−/−</sup> (right panels) mice at E13.5 and E15.5. In each case, four planes of hemisections are shown (rostral to caudal). Gucy1A3 expression in the MGE is restricted to the dMGE. By E15.5, Gucy1A3 expression is detected in scattered cells in the cortical SVZ (higher magnification in I–J below); this expression is reduced in the Dlx1/2<sup>−/−</sup> mutants (I’, J’, arrows). Dlx1/2<sup>−/−</sup> mutants also have reduced Gucy1A3 expression in the SVZ of the LGE and MGE (E13.5 and E15.5); in addition, at E15.5, the mutants have ectopic Gucy1A3 expression in the caudalventral CGE/MGE (I’, J’, arrows), the region that has ectopic interneuron migration (Long et al., 2009b). Gucy1B3 and Nos1 expression is also greatly reduced in the basal ganglia anlage of the Dlx1/2<sup>−/−</sup> mutants. CGE, Caudal ganglionic eminence; CX, cortex; GP, globus pallidus; Str, striatum. Scale bars: A–D, I–J, 500 μm; I’–J’, 120 μm.

from Sigma. C3 transferase, calpeptin, and EGF were all purchased from Calbiochem.
In situ RNA hybridization analysis of Gucy1A3 expression in the telencephalon of Lhx6^−/-GFP (heterozygous) (left panels) and Lhx6^GAPAPAP (homozygote mutants) (right panels) mice at E13.5 (A–D’) and E15.5 (E–H’). In each case, four planes of hemisections are shown (rostral to caudal). Expression of Gucy1A3 is specifically lost from the dMGE (B’, F’), I–T’, Expression of NOS1 (NOS1, NOS2, and NOS3 in the developing basal ganglia of WT and Dlx1/2^−/− E15.5 mice. In situ RNA hybridization analysis of NOS1 (I–L’, NOS2 (M–P’), and NOS3 (Q–T’) expression in the telencephalon of WT (left panels) and Dlx1/2^−/− (right panels) mice at E15.5. In each case, four planes of hemisections are shown (rostral to caudal). Scale bars: A–T’, 500 μm.

Figure 2. Lhx6 regulates the expression of Gucy1A3 in the dorsal MGE. In situ RNA hybridization analysis of Gucy1A3 expression in the telencephalon of Lhx6^−/− (heterozygotes) (left panels) and Lhx6^GAPAPAP (homozygote mutants) (right panels) mice at E13.5 (A–D’) and E15.5 (E–H’). In each case, four planes of hemisections are shown (rostral to caudal). Expression of Gucy1A3 is specifically lost from the dMGE (B’, F’), I–T’, Expression of NOS1 (NOS1, NOS2, and NOS3 in the developing basal ganglia of WT and Dlx1/2^−/− E15.5 mice. In situ RNA hybridization analysis of NOS1 (I–L’, NOS2 (M–P’), and NOS3 (Q–T’) expression in the telencephalon of WT (left panels) and Dlx1/2^−/− (right panels) mice at E15.5. In each case, four planes of hemisections are shown (rostral to caudal). Scale bars: A–T’, 500 μm.

Each experiment. All results are expressed as the mean ± SE of sample size n = 3. Data were analyzed using Student’s t test or one-way ANOVA followed by Bonferroni post test to determine the level of significance. p ≤ 0.05 was considered significant, and p ≤ 0.01 was considered highly significant.

Primary cell culture. Primary cultures were made from WT, CD-1, or Lhx6-GFP animals (of either sex) dissociated anterior MGE (VZ/SVZ) as described previously (Xu et al., 2004; Cobos et al., 2005). After a day in culture, the cells were exposed to DMSO or drug (e.g., ODQ); 30 min later, the cells were fixed and studied by immunofluorescence.

Immunocytochemistry. Immunofluorescence on dissociated cells (Xu et al., 2004) using the following primary antibodies: mouse anti-βIII-tubulin (TuJ1) (1:1000; Covance), chicken anti-GFP (1:1000; Aves Laboratories). Secondary antibodies were as follows: Alexa-488 goat anti-mouse (1:500), Alexa-488 goat anti-chicken (1:500; Invitrogen).

Boyden chamber assay. Dissociated MGE cells (E13.5 animals of either sex) were plated on a filter of Boyden chamber inserts (BD Biosciences) with an 8 μm pore size in a 24-well format. A total of 7 × 10^3 cells in 200 μl Neurobasal medium supplemented with B27 were added to the top chamber. Cell migration was promoted by adding 10% FBS to the lower compartment of the chamber. Cultures were incubated for 19–22 h with different pharmacological inhibitors/analogues in both chambers. Cells that had remained on the top of the filter were eliminated with a cotton tip. The filters were then stained using HEMA3 stain pack (Protocol). The filter’s lower side was imaged on a Nikon ECLIPSE 80i microscope (Nikon Instruments) and counted using ImageJ software (National Institutes of Health) in five randomly selected fields. For Dlx1/2^−/− mutants, we used 3 WT and 3 mutant embryos per experiment. For all other Boyden assays, we used 7 WT embryos per experiment. Three independent experiments were performed. Cell migration is displayed as percentage of cells migrating normalized to control or DMSO-treated wells. All results are expressed as the mean ± SE of sample size n = 3. Data were analyzed using Student’s t test or one-way ANOVA followed by Bonferroni post test to determine the level of significance. p ≤ 0.05 was considered significant, and p ≤ 0.01 was considered highly significant.

