Report

Neuron

Inside-Out Radial Migration Facilitates Lineage-Dependent Neocortical Microcircuit Assembly

Highlights

- Radial glial progenitors form strong electrical synapses with their progeny
- Sister excitatory neurons form electrical synapses at embryonic stages
- Inside-out radial migration is required for sister excitatory neuron coupling
- Lateral dispersion of sister excitatory neurons disrupts their coupling

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In Brief

He et al. show that electrical synapses preferentially form between progenitor and newborn progeny, and between sister excitatory neurons, in the embryonic neocortex. Moreover, disruption of the birth-date-dependent inside-out radial migration impairs preferential electrical coupling between sister excitatory neurons.

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Report

Inside-Out Radial Migration Facilitates Lineage-Dependent Neocortical Microcircuit Assembly

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SUMMARY

Neocortical excitatory neurons migrate radially along the glial fibers of mother radial glial progenitors (RGPs) in a birth-date-dependent inside-out manner. However, the precise functional significance of this well-established orderly neuronal migration remains largely unclear. Here, we show that strong electrical synapses selectively form between RGPs and their newborn progeny and between sister excitatory neurons in ontogenetic radial clones at the embryonic stage. Interestingly, the preferential electrical coupling between sister excitatory neurons, but not that between RGP and newborn progeny, is eliminated in mice lacking REELIN or upon clonal depletion of DISABLED-1, which compromises the inside-out radial neuronal migration pattern in the developing neocortex. Moreover, increased levels of Ephrin-A ligand or receptor that laterally disperse sister excitatory neurons also disrupt preferential electrical coupling between radially aligned sister excitatory neurons. These results suggest that RGP-guided inside-out radial neuronal migration facilitates the initial assembly of lineage-dependent precise columnar microcircuits in the neocortex.

INTRODUCTION

Radial glial progenitors (RGPs) in the ventricular zone (VZ) produce nearly all excitatory neurons in the neocortex (Anthony et al., 2004; Englund et al., 2005; Haubensak et al., 2004; Heins et al., 2002; Kriegstein and Alvarez-Buylla, 2009; Malatesta et al., 2000; Miyata et al., 2004; Noctor et al., 2001, 2004). They exhibit a characteristic bipolar morphology with a short apical ventricular endfoot and a long basal radial glial fiber that reaches the pial surface and supports neuronal migration (Hatten, 1999; Rakic, 1971). During the peak phase of neurogenesis, RGPs actively divide at the VZ surface to produce neurons directly or indirectly through transient amplifying progenitors, such as intermediate progenitors (IPs) (Haubensak et al., 2004; Hevner, 2006; Miyata et al., 2004; Noctor et al., 2004), outer subventricular zone radial glial progenitors (Fietz et al., 2010; Hansen et al., 2010; Martínez-Cerdeño et al., 2012; Reillo et al., 2011; Shitamukai et al., 2011; Wang et al., 2011), or short neural precursors (Gal et al., 2006). Newborn neurons then migrate radially along the radial glial fibers of mother RGPs to constitute the future neocortex (Rakic, 1988). It is well-established that radial neuronal migration occurs in a birth-date-dependent inside-out manner; that is, early-born neurons migrate to occupy deep layers, whereas late-born neurons migrate past early-born neurons to occupy superficial layers (Angevine and Sidman, 1961; Greig et al., 2013; Rakic, 1974). Despite its discovery over 50 years ago, little is known about the precise significance of this orderly neuronal migration for the functional development of the neocortex.

Recently, we found that radially situated sister excitatory neurons arising from the same RGPs preferentially develop electrical synapses with each other at the neonatal stage, which facilitates subsequent formation of specific chemical synapses and emergence of functional columnar circuits with defined physiological properties (Li et al., 2012; Yu et al., 2009, 2012). While these studies provide crucial insights into the early steps of functional development of the neocortex (Dupont et al., 2006; Yuste et al., 1992), the developmental origin and cellular basis of lineage-dependent preferential electrical synapse formation remain unknown. In this study, we investigated the influence of the early processes of neuronal production and migration on precise electrical coupling and microcircuit assembly in the developing mouse neocortex.

RESULTS

Preferential Electrical Coupling between RGPs and Their Progeny

Previous dye injection experiments suggested that RGPs form extensive gap junctions with nearby RGPs in the VZ (Lo Turco and Kriegstein, 1991; Noctor et al., 2001). Moreover, gap-junction-mediated adhesive force between RGPs and nascent neurons is thought to be critical for neuronal migration (Cina et al., 2009; Elias et al., 2007). Yet it is unclear whether RGPs are functionally coupled with their progeny including migrating neurons and, if so, whether such electrical coupling exhibits a

