1 # Radial glial fibers support neuronal migration and regeneration
2 # after neonatal brain injury
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Summary

Radial glia (RG) are embryonic neural stem cells (NSCs) that produce neuroblasts and provide fibers that act as a scaffold for neuroblast migration during embryonic development. Although they normally disappear soon after birth, here we found that RG fibers can persist in injured neonatal mouse brains and act as a scaffold for postnatal ventricular-subventricular zone (V-SVZ)-derived neuroblasts that migrate to the lesion site. This injury-induced maintenance of RG fibers has a limited time window during postnatal development and promotes directional saltatory movement of neuroblasts via N-cadherin-mediated cell-cell contacts that promote RhoA activation. Transplanting an N-cadherin-containing scaffold into injured neonatal brains likewise promotes migration and maturation of V-SVZ-derived neuroblasts, leading to functional improvements in impaired gait behaviors. Together these results suggest that RG fibers enable postnatal V-SVZ-derived neuroblasts to migrate toward sites of injury, thereby enhancing neuronal regeneration and functional recovery from neonatal brain injuries.

1 **Keywords**
2 Neonatal brain injury, postnatal neurogenesis, ventricular-subventricular zone, radial
3 glial cell, neuronal migration, neuronal regeneration, gait behavior, N-cadherin
4

Introduction

Neonatal brain injury, such as hypoxia-ischemia, is a primary cause of childhood mortality and lifelong disability. However, there is currently no therapy to repair the injured brain tissues. The ventricular-subventricular zone (V-SVZ) is a neural stem cell (NSC) niche in the postnatal vertebrate brain and continuously supplies new neurons (Kaneko et al., 2017). Notably, the human neonatal V-SVZ has a remarkable neurogenic capacity (Paredes et al., 2016; Sanai et al., 2011), raising the possibility that the V-SVZ could be a source for endogenous neural regeneration after neonatal brain injury.

In rodents, neuroblasts use various migratory scaffolds. In the injured adult brain, V-SVZ-derived neuroblasts migrate along blood vessels (Yamashita et al., 2006) toward the lesion. Transplanting blood-vessel-mimetic scaffolds into the injured adult brain promotes neuroblast migration to the lesion (Ajioka et al., 2015; Fujioka et al., 2017). Compared to the adult brain, the neurogenic niche of the neonatal brain contains larger numbers of neuroblasts that efficiently migrate toward a lesion (Covey et al., 2010). However, the neonatal scaffolds that guide neuroblasts toward injured areas have not been fully investigated. Radial glia (RG) are embryonic NSCs that extend thin fibers to the pial surface from their soma, which is located in the ventricular zone (Rakic, 1972). In the embryonic cerebral cortex, newly generated neuroblasts use the RG fibers as a scaffold for migration (Kawauchi et al., 2010). In this process, the RG fibers form adherens junction (AJ)-like structures with the neuroblasts and guide them appropriately to form the cortical layers (Franco et al., 2011; Rakic, 1972). Soon after birth, the RG transform into astrocytes or ependymal cells (Kriegstein and Alvarez-Buylla, 2009). Therefore, it remains unknown how migrating neuroblasts are guided after neonatal brain injury.

Here, we show the functional significance of RG fibers for endogenous neuronal regeneration and functional recovery after neonatal brain injury.

Results

Neonatal RG cells maintain their fibers after brain injury

We performed cryogenic injury on postnatal day 2 (P2) and analyzed the dynamics of RG fiber disappearance. In the contralateral (uninjured) side, the density of Nestin+ RG fibers gradually decreased (Figures S1A and S1E), consistent with previous observations (Kriegstein and Alvarez-Buylla, 2009). On the other hand, the density of RG fibers in the ipsilateral cortex was significantly higher than that in the contralateral one at all of these time points, although it was highest at 7 day post injury (dpi) and decreased thereafter (Figures S1A and S1E). In addition, the RG fibers were longer in the injured than in the uninjured brain (Figures S1I and S1J). These results suggested that neonatal brain injury promotes the maintenance of RG fibers.

To examine the effects of an injury caused in later stages on RG fibers, we performed the cryogenic injury in P4, P14, and 8-week-old (8w, adult) mice and analyzed the fibers 7 days later. The Nestin+ fibers were retained in the P4 model, although the fiber density in the P4 model was significantly lower than in the P2 model (Figures S1A, S1B, and S1E). No clear Nestin+ RG fibers were observed in the P14 or 8w injury models (Figures S1C–S1E). These results suggested that RG fibers have the potential to be retained after injury only during the neonatal stages. Time-lapse imaging of cultured brain slices revealed that the diminished fibers could regrow in response to injury (Figure S1K). Consistently, these fibers in the injured brain were significantly longer than those in the uninjured brain in the P14 and 8w models (Figure S1F). RG fibers were also observed in the neonatal mouse brain after hypoxic and ischemic injury (Figures S1G and S1H). Taken together, these results suggested that the neonatal brain has the potential to maintain RG fibers after injury.

Neonatal RG cells provide a migratory scaffold for V-SVZ-derived neuroblasts after brain injury

We performed a cryogenic injury at P2 and studied injury-induced neurogenesis at P9 (Figure 1A). A large number of doublecortin (Dcx+) cells with the typical morphology for migrating neuroblasts appeared around the lesion (Figure 1B). These neuroblasts, which were at least partly derived from the V-SVZ (Figure 1C), were observed to be associated with Nestin+ fibers (Figures 1B–1D). To label RG fibers specifically, Cre-encoding adenovirus (Ad-Cre) was injected into the cortical surface of P0 *R26-tdTomato;Dcx-EGFP* mice (Merkle et al., 2007) (Figures 1A, 1E, and S1I). The tdTomato fluorescence clearly labeled fiber-bearing cells that expressed the RG cell markers Pax6, Nestin, and ErbB4 (Schmid et al., 2003), and whose cell bodies were observed in the V-SVZ and corpus callosum (CC), in addition

to astrocytes and oligodendrocytes (Figures 1E' and S2A-S2D). We found that 55.5% ± 3.1% of the *Dcx-EGFP*+ neuroblasts migrated radially (toward the lesion) and that 96.0% ± 0.3% of these migrating neuroblasts were associated with tdTomato+Nestin+ RG fibers (Figures S2E and S2F). Notably, 34.8% ± 4.7% of the *Dcx-EGFP*+ neuroblasts aligned their whole cell body with fibers (Figures 1F, 1G, S2E, and S2F). These results suggested that V-SVZ-derived neuroblasts that migrate radially toward the lesion after neonatal brain injury are associated with RG fibers.

N-cadherin, a protein involved in regulating cell-cell adhesion, is involved in RG-guided neuroblast migration in the embryonic cortex (Kawauchi et al., 2010). We observed N-cadherin expression in both neonatal RG fibers and migrating neuroblasts after injury (Figure 1D). To inactivate the function of N-cadherin in the radial fibers, RG cells were infected with an adenovirus vector encoding a dominant-negative form of N-cadherin (DN-N-cadherin) and Cre at P0 (Figures 1A and 1F). The DN-N-cadherin expression in RG did not affect the morphology or density of their fibers at 7 dpi (Figures S2G and S2H). However, the proportion of neuroblasts associated with the DN-N-cadherin-expressing fibers was significantly lower than that in the control group (Figures 1F, 1G, S2E, and S2F). Furthermore, the neuroblast density was significantly decreased in the DN-N-cadherin-virus-infected area and increased in the non-infected area, compared with those areas in control mice (Figure 1H), suggesting that the neuroblasts preferred the fibers without DN-N-cadherin for their migration. Specific downregulation of the N-cadherin expression in RG using an adenoviral knockdown (KD) vector also decreased the proportion of neuroblasts associated with fibers and the neuroblast density (Figures 1I and 1J). These results suggested that radial glial N-cadherin is involved in the fiber-guided migration of neuroblasts after injury. The KD of FAK and L1-CAM, which are involved in fiber-guided neuroblast migration in the embryonic cortex (Tonosaki et al., 2014; Valiente et al., 2011), did not affect the association of neuroblasts with KD fibers (Figures S2M-S2P). Transmission electron microscopy (TEM) analyses revealed direct contacts between the neuroblasts and fibers, in which AJ-like electron-dense structures were occasionally observed (Figures 1K–1K", red arrows). The expression of DN-N-cadherin in the fibers decreased the density of such structures and increased the proportion of irregular contacts, in which the membranes of the neuroblasts and fibers were not parallel (Figures 1L–1M, blue arrowheads). These observations suggested that the N-cadherin in RG is involved in forming the proper cell adhesion between fibers and migrating neuroblasts in the neonatal brain. Taken together, these results indicate that neonatal RG associate with the V-SVZ-derived neuroblasts that migrate toward the lesion after brain injury.

N-cadherin promotes the RhoA activation and saltatory movement of neuroblasts migrating along RG fibers

To examine whether the neuroblasts utilize RG fibers as a migratory scaffold toward a lesion, we monitored them by live imaging in cultured brain slices of adenovirus-infected *R26-tdTomato;Dcx-EGFP* mice at 4–5 dpi. *Dcx-EGFP*+ neuroblasts extended their leading process along tdTomato+ RG fibers and translocated their soma in the direction of the lesion in a saltatory manner (Figures 2A and 2A'). Neuroblasts migrating along DN-N-cadherin-expressing RG showed a significantly lower migration speed (Figures 2A and 2B) and detached from the fibers more frequently (Figures 2A, 2A', and 2C). Consistent with the histological analyses (Figures 1G, 1I, S2E, and S2F), the proportion of neuroblasts that was not attached to fibers was significantly increased in the DN-N-cadherin group (Figure 2D). These results suggested that radial glial N-cadherin is involved in the efficient and continuous migration of neuroblasts along RG fibers toward a lesion.

The migration speed of neuroblasts is determined by the somatic stride length, somatic stride frequency, and length of the pause (“resting” phase) (Ota et al., 2014). DN-N-cadherin expression in RG significantly decreased the somatic stride length and increased the duration of the resting phase and of each migration cycle in the neuroblast migration (Figures 2E–2G). Collectively, these results suggested that the RG-fiber guided neuronal migration in the injured neonatal brain depends on N-cadherin-mediated adhesion, which increases the somatic stride length and frequency of the neurons' saltatory movement.