Real-time imaging. Coronal E15.5 Lhx6-GFP brain slices (200 μm, from either males or females) were treated with DMSO or ODQ for 12 h. After drug treatment, the slices were placed onto nucleopore membrane filters over 35 mm glass-bottom dishes containing MEM (Invitrogen) and 10% FBS. Mounted slices were immediately transferred to a 37°C/5% CO2 live tissue incubation chamber attached to a Zeiss inverted microscope and a PASCAL confocal laser scanning system and imaged repeatedly every 12 min for up to 20 h. Real-time interneuronal migration patterns were quantified using Zeiss LSM Image Browser software. Statistical analysis was performed with the Student’s t test using Prism4 software (GraphPad Software).

Protein preparation and Western blotting. MGE cells were lysed in RIPA buffer containing protease inhibitors (Roche). Western blot was performed as described previously (Cobos et al., 2007). Each experiment was repeated at least three times. The intensity of the bands was quantified using the histogram feature in Adobe Photoshop (CS3). Using the marquee tool in Photoshop, a box was drawn covering one band, and the mean intensity of the value was recorded. The size of the box was consistent throughout the analysis. The mean value was then subtracted from the background. Results were presented as either absolute mean values or percentage of relative protein levels, normalized to control or DMSO-treated cell extracts. Primary antibodies used were obtained from Cell Signaling Technology: rabbit vasodilator-stimulated phosphoprotein (VASP) (1:1000); rabbit anti-pVASP-Ser239 (1:1000), and rabbit anti-phospho-myosin light chain 2 (Ser19) (1:400).

Quantification of neurite length. Quantification of neurite length was performed in immature interneurons from brain slices (200 μm) or MGE primary cultures prepared with Lhx6-GFP or WT embryos (from either male or females), immunostained for GFP or Tuj1, respectively. Results were expressed as length of the longest neurite normalized to DMSO control. Data were analyzed using Student’s t test or one-way ANOVA followed by Bonferroni post test to determine the level of significance. p ≤ 0.05 was considered significant, and p ≤ 0.01 was considered highly significant.

Statistical analysis was performed with the Student’s t test or one-way ANOVA followed by Bonferroni post test to determine the level of significance. p ≤ 0.05 was considered significant, and p ≤ 0.01 was considered highly significant.
Statistical analysis. All results are expressed as the mean ± SE of sample size n = 3. Data were analyzed using a Student’s t test or one-way ANOVA followed by Bonferroni post test to determine the level of significance. p ≤ 0.05 was considered significant (*), and p ≤ 0.01 was considered highly significant (**).

Results
Soluble guanylate cyclase and NO synthase (NOS) expression in the developing telencephalon
To elucidate the molecular mechanisms underlying the subpallial migration defects in the Dlx1/2−/− mutants (Anderson et al., 1997a, b; Cobos et al., 2007; Long et al., 2007), we previously identified dysregulated genes in the E15.5 GEs using RNA expression array analysis (Cobos et al., 2007; Long et al., 2009a, b). One of the most prominent downregulated genes was Gucy1A3, encoding the a1 subunit of sGC. Gucy1A3 RNA was reduced ~3.5-fold in the Dlx1/2−/− mutant, which was confirmed by in situ RNA hybridization at E13.5 and E15.5 (Fig. 1A–H).

In WT mice, Gucy1A3 RNA was expressed in most cells of the E13.5 SVZ of the lateral ganglionic eminence (LGE), MGE, and CGE. Within the MGE, its expression was largely restricted to the dorsal MGE (dMGE), the part of the MGE that is largely responsible for generating pallial interneurons (Fig. 1B, B′) (Fogarty et al., 2007; Flandin et al., 2010). On the other hand, the ventral MGE (vMGE), which generates the globus pallidus (Flandin et al., 2010), had low Gucy1A3 expression (Fig. 1C, C′). In the E13.5 Dlx1/2−/− mutant, Gucy1A3 expression was greatly reduced (Fig. 1A′–D′).

At E15.5, Gucy1A3 expression was maintained in the GEs (again at very low levels in the vMGE) and was more prominently expressed in the subpallial mantle zone (MZ), particularly in the striatal neurons (Gucy1A3 expression was not detected in the globus pallidus). In the Dlx1/2−/− mutant, SVZ expression was strongly reduced and MZ expression was moderately decreased (Fig. 1E–H′). Ectopic subpallial expression (E′) (Fig. 1F′) was observed in a caudal ventral region; previous analysis showed that this region expressed markers of immature interneurons (e.g., Lhx6) that had failed to migrate to the cortex (Long et al., 2009b).

Lhx6 and Lhx6/8 mutants have defects in interneuron migration, including a prominent dMGE ectopia corresponding to a collection of unmigrated cells (Zhao et al., 2008; Flandin et al., 2011). In the Lhx6 null mutant (E13.5 and E15.5), Gucy1A3 expression in the dMGE was greatly reduced (Fig. 2A–H′), thus providing further evidence for a link between Gucy1A3 and interneuron migration.

In the pallium of WT mice, Gucy1A3 was expressed in cortical plate (CP) and in scattered cells in the SVZ whose location is consistent with tangentially migrating interneurons. Indeed, in the Dlx1/2−/− mutant, pallial SVZ expression was nearly eliminated (Fig. 1I′, J′), supporting the idea that Gucy1A3 is expressed in immature interneurons in the SVZ of the dMGE and as they tangentially migrate to the cortex. These interneurons could also be derived from the CGE, which also has high levels of Gucy1A3 expression (Fig. 1D, H).