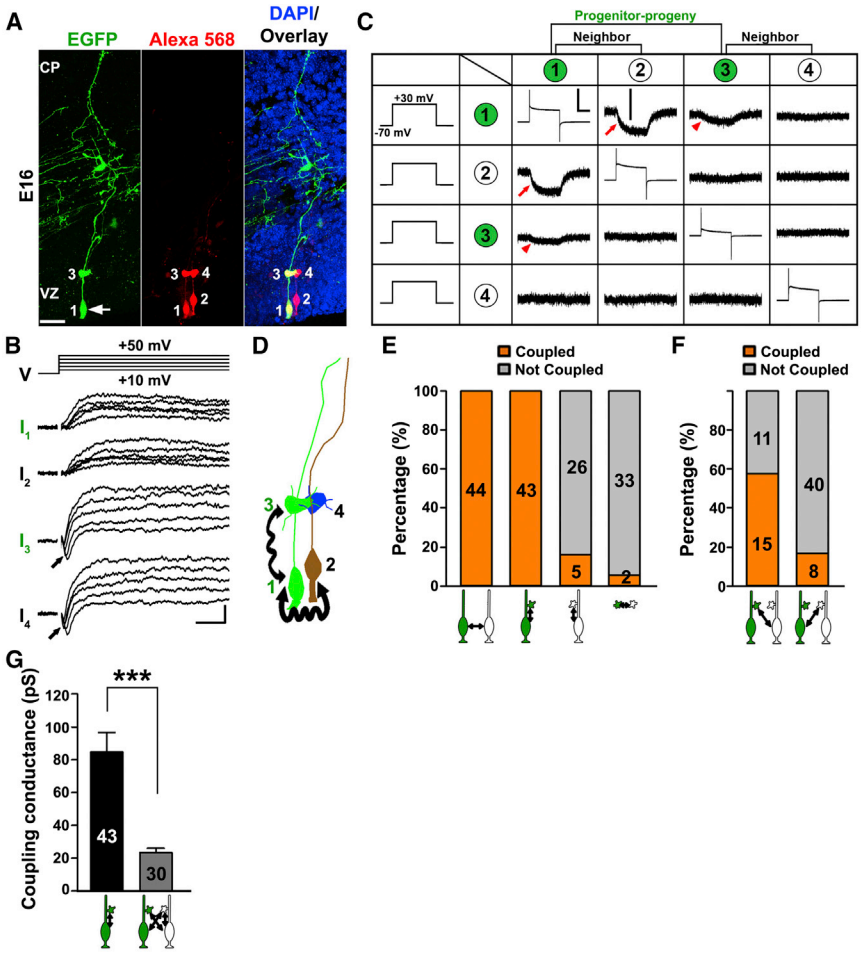

Figure 1. Preferential Formation of Strong Electrical Coupling between RGP and Its Progeny

(A) Representative images of an EGFP-expressing RGP in the VZ (cell 1, arrow) and its progeny in the SVZ (cell 3) (green) and a nearby non-EGFP-expressing RGP in the VZ (cell 2) and a nearby non-EGFP-expressing SVZ cell (cell 4) on the same side in the E16 neocortex. Cells were subjected to quadruple whole-cell recording, filled with Alexa Fluor 568 hydrazide (red), and stained with 4′,6-diamidino-2-phenylindole (DAPI, blue). Scale bar: 30 μm.

(B) Example current traces of the four cells evoked by 10 mV step changes in membrane potential from +10 mV to +50 mV. Note that inward voltage-dependent sodium-channel-mediated currents are elicited in cells 3 and 4 (arrows), but not in cells 1 and 2. Scale bars: 50 pA, 5 ms.

(C) Example traces of currents elicited by sequential depolarization of one of the four cells from −70 mV to +30 mV. Green circles indicate an EGFP-expressing RGP and its progeny in the SVZ, whereas white circles indicate a nearby non-EGFP-expressing RGP and a nearby non-EGFP-expressing SVZ cell. The average traces are shown in corresponding rectangles with the driver cell at the main diagonal. Red arrows and arrowheads indicate detectable junctional currents between RGPs and between RGP and SVZ progeny, respectively. Similar symbols and panel display are used in subsequent figures. Scale bars: 500 pA, 100 ms, and 5 pA.

(D) Morphological reconstruction of the four cells. The wavy lines with arrows indicate electrical coupling, and the thickness of the line reflects the coupling strength.

(E) Summary of the rate of electrical coupling observed between nearby RGPs, between EGFP-expressing RGPs and their progeny, between non-EGFP-expressing RGPs and SVZ cells adjacent to EGFP-expressing cells on the same side, and between nearby non-sister SVZ cells.

(F) Summary of the rate of electrical coupling observed between EGFP-expressing SVZ cells and non-EGFP-expressing RGPs adjacent to EGFP-expressing RGPs and between EGFP-expressing RGPs and non-EGFP-expressing SVZ cells adjacent to EGFP-expressing SVZ cells.

(G) Quantification of the coupling conductance observed between EGFP-expressing RGP and progeny pairs and between RGP and non-progeny SVZ cell pairs at E16–E18. Data are presented as mean ± SEM; ***, $p < 0.001$. See also Figure S1.

lineage-dependent preference. To address these questions, we injected low-titer enhanced GFP (EGFP)-expressing retroviruses into the lateral ventricle of embryonic day 12 (E12) mouse embryos in utero to label individual dividing RGPs and their progeny (Noctor et al., 2001; Yu et al., 2009) (see Supplemental Experimental Procedures). As expected, we frequently observed individual EGFP-expressing clones containing a single bipolar RGP (arrow) and several daughter cells radially aligned along the mother RGP at E16 (Figure 1A, green). Under the visual guidance of infrared differential interference contrast (IR-DIC) and epi-fluorescence illumination, we performed quadruple whole-cell patch clamp recordings on an EGFP-expressing bipolar RGP in the VZ and its multipolar daughter cell in the subventricular zone (SVZ) (cells 1 and 3), as well as on a nearby non-EGFP-expressing RGP (cell 2) and a nearby non-EGFP-expressing non-daughter cell (cell 4) located on the same side in the VZ and the SVZ, respectively (Figures 1A–1D).