Since RhoA signaling in the swelling of migrating neuroblasts is known to promote their saltatory movement (Ota et al., 2014), we next monitored their RhoA activity using fluorescent resonance energy transfer (FRET) imaging in an N-cadherin-Fc-coated stripe assay. The RhoA activity in the swelling of migrating neuroblasts was significantly increased when they migrated on an N-cadherin-containing scaffold (Figures 2H–2J).

N-cadherin can interact with various signaling molecules. Next, to examine whether the N-cadherin in neuroblasts was also involved in their migration on an N-cadherin-containing scaffold, we introduced N-cadherin KD plasmids (Figures S2I–S2L) into cultured neuroblasts and analyzed their migratory behaviors in a stripe assay (Figures 2K–2P). The neuroblasts significantly increased their migration speed when they entered an N-cadherin-Fc stripe (Figures 2K and 2L). The increased migration speed appeared to be due to both an increased somatic stride length and a decreased time spent in the resting phase in each migration cycle (Figures 2K–2O), consistent with the effects of DN-N-cadherin-expression in RG (Figures 2A–2G). When neuroblasts reached the border of an N-cadherin-Fc stripe, most of the control

cells changed the direction of their leading process to remain on the N-cadherin-Fc-coated area. The percentage of cells that showed this behavior was significantly decreased by N-cadherin-KD (Figures 2K and 2P; Movie S1), suggesting that the neuroblast's N-cadherin helps to maintain the directional neuroblast migration on an N-cadherin-containing scaffold. Taken together, these results suggested that N-cadherin promotes RhoA activation and saltatory movement of neuroblasts migrating along RG fibers.

N-cadherin-containing scaffold promotes the recovery of neurological functions by increasing V-SVZ-derived neuronal migration and regeneration after neonatal brain injury

The neonatal V-SVZ supplies new mature neurons to the cerebral cortex under physiological conditions (Le Magueresse et al., 2011) and to the injured striatum and cortex after brain injury (Yang et al., 2007., 2008). Cryogenic injury increased the number of neuronal progenitor cells (Figures S3A and S3D). The number of Dlx2+Dcx+ but not of Tbr2+Dcx+ neuroblasts was increased after injury (Figures S3B, S3C, S3E, and S3F), suggesting that GABAergic neuroblasts are recruited to the injured cortex. Furthermore, neonatal cryogenic injury significantly increased the number of EmGFP+NeuN+ mature neurons, which were mostly GAD67+ and less frequently Parvalbumin (PV)+ or Calretinin (CR)+ (Figures S3G–S3M), indicating that they were V-SVZ-derived cortical interneurons. Over 60% of these neurons were located in cortical layers IV–VI (Figure S3I). The number of V-SVZ-derived mature neurons in the cortex was significantly decreased by expressing DN-N-cadherin in the neonatal RG (Figures 3A–3C), suggesting that RG fibers contribute to the migration and maturation of V-SVZ-derived neuroblasts in the injured neonatal cortex.

Next, to test whether an N-cadherin-containing artificial scaffold would promote V-SVZ-derived neuronal migration after brain injury, we developed polyethylene terephthalate (PET) fibers and gelatin sponges conjugated with Fc or N-cadherin-Fc (control or N-cadherin fibers/sponges, respectively). The migration speed of the V-SVZ-derived neuroblasts increased when they made contact with the N-cadherin fibers and sponges in vitro (Figures 3D, 3E, 3F, and S4A–S4D; Movie S2). We then transplanted N-cadherin fibers or sponges into the injured cortex (Figure 3F). While there was no significant difference in the density of Dcx+ neuroblasts between the control and N-cadherin fibers (Figures S4E and S4F), the density of neuroblasts within the sponges was increased in the mice treated with the N-cadherin sponge (Figures 3G–3I), suggesting that N-cadherin sponges support neuroblast migration more efficiently than do N-cadherin fibers in vivo, under our experimental conditions.

To investigate whether N-cadherin sponges promote neuroblast migration in older brains that lack RG fibers, we performed the cryogenic injury at P14 or 8w and transplanted N-cadherin sponges (Figure 3F). The number of neuroblasts reaching the lesion in the control-sponge groups was significantly smaller in the P14 and 8w models compared with P2, supporting the concept that RG fibers are important scaffolds for neuroblast migration toward the lesion (Figures 3H and 3I). Consequently, however, the effect of N-cadherin sponge on the promotion of neuroblast migration was more obvious in the older brains (Figure S4G), even though the absolute number of neuroblasts in the N-cadherin sponge was highest in the P2 model and decreased with age (Figures 3H and 3I).

Furthermore, we transplanted N-cadherin sponge at 10 dpi into the P2 injury model and compared the number of neuroblasts in the sponges with that in the 3 dpi transplantation group (Figure 3F). At 4 dpt, the density of neuroblasts was higher in the brains with transplantation at P5 than in those with transplantation at P12 (Figure 3I), suggesting that early sponge transplantation had the most beneficial effect on neuroblast recruitment after neonatal brain injury.

To examine the effect of N-cadherin-sponge transplantation on neuronal regeneration, V-SVZ cells were labeled by electroporation, and their fate was analyzed at 28 dpi (Figures 3F, 3J, and 3K). The number of V-SVZ-derived NeuN+ mature neurons in and around the lesion was significantly greater in the mice treated with the N-cadherin sponge than in those treated with the control sponge (Figures 3J and 3K). Moreover, the proportion of V-SVZ-derived NeuN+ neurons in the upper cortical layers was significantly increased by transplanting N-cadherin sponge (Figure 3K). These results suggested that the N-cadherin-containing scaffold promoted V-SVZ-derived neuronal regeneration after neonatal brain injury.

Finally, we investigated the effects of N-cadherin-sponge transplantation on functional recovery at 28 dpi. To analyze the spontaneous gait behaviors, we used CatWalk analyses. The brain injury caused a decrease in the contact area of the front paws (‘‘Max contact area’’ and ‘‘Print area’’) and an increase in the width between the front paws (‘‘Base of support’’) (Figures 4A–4C; Table S1). Control-sponge transplantation did not worsen these gait behaviors (Figures 4A–4C; Table S1), suggesting that the sponge transplantation did not have any adverse effects. Notably, N-cadherin-sponge transplantation improved the defects in these gait parameters (Figures 4A–4C; Table S1), suggesting that the N-cadherin sponge promoted functional recovery in addition to neuronal regeneration after neonatal brain injury.

Next, we performed the foot-fault test (Barth et al., 1990). Cryogenic injury induced left-right asymmetry of the foot-fault ratio at 28 dpi in the P2 injury model, which was recovered by the transplantation of N-cadherin but not control sponge

(Figure 4D). N-cadherin-sponge transplantation also led to a clear improvement in the neurological score in the P14 but not in the 8w model (Figure 4D). Thus, although neuroblast migration can be enhanced by N-cadherin sponge even in the adult brain, the time window for functional recovery appears to be more limited.

To further determine the contribution of V-SVZ-derived endogenous neuronal regeneration on functional recovery, we intraventricularly injected Ad-Cre into P0 *neuron-specific enolase (NSE)-diphtheria toxin fragment A (DTA)* mice (Imayoshi et al., 2008; Kobayakawa et al., 2007), which eliminates neuronal progenies (Figures 4E–4G). The improvement in the foot-fault ratio by N-cadherin-sponge transplantation was not observed in the Ad-Cre-infected *NSE-DTA* mice (Figure 4H). Taken together, these results suggested that an N-cadherin-containing scaffold promoted the functional recovery after neonatal brain injury, and that V-SVZ-derived neuronal regeneration contributed to this recovery.

Discussion

We demonstrated that, after neonatal brain injury in mice, RG support the radial migration of neuroblasts toward the lesion by providing a migratory scaffold. N-cadherin plays a critical role in forming the appropriate cell adhesion structures to maintain the neonatal RG-guided neuroblast migration. Furthermore, the transplantation of an N-cadherin-containing scaffold into the injured cortex promoted neuroblast migration, and the recovery of neurological dysfunction.

RhoA activity in the proximal leading process promotes neuronal somal translocation and directional migration (Ota et al., 2014), which we observed to increase on an N-cadherin-containing scaffold. Since N-cadherin-dependent cell-cell contacts activate RhoA in the neuron-RG interaction in the embryonic period (Xu et al., 2015), it is possible that, N-cadherin stimulates RhoA activity of V-SVZ-derived migrating neuroblasts, to maintain the driving force for the neuronal saltatory movement along fibers. Reelin and MCP-1 are upregulated after brain injury (Courte`s et al., 2011; Yang et al., 2007), and may be needed for the directional migration of neuroblasts toward the lesion. These molecules in combination with N-cadherin-mediated cell adhesion could contribute to the efficient fiber-guided neuroblast migration toward injured areas.

The morphology of embryonic RG cells is controlled by neuregulin-ErbB signaling, FGF, and several polarity genes (Schmid et al., 2003; Ganat et al., 2002; Yokota et al., 2010). We found that ErbB4 was expressed in RG fibers and that its ligand neuregulin was expressed in the injured cortex (Figures S2A, S2Q and S2R). Furthermore, the length of fibers increased after injury, similar to the previously reported fiber extension induced by a constitutively active form of ErbB2 (Ghashghaei et al., 2007). Therefore, it is possible that neuregulin-ErbB signaling contributes to the maintenance of RG fibers after neonatal brain injury.