Next, we assessed whether other guanylate cyclase subunits were expressed in the E15.5 telencephalon. Similar to Gucy1A3, RNA expression of Gucy1B3 was easily detected at E13.5 and E15.5, where it was largely restricted to the LGE (SVZ and MZ) and CP, with much lower expression in the CGE and MGE (Fig. 1N, R, L, M, P, Q). In the Dlx1/2−/− mutant, subpallial Gucy1B3 expression was nearly eliminated (Fig. 1K′–N′, O′–R′). While Gucy1A3 and Gucy1B3 were the only sGC subunits with strong in situ hybridization expression in the E15.5 subpallium, gene expression array analyses of E12.5 MGE, E15.5 MGE, and P0 cell-sorted purified cortical interneurons detected expression of Gucy1B2, Gucy1B3, and Gucy1A3, whereas Gucy1A2 expression was not detected (Cobos et al., 2007; Stanco et al., unpublished...
were expressed at similar levels (expression in the MGE was lost (Fig. 1). The vMGE (Fig. 2), zones of the cortex, LGE, dMGE, and CGE, with lower expression in the telencephalon; however, there appeared to be expression in the ventricular ground; however, there appeared to be expression in the ventricular

Figure 4. A–D’, Slice electroporation migration assay. Cells were visualized by transfecting a GFP expression vector into the MGE in either the WT (A–A’, C–C’) or the Dlx1/2−/− mutants (B–B’, D–D’) treated with DMSO or 8-Br-cGMP. Migration into the LGE and cortex was assessed after 24, 36, and 48 h. E, Histogram showing quantification of the number of cells migrating to the cortex from MGE in slices. Data are the mean ± SEM. *p ≤ 0.05 (paired Student’s t test). **p ≤ 0.01 (paired Student’s t test). A’, C’, p = 0.018; A”, C”, p = 0.0006. Slices were grown in either DMSO or 8-Br-cGMP (500 μM). CX, Cortex. Scale bars: A–D’, 500 μm.

observations). For instance, in P0 interneurons, Gucy1A3 was the most robustly expressed (~5000 units), and Gucy1B2 and Gucy1B3 were expressed at similar levels (~450 and ~1150 units, respectively).

sGCs are activated by NO produced by NOSs (Knowles and Moncada, 1994; Fribe and Koesling, 2003; Stothers et al., 2003; Garthwaite, 2008). There are three NOS genes: NOS1 (nNOS), NOS2, and NOS3; we studied their expression by in situ hybridization. At E13.5, NOS1 expression was detected in a ventral–dorsal gradient in the MGE SVZ, in addition to expression in the cortical plate (Fig. 1S–V). At E13.5 Dlx1/2−/− mutant, NOS1 expression in the MGE was lost (Fig. 1U). At E15.5, NOS1 expression in the WT subpallium was greatly reduced, with modest expression in the MGE SVZ (Fig. 2K), whereas cortical expression was strong, particularly in the SVZ and cortical plate (Fig. 2L–L’). NOS2 expression was difficult to discern because of high background; however, there appeared to be expression in the ventricular zones of the cortex, LGE, dMGE, and CGE, with lower expression in the vMGE (Fig. 2M–P). In the Dlx1/2−/− mutants, NOS2 expression increased in the VZ of the LGE and dMGE (Fig. 2M’–P’).

NOS3 expression was prominent in blood vessels throughout the telencephalon; this expression did not change in the Dlx1/2−/− mutants (Fig. 2Q–T’). Thus, subpallial NO could be produced in the VZ by NOS2, in the MGE SVZ by NOS1, and scattered throughout the subpallium by NOS3 in blood vessels. CGE explants suggesting the GMP is a positive regulator of migration (Fig. 3B,D). These results are quantified in Figure 3E,F. In the Boyden chamber assay, 8-Br-cGMP did not stimulate migration of WT MGE cells but did stimulate migration of the Dlx1/2−/− mutant MGE cells (Fig. 3G). In the slice migration assay, 8-Br-cGMP stimulated MGE tangential migration to the cortex approximately twofold in WT slices (Fig. 4C–C’, whereas it did not rescue migration of Dlx1/2−/− mutants (Fig. 4D–D’), probably because of the many defects in the Dlx1/2−/− mutants. These results are quantified in Figure 4E.

Inhibition of sGC and PKG blocks migration from the MGE and CGE

To investigate the role of sGCs in the tangential migration of MGE- and CGE-derived interneurons, we used a specific inhibitor of sGCs named ODQ (Boulton et al., 1995; Garthwaite et al., 1995; Zhao et al., 2000; Cechova and Pajewski, 2004). We analyzed the effect of ODQ using slice (telencephalic), explants, and Boyden chamber migration assays. In all three assays, ODQ inhibited migration in a dose-dependent manner.

The slice migration assay (using electroporation of GFP into either the MGE or CGE) showed partial inhibition of tangential migration with 25 μM ODQ (Fig. 5B,B’, F,F’,I) and nearly complete inhibition with 50 μM ODQ (Fig. 5C,C’,G,G’,I). The inhibition of tangential migration in both MGE and CGE slices at 50

Of these, only NOS1 expression was lost in the Dlx1/2−/− mutants. In summary, Dlx1/2−/− mutants lose subpallial expression of Gucy1A3, Gucy1B3, and NOS1, and thus could have an alteration of cGMP-mediated cellular processes. To investigate whether these gene expression changes could alter the properties of immature cells in the GEs, we studied mouse mutants (Dlx1/2−/−, Gucy1A3−/−, and NOS1−/−) and inhibitors of sGCs (ODQ), NOS (NAME86), and PKG (a kinase activated by cGMP) (KT5823).