Previous studies suggest that progenitors in the VZ exhibit distinct membrane properties, including relatively low input resistance and a lack of voltage-dependent sodium conductance (Lo Turco and Kriegstein, 1991; Noctor et al., 2001). Indeed, we found that recordings from bipolar RGPs in the VZ showed low membrane resistance (205.4 ± 9.2 MΩ, n = 43), small voltage-dependent outward currents, and no voltage-dependent inward current (Figure 1B, I_1 and I_2). In comparison, recordings from multipolar cells in the SVZ showed high membrane resistance (1,254.2 ± 86.5 MΩ, n = 47), large voltage-dependent outward currents, and obvious voltage-dependent inward sodium currents (arrows) (Figure 1B, I_3 and I_4), indicative of the neuronal identity. Notably, EGFP expression did not affect the properties of RGPs (outward current: $EGFP^+$, 63.0 ± 4.0 pA, n = 21; $EGFP^-$, 66.1 ± 5.9 pA, n = 21; p = 0.7) and SVZ progeny (inward current: $EGFP^+$, 65.2 ± 8.0 pA, n = 21; $EGFP^-$, 64.2 ± 10.0 pA, n = 21; p = 0.9).

We then examined electrical coupling among the four cells. Under voltage-clamp conditions, we sequentially depolarized one of the four cells by a voltage step (−70 mV to +30 mV for 250 ms), which elicited a large outward current in the stimulated cell (driver cell) (Figure 1C, main diagonal). Should a cell be electrically coupled to the driver cell, a simultaneous inward current would be detected as a result of gap-junction-mediated electrotonic propagation. We found that depolarization of one RGP triggered a prominent simultaneous current in the other RGP (cell 1 and 2; Figure 1C, red arrows), suggesting a strong electrical coupling between nearby RGPs, consistent with previous dye injection experiments (Lo Turco and Kriegstein, 1991; Noctor et al., 2001). Interestingly, depolarization of the EGFP-expressing RGP (cell 1) also elicited a simultaneous current in the EGFP-expressing daughter cell (cell 3), but not in the nearby non-EGFP-expressing daughter cell (cell 4), in the SVZ (Figure 1C, red arrowhead). This electrical transmission was reciprocal, as depolarization of the EGFP-expressing daughter cell (cell 3) elicited a simultaneous current in the EGFP-expressing RGP (cell 1), but not in the nearby non-EGFP-expressing RGP (cell 2), in the VZ (Figure 1C, red arrowhead). Together, these results suggest that besides the nearby RGP, an RGP is preferentially coupled with its daughter cell, but not the nearby non-daughter cell, located in the SVZ. Newborn progeny of RGPs in the SVZ include neurons as well as IPs that express the T-box transcription factor TBR2 and undergo symmetric division to produce neurons (Haubensak et al., 2004; Hevner, 2006; Miyata et al., 2004; Noctor et al., 2004). We also detected electrical coupling between the RGP and its TBR2-expressing IP progeny in the SVZ with no detectable inward sodium current (Figures S1A–S1C). Notably, the recorded currents propagating between electrically coupled RGPs and SVZ progeny often exhibited directional differences, likely reflecting the difference in their membrane properties and the high series resistance of recording electrodes.

We recorded a total of 43 pairs of EGFP-expressing RGPs and their SVZ progeny, and all were electrically coupled (Figure 1E). In addition, all the examined pairs of EGFP-expressing RGPs and nearby non-EGFP-expressing RGPs were electrically coupled. In contrast, only 16.1% of radially situated non-EGFP-expressing RGP and non-EGFP-expressing progeny pairs were coupled, and only 5.7% of nearby non-sister SVZ progeny pairs (one EGFP-expressing cell and one non-EGFP-expressing cell in the SVZ) were coupled. These results suggest that RGPs are effectively coupled to their progeny in the SVZ and neighboring RGPs in the VZ. The effective coupling between an RGP and its progeny as well as the neighboring RGP raises the possibility of indirect coupling between an RGP and the progeny derived from a nearby RGP in the SVZ. Consistent with this, we observed a much higher rate of coupling in EGFP-expressing SVZ cell and non-EGFP-expressing RGP pairs (57.7%) than in EGFP-expressing RGP and non-EGFP-expressing SVZ cell pairs (16.7%) (Figures 1F and S1D). Notably, the coupling conductance between a lineage-related EGFP-expressing RGP and its SVZ progeny was substantially larger than that between the RGP and the radially located SVZ cell that were unlikely to be lineage related (one EGFP-expressing and one non-EGFP-expressing, or non-EGFP-expressing pairs) (Figure 1G). In addition, the coupling conductance between nearby RGPs was generally larger than that between the RGP and its SVZ progeny, and appeared inversely correlated to the distance between their endfeet at the VZ surface (Figures S1E–S1G). Taken together, these results showed that the SVZ daughter cells are preferentially and strongly coupled to their mother RGPs in the VZ.