The provision of migratory scaffolding is a promising strategy for improving neural regeneration. We showed that expressing DN-N-cadherin, which lacks the extracellular (EC) domain of N-cadherin, disrupted the neuron-RG interaction, suggesting that N-cadherin-mediated cell adhesion is involved in fiber-guided migration. Based on this finding, we generated artificial scaffolds using N-cadherin-Fc, which contains the N-cadherin EC domain, to mimic the N-cadherin expression on RG fibers. N-cadherin-PET fibers did not promote neuroblast migration into the injured cortex in vivo, due to the technical difficulty of orienting these fibers radially within the lesion. Although N-cadherin sponges lack radial organization, they provide a continuous surface area with which migrating neuroblasts can make contact, thereby enabling their continuous outward migration. Furthermore, the sponge is made of

1 gelatin, which is reported to adapt to the brain environment without causing further
2 inflammation after injury (Ajioka et al., 2015). N-cadherin-sponge transplantation
3 promoted neuroblast migration even in the adult stages when the brain lacks RG
4 fibers, although it showed a limited time window for functional recovery, probably
5 partly due to an insufficient number of migrating neuroblasts. Providing an appropriate
6 migratory scaffold such as a blood-vessel mimetic (Ajioka et al., 2015; Fujioka et al.,
7 2017) or radial-fiber mimetic (this study) is a promising strategy for neural
8 regeneration in the injured neonatal brain. It will be important to develop methods for
9 transplanting gelatin-based fibers conjugated with N-cadherin-Fc radially within
10 damaged brain tissue.
11 Our results demonstrated that neonatal brain injury enhanced the supply of
12 V-SVZ-derived GABAergic interneurons in and around the lesion in the
13 somatosensory and primary motor cortex by causing RG fibers to be sustained. The
14 genetic ablation of new neurons decreased the functional recovery by
15 N-cadherin-sponge transplantation, indicating that V-SVZ-derived neurogenesis in the
16 injured neonatal brain contributed to the functional recovery, suggesting that
17 interneuron regeneration is important for functional recovery after cortical injury.
18 Catwalk parameters consist of four groups: (1) spatial parameters related to
19 individual paws, (2) spatial relationships between different paws, (3) interlimb
20 coordination, and (4) temporal parameters (Neumann et al., 2009). Among
21 parameters in group 1 and 2, ‘‘Max contact area,’’ ‘‘Print area,’’ and ‘‘Base of support,’’
22 in which we found significant improvement in the sponge-transplanted group, have
23 been used as important outputs for unsteady gait behaviors caused by injury in the
24 sensorimotor cortex (Williams et al., 2009). Changes in the ‘‘Max contact area’’ and
25 “Print area’’ are likely to be derived from injury-induced altered plantar use and
26 muscular weakness of the limbs. In addition, increasing the ‘‘Base of support’’, rather
27 than the stride length, could be more effective for the animal to compensate for
28 injury-induced left-right asymmetry. Since the cryogenic injury that we used produces
29 a local lesion only in the cortex, and not in central networks that regulate coordinated
30 locomotion, such as the central pattern generators in the spinal cord and
31 mesencephalic locomotor region in the brain stem (Rossignol and Frigon, 2011), it is
32 reasonable that the interlimb coordination and temporal parameters (groups 3 and 4)
33 were not affected by the injury in our study.
34 Our results could provide a foundation for treatment strategies for neonatal human
35 brain injury. In humans, RG cells exist at least until the late stage of pregnancy (Malik
36 et al., 2013), raising the possibility that neonatal infants with brain injuries maintain
37 radial glial fibers. Recent studies have unraveled the molecular machineries involved
38 in the formation and function of human outer RG (Ostrem et al., 2017), some of which

1 could play a role in the maintenance of RG cells after neonatal human brain injury.
2 Considering the strong neurogenic potential in the neonatal human brain (Sanai et al.,
3 2011; Paredes et al., 2016), the placement of a directional migratory scaffold into a
4 lesion or the activation of RG-maintaining factors are promising treatment strategies
5 for neonatal brain injury using endogenous NSCs.
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Supplemental information

Supplemental Information includes four figures, two tables, and two movies and can be found with this article online at https://doi.org/10.1016/j.stem.2017.11.005.

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Author contributions

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Legends to Figures

Figure 1. RG retain their fibers and provide a migratory scaffold for V-SVZ-derived neuroblasts after neonatal brain injury.

(A) Experimental scheme.

(B) Coronal section of the cortex in *Dcx-EGFP* mice stained for GFP (green) and Nestin (red) at 7 dpi. Arrowheads, GFP+ neuroblasts associated with Nestin+ fibers (B1–B4).

(C) Coronal section of the cortex in wild-type (WT) mice, in which EmGFP-expressing plasmids were electroporated into the V-SVZ, stained for GFP (green), Dcx (red), and Nestin (white).

(D) Expression of N-cadherin (red) in Dcx+ (green) neuroblasts (asterisks) and Nestin+ (white) RG fibers (arrows).

(E) Neonatal RG fibers after injury, targeted by adenovirus. Coronal section of the cortex in *R26-tdTomato* mice stained for DsRed (red) and Nestin (white). Yellow and white arrows indicate RG cells located in the V-SVZ and CC, respectively (E').

(F–J) Effect of expressing DN-N-cadherin (F–H) or N-cadherin-KD (I and J) in RG cells on neuroblast attachment to fibers (F, G, and I) and migration toward the lesion (F, H, and J). Coronal section of the cortex in *R26-tdTomato;Dcx-EGFP* mice stained for GFP (green), DsRed (red), and Nestin (white) (F). (G and I) Proportion of total neuroblasts located along fibers (''whole-cell association'' in Figure S2F).

(K and L) TEM images of neuroblasts (N, green), control (K), and DN-N-cadherin-expressing (L) RG fibers (RGF, red). Red arrows and blue arrowheads indicate AJ-like electron-dense structures and irregular contacts, respectively.

(M) Contact density and proportion of irregular contact regions at neuroblast-fiber adhesion points.

Scale bars, 10 mm (B), 50 mm (E), 5 mm (C, D, and F), 500 nm (K and L). Error bars, mean ± SEM. See also Figures S1 and S2.

Figure 2. N-cadherin scaffold promotes RhoA activation and saltatory movement in neuroblasts migrating along RG fibers.

(A) Time-lapse images of GFP+ neuroblasts (green) migrating along control and DN-N-cadherin-expressing tdTomato+ fibers (purple) in an injured cortex slice at 5 dpi. Arrows and arrowheads indicate a neuroblast's leading tip and a RG fiber, respectively.

(B–G) Migration speed (B), proportion of time spent in the fiber-attached phase (C), proportion of neuroblasts not attached to fibers (D), stride length (E), proportion of time spent in resting phase (F), and migration cycle time (G) of neuroblasts.

(H and I) Time-lapse FRET ratiometric images of RhoA activity (pseudocolors) in a cultured neuroblast (H). Magnified images are shown in (I).
(J) RhoA activation.
(K–P) Migratory behaviors of cultured neuroblasts on N-cadherin-Fc stripes. (K) Time-lapse images of tdTomato+ neuroblasts (red). Migration speed (L), proportion of time spent in resting phase (M), stride length (N), and migration cycle time (O) of neuroblasts. (P) Preference for the N-cadherin-Fc stripes. Dashed lines (H and K) indicate the stripe borders.
Scale bars, 10 mm. Error bars, mean ± SEM. See also Figure S2 and Movie S1.

Figure 3. N-cadherin-containing scaffold promotes the migration and maturation of V-SVZ-derived neuroblasts after neonatal brain injury.
(A and B) Coronal sections of the cortex in control (A) and DN-N-cadherin (B) groups stained for EmGFP (green). These are composite images of eight separate fields (two vertical and four horizontal tiles).
(C) The number of EmGFP+NeuN+ cells in the injured cortex.
(D) Time-lapse images of cultured neuroblasts migrating along control and N-cadherin sponge (Sp).
(E) Speed of cultured neuroblasts.
(F) Experimental scheme.
(G) EmGFP+ (green) V-SVZ-derived Dcx+ (red) neuroblast within the N-cadherin sponge (orange).
(H) Coronal sections of the cortex in WT mice (P2, P14, and 8w models) treated with sponge (yellow-green), stained for Dcx (red). Arrows, Dcx+ cells along the sponge.
(I) Density of Dcx+ cells within the sponges.
(J and J') Coronal sections of the cortex of P30 WT mice into which a sponge had been transplanted, stained for EmGFP (green) and NeuN (red). Arrows, EmGFP+NeuN+ neurons.
(K) Number (left) and distribution (right) of EmGFP+NeuN+ neurons in the injured cortex.
Scale bars, 50 mm (A, B, H, and J) and 10 mm (D and G). Error bars, mean ± SEM. See also Figures S3 and S4 and Movie S2.

Figure 4. N-cadherin-containing scaffold improves functional recovery by promoting V-SVZ-derived neuronal regeneration after neonatal brain injury.
(A–C) Catwalk analysis at P30. ‘‘Max contact area’’ (A), ‘‘Print area’’ (B), and ‘‘Base of support’’ (C) of the front paws.
(D) Foot-fault test. Percentage of left foot faults in P2, P14, and 8w injury models.

(E) Experimental scheme.
(F) Strategy for eliminating V-SVZ-derived new neurons.
(G) Number of EmGFP+NeuN+ new neurons in the injured cortex at P30.
(H) Foot-fault test in Ad-Cre;*NSE-DTA* mice into which N-cadherin sponge had been transplanted.
Error bars, mean ± SEM. See also Table S1.