Migration defects of Dlx1/2−/− mutant MGE and CGE cells are partially rescued with 8-Br-cGMP, a cGMP analog

Given the reduced subpallial expression of Gucy1A3, Gucy1B3, and NOS1 in the Dlx1/2−/− mutants, we hypothesized that some of their cellular defects, such as migration, could be the result of changes in cGMP signaling. To test this, we used two migration assays (explant culture and Boyden chamber) to determine whether exposing the cells to the cell-permeable cGMP analog (8-Br-cGMP) would partially rescue their migration defect. Using the explant assay, we confirmed that Dlx1/2−/− mutant MGE and CGE (E13.5) had greatly reduced cellular migration (Fig. 3A’,C’), compared with WT explants (Fig. 3A,C). Addition of 8-Br-cGMP (500 μM) to the Dlx1/2−/− mutant explants induced outgrowth from the mutant MGE and CGE (Fig. 3B’,D’). 8-Br-cGMP also stimulated migration from WT MGE and

Migration defects of Dlx1/2−/− mutant MGE and CGE cells were fully rescued with 8-Br-cGMP in the slice migration assay (explant culture and in situ hybridization). Dlx1/2−/− mutant MGE and CGE (E13.5) explants in the slice migration assay (explant culture and in situ hybridization) showed complete rescue of migration defects with 8-Br-cGMP (500 μM) compared with WT explants (Fig. 3A’,C’). Addition of 8-Br-cGMP (500 μM) to the Dlx1/2−/− mutant explants induced outgrowth from the mutant MGE and CGE (Fig. 3B’,D’). 8-Br-cGMP also stimulated migration from WT MGE and
Figure 5. Inhibition of soluble guanylate cyclase activity with ODQ reduces interneuron process length and migration from the MGE and CGE to the cortex in E13.5 telencephalic slices. Movies 1 and 2 show additional data. A–H, Slice electroporation migration assay. Cells were visualized by transfecting a GFP expression vector into either the MGE (A–D) or CGE (E–H) by electroporation; migration into the LGE and cortex was assessed after 24 and 48 h. I, Histogram showing quantification of the number of cells migrating to the cortex from MGE or CGE in slices. Slices were grown in DMSO, 25 μM ODQ, 50 μM ODQ, or 100 μM ODQ. Data are the mean ± SEM. *p ≤ 0.05 (one-way ANOVA followed by Bonferroni post test). **p ≤ 0.01 (one-way ANOVA followed by Bonferroni post test).

A, A', p = 1.1 × 10^-6; B, B', p = 0.00001; A', C', p = 0.00158; A', D', p = 0.00142; E, E', p = 1.3 × 10^-6; F, F', p = 0.00003; E', G', p = 0.00016; E', H', p = 0.00037. CX, Cortex. Scale bars: A–H, 500 μm. J, K, Confocal image showing Lhx6-GFP expression in an E15.5 brain slice (200 μm) treated with either DMSO or ODQ (100 μM) for 1 h. L–O, Interneurons from the CP, oriented in a radial (L, M) or tangential (N, O) direction, are aligned by their cell soma (yellow arrowhead). The leading process length was assessed from the base of the soma to the tip of the leading process (red arrowhead). P, Quantification of rates of tangential migration through the MZ and SVZ are based on live imaging of E13.5 Lhx6-GFP *+/− ODQ-treated and DMSO-treated control cortices for 12 h. MZ, p = 4.01 × 10^-7; SVZ, p = 1.84 × 10^-8. Q, Quantification of the number of motile cells (i.e., cells migrating at a rate of >5 μm/h) within the CP of ODQ-treated and DMSO-treated control cortices (p = 0.00021). R, S, Quantification of leading process length of interneurons oriented in either radial or tangential direction (N = 5). *p < 0.001 (Student’s t test). R, p = 1.16 × 10^-5. S, p = 0.00043. Scale bars: J, K, 110 μm; L, M, 28 μm; N, O, 30 μm. T, Matrigel explant cell migration assays of E13.5 MGE showing the outgrowth of neurites/cell processes (β-III-tubulin *+/−) (U, V) and migration of the soma/nucleus (Hoechst *+/−) (U', V') treated with DMSO (U, U') or ODQ (100 μM) (V, V'). Bottom, Histograms reporting the percentage change in the MGE explant outgrowth. Data are the mean ± SEM. *p < 0.05 (paired Student’s t test). **p < 0.01 (paired Student’s t test).
and 100 μM ODQ at 48 h was statistically significant as quantified in Figure 5F. Similar results were obtained with the explant culture assay (~70% inhibition with 50 μM ODQ) (Fig. 6A–I); more modest inhibition was detected with the Boyden chamber assay (~30% inhibition with 50 μM ODQ, ~60% with 100 μM ODQ) (Fig. 6K). Next, we assessed whether we could rescue the ODQ-induced migration deficits by adding 8-Br-cGMP (500 μM) to ODQ in the Boyden chamber assay. 8-Br-cGMP partially counteracted the effect of 50 and 100 μM ODQ. However, the rescue in migration by 8-Br-cGMP was statistically significant in 100 μM ODQ (Fig. 6L).