Preferential Electrical Coupling of Sister Excitatory Neurons at Embryonic Stages

We next examined the emergence of electrical coupling between sister excitatory neurons in the cortical plate ([CP], the future neocortex) where newborn neurons progressively migrate. We performed dual whole-cell recordings of radially aligned EGFP-expressing sister excitatory neurons in the developing neocortex at different embryonic stages (E16–E18) and assessed their electrical coupling by sequential depolarization (Figures 2A and 2B). The rate of electrical coupling between sister neurons was 11.4% at E16, 12.9% at E17, and 21.4% at E18 (Figure 2C), suggesting a progressive formation of gap junctions between sister excitatory neurons during embryonic neocortical development.

To test whether sister excitatory neurons preferentially form gap junctions with each other at the embryonic stage, we performed quadruple whole-cell recordings on two EGFP-expressing sister excitatory neurons and two non-EGFP-expressing excitatory neurons adjacent to the EGFP-expressing neurons on the same side at E18 (Figures 2D and 2E). We analyzed a total of 42 pairs of sister neurons and found that 21.4% were electrically coupled (Figure 2F). In contrast, virtually none of the non-sister neuron pairs were coupled (Figure 2F), consistent with the previous observation of no obvious electrical coupling between randomly selected excitatory neuron pairs (Bittman et al., 1997; Pangratz-Fuehrer and Hestrin, 2011). These results strongly suggest that sister excitatory neurons in the embryonic neocortex preferentially form gap junctions with each other, but not with nearby non-sister excitatory neurons.

Inside-Out Radial Migration Is Required for Sister Excitatory Neuron Electrical Coupling

Excitatory neuron migration in the developing neocortex occurs in a birth-date-dependent "inside-out" manner; that is, the late-born neurons migrate past the early-born neurons and progressively occupy the superficial layers (Angevine and Sidman, 1961; Rakic, 1974). This orderly neuron migration facilitates encounters between sister excitatory neurons that migrate along a similar path (Rakic, 1971), thereby promoting gap junction formation between them. To test this, we took advantage of *Reeler* mice that lack REELIN (D'Arcangelo et al., 1995; Hirotsune et al., 1995; Ogawa et al., 1995), a large secreted protein that regulates neuronal migration and positioning in the developing brain (Caviness and Rakic, 1978; Rice and Curran, 2001). In the *Reeler* neocortex, the "inside-out" neuronal migration is disrupted, and the neurons are organized in an overall inverted fashion ("outside-in") (Figures S2A and S2B). While CUX1- and CTIP2-expressing neurons occupied the superficial and deep layers, respectively, in the wild-type neocortex, their localizations were largely reversed in the *Reeler* neocortex. Previous studies suggested that the REELIN pathway is not required for glia-guided neuronal migration (Britto et al., 2011; Franco et al.,

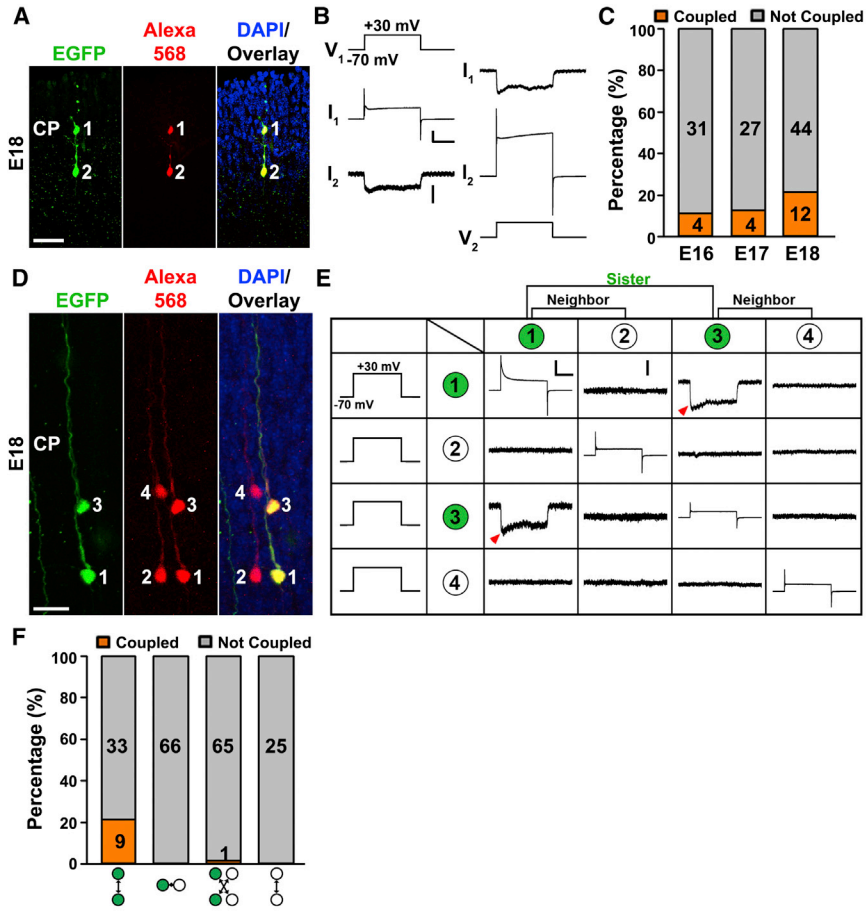

Figure 2. Progressive and Preferential Electrical Coupling between Radially Situated Sister Excitatory Neurons in the Embryonic Neocortex