1 # STAR Methods

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3 ## KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-Doublecortin	Cell Signaling Technology	Cat#4604; RRID: AB_561007
Guinea pig polyclonal anti-Doublecortin	Millipore	Cat#AB2253; RRID: AB_1586992
Goat polyclonal anti-Doublecortin (C-18)	Santa Cruz Biotechnology	Cat#8066; RRID: AB_2088494
Rat monoclonal anti-GFP	Nacalai Tesque	Cat#04404-84; RRID: AB_10013361
Chicken polyclonal anti-Nestin	Aves Labs	Cat#NES; RRID: AB_2314882
Rabbit polyclonal anti-DsRed	Clontech, Laboratories	Cat#632496; RRID: AB_10013483
Mouse monoclonal anti-NeuN (clone A60)	Millipore	Cat#MAB377; RRID: AB_2298772
Mouse monoclonal anti-Calretinin antibody	Millipore	Cat#MAB1568; RRID: AB_94259
Mouse monoclonal anti-Parvalbumin	Sigma-Aldrich	Cat#P3088; RRID: AB_477329
Mouse monoclonal anti-MASH1	BD Biosciences	Cat#556604; RRID: AB_396479
Rabbit polyclonal anti-TBR2 / Eomes	Abcam	Cat#ab23345; RRID: AB_778267
Rabbit polyclonal anti-Pax6	Covance Research Products Inc.	Cat#PRB-278P-100; RRID: AB_291612
Mouse monoclonal anti-N-Cadherin	BD Biosciences	Cat#610921; RRID: AB_398236
Mouse monoclonal anti-Glial Fibrillary Acidic Protein (GFAP)	Sigma-Aldrich	Cat#G3893; RRID: AB_477010
Rabbit polyclonal anti-Human Olig2	Immuno-Biological Laboratories	Cat#18953; RRID: AB_2267671
Guinea pig anti-Dlx2	Kazuaki Yoshikawa; Kuwajima et al., 2006	Cat#DLX2; RRID: AB_2314328

Mouse monoclonal anti-PSA-NCAM	Tatsunori Seki; Seki et al., 1991	Cat#PSA-NCAM; RRID: AB_2315216
Hoechst 33342	Thermo Fisher Scientific	Cat#62249
Mouse monoclonal anti-GAD67	Millipore	Cat#MAB5406; RRID: AB_2278725
Rabbit polyclonal anti-ErbB4	Abcam	Cat#ab15137
Rabbit polyclonal anti-Neuregulin-1α/β1/2 (C-20)	Santa Cruz Biotechnology	Cat#sc-348; RRID: AB_675753
Rabbit polyclonal anti-FAK	Millipore	Cat#06-543; RRID: AB_310162
Mouse monoclonal anti-L1-CAM (2C2)	Abcam	Cat#ab24345; RRID: AB_448025
Alexa Flour 488 donkey anti-mouse IgG (H+L)	Invitrogen	Cat#A21202; RRID: AB_141607
Alexa Flour 488 donkey anti-rat IgG (H+L)	Invitrogen	Cat#A21208; RRID: AB_141709
Alexa Flour 488 donkey anti-rabbit IgG (H+L)	Invitrogen	Cat#A21206; RRID: AB_141708
Alexa Flour 568 donkey anti-mouse IgG (H+L)	Invitrogen	Cat#A10037; RRID: AB_2534013
Alexa Flour 568 donkey anti-rabbit IgG (H+L)	Invitrogen	Cat#A10042; RRID: AB_2534017
Alexa Flour 568 goat anti-guinea pig IgG (H+L)	Invitrogen	Cat#A11075; RRID: AB_141954
Alexa Flour 568 donkey anti-goat IgG (H+L)	Invitrogen	Cat#A11057; RRID: AB_142581
Alexa Flour 647 donkey anti-rabbit IgG (H+L)	Invitrogen	Cat#A31573; RRID: AB_2536183
Alexa Flour 647 donkey anti-mouse IgG (H+L)	Invitrogen	Cat#A31571; RRID: AB_162542
Cy5-AffiniPure donkey anti-chicken IgY (IgG) (H+L)	Jackson ImmunoResearch	Cat#703-175-155; RRID: AB_2340365
Cy3-AffiniPure donkey anti-chicken IgY (IgG) (H+L)	Jackson ImmunoResearch	Cat#703-165-155; RRID: AB_2340363
Cy2-AffiniPure donkey anti-chicken IgY (IgG) (H+L)	Jackson ImmunoResearch	Cat#703-225-155; RRID: AB_2340370

Biotin-SP AffiniPure Fab Fragment donkey anti-rabbit IgG (H+L)	Jackson ImmunoResearch	Cat#711-066-152; RRID: AB_2340594
Peroxidase-AffiniPure donkey anti-rat IgG (H+L)	Jackson ImmunoResearch	Cat#712-035-153; RRID: AB_2340639
Peroxidase-AffiniPure goat anti-mouse IgG (H+L)	Jackson ImmunoResearch	Cat#115-035-146; RRID: AB_2307392
Peroxidase-AffiniPure goat anti-rabbit IgG (H+L)	Jackson ImmunoResearch	Cat#111-035-144; RRID: AB_2307391
AffiniPure Fab Fragment Donkey anti-Rabbit IgG (H+L)	Jackson ImmunoResearch	Cat#711-007-003; RRID: AB_2340587
AffiniPure Fab Fragment Donkey anti-Mouse IgG (H+L)	Jackson ImmunoResearch	Cat#715-007-003; RRID: AB_2307338
Rat monoclonal anti-HA High Affinity	Roche	Cat#11867423001; RRID: AB_10094468
Mouse monoclonal anti-actin	Millipore	Cat#MAB1501; RRID: AB_2223041
Anti-Human IgG (Fc specific)-FITC antibody produced in goat	Sigma-Aldrich	Cat#F9512; RRID: AB_259808
Anti-Human IgG (Fc specific) antibody produced in goat	Sigma-Aldrich	Cat#I2136; RRID: AB_260147
Bacterial and Virus Strains		
Ad-CMV-iCre	Vector BioLabs	Cat#1045
Ad-CMV-DN-N-cadherin-IRES-Cre	This paper	N/A
Ad-CMV-tdTomato-miR-N-cadherin	This paper	N/A
Ad-CMV-tdTomato-miR-lacZ	This paper	N/A
Chemicals, Peptides, and Recombinant Proteins		
BamHI	New England Biolabs	Cat#R0136S
SalI	New England Biolabs	Cat#R0138S
BspMI	New England Biolabs	Cat#R0502S
Fast green	Sigma-Aldrich	Cat#F7252
Normal donkey serum	Millipore	Cat#S30-100ML
Immobilon-P membrane PVDF	Millipore	Cat# IPVH00010
beMatrix Gelatin LS-H	Nitta Gelatin	Cat#633-25751
1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide	Wako	Cat#348-03631
Neurobasal medium	Gibco	Cat#21103049
N-cadherin-Fc (IgG-Fc fused with the extracellular domain of mouse N-cadherin)	Toshihiro Akaike; Yue et al., 2010	N/A

IgG-Fc	Toshihiro Akaike; Yue et al., 2010	N/A
HBSS (Hank's Balanced Salt Solution)	Gibco	Cat#14170-112
trypsin-EDTA	Invitrogen	Cat#25300-054
Leibovitz's L-15	Gibco	Cat#11415-064
DNase I	Roche	Cat#10104159001
RPMI-1640	Wako	Cat#189-02145
Matrigel	BD Biosciences	Cat#354234
Supernatant B-27 Plus	Invitrogen	Cat#130-093-566
L-glutamine	Gibco	Cat#25030-081
Penicillin-Streptomycin	Gibco	Cat#15140-122
Raichu-1298X	Michiyuki Matsuda; Yoshizaki et al., 2003	N/A
Gateway LR Clonase II Enzyme Mix	Invitrogen	Cat#11791-020
Critical Commercial Assays		
Mouse Neural Stem Cell Nucleofector Kit	Lonza	Cat#VPG-1004
PureLink HiPure Plasmid Maxiprep Kit	Invitrogen	Cat#K2100-06
ViraPower Adenoviral Gateway Expression Kit	Invitrogen	Cat#K4930-00
Vectastain Elite ABC-peroxidase kit	Vector Laboratories	Cat#PK-6100; RRID: AB_2336819
TSA Cyanine 3 System antibody amplification kit	PerkinElmer Inc.	Cat#NEL704A001KT; RRID: AB_2572409
Amersham ECL Prime Western Blotting Detection Reagent	GE Healthcare	Cat#RPN2232
Deposited Data		
Catwalk raw data	This paper; Mendeley Data	http://dx.doi.org/10.17632/t4r38rrf3d.1
Experimental Models: Organisms/Strains		
Mouse: ICR	Japan SLC	N/A
Mouse: C57BL6/J	Japan SLC	N/A
Mouse: R26-tdTomato	The Jackson Laboratory	Stock#007914; RRID: IMSR_JAX:007914
Mouse: Neurog2-d4Venus	Takaki Miyata; Kawaue et al., 2014	N/A

Mouse: NSE-DTA	Shigeyoshi Itohara; Kobayakawa et al., 2007; Imayoshi et al., 2008	N/A
Mouse: Dcx-EGFP	Mutant Mouse Research Resource Center (MMRRC)	Cat#000244-MU; RRID: MMRRC_000244-MU
Oligonucleotides		
See Table S2 for oligonucleotide sequences	This study	N/A
Recombinant DNA		
pLV-CMV-tdTomato-IRES-Cre	Magdalena Götz; Robel et al., 2011	N/A
pCAG-MCS2-DN-N-cadherin	Richard L. Huganir; Nuriya et al., 2006	N/A
pCAG-MCS2-HA-N-cadherin	Richard L. Huganir; Nuriya et al., 2006	N/A
pEGFPC1-FAK	Kozo Kaibuchi; Itoh et al., 2010	N/A
pCMV6-L1-CAM	Origene Technologies, Inc.	Cat#MC203533
pENTR4-H1	RIKEN	Cat#RDB04395
BLOCK-iT Pol II miR RNAi Expression Vector Kit with EmGFP	Invitrogen	Cat#K493600
ptdTomato-N1	Clontech Laboratories	Cat#632532
pCAGGS-EmGFP	Kazunobu Sawamoto; Ota et al., 2014	N/A
pCAGGS-tdTomato-miR-N-cadherin	This paper	N/A
pCAGGS-DsRed-miR-FAK	This paper	N/A
pCAGGS-DsRed-miR-L1-CAM	This paper	N/A
pCAGGS-tdTomato-miR-lacZ	This paper	N/A
pAd/CMV/V5-DEST Gateway Vector Kit	Invitrogen	Cat#V493-20
Software and Algorithms		
ImageJ	National Institutes of Health	http://imagej.nih.gov/ij/

Neurolucida	MBF Bioscience	http://www.mbfbioscience.com/neurolucida
Stereo Investigator	MBF Bioscience	http://www.mbfbioscience.com/stereo-investigator
MetaMorph	Molecular Devices	https://www.moleculardevices.com/systems/metamorph-research-imaging/metamorph-microscopy-automation-and-image-analysis-software
ZEN	Carl Zeiss	https://www.zeiss.com/microscopy/int/products/microscope-software/zen.html
Other		
Silicon matrices (50-μm wide)	Martin Bastmeyer (Karlsruhe Institute of Technology)	http://znbio.zoo.kit.edu
Wiretrol I (5 μl)	Drummond Scientific Company	Cat#5-000-1005