To assess the effect at the single-cell level of reducing Gucy function, we treated slices from an E15.5 Lhx6-GFP-Bac mouse and 100 μM ODQ at 48 h was statistically significant as quantified in Figure 5F. Similar results were obtained with the explant culture assay (~70% inhibition with 50 μM ODQ) (Fig. 6A–I); more modest inhibition was detected with the Boyden chamber assay (~30% inhibition with 50 μM ODQ, ~60% with 100 μM ODQ) (Fig. 6K). Next, we assessed whether we could rescue the ODQ-induced migration deficits by adding 8-Br-cGMP (500 μM) to ODQ in the Boyden chamber assay. 8-Br-cGMP partially counteracted the effect of 50 and 100 μM ODQ. However, the rescue in migration by 8-Br-cGMP was statistically significant in 100 μM ODQ (Fig. 6L).

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First, we tested whether ODQ (50 μM) inhibition of neurite length was reversible when ODQ was removed from the media (Fig. 11A–S). Furthermore, inhibition of tangential migration from the MGE to the cortex was also partially reversible when ODQ was removed from the media (Fig. 11A–S). Therefore, inhibition of neurite length was reversible when ODQ was removed from the media in a slice electroporation experiment (Fig. 11J–S). Thus, inhibition of sGC rapidly reduced neurite length and could underlie the defects in migration.

cGMP is known to modulate the cytoskeleton in several ways (see Fig. 14). Increased cGMP levels inactivate the RhoA signaling pathway (Sawada et al., 2001; Begum et al., 2002; Gudi et al., 2002; Sauzeau et al., 2003, 2000). Furthermore, activated RhoA signaling leads to neurite retraction, whereas inhibition of RhoA signaling leads to neurite elongation (Hirose et al., 1998; Togashi et al., 2000). Thus, it is possible that ODQ-mediated neurite retraction could be due to activated RhoA signaling. To test this hypothesis, we performed a series of in vitro pharmacological assays on MGE neurite length (Figs. 13A–J and 14).

First, we tested whether ODQ (50 μM) inhibition of neurite length was counteracted by 8-Br-cGMP. Indeed, neurite length was intermediate in cells exposed to 8-Br-cGMP and ODQ (Fig. 13 B, D, E, J). Addition of 8-Br-cGMP to DMSO or ODQ-treated MGE cells appeared to increase PKG activity (a measure of cGMP levels), as indicated by increased levels of phospho-VASP Ser239 by Western blot analysis (Fig. 12I); phospho-VASP Ser239 is a recognized indicator of active PKG (Smolenski et al., 1998; Oelze et al., 2000; Lawrence and Pryzwansky, 2001).

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Inhibition of sGC rapidly alters the shape of MGE neurons via the activation of the Rho signaling pathway

To understand the mechanisms by which ODQ inhibition of sGC impeded neuronal migration, we studied the shape of β-III-tubulin+ (TuJ1) MGE-derived neurons 30 min after ODQ treatment. ODQ induced a clear dose-dependent attenuation of neurite length (Fig. 11A–E), similar to what we observed in cortical slice culture (Fig. 5J–S), and dissociated MGE culture of Lhx6-GFP+ neurons (Fig. 12A–D). Importantly, ODQ attenuation of neurite length was reversible when ODQ was removed from the media (Fig. 11F–J). Furthermore, inhibition of tangential migration from the MGE to the striatum was also partially reversible when ODQ was removed from the media in a slice electroporation experiment (Fig. 11J–S). Thus, inhibition of sGC rapidly reduced neurite length and could underlie the defects in migration.

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Figure 7. Inhibition of PKG activity with KT5823 reduces interneuron migration from the MGE to the cortex in E13.5 telencephalic slices. **A–B**, Slice electroporation migration assay. Cells were visualized by transfecting a GFP expression vector into the MGE (**A–B**) by electroporation; migration into the LGE and cortex was assessed after 7, 19, and 41 h. **C**, Histogram showing quantification of the number of cells migrating to the cortex from MGE in slices. Slices were grown in either DMSO or 25 μM KT5823. CX, Cortex. Data are the mean ± SEM. *p < 0.05 (paired Student’s t test), **p < 0.01 (paired Student’s t test). **A’, B’, p = 7.5 × 10^{-6}. Additionally, inhibition of PKG activity with KT5823 inhibits cell migration from explants of the E13.5 MGE and CGE, assessed using Matrigel explants and Boyden chamber assays. **D–I**, Matrigel explant cell migration assays of E13.5 MGE and CGE comparing DMSO (**D, G**), 100 μM ODQ (**E, H**), and 25 μM KT5823 (**F, I**). **J, K**, Histograms reporting the percentage change in MGE (**J**) and CGE (**K**) explant outgrowth as a function of ODQ and KT5823 using one-way ANOVA followed by Bonferroni post test. DMSO versus ODQ, p = 3.26E-07; DMSO versus KT, p = 6.06E-07. **K**, DMSO versus ODQ, p = 3.57E-05; DMSO versus KT, p = 7.47E-05. **L**, Boyden chamber assay showing that both ODQ and KT5823 inhibit migration. Data are the mean ± SEM. *p < 0.05 (one-way ANOVA followed by Bonferroni post test), **p < 0.01 (one-way ANOVA followed by Bonferroni post test). DMSO versus ODQ, p = 1.4 × 10^{-6}; DMSO versus KT, p = 2.1 × 10^{-5}. Scale bars: **A–I**, 500 μm.
mediates its effects through several pathways, including repression of Rac and CDC42 signaling (Sander et al., 1999; Wahl et al., 2000; Sugimoto et al., 2003; Xu et al., 2003; Seasholtz et al., 2004; Rosenfeldt et al., 2006; Burridge and Doughman et al., 2006) and activation of Rho kinase (ROCK) (Ishizaki et al., 1996; Leung et al., 1996, 1995; Matsui et al., 1996). Thus, we tested the effect of pharmacological inhibition and activation of RhoA, activation of Rac/CDC42, and inhibition of ROCK on MGE neurite length.