(A) Representative images of a pair of EGFP-expressing sister excitatory neurons (green, cells 1 and 2) in the CP of the E18 neocortex subjected to dual whole-cell recording, filled with Alexa Fluor 568 hydrazide (red), and stained with DAPI (blue). Scale bar: 50 μm.

(B) Example traces of currents elicited in both cells by sequential depolarization of one of the two cells. Scale bars: 500 pA, 100 ms, and 10 pA.

(C) Summary of the rate of electrical coupling between sister neurons at different embryonic stages.

(D) Representative images of two radially situated EGFP-expressing sister excitatory neurons (cells 1 and 3, green) and two adjacent non-EGFP-expressing control excitatory neurons on the same side (cells 2 and 4) in the CP of E18 neocortex subjected to quadruple whole-cell recording. Scale bar: 25 μm.

(E) Example traces of currents in the four neurons elicited by sequential depolarization of one of the four cells. Green circles indicate two EGFP-expressing sister excitatory neurons, and white circles indicate two adjacent non-EGFP-expressing non-sibling control excitatory neurons. Average currents are shown in corresponding rectangles with the driver cell at the main diagonal. Red arrowheads indicate detectable junctional currents. Similar symbols and panel display are used in subsequent figures. Scale bars: 500 pA, 100 ms, and 5 pA.

(F) Summary of the rate of electrical coupling observed between sister excitatory neurons and their adjacent non-sibling excitatory neurons.

2011; Jossin and Cooper, 2011; Sanada et al., 2004). Consistent with this, we found that the progeny of RGPs remained closely associated with the mother RGPs in the *Reeler* neocortex throughout the embryonic stages (Figure S3A). In the embryonic *Reeler* neocortex, the lateral distances between the progeny and the radial glial fiber of the corresponding mother RGP, as well as between the clonally related neuronal progeny, were comparable to those in the wild-type neocortex (Figures S3B and S3C). In addition, we observed no obvious differences in the clone size (Figure S3D) or the membrane properties of RGPs and their progeny (Figure S3E), indicating that lineage progression and early neuronal differentiation are not impaired in the *Reeler* neocortex, as previously suggested (Rice and Curran, 2001). Moreover, we found that RGPs remained preferentially electrically coupled to their SVZ progeny in the *Reeler* neocortex, similar to those in the wild-type neocortex (Figures 3A–3D, S3F, and S3G). These results suggest that lineage-dependent preferential interaction between RGPs and their progeny are not disrupted in the *Reeler* neocortex.

Next, we examined gap junction formation between sister excitatory neurons in the *Reeler* neocortex. To test whether the radial position of sister neurons is indeed inverted regarding their birth dates, we performed sequential birth dating experiments in animals that received in utero intraventricular injection of low-titer EGFP-expressing retrovirus at E12. Single doses of 5-ethynyl-2′-deoxyuridine (EdU) and 5-bromo-2-deoxyuridine (BrdU) were administrated to pregnant dams at E12 and E14, respectively, and animals were recovered at postnatal day (P) 1 for analysis. Consistent with an ‘‘inside-out’’ migration pattern (Angevine and Sidman, 1961; Rakic, 1974), we found that the late-born sister neurons (EGFP+/BrdU+, cells 2 and 3) were located above the early-born sister neuron (EGFP+/EdU+, cell 1) in the wild-type neocortex (Figure S2C, top). In contrast, the late-born sister neuron (EGFP+/BrdU+, cell 1) was located below the early-born neuron (EGFP+/EdU+, cell 2) in the *Reeler* neocortex (Figure S2C, bottom), indicating an inverted neuronal migration.

Quadruple whole-cell recordings were then performed to probe electrical coupling between radially aligned sister excitatory neurons with pia-directed principal dendrites and their nearby non-sister excitatory neurons in the developing neocortex at the embryonic (E17 and E18) and neonatal (P1–P4) stages (Figures 3E–3H). Compared to the wild-type littermate control, the rate of electrical coupling between sister neurons was drastically decreased in the *Reeler* neocortex (Figures 3I and 3J). We did not observe any significant changes in the rate of electrical coupling between non-sister neuron pairs or radially situated non-EGFP-expressing neuron pairs (Figures 3I and 3J).