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CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Kazunobu Sawamoto (sawamoto@med.nagoya-cu.ac.jp).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals

All of the experiments involving live animals were performed in accordance with the guidelines and regulations of Nagoya City University and approved by the President of Nagoya City University. Animals were housed in cages lined up with chip bedding in a controlled environment (23 ± 1°C, 12 h light/dark cycle changed 8:00 o'clock) with *ad libitum* access to water and food (MF, Oriental Yeast, Tokyo, Japan) in a specific-pathogen-free facility. Wild-type (WT) ICR and C57BL6/J mice were purchased from Japan SLC (Shizuoka, Japan). The following transgenic mouse lines were used: *R26-tdTomato* mice (Stock No. 7914, the Jackson Laboratory) provided by Dr. Masahiro Yamaguchi (Kochi Medical School, Japan), *Neurog2-d4Venus* mice (Kawaue et al., 2014), *NSE-DTA* mice (Imayoshi et al., 2008; Kobayakawa et al., 2007) provided by Dr. Shigeyoshi Itohara (RIKEN, Japan), and *Dcx-EGFP* mice (Gong et al., 2003) provided by the Mutant Mouse Research Resource Center (MMRRC. RRID: MMRRC_000244-MU). The *R26-tdTomato* and *NSE-DTA* lines were on a C57BL6/J genetic background. The *Dcx-EGFP* mouse line was intercrossed with the *R26-tdTomato* reporter mouse line (homozygous). Genotypes were confirmed by PCR on mouse tail clippings. Cre-mediated recombination of the lox-stop-lox cassettes by adenoviral vectors (Ad-CMV-Cre and Ad-CMV-DN-N-cadherin-IRES-Cre) in the *R26-tdTomato* line leads to permanent tdTomato expression ubiquitously. Cre-mediated recombination of the lox-stop-lox cassettes by Ad-CMV-Cre in the *NSE-DTA* line leads to permanent DTA expression under the control of the *NSE* gene promoter, which eliminates neuronal progenies. Mice were age-matched in each experiment. Before delactation, littermates were housed with their mother or foster mouse. After delactation, the animals were divided by gender and group-housed (up to 7 mice per cage). In experiments using adult mice, 8-week-old healthy male mice were used. In other experiments using animals, both male and female healthy mice were used. Littermates were randomly assigned to experimental groups.

Culture of V-SVZ cells

The neonatal V-SVZ was dissected from WT ICR P0-1 pups and dissociated with trypsin-EDTA (Invitrogen, Carlsbad, CA). Both male and female pups were used. The cells were washed twice with L-15 medium (GIBCO, Big Cabin, OK) containing 40 µg/mL DNase I (Roche) and then transfected with 2 µg plasmid DNA using the Amaxa Nucleofector II system (Lonza, Geneva, Switzerland). The transfected cells were suspended in RPMI-1640 medium (Wako, Osaka, Japan), incubated for 15 min at 37°C, and allowed to aggregate, and the aggregates were then cut into blocks (150-200 µm in diameter), mixed with 50% Matrigel (BD Biosciences, Franklin Lake, NJ) in L-15 medium, and plated on dishes. The dishes were maintained in a humidified incubator at 37°C with 5% CO2. The gel containing the aggregates was cultured in serum-free Neurobasal medium (GIBCO) containing 2% B-27 supernatant (Invitrogen), 2 mM L-glutamine (GIBCO), and 50 U/mL penicillin-streptomycin (GIBCO) for 48 h.

METHOD DETAILS

Brain injuries

Postnatal day 2 (P2), P4, P14, and 8-week-old mice were subjected to cryogenic injury as described previously (Ajioka et al., 2015). Briefly, the mice were deeply anesthetized by spontaneous inhalation of isoflurane, and the parietal skull was exposed through a scalp incision. A metal probe (1.5-mm diameter) cooled by liquid nitrogen was stereotaxically placed on the right skull (0.5-mm anterior and 1.2-mm lateral to the bregma), for 30, 60, and 120 s in the P2 and P4, P14, and 8-week-old mice, respectively. The scalp was immediately sutured, and the mice were returned to the home cage. This procedure reproducibly yielded lesions that were 500-600-µm deep.

Hypoxic ischemic injury was induced in P5 mice. During surgery, the mice were deeply anesthetized by spontaneous inhalation of isoflurane. The right common carotid artery was cauterized under a dissecting microscope, followed by a 1-h recovery period, and then by systemic hypoxia (oxygen/nitrogen, 8/92%) for 20 min in a plastic box at 37°C in a humidified atmosphere. After this procedure, the mice were returned to the home cage..

Adenoviral vectors and RNAi constructs

To generate pENTR4-DN-N-cadherin-IRES-Cre, the IRES-Cre fragment from pLV-CMV-tdTomato-IRES-Cre (Dr. Magdalena Götz [Helmholtz Zentrum München]) (Robel et al., 2011) and DN-N-cadherin fragment from pCAG-MCS2-DN-N-cadherin

(Drs. Takeshi Kawauchi [Keio University] and Richard L. Huganir [Johns Hopkins University School of Medicine]) (Nuriya and Huganir, 2006) were amplified by PCR and inserted into the BamHI and SalI sites of pENTR4-H1 (RIKEN), respectively. For N-cadherin knockdown (KD) experiments using adenoviral vectors, the target sequence of the mouse N-cadherin gene was inserted into a modified Block-iT Pol II miR RNAi expression vector containing EmGFP (Invitrogen). As a control, a lacZ target sequence was used as described previously (Ota et al., 2014). To generate pENTR-tdTomato-miR-lacZ and -N-cadherin, the fragment encoding EmGFP in the pENTR-EmGFP-RfA plasmid was removed between BspMI sites, and a tdTomato fragment amplified by PCR from ptdTomato-N1 (Clontech Laboratories, Inc., Mountain View, CA) was inserted. The Gateway system (Invitrogen) was used to generate the following adenoviral vectors: pAd-CMV-DN-N-cadherin-IRES-Cre, pAd-CMV-tdTomato-miR-N-cadherin, and pAd-CMV-tdTomato-miR-lacZ. These vectors were transfected into HEK293A cells to produce adenoviral particles, according to the manufacturer's instructions (Invitrogen). Adenoviral particles were concentrated by cesium chloride density-gradient centrifugation at 25,000 g for 2 h at 4 ºC, followed by 30,000 g for 3 h at 4 ºC, in an ultracentrifuge (himac CP100WX, Hitachi). As a control for Ad-CMV-DN-N-cadherin-IRES-Cre, Ad-CMV-Cre (Vector BioLabs, Malvern, PA) was used.

For the N-cadherin KD experiments using electroporation, the DNA cassettes (tdTomato-miR-N-cadherin and tdTomato-miRlacZ) were cloned into a modified pCAGGS vector using the Gateway system (Invitrogen). For other KD experiments (FAK-KD and L1-CAM-KD), the target sequence of the mouse FAK or L1-CAM gene was inserted into a modified Block-iT Pol II miR RNAi expression vector. The DNA cassettes were cloned into a modified pCAGGS vector using the Gateway system (Invitrogen). All plasmids were prepared using a PureLink HiPure Plasmid Maxiprep Kit (Invitrogen), and the sequences were confirmed by DNA sequencing.

Injection of adenoviral vectors

Since radial glial cells are located at the ventricular surface and extend a long radial fiber toward the pial surface, the injection of a small volume of Ad-Cre into the cortical surface of a reporter mouse leads to retrograde infection through the fibers. Consequently, Cre-loxP-mediated recombination results in the specific and continuous labeling of radial glial cells in the neonatal brain (Merkle et al., 2007). Radial glial cells were labeled using P0 *R26-tdTomato;Dcx-EGFP*, *Dcx-EGFP* or *R26-tdTomato* mice as described previously (Merkle et al., 2007) with some modifications. Briefly, P0 mice were anesthetized by hypothermia (5 min) or spontaneous inhalation of isoflurane, and positioned on the platform of a stereotaxic

apparatus (David Kopf Instruments, Tujunga, CA) by a craniophore. After the parietal skull was exposed through a scalp incision, a 20-nL volume of adenoviral suspension was injected from straight above into the surface of the cerebral cortex, using the following stereotaxic coordinates: +0.5 mm anterior, +1.0 mm lateral from bregma, and +0.3 mm deep from the skull surface. The injection was made with a beveled pulled glass micropipette (Wiretrol 5 µl, Drummond Scientific Company, Broomall, PA). After injection, the scalp was immediately sutured and the mice were returned to their mothers and monitored until they had resumed nursing. To assess the neuronal maturation, a 60-nL volume of adenoviral suspension was injected into P0 mice as described above using the following stereotaxic coordinates: +0.8, +0.5, and +0.2 mm anterior, +1.0 mm lateral from bregma, and +0.3 mm deep from the skull surface, to label radial glial cells in the cortex beyond the injured region. To label V-SVZ cells, a 1 µL volume of adenoviral suspension (Ad-CMV-Cre) was injected into the lateral ventricle of P0 *NSE-DTA* or C57BL6/J mice as described above, using the following stereotaxic coordinates: +1.8 mm anterior, +1.1 mm lateral from Lambda, and +2.0 mm deep from the skull surface. The labeling efficiencies were as follows: Control (Ad-CMV-Cre) at P2, 97.7 ± 0.5% of Nestin+ fibers (n = 3 mice); DN-N-cadherin at P2, 98.0 ± 0.6% (n = 3 mice); $p > 0.05$, unpaired t test; Control (Ad-CMV-Cre) at P9, 99.2 ± 0.2% (n = 4 mice); DN-N-cadherin at P9, 99.0 ± 0.2% (n = 3 mice); $p > 0.05$, unpaired t test; Control (Ad-tdTomato-miR-lacZ) at P9, 98.7 ± 0.4% of Nestin+ fibers (n = 4 mice); N-cadherin-KD at P9, 97.9 ± 0.4% (n = 4 mice); $p > 0.05$, unpaired t test.