**Figure 8.** *Gucy1A3*<sup>−/−</sup> mice have reduced numbers of calbindin<sup>+</sup> cortical interneurons at E15.5. Calbindin immunohistochemistry staining of E15.5 coronal hemisections of WT (A–C) and *Gucy1A3*<sup>−/−</sup> (A′–C′) telencephalons showing migrating immature cortical interneurons in rostral (A, A′), middle (B, B′), and caudal (C, C′) planes of sections. D, Histogram showing quantification of calbindin<sup>+</sup> cell numbers in the cortical SVZ and cortical plate. Data are the mean ± SEM. *p ≤ 0.05 (one-way ANOVA followed by Bonferroni post test). **p ≤ 0.01 (one-way ANOVA followed by Bonferroni post test). SVZ, WT versus *Gucy1A3*<sup>−/−</sup> (rostral, p = 0.00317; medial, p = 0.00202; caudal, p = 0.00344); cortical plate (caudal, p = 0.00745). Scale bars: A–C, 200 μm.

**Figure 9.** Inhibition of NOS activity with NAME86 inhibits interneuron migration from E13.5 explants of the MGE and CGE, assessed using electroporation slice culture migration assays. Cells were visualized by transfecting a GFP expression vector into either the MGE (A–C′) or CGE (D–F′) by electroporation; migration into the LGE and cortex was assessed after 24 and 48 h. Slices were grown in DMSO, 100 μM ODQ, or 100 μM NAME86. G, Histogram quantifying the number of cells migrating to the cortex from MGE or CGE slices. Data are the mean ± SEM. *p ≤ 0.05 (one-way ANOVA followed by Bonferroni post test). **p ≤ 0.01 (one-way ANOVA followed by Bonferroni post test). A′, B′, p = 1.3 × 10⁻⁵; A, A′, C, p = 0.00014; D′, E′, p = 0.00215; D′, F′, p = 0.0396. H, NOS1<sup>−/−</sup> expression in E14.5 WT sections. Arrows in the 20 × magnification image indicate NOS1<sup>−/−</sup> in corticofugal axons entering the striatum. CX, Cortex. Scale bars: A–F, 200 μm; H (left), 500 μm; H (right), 100 μm.
The ROCK inhibitor alone (Y-27632) (Sahai et al., 1999; Ishizaki et al., 2000) slightly increased neurite length, although this was not statistically significant compared with DMSO. ODQ in combination with Y-27632 (Fig. 13C,D,F,J) resulted in intermediate to cells exposed only to ODQ or Y-27632 (Fig. 13C,D,F,J). Thus, the partial rescue of ODQ-mediated neurite retraction by 8-Br-cGMP promotes evidence that cGMP signaling promotes neurite length. Furthermore, the partial rescue of ODQ-mediated neurite retraction by inhibition of the Rho pathway (by the ROCK inhibitor, Y-27632) provides evidence that Rho signaling inhibits neurite length.

To further investigate the role of RhoA signaling on MGE neuron neurite length, we tested the effect of pharmacological activation or inhibition of RhoA. The Rho inhibitor C3 transferase (Nwariaku et al., 2003; Krijnen et al., 2010) increased neurite length, whereas the Rho activator calpeptin (Castellone et al., 2011; Xiang et al., 2011) reduced neurite length (Fig. 13G,H,J). Thus, as predicted by the effect of ROCK inhibition, RhoA activation repressed neurite extension of immature MGE neurons consistent with the model in Figure 14.

As RhoA can repress Rac activity (Burridge and Doughman et al., 2006), we explored whether the effects on MGE neuronal morphology, which occurred in response to changes in RhoA activity, were acting through Rac. Thus, we tested the effect of activating Rac/CDC42 using EGF (Kurokawa et al., 2004). However, EGF treatment did not have a discernible effect on neurite length (Fig. 13I,J). Thus, whereas activation of RhoA shortened neurites, activation of Rac did not affect MGE neurite length (at least through EGF). Therefore, we have evidence that cGMP can act through PKG repression of RhoA signaling to increase neurite length of immature MGE neurons.

To test this model, we expressed various forms of RhoA in the slice migration assay. Consistent with our hypothesis, a dominant active RhoA (DA RhoA) inhibited tangential migration (Fig. 13N,N’,O), whereas the dominant-negative RhoA (DN RhoA) and the ROCK inhibitor (Y-27632) did not inhibit MGE tangential migration (Fig. 13L,L’,M,M’,O), compared with DMSO-treated slices (Fig. 13K,K’,O). Furthermore, the constitutively active RhoA induced approximately twofold attenuation in the neurite length of MGE immature neurons (Fig. 13R). Similar migration results were obtained with the Boyden chamber assay (approximately threefold inhibition with 50 μM ODQ, approximately sevenfold inhibition with the Rho activator calpeptin, and approximately threefold increase in migration with 10 μM Y-27632 (Fig. 13T). To begin to understand how cGMP modulates MGE neuron migration, we investigated whether cGMP’s known effects on the cytoskeleton also affected immature MGE neurons. In smooth muscle cells, increases in cGMP levels increase the activity of MLCP. MLCP dephosphorylates, and thereby reduces, the activity of the regulatory MLC, which results in reduced myosin activity and smooth muscle relaxation (Gong et al., 1996; Otto et al., 1996; Fujihara et al., 1997; Uehata et al., 1997; Seko et al., 2003; Harshthorne et al., 2004; Nakamura et al., 2007; Somlyo, 2007). Thus, cGMP-dependent phosphorylation of MLCP reduces myosin activity and leads to smooth muscle relaxation.