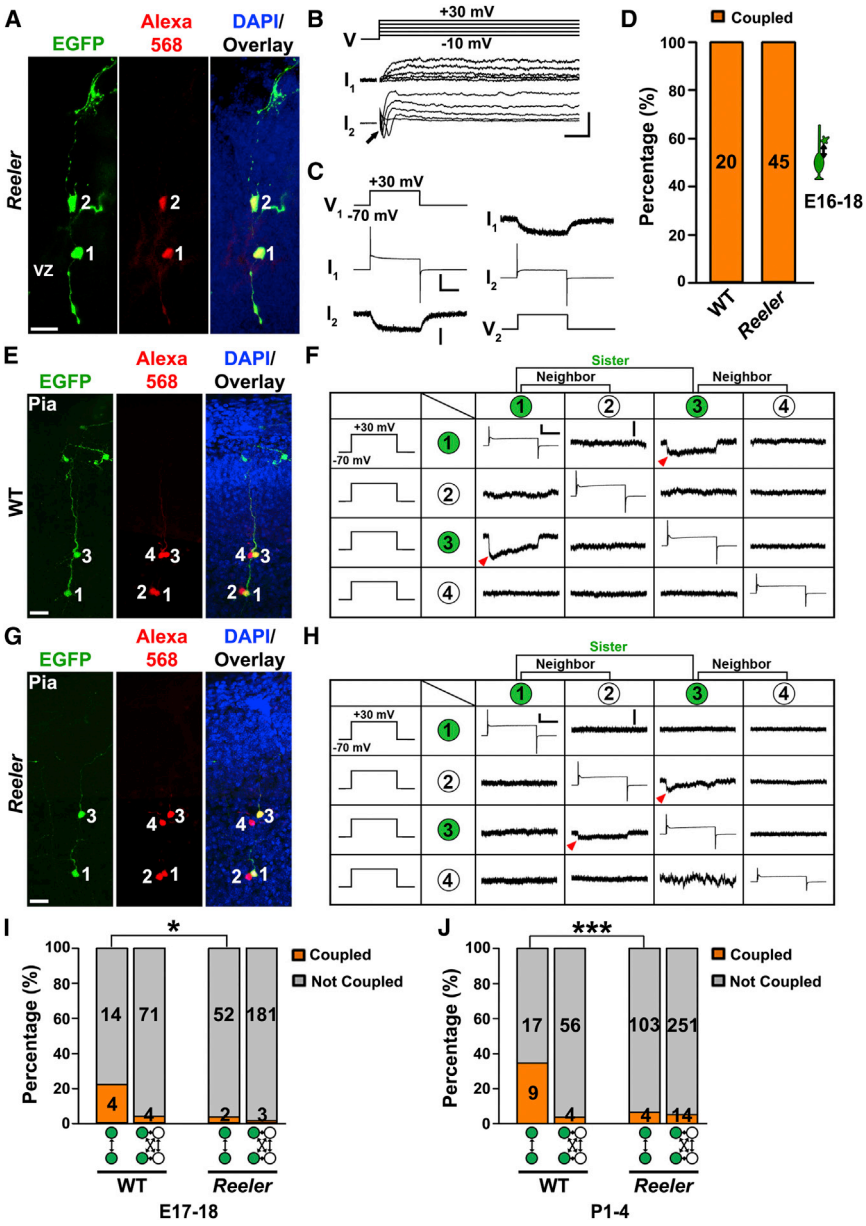

Figure 3. Preferential Electrical Coupling between Sister Excitatory Neurons, but Not between RGP and progeny, Is Abolished in the Neocortex of *Reeler* Mice

(A–C) Dual whole-cell recording of an EGFP-expressing RGP (cell 1, green, [A]) and its neuronal progeny in the SVZ (cell 2, green, [A]) with obvious sodium currents (arrow, [B]) in the embryonic *Reeler* neocortex. Depolarization of one cell evoked currents simultaneously in both cells (C), indicating electrical coupling between the RGP and its neuronal progeny. Scale bars: 20 μm (A); 100 pA and 10 ms (B); 200 pA, 100 ms, and 4 pA (C).

(D) Summary of the rate of electrical coupling between RGPs and their neuronal progeny in the SVZ of the wild type (WT) or the *Reeler* neocortex.

(E–H) Quadruple whole-cell recordings of two EGFP-expressing sister excitatory neurons (1 and 3, green) and two adjacent non-EGFP-expressing control excitatory neurons (2 and 4) in the embryonic and neonatal WT ([E] and [F]) and *Reeler* ([G] and [H]) neocortices. Scale bars: 25 μm ([E] and [G]); 250 pA, 100 ms, 10 pA ([F] and [H]).

(I and J) Summary of the rate of electrical coupling observed between sister excitatory neurons and their adjacent non-sister excitatory neurons in the WT and *Reeler* neocortices at E17 and E18 (I) and P1–P4 (J). *, $p < 0.05$; ***, $p < 0.001$. See also Figures S2 and S3.

These results suggest that the preferential electrical coupling between radially aligned sister excitatory neurons is largely abolished in the *Reeler* neocortex, where the inside-out order of neuronal migration is disrupted.