Postnatal electroporation

The V-SVZ cells in P0 ICR, C57BL6/J, *R26-tdTomato*, and *NSE-DTA* mice were labeled by electroporation as described previously (Ota et al., 2014) with some modifications. Briefly, the mice were anesthetized by hypothermia (5 min) or spontaneous inhalation of isoflurane and fixed to the platform of a stereotaxic injection apparatus (David Kopf Instruments) by a craniophore. A solution containing EmGFP-expressing pCAGGS plasmid (7.5 µg/mL per pup) and 0.01% fast green was injected into the lateral ventricles of the right hemisphere (1.8 mm anterior, 1.25 mm lateral to lambda, and 2.0 mm deep), and introduced into V-SVZ cells by electronic pulses (70 V, 50 msec, four times) using an electroporator (CUY-21SC; Nepagene, Chiba, Japan) with a forceps-type electrode (CUY650P7). V-SVZ-labeled pups were randomly subjected to cryogenic injury and sponge transplantation. If both adenovirus injection and electroporation were performed on a mouse on the same day (P0), the adenovirus was injected first, and then electroporation was performed at least 8 hours later. The labeling efficiency of V-SVZ cells by pCAGGS-EmGFP electroporation was not statistically different between experimental groups at P2 (Control, 6.3 ± 1.2% of

V-SVZ cells, n = 3 mice; Injury, 6.3 ± 1.7% of V-SVZ cells, n = 3 mice; $p > 0.05$, unpaired t test) or at P30 (Ad-Cre;control, 2.7 ± 0.1%, n = 3 mice; Ad-Cre;NSE-DTA, 2.5 ± 0.0%, n = 3 mice; $p > 0.05$, unpaired t test). The labeling efficiency of DCX+ cells by pCAGGS-EmGFP electroporation (GFP+DCX+ / DCX+ cells) in the injured cortex at P9 was 4.0 ± 0.7% of the DCX+ cells (n = 5 mice). For KD experiments (N-cadherin-KD, FAK-KD, and L1-CAM-KD), plasmid solution (7.5 μg/mL per pup) containing 0.01% fast green was injected into the lateral ventricles of the right hemisphere (1.8 mm anterior, 1.25 mm lateral to lambda, and 2.0 mm deep), and electronic pulses (70 V, 50 msec, four times) were applied by an electroporator (CUY-21SC) with a forceps-type electrode (CUY650P7) in the dorsoventral direction.

Immunoblotting

Immunoblot analysis was performed as described previously (Ota et al., 2014). To check the knockdown efficiency of the miRNAs (N-cadherin, FAK, and L1-CAM), plasmids expressing cDNA (N-cadherin, FAK, and L1-CAM) and miRNA were co-transfected into HEK293T cells using polyethylenimine. pCAG-MCS2-HA-N-cadherin was provided by Drs. Takeshi Kawauchi (Keio University) and Richard L. Huganir (Johns Hopkins University School of Medicine) (Nuriya and Huganir, 2006). pEGFPC1-mouse FAK was provided by Dr. Kozo Kaibuchi (Nagoya University) (Itoh et al., 2010). pCMV6-mouse L1-CAM was purchased from OriGene Technologies, Inc. (Rockville, MD). Forty-eight hours after transfection, the cells were lysed in lysis buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 1% NP-40, 0.01% SDS, 10 μg/mL leupeptin). To check the expression of neuregulin-1α/1β/2, cortex tissues were dissected from WT ICR P6 (4 day post injury) mice, and homogenized in lysis buffer. The lysates were briefly sonicated and cleared by centrifugation. The proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). The membranes were blocked in 5% skim milk in Tris Buffered Saline (TBS) containing 0.01% Tween-20, followed by incubation with primary antibodies at 4°C overnight, and horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Inc., West Grove, PA) at room temperature for 1 h. Signals were detected and measured with enhanced luminal-based chemiluminescent western blotting reagent (GE Healthcare) using a cooled CCD camera (LAS 3000mini, Fujifilm, Tokyo, Japan). The following primary antibodies were used: rat anti-HA antibody (1:1,000, Roche), mouse anti-L1-CAM antibody (1:1,000, Abcam, Cambridge, UK), rat anti-GFP antibody (1:1,000, Nacalai Tesque, Kyoto, Japan), rabbit anti-Neuregulin-1a/1b/2 antibody (1:1000, Santa Cruz Biotechnology, Dallas, TX), and mouse anti-actin antibody

(1:10,000, Millipore). Intensities of signal expressions were calculated using ImageJ software.

Immunohistochemistry

Immunohistochemistry was performed as described previously (Ota et al., 2014). Briefly, the brain was fixed by transcardiac perfusion with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB), and postfixed in the same fixative overnight at 4°C. Floating 60-μm-thick coronal sections were prepared using a vibratome sectioning system (VT1200S, Leica, Heidelberg, Germany). The sections were incubated for 40 min at room temperature (RT) in blocking solution (10% normal donkey serum [Millipore] and 0.2% Triton X-100 in phosphate-buffered saline [PBS]), overnight at 4°C with primary antibodies, and then for 2 h at room temperature with Alexa Fluor-conjugated secondary antibodies (1:500; Invitrogen). For the anti-Nestin antibody, AffiniPure donkey anti-chicken IgY secondary antibodies (Jackson ImmunoResearch Laboratory Inc.) were used. In the sponge transplantation experiments (Figures 3J, 3K, and 4G), 200-μm-thick coronal sections were treated with 100% methanol for 30 min at -30°C, acetone for 30 min at -30°C, 0.3% H2O2 in methanol for 2 h at RT, and 50% methanol for 15 min at RT before incubation in blocking solution (10% normal donkey serum and 0.5% Triton X-100 in PBS). Signal amplification was performed with biotinylated secondary antibodies (Jackson ImmunoResearch Laboratory Inc.) and the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA), and the signals were visualized using the TSA Fluorescence System (PerkinElmer, Waltham, MA). For Mash1 staining, the sections were treated with acetone for 60 s on ice. For double staining using anti-Pax6, anti-ErbB4 or anti-Olig2 and anti-DsRed antibodies, sequential immunostaining was performed with AffiniPure Fab Fragment Donkey Anti-Rabbit IgG (H+L) (Jackson ImmunoResearch Laboratories, Inc.). For double staining using anti-palvalbumin (PV), anti-calretinin (CR), or anti-GAD67 and anti-NeuN antibodies, AffiniPure Fab Fragment Donkey Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, Inc.) was used. The following primary antibodies were used: rabbit anti-Dcx (1:200, Cell Signaling Technology, Beverly, MA), guinea pig anti-Dcx (1:3,000, Millipore), goat anti-Dcx antibody (1:500, Santa Cruz Biotechnology), rat anti-GFP (1:500, Nacalai), chicken anti-Nestin (1:1,000, Aves Labs, Tigard, OR), rabbit anti-DsRed (1:1,000, Clontech), mouse anti-NeuN antibody (1:200, Millipore), mouse anti-CR (1:3,000, Millipore), mouse anti-PV (1:2,000, Sigma, St. Louis, MO), mouse anti-Mash1 (1:100, BD), rabbit anti-Tbr2 (1:200, Abcam), rabbit anti-Pax6 (1:100, Covance, Princeton, NJ), mouse anti-N-cadherin (1:200, BD), mouse anti-glial fibrillary acidic protein (GFAP) (1:500, Sigma-Aldrich), rabbit anti-Olig2 (1:200, IBL, Gunma, Japan), mouse

anti-GAD67 (1:800, Millipore), rabbit anti-ErbB4 (1:300, Abcam), rabbit anti-FAK (1:100, Millipore), and mouse anti-L1-CAM (1:1,000, Abcam). The guinea pig anti-Dlx2 antibody (1:3,000) was kindly provided by Dr. Kazuaki Yoshikawa (Osaka University) (Kuwajima et al., 2006). For nuclear staining, Hoechst 33342 (1:3,000, Thermo Fisher Scientific, Waltham, MA) was used.

Images of neuronal progenitors, radial glial fibers, mature neurons, and migrating neuroblasts associated with radial glial fibers, sponges or polyethylene terephthalate (PET) fibers were acquired by scanning at 1-μm intervals using an LSM 700 confocal laser-scanning microscope (Carl Zeiss, Jena, TH, Germany) with a 20×3 and 40×3 objective lens. In Figure 3 (A and B), composite images of eight separate fields (two vertical and four horizontal tiles) were acquired using the tile-scan feature of ZEN software (Carl Zeiss) with a 20×3 objective. To characterize the Dcx+, CR+, PV+, GAD67+, or NeuN+ neurons, the co-localization of signals in the cortex was confirmed by scanning at 1-μm intervals. To quantify the EmGFP+ cells in the V-SVZ and neuroblasts in the injured cortex, the cells were counted stereologically using a Stereo Investigator system (MBF Bioscience, Williston, VT). After adenoviral injection and electroporation, the mice were randomly subjected to cryogenic injury and sponge transplantation. For the analyses of neuronal progenitors and migrating neuroblasts, the actual number of cells in every sixth 60-μm-thick coronal section was counted, and then the total number was estimated by multiplying the sum of the counted cells by six. To examine the radial glial fiber length and morphology, three sequential 60-μm-thick coronal sections were analyzed. In the analysis of neuroblast and radial glial fiber associations, an ‘‘association’’ was defined as ‘‘less than 2 μm between the neuroblast and fiber,’’ based on previous studies (Shikanai et al., 2011). For the mature neuron analyses, all of the EmGFP+NeuN+ cells in the injured sensory and motor cortex (M2/M1/S1HL/S1FL/MPtA/LPtA/S1Tr) (Paxinos et al., 2007) were analyzed. The actual number of cells in every second 60-μm-thick coronal section was counted, and then the total number was estimated by multiplying the sum of the counted cells by two. In the sponge transplantation experiments (Figures 3K and 4G), 200-μm-thick coronal sections were used to preserve the sponge in the injured regions. The morphology of tdTomato+ radial glial cells was reconstructed and quantified using Neurolucida (MBF Bioscience).