To explore whether immature MGE neurons respond similarly to changes in cGMP levels, we studied the phosphorylation state of MLC in response to ODQ. We observed increased MLC phosphorylation with 50 μM ODQ (Fig. 13C, compared with Y-27632 (Fig. 13D). Thus, the observed increase in neurite length with ODQ was independent of Rac activation. The ROCK inhibitor calpeptin, however, inhibited neurite length, which suggests that ROCK signaling was not required for the observed increase in neurite length. Thus, the observed increase in neurite length with ODQ may be due to ROCK inhibition, which results in reduced myosin activity and smooth muscle relaxation.
phosphorylation (p-MLC Ser19) after ODQ treatment using immunofluorescence and Western blot analysis (Fig. 13).

ODQ increased the amount of p-MLC at the distal end of large neurites (Fig. 13), in contrast to cells treated with 8-Br-cGMP (Fig. 13) or Y-27632 (Fig. 13).

Given that ROCK can repress the activity of MLC phosphatase (Wang et al., 2011), we suggest that ODQ-mediated reduced cGMP is increasing p-MLC through activating the RhoA signaling pathway, as described above (Fig. 14, summary diagram).

**Discussion**

Toward identifying genes that are candidates for regulating MGE neuronal differentiation and migration, we have investigated gene expression changes in the Dlx1/2−/− and Lhx6−/−...
mouse mutants (Zhao et al., 2008; Long et al., 2009a,b; Flandin et al., 2011; McKinsey et al., 2013). Here we have focused on Gucy1A3, whose expression in the SVZ of the rostrodorsal MGE is greatly reduced in both mutants (Figs. 1 and 2). Gucy1A3 encodes a sGC whose activity is induced by NO to generate cGMP (Denninger and Marletta, 1999; Lincoln et al., 2001; Ghalayini, 2004; Evgenov et al., 2006; Stasch et al., 2011). However, as is common in mammalian genetic studies, genetic redundancy could compensate for the functional loss of one member of the gene family. Indeed, RNA arrays detected Gucy1B2, Gucy1B3, and Gucy1A3 expression in the E12.5 and E15.5 MGE, as well as in cell-sorter-purified P0 cortical interneurons (Cobos et al., 2007; Stanco et al., unpublished observations). Therefore, it is likely that the mild phenotype of the Gucy1A3−/− mutant is due to genetic compensation.

NO activates cGMP production by sGCs. Pharmacological inhibition of NO production reduced migration in slice and explant cultures, as well as the Boyden chamber assay (Figs. 9A–F,G and Fig. 10A–I). This provides supportive and independent evidence that sGC function is important for MGE migration. Whereas the Nos1−/− mutant mouse did not show a clear defect in MGE interneuron migration (data not shown), Nos2 and Nos3 are also expressed in the developing telencephalon (Fig. 2M–T). These homologs may generate sufficient NO to support cGMP synthesis by sGC to promote neurite extension and cell migration.