Neuronal migration and lamination are globally affected in the *Reeler* neocortex. To test whether the impairment of preferential electrical coupling between sister excitatory neurons is indeed due to defective inside-out radial migration at the clonal level, we developed retroviruses expressing EGFP together with short hairpin RNAs (shRNAs) against *Disabled-1* (*Dab1*), which encodes an essential downstream effector of the REELIN pathway, as previously described (Howell et al., 1997; Rice et al., 1998). Indeed, we found that expression of *Dab1* shRNA-3 (but not vector alone, *Dab1* shRNA-2, or *Dab1* shRNA-1) suppressed the expression of DAB1 (Figure S4A) and disrupted the radial migration of neocortical neurons (Figures S4B and S4C). Notably, removal of DAB1 does not prevent newborn neurons from associating with their mother radial glia during the course of radial migration (Franco et al., 2011; Sanada et al., 2004). We then performed quadruple whole-cell recordings to examine the preferential electrical coupling between sister excitatory neurons expressing the vector alone, *Dab1* shRNA-1, or shRNA-3 at P1–P4 (Figures 4A–4D). While sister neurons expressing the vector alone or *Dab1* shRNA-1 exhibited a similar rate of electrical coupling as those expressing EGFP, sister neurons expressing *Dab1* shRNA-3 were no longer preferentially coupled (Figure 4E). No significant change was observed in the rate of electrical coupling between non-sister neuron pairs or between radially situated non-EGFP-expressing neuron pairs (Figure 4E). Knockdown of DAB1 did not affect the RMP (vector only, −59.8 ± 0.8 mV, n = 25; shRNA-1, −59.3 ± 0.9 mV, n = 25; shRNA-3, −61.0 ± 1.0 mV, n = 25) or morphology (Figures 4A and 4C) of neurons at this stage. These results further support the notion that the inside-out radial migration of clonally related excitatory neurons is essential for the preferential formation of gap junctions between them. Notably, the preferential chemical synapse formation between sister excitatory neurons at the late postnatal stage was also impaired when the inside-out radial migration was disrupted (Figures S3H and S4D).

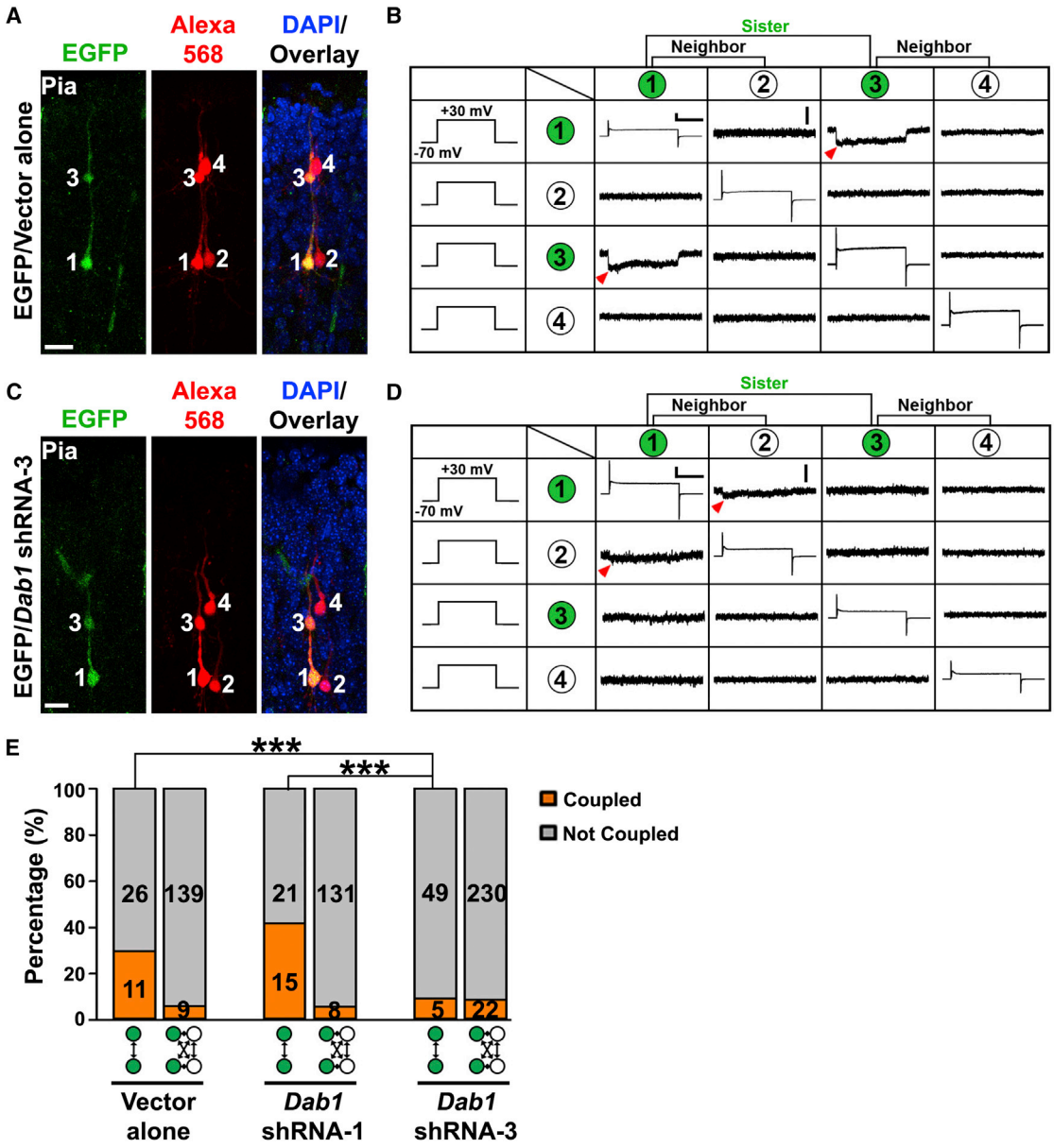

Figure 4. Preferential Electrical Coupling between Sister Excitatory Neurons Is Disrupted by Clonal Knockdown of DAB1