Transmission electron microscopy

P9 mouse brain infected with control or DN-N-cadherin-expressing adenovirus was fixed by transcardiac perfusion with 2.5% glutaraldehyde (GA) and 2% PFA in 0.1 M PB (pH 7.4) at 4°C. The excised brain tissue was cut into 200-μm coronal sections on a vibratome (VT1200S, Leica). The sections were treated with 2%

OsO4 in the same buffer for 2 h at 4°C. The brain tissue was then dehydrated in a graded ethanol series, placed in propyleneoxide, and embedded in Durcupan resin for 72 h at 60°C to ensure polymerization. Semi-thin sections (1.5-μm-thick) were sequentially cut and stained with 1% toluidine blue, and then sections of interest were identified by light microscopy. Ultra-thin sections (60-70 nm) were then cut from the semi-thin sections using an ultramicrotome (UC6, Leica) with a diamond knife, and stained with 2% uranyl acetate in distilled water for 15 min and with modified Sato's lead solution for 5 min. The sections were analyzed with a transmission electron microscope (JEM-1400plus; JEOL, Tokyo, Japan). The lengths of the AJ-like electron-dense adhesion structures and irregular contacts were quantified using ImageJ software (National Institutes of Health). Neuroblasts were identified by their dark cytoplasm with many free ribosomes and electron-dense nucleus, and radial glial cells were identified by their electron-lucent nuclei, and light cytoplasm with glycogen granules and abundant intermediate filaments. The numbers of analyzed cells were as follows: control, 21 cells from 2 mice; DN-N-cadherin, 17 cells from 2 mice.

Time-lapse imaging of injured brain slices

Brain slices were prepared for time-lapse imaging from neonatal 4-5 d-post-injury *R26-tdTomato;Dcx-EGFP* mice after the injection of adenoviral vectors (at P0). Briefly, the brain was dissected and cut into coronal slices (200-μm thick) using a vibratome (VT1200S, Leica). The slices were placed on a stage-top imaging chamber (Warner Instruments, Hamden, CT, USA) and kept under continuous perfusion with artificial cerebrospinal fluid (aCSF, 1 mL/min, containing 125 mM NaCl, 26 mM NaHCO3, 3 mM KCl, 2 mM CaCl2, 1.3 mM MgCl2, 1.25 mM NaH2PO4, and 20 mM Glucose, pH 7.4, maintained at 38°C, bubbled with 95% O2 and 5% CO2) during the imaging. Using a confocal laser microscope (LSM710, Carl Zeiss) equipped with a gallium arsenide phosphide detector, z stack images (4 z sections with 3–5-μm step sizes) were captured every 10 min for 6-16 h. The attachment of migrating neuroblasts to fibers was evaluated as the proportion of time spent in the fiber-attached phase during the migration process. To quantify the speed, stride length, resting phase, and cycle of neuroblast migration along radial glial fibers in captured images, neuroblasts in the cortex with a monopolar or bipolar shape were traced using ImageJ software (manual tracking plugin). The speed of the fiber extension was analyzed using ImageJ software. All of the neuroblasts that could be continuously tracked for at least 60 min were used for this analysis. For the assessment of migration cycle, all of the neuroblasts that could be continuously tracked for at least 1 cycle of saltatory movement were used. We defined cells in the 'resting phase' as those in which the soma moved slower than 12 μm/h. In Figures 2A and S1K,

numbers indicate minutes from the first frame.

Preparation of N-cadherin-Fc-sponge

The gelatin (GE) sponge was prepared as described previously (Ajioka et al., 2015) with some modification. Fifty microliters of 3% GE beMatrix Gelatin LS-H (Nitta Gelatin, Osaka, Japan) was added to each well of a 384-well plate and frozen at -20°C. The frozen GE samples were then lyophilized at 25°C, with centrifugation at 400 rpm (VC-96W; Taitek, Saitama, Japan). The freeze-dried GE samples were then crosslinked with 25 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (Wako) in 90% acetone at room temperature overnight. After washing five times with double distilled water, the GE sponge was incubated in Neurobasal medium (GIBCO) for 3 h. Then, GE sponge was cut into blocks (1.2 x 1.2 x 1.2 mm^3) and conjugated with 10μg/mL N-cadherin-Fc (IgG-Fc fused with the extracellular domain of mouse N-cadherin) or Fc solutions (Yue et al., 2010) for 24 h at 4°C.

Preparation of N-cadherin-PET fibers

PET fibers 24 μm in diameter were kindly supplied by Toray Industries (Shiga, Japan) (Inoue et al., 2009). PET fibers were coated with N-cadherin-Fc (IgG-Fc fused with the extracellular domain of mouse N-cadherin) or with Fc solution (Yue et al., 2010) for 1 h at 37°C, then rinsed with PBS 5 times.

Transplantation of N-cadherin-sponges or -fibers

N-cadherin-Fc-sponge or control Fc-sponge, or N-cadherin-Fc-PET fibers or control Fc-PET fibers, was transplanted as described previously (Ajioka et al., 2015). Briefly, 3 or 10 days after cryogenic injury induction, the mice were anesthetized by spontaneous inhalation of isoflurane. The previous incision was cut to expose the injured parietal skull and opened by tweezers. N-cadherin-Fc- or Fc-sponge (1.2 x 1.2 x 1.2 mm^3) was placed into the cavity by tweezers. In the PET-fiber transplantation, fibers at a density of about 1.2 x 1.2 x 1.2 mm^3 N-cadherin-Fc- or control-Fc-fibers (1.2 mm in length) were implanted into the cavity by tweezers. After transplantation, the sponge was covered with parietal skull, and the scalp skin was sealed. After transplantation, the mice were placed on a warm heater to recover.

In vitro cell culture

A stripe assay enabled us to analyze the migratory behaviors of single neuroblasts crossing the border between control-Fc and N-cadherin-Fc stripes. For the first stripes, 10 μg/mL N-cadherin-Fc was combined with 3 μg/mL

FITC-conjugated anti-human-IgG Fc antibody (Sigma) in Hank's balanced salt solution (HBSS). For the second (control) stripes, 10 µg/mL Fc was combined with 3 µg/mL anti-human-IgG Fc antibody (Sigma) in HBSS. After preincubating both stripe solutions for 30 min under moderate agitation at 4°C, 100 µL of the first stripe solution was injected into silicon matrices (50-µm wide, from Prof. Martin Bastmeyer [Cell- and Neurobiology at the Karlsruhe Institute of Technology]) placed on glass-bottom 35-mm Petri dishes. After a 30-min incubation at 37°C, the dishes and matrices were rinsed with 500 µL of HBSS, and the matrices were carefully removed. The dishes were then coated with 100 µL of the second stripe solution. After a 30-min incubation at 37°C, the dishes were washed three times with HBSS. The neonatal V-SVZ was dissected from WT ICR P0-1 pups and dissociated with trypsin-EDTA (Invitrogen). The cells were washed twice with L-15 medium (GIBCO) containing 40 µg/mL DNase I (Roche) and then transfected with 2 µg plasmid DNA (pCAGGS-tdTomato-miR-N-cadherin or -LacZ miRNA) using the Amaxa Nucleofector II system (Lonza). The transfected cells were suspended in RPMI-1640 medium (Wako) and allowed to aggregate, and the aggregates were then cut into blocks (150-200 µm in diameter), mixed with 50% Matrigel (BD Biosciences) in L-15 medium, and plated on the stripes.

For the neuronal culture with N-cadherin-Fc-sponge or N-cadherin-Fc-fibers, the V-SVZ cell aggregates were placed next to N-cadherin-Fc- or control-Fc-sponge, or N-cadherin-Fc- or control-Fc-fibers in 50% Matrigel. The dishes were maintained in a humidified incubator at 37°C with 5% CO_2. The gel containing the aggregates was cultured in serum-free Neurobasal medium (GIBCO) containing 2% B-27 supernatant (Invitrogen), 2 mM L-glutamine (GIBCO), and 50 U/mL penicillin-streptomycin (GIBCO) for 48 h.

Time-lapse video recordings were obtained using an inverted light microscope (Axio-Observer, Carl Zeiss) equipped with the Colibri light-emitting diode light system, using ×20 dry objective lens. Images were obtained automatically every 3 min (Figures 3D, 3E, S4A, S4C, and S4D) or 5 min (Figures 2K–2P), for 24 h. The migration speeds were quantified using ImageJ software. All of the neuroblasts that could be continuously tracked for at least 60 min were used for this analysis. For the assessment of the migration cycle, all of the neuroblasts that could be continuously tracked for at least 1 cycle of saltatory movement were used. We defined cells in the 'resting phase' as those in which the soma moved slower than 12 µm/h. In Figures 2K, 3D, and S4A, numbers indicate minutes from the first frame.

Immunocytochemistry

Cultured neurons on coverslips were rinsed in PBS (pH 7.4) and fixed with 4%

PFA in 0.1 M PB at room temperature for 30 min. After a 40-min pre-incubation in blocking solution (10% normal donkey serum [Millipore] and 0.2% Triton X-100 in PBS), the cells were incubated with primary antibodies at 4°C overnight. The following primary antibodies were used: rabbit anti-Dcx (1:200, Cell Signaling Technology), rabbit anti-DsRed (1:1,000, Clontech), and mouse anti-N-cadherin (1:200, BD). The mouse anti-PSA-NCAM antibody (1:1,000) was a kind gift from Dr. Tatsunori Seki (Tokyo Medical University) (Seki and Arai, 1991). For nuclear staining, Hoechst 33342 (1:3,000, Thermo Fisher Scientific) was used. The multi-labeled cultured cells were analyzed with an LSM700 confocal laser-scanning microscope (Carl Zeiss) (Figures S2K and S2L), and more than 3 random fields were chosen under a 40x objective from each coverslip for quantification. The cell bodies of PSA-NCAM+tdTomato+ neuroblasts were traced, and the intensity of N-cadherin expression was calculated using ZEN software (Carl Zeiss). At least three independent experiments were performed for each quantification.