Figure 12. Inhibition of sGC rapidly alters the neurite length of MGE neurons that were labeled by GFP expression from an Lhx6-GFP-Bac transgene. A–H, Dose-dependent attenuation of MGE neurite length comparing DMSO (A), 25 µM ODQ (B), 50 µM ODQ (C), and 100 µM ODQ (D) followed by the partial rescue in ODQ-induced neurite length by the addition of 8-Br-cGMP (E–H). Scale bars: A–H, 50 µm. I, Western blot analysis showing increased levels of phospho-VASP Ser239 (a measure of PKG activity) in MGE cells treated with either 8-Br-cGMP alone or in combination with ODQ compared with DMSO or ODQ alone treated cells. Total VASP (tVASP) was used as the loading control. J, Quantification of the Western blot bands using the histogram tool in Adobe Photoshop (CS3). Results are presented as relative protein levels, expressed in percentage normalized to DMSO-treated cell extracts.
Figure 13. Inhibition of sGC activity with ODQ mediates neurite retraction via the activation of Rho signaling pathway. A–F, ODQ inhibition of neurite length (D) is counteracted by either 8-Br-cGMP (B, E, J) or the ROCK inhibitor Y-27632 (C, F, J). The RhoA inhibitor C3 transferase (G) and the Rac activator EGF (I) increased neurite length. On the other hand, the RhoA activator calpeptin reduced neurite length (H). Scale bars: A–I, 50 μm. G–J, The RhoA inhibitor C3 transferase (G) and the Rac activator EGF (I) increased neurite length. On the other hand, the RhoA activator calpeptin reduced neurite length (H). Scale bars: A–I, 50 μm. Results are quantified in J. J, DMSO versus ODQ, p = 6.855 x 10^{-6}; ODQ versus ODQ + 8-Br, p = 0.0296; ODQ versus ODQ + Y, p = 1.288 x 10^{-4}; DMSO versus calpeptin, p = 8.756 x 10^{-12}. K–N, Slice electroporation migration assay. Cells were visualized by transfecting a GFP expression vector (K, L, M, N) or cotransfecting a GFP expression vector with constitutively active RhoA (CA RhoA) (N, S) into the MGE (K–N) by electroporation; migration into the LGE and cortex was assessed after 7 and 40 h. O, Histogram showing quantification of the number of cells migrating to the cortex from MGE in slices. Slices were grown in either DMSO or 10 μM Y-27632 as indicated. Expression of a constitutively active RhoA (CA RhoA) inhibited tangential migration (N, S) significantly, whereas the dominant-negative RhoA (DN RhoA) and the ROCK inhibitor (Y-27632) did not inhibit MGE tangential migration (L, M, S). Instead, there was a significant increase in the migration. K, K, p = 3.9 x 10^{-10}; L, L', p = 4.41 x 10^{-8}; M, M', p = 1.23 x 10^{-8}; L', L', p = 0.00042; K, K', p = 0.01142; K*, K', p = 3.76 x 10^{-9}. CX, Cortex. Scale bars: K–N, 500 μm. P–R, Expression of a constitutively active RhoA (CA RhoA) (R) construct in MGE neurons reduced the neurite length significantly compared with control (P) or dominant-negative RhoA (DN RhoA) construct (Q). S, Histogram showing the effect on neurite length when dominant active or negative RhoA constructs were expressed in MGE neurons. P versus R, p = 0.00031. Scale bars: P–R, 50 μm. T, Boyden chamber assay showing that both ODQ and the Rho activator (calpeptin) inhibit migration in contrast to Y-27632 (ROCK inhibitor). DMSO versus ODQ, p = 0.0002; DMSO versus Y, p = 0.00039; DMSO versus Rho Act, p = 5.1 x 10^{-7}. (Figure legend continues.)
Signals other than NO may also regulate sGC activity in MGE-derived neurons. For instance, Sema3A can activate sGC (Schmidt et al., 2002; Nishiyama et al., 2003, 2008, 2011; Togashi et al., 2008). These studies of Sema3A-mediated increased cGMP levels have focused on axon guidance and axon/dendrite specification, where cGMP regulates the neuron's response through activating cyclic nucleotide-gated calcium channels (Nishiyama et al., 2008, 2011; Togashi et al., 2008). Thus, we assessed whether repressing sGC activity in MGE neurons by blocking RhoA/ROCK activity (Figs. 12, 13, and 14). This effect was partially reversed using 8-Br-cGMP (Figs. 12E–H and 13B, E, F), providing evidence that neurite retraction was caused by inhibiting cGMP production (and thereby reducing active PKG). Furthermore, ODQ’s effect was also partially reversed by the ROCK inhibitor Y-27632 (Fig. 13C, F, J), suggesting that neurite retraction was caused by activation of RhoA/ROCK signaling. This hypothesis was supported by results obtained by expression of dominant active RhoA that reduced neurite length and tangential migration of MGE immature neurons (Fig. 13P–S). We summarize our postulation of the pathway in a schema (Fig. 14). Most information on the mechanisms of how NO/cGMP signaling controls the cytoskeleton and cellular movement comes from work in non-neural cells, particularly smooth muscle (for review, see Pfitzer, 2001), ROCK increases the phosphorylation of the regulatory subunit of myosin (pMLC), through inhibiting MLCP (for review, see Pfitzer, 2001). Increased levels of pMLC increase myosin II activity. For instance, RhoA/ROCK signaling in cerebellar granule neurons activated myosin II activity, which in turn inhibited neurite outgrowth (Kubo et al., 2008).

These results are in concordance with our findings in immature MGE neurons. We found that inhibition of cGMP signaling with ODQ reduced neurite length (Figs. 5, 11, 12, and 13). This effect was partially reversed with the ROCK inhibitor Y-27632 (Fig. 13). Furthermore, whereas 8-Br-cGMP decreased the level of pMLC, ODQ increased pMLC levels (Fig. 13V, U). Thus, we propose that sGC generation of cGMP promotes neurite extension and migration of subpallial-derived interneurons by blocking RhoA/ROCK activity (Figs. 12, 13, and 14). This model, summarized in Figure 14, has several ramifications, as interneuron migration is regulated by repulsive cues that cause neurite retraction. For instance, there is evidence that semaphorin3A/3F expression in the striatum and cerebral cortex repel migrating interneurons (Marín et al., 2001; Tamamaki et al., 2003). Thus, we predict that cGMP could counteract semaphorin-mediated repulsion. Another prediction is that NO-producing tissues would promote interneuron migration. NOSI is expressed in the cortical plate (Fig. 1S–V); thus cortical plate

![Figure 14](image.png)
neurons and their processes (including axons projecting through the basal ganglia; Fig. 9H) could be a source of NO that would promote interneuron neurite extension and migration.

Our analysis of sGC function during the development of the subpallium, and its derivatives such as cortical interneurons, adds new insights into the molecular mechanisms underlying the cellular phenotypes of cells lacking Dlx1/2 or Lhx6. We suggest that sGC regulation of cGMP levels is integrated with the other signaling systems that control the differentiation and migration of cortical interneurons, including those downstream of CXCR4, CXCR7, ErbB4, Neuropilin, and Pak3 (Marin et al., 2001; Tamamaki et al., 2003; Flames et al., 2004; Cobos et al., 2007; Fogarty et al., 2007; Nóbrega-Pereira et al., 2008; Sánchez-Alcañiz et al., 2011; Wang et al., 2011). It is clear that no single signaling system regulates each step in the maturation and migration of subpallial cells, explaining why the phenotypes of Dlx1/2 or Lhx6 mutants are more severe and, in some cases, qualitatively distinct from the effects of mutations, or pharmacological inhibition of sGCs or single components of the other signaling systems.

References

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