(A–D) Quadruple whole-cell recordings of two sister excitatory neurons expressing EGFP/Vector alone ([A] and [B]) or EGFP/*Dab1* shRNA-3 ([C] and [D]) (1 and 3, green) and two adjacent non-EGFP-expressing control excitatory neurons (2 and 4) in the neonatal neocortex. Scale bars: 20 μm ([A] and [C]); 500 pA, 100 ms, and 5 pA ([B] and [D]).

(E) Summary of the rate of electrical coupling observed between sister excitatory neurons expressing vector alone, *Dab1* shRNA-1, or shRNA-3 and their adjacent non-sister excitatory neurons in the neocortex. ***, $p < 0.001$. See also Figure S4.

Lateral Dispersion of Sister Excitatory Neurons Disrupts Preferential Electrical Coupling

To further test the importance of radial migration of sister excitatory neurons along a similar path in controlling preferential electrical synapse formation, we exploited the recent finding that Ephrin-A (EFNA) and Ephrin-A receptor (EPHA) signaling regulates the lateral dispersion of clonally related neurons in the developing neocortex (Torii et al., 2009). In line with the previous study, we found that overexpression of EFNA5 or EPHA7 substantially increased the lateral/tangential dispersion of excitatory neuron clones (Figure S5).

Interestingly, the rate of electrical coupling between radially situated sister excitatory neurons expressing EFNA5 or EPHA7 at P1–P4 was significantly reduced, compared to control sister neuron pairs expressing EGFP alone (Figures S6A–S6E). The rate of electrical coupling between non-sister neuron pairs or radially situated non-EGFP-expressing neuron pairs did not change significantly (Figure S6E). Overexpression of EFNA5 or

EPHA7 had no effect on neuronal RMPs (P1-2: EFNA5, −59.7 ± 0.5 mV, n = 57; neighbor control, −59.6 ± 0.6 mV, n = 57; P1 and P2: EPHA7, −59.5 ± 0.5 mV, n = 66; neighbor control, −59.7 ± 0.5 mV, n = 66), indicating that neuronal maturation is not perturbed. Together, these results suggest that increased lateral dispersion of neuronal clones prevents preferential electrical coupling between sister excitatory neurons.

DISCUSSION

Preferential electrical coupling between sister excitatory neurons represents one of the very first steps of functional development of the neocortex (Li et al., 2012; Yu et al., 2009, 2012). In this study, we defined the developmental origins of this lineage-dependent electrical synapse formation and precise microcircuit assembly. While RGPs form extensive gap junctions with neighboring RGPs in the VZ, they preferentially form gap junctions with their progeny in the SVZ, suggesting that a lineage-specific functional interaction between RGPs and their progeny occurs as the progeny are produced and migrate radially. Moreover, we found that sister excitatory neurons progressively and selectively develop gap junctions with each other while they migrate to reach their final destination in the embryonic and neonatal neocortex. Interestingly, this precise electrical synapse formation depends on the inside-out radial migration of sister excitatory neurons along a similar path, which likely promotes the interaction of sister excitatory neurons. Removal of REELIN or knockdown of its downstream signaling mediator DAB1 disrupts clonal inside-out neuronal migration and impairs the preferential electrical synapse formation between sister excitatory neurons. In addition, elevated levels of EFNA/EPHA-mediated signaling, which laterally disperse clonally related excitatory neurons, prevent preferential electrical coupling between them. These results suggest that migration and spatial localization of neurons in the developing neocortex serve as a determinant of highly specific neuronal connectivity, as previously shown in the spinal cord (Jessell et al., 2011; Sürmeli et al., 2011).

Importantly, our findings establish a clear link between the developmental processes of neurogenesis, neuronal migration, and precise microcircuit assembly in the neocortex. This may represent a highly efficient developmental program encoding the construction of repetitive columnar circuits for information processing in the neocortex, as postulated over 40 years ago (Hubel and Wiesel, 1974). In addition, recent studies suggest that neocortical expansion during evolution is tightly associated with expanded germinal zones with diverse progenitor cell types and a rearranged neuronal radial migration scaffold (Florio and Huttner, 2014; Lui et al., 2011). It will therefore be interesting to understand neurogenesis and neuronal migration in different species and their relationship to functional development of the neocortex under normal and disease conditions.

EXPERIMENTAL PROCEDURES

Animals were maintained and handled according to the protocols approved by the Institutional Animal Care and Use Committee at the Memorial Sloan Kettering Cancer Center.

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2015.05.002.

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