FRET imaging

FRET imaging of the RhoA activity in cultured migrating neuroblasts was performed as described previously (Ota et al., 2014). The FRET probe for RhoA (Raichu-1298X) (Yoshizaki et al., 2003), a gift from Dr. Michiyuki Matsuda (Kyoto University), was introduced into cultured V-SVZ-derived neuroblasts by electroporation using the Amaxa Nucleofector II system. Time-lapse imaging of the FRET-probe-expressing neuroblasts was performed using an LSM710 laser-scanning confocal microscope (Carl Zeiss) with a 40× water-immersion objective lens. The FRET ratio (intensity of FRET/CFP) was calculated, and the final images were generated using the MetaMorph software ratio image function (Molecular Devices, Sunnyvale, CA). The baseline RhoA activity was calculated by averaging the basal activities in the leading shaft, and defining the average in each cell as 1.0. The extent of RhoA activation in the proximal leading process in a circular region of interest (ROI) (= $RhoA^{prox}$) was measured using the MetaMorph software Region measurements function, and normalized to the baseline activity in each frame (RhoA activation = $RhoA^{prox}$ -1). All of the probe-expressing bipolar neuroblasts were analyzed in each experiment. Three independent experiments were performed. In Figures 2H and 2I, numbers indicate minutes from the first frame.

Behavior tests

Mice were subjected to quantitative neurological testing at P30. The body weight was not statistically different among experimental groups. We analyzed the gait behaviors on an elevated wire hexagonal grid (Foot-fault test), in which the motor

function involved in accurate limb placement, which is integrated with sensory feedback from the planta, is assessed (Barth et al., 1990). The foot-fault test was performed at 23 ± 1°C. Briefly, mice were placed on an elevated wire hexagonal grid with 40-mm wide openings, and allowed to roam freely. A misstep was recorded as a foot fault when the mouse slipped or fell with one of its limbs dropping into an opening in the grid. The number of foot-faults for each limb was separately counted for 5 min, and then the ratio of the number of contralateral (left) fore- and hindlimb faults to the total number for the four limbs was calculated as a percentage. The test was performed twice, and the values were averaged.

Gait analysis was performed using the Noldus CatWalk XT (Noldus Information Technology, Wageningen, the Netherlands), an automated gait analysis system, according to the manufacturer's instructions. Briefly, in a dark environment at 23 ± 1°C, the mice were allowed to walk across a glass walkway illuminated with a green light that was completely reflected internally except at the points receiving pressure. The contact point of each paw on the glass was illuminated, which was recorded with a high-speed video camera. The footprints recorded during each trial were analyzed using the CatWalk XT 10.5 software to generate a series of parameters. At least three successful sustained walk recordings for each mouse were used for each analysis, and the average of the runs was reported. All of the parameters measured by Catwalk are shown in Table S1.

Experimental design

The number of mice, cells, and experimental replication can be found in the respective figure legend. No specific strategy for randomization was employed, and no blinding was used, except for the stereological counting of EmGFP+ cells in the V-SVZ and neuroblasts in the injured cortex using a Stereo Investigator system. No statistical calculation was used to estimate the sample size. Sample sizes for experiments were determined according to previous studies (Ota et al., 2014; Fujioka et al., 2017). We included animals with cryogenic lesions that were 500-600-μm deep in the analyses.

QUANTIFICATION AND STATISTICAL ANALYSIS

All data are shown as the mean ± SEM (standard error of the mean). Two groups were compared using a two-tailed paired or unpaired t test, Wilcoxon signed-rank test, and Mann-Whitney U-test. Multiple group comparisons were performed by one-way ANOVA followed by a Tukey multiple comparison test or Dunnett test, or by a Kruskal-Wallis test followed by a Steel-Dwass multiple comparison test or Steel test. A Shapiro-Wilk test was used to assess normality. A

P-value less than 0.05 was considered to be statistically significant. The statistical test used and the statistical parameters are as below: Figures 1G and 1I, (G) n = 3 mice each; unpaired t-test, *p < 0.05; (I), n = 4 mice each; unpaired t-test, ***p < 0.005. Figures 1H and 1J, (H) control, n = 4 mice; DN-N-cad, n = 5 mice; paired and unpaired t-test, ***p < 0.005; (J) control, n = 4 mice; N-cad-KD, n = 4 mice; paired and unpaired t-test, *p < 0.05, *p < 0.01. Figure 1M, control, n = 21 cells; DN-N-cad, n = 17 cells; unpaired t-test, **p < 0.01, ***p < 0.005. Figure 2B, 2E, and 2F, control, n = 42 cells from 8 mice; DN-N-cad, n = 60 cells from 12 mice; Mann-Whitney U-test, ***p < 0.005. Figure 2C, control, n = 63 cells from 8 mice; DN-N-cad, n = 107 cells from 12 mice; Mann-Whitney U-test, ***p < 0.005. Figure 2D, control, n = 63 cells from 8 mice; DN-N-cad, n = 107 cells from 12 mice; Fisher's exact test, ***p < 0.005. Figure 2G, control, n = 39 cells from 8 mice; DN-N-cad, n = 37 cells from 10 mice; Mann-Whitney U-test, ***p < 0.005. Figure 2J, n = 9 cells, three independent experiments, paired t-test, *p < 0.05. Figure 2L, control, n = 15 cells (five independent experiments); N-cad-KD, n = 27 cells (six independent experiments), paired t-test, ***p < 0.005. Figure 2M; control, n = 15 cells (five independent experiments); N-cad-KD, n = 27 cells (six independent experiments), paired t-test and Wilcoxon signed-rank test, ***p < 0.005. Figure 2N, control, n = 16 cells (five independent experiments); N-cad-KD, n = 26 cells (six independent experiments), Wilcoxon signed-rank test, ***p < 0.005. Figure 2O, control, n = 16 cells (five independent experiments); N-cad-KD, n = 23 cells (five independent experiments), Wilcoxon signed-rank test, ***p < 0.005. Figure 2P, control, n = 27 cells (five independent experiments); N-cad-KD, n = 18 cells (four independent experiments), Chi-squared test with Yates' continuity correction. *p < 0.05. Figure 3C, control, n = 6 mice; DN-N-cad, n = 7 mice; unpaired t-test, *p < 0.05. Figure 3E, control-non-contact,

n = 14 cells; control-contact, n = 19 cells; N-cad-non-contact, n = 19 cells; N-cad-contact, n = 28 cells; three independent experiments; unpaired t-test, ***p < 0.005. Figure 3I, P2 (3 dpi), control, n = 7 mice, N-cad, n = 7 mice; P14 (3 dpi), control, n = 6 mice, N-cad, n = 5 mice; 8w (3 dpi), control, n = 7 mice, N-cad, n = 7 mice; P2 (10 dpi), control, n = 4 mice, N-cad, n = 5 mice; unpaired t-test, **p < 0.01, ***p < 0.005; control, P2 (3 dpi) vs P14 (3 dpi) or 8w (3 dpi), one-way ANOVA followed by Tukey multiple comparison test, ###p < 0.005; N-cad, (P2 [3 dpi] vs P14 [3 dpi], 8w [3 dpi], or P2 [10 dpi], ##p < 0.01, ###p < 0.005), (P14 [3 dpi] vs 8w [3 dpi], §§p < 0.01); one-way ANOVA followed by Tukey test. Figure 3K, control, n = 10 mice; N-cad, n = 8 mice; left, unpaired t-test; right, Chi-squared test, *p < 0.05. Figures 4A–4C, n = 10 mice; one-way ANOVA followed by Tukey test, except for (A) right (Kruskal-Wallis test followed by Steel-Dwass test), *p < 0.05, **p < 0.01, ***p < 0.005. Figure 4D, P2 model, control, n = 11 mice; injury, n = 10 mice; injury + control-sp, n = 13 mice; injury

+ N-cad-sp, n = 14 mice, Kruskal-Wallis test followed by Steel-Dwass test; P14 and 8w models, n = 7 mice each, one-way ANOVA followed by Tukey test. *$p < 0.05$, ***$p < 0.005$. Figure 4G, control, n = 5; NSE-DTA, n = 4; unpaired t-test, *$p < 0.05$. Figure 4H, control, n = 11 mice; NSE-DTA, n = 7 mice; unpaired t-test, ***$p < 0.005$. All statistical data, including the statistical tests used, mean ± SEM, and P values are indicated in the text, figure legends, figures, Table S1, and STAR Methods (method details and this sections). Values with error bars in the figures indicate mean ± SEM. Littermates were randomly assigned to experimental groups.

DATA AND SOFTWARE AVAILABILITY

All software was commercially or freely available, and is listed in the STAR Methods description and Key Resource Table. The accession number for the Catwalk raw data reported in this paper is Mendeley data: http://dx.doi.org/10.17632/t4r38rrf3d.1.

Supplemental items

Table S1. Catwalk parameters, Related to Figure 4.

Group1, spatial parameters related to individual paws; Group 2, relative spatial relationships between different paws; Group 3, interlimb coordination; Group 4, temporal parameters (Neumann et al., 2009). LF, left frontpaw; RF, right frontpaw; LH, left hindpaw; RH, right hindpaw. *$p<0.05$, **$p<0.01$, ***$p<0.005$ for Injury compared to Control; †$p<0.05$, ††$p<0.01$, †††$p<0.005$ for Injury + control-sponge compared to Control; ‡$p<0.05$, ‡‡$p<0.01$, ‡‡‡$p<0.005$ for Injury + N-cadherin-sponge compared to Control; §$p<0.05$ for Injury + control-sponge compared to Injury; ‖$p<0.05$, ‖‖$p<0.01$, ‖‖‖$p<0.005$ for Injury + N-cadherin-sponge compared to Injury; ¶$p<0.05$, ¶¶¶$p<0.005$ for Injury + N-cadherin-sponge compared to Injury + control-sponge.

Movie S1. Migratory behaviors of cultured neuroblasts on Fc- and N-cadherin-Fc stripes, Related to Figure 2.

The behavior of migrating neuroblasts (red) was recorded at 5-min intervals. Green color shows N-cadherin-Fc stripes. Sequential images of these neuroblasts are shown in Figure 2K.

Movie S2. Time-lapse imaging of cultured neuroblasts migrating along control and N-cadherin-sponge, Related to Figure 3.

The behavior of migrating neuroblasts (red) was recorded at 3-min intervals. Sequential images of these neuroblasts are shown in Figure 3D.