

SHORT
COMMUNICATION

Rac1-mediated indentation of resting neurons promotes the chain migration of new neurons in the rostral migratory stream of post-natal mouse brain

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New neurons generated in the ventricular-subventricular zone in the post-natal brain travel toward the olfactory bulb by using a collective cell migration process called ‘chain migration.’ These new neurons show a saltatory movement of their soma, suggesting that each neuron cycles through periods of ‘rest’ during migration. Here, we investigated the role of the resting neurons in chain migration using post-natal mouse brain, and found that they undergo a dynamic morphological change, in which a deep indentation forms in the cell body. Inhibition of Rac1 activity resulted in less indentation of the new neurons *in vivo*. Live cell imaging using a Förster resonance energy transfer biosensor revealed that Rac1 was activated at the

sites of contact between actively migrating and resting new neurons. On the cell surface of resting neurons, Rac1 activation coincided with the formation of the indentation. Furthermore, Rac1 knockdown prevented the indentation from forming and impaired migration along the resting neurons. These results suggest that Rac1 regulates a morphological change in the resting neurons, which allows them to serve as a migratory scaffold, and thereby non-cell-autonomously promotes chain migration.

Keywords: adult neurogenesis, collective cell migration, Rho family small GTPase.

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Neuronal migration is critical, not only for embryonic development but also in the post-natal and adult brain (Hatten 2002; Marin and Rubenstein 2003; Ghashghaei *et al.* 2007; Sawada *et al.* 2011). In the olfactory system, new neurons generated in the ventricular-subventricular zone (V-SVZ) form elongated cell aggregates called ‘chains,’ which enter a highly restricted migratory route termed the rostral migratory stream (RMS) (Altman 1969; Lois and Alvarez-Buylla 1994; Wichterle *et al.* 1997; Kaneko *et al.* 2010). The cell chains move toward the olfactory bulb (OB) by ‘chain migration,’ providing a unique model for studying stream-type collective cell migration (Rorth 2009). The neurons within the chains migrate along each other and do not require radial glial fibers or pioneer axons as a scaffold (Lois and Alvarez-Buylla 1994; Wichterle *et al.* 1997). Previous studies have shown that well-organized intercellular association among the new neurons themselves is important for efficient chain migration (Ono *et al.* 1994; Chazal *et al.* 2000; Battista and Rutishauser 2010). However, how new

neurons control this intercellular association is largely unknown.

Rho-family GTPases play crucial roles in the regulation of cell migration, which involves cytoskeletal and adhesion proteins (Luo 2000; Fukata *et al.* 2003; Govek *et al.* 2005; Jaffe and Hall 2005). The small GTPase Rac1 is critical for cell migration, membrane ruffling, actin polymerization, and cell polarization (Ridley *et al.* 1992; Ehrlich *et al.* 2002;

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Abbreviations used: Dcx, doublecortin; EmGFP, emerald green fluorescent protein; FRET, Förster resonance energy transfer; OB, olfactory bulb; RMS, rostral migratory stream; V-SVZ, ventricular-subventricular zone.

Pankov *et al.* 2005), and plays a key role in the collective cell migration that precedes organ development during embryogenesis (Migeotte *et al.* 2010; Wang *et al.* 2010; Dumortier *et al.* 2012; Woo *et al.* 2012; Ramel *et al.* 2013). Previous studies have shown that Rac1 is involved in the cell survival and proliferation of progenitor cells in the V-SVZ and in the neurite outgrowth of V-SVZ-derived neurons (Khodosevich and Monyer 2010; Leone *et al.* 2010). However, the function and spatiotemporal regulation of Rac1 in chain migration are largely unknown.

Here, we report that Rac1 regulates the morphology of 'resting' neurons to promote efficient chain migration. We found that new neurons in the resting phase form a large indentation in their cell body. Inactivating Rac1 in the resting neurons caused impaired indentation and decreased the speed of the migrating neurons. We propose that, during chain migration, the resting-phase neurons change their morphology via Rac1 activity, to create a path for the active-phase migratory neuronal chains, and thus serve as a migratory scaffold.

Methods

Animals

Institute of Cancer Research (ICR) mice were purchased from SLC (Shizuoka, Japan). All the experimental procedures involving mice complied with the ARRIVE Guidelines, were reviewed by the Institutional Laboratory Animal Care and Use Committee, and were approved by the President of Nagoya City University. In all experiments, mice of either sex were used.

Antibodies

The antibodies used in this study included mouse anti-Rac (Sigma, St. Louis, MO, USA), mouse anti- α Tubulin (DM1A) (Sigma), goat anti-Doublecortin (Dcx) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and rat anti-green fluorescent protein (GFP) (Nacal Tesque, Kyoto, Japan) antibodies.

Immunohistochemistry

Brains were fixed by transcardiac perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer and post-fixed overnight in the same fixative, and 60 μ m thick floating sagittal sections were prepared using a vibratome (VT1200S, Leica, Heidelberg, Germany) as reported previously (Kaneko *et al.* 2010). For immunostaining, the sections were incubated for 1 h in blocking solution (10% donkey serum and 0.2% Triton X-100 in phosphate-buffered saline) overnight at 4°C with the primary antibodies, and for 2 h at 25°C with Alexa Fluor-conjugated secondary antibodies (Invitrogen, Carlsbad, CA, USA). Nuclei were stained with Hoechst33342 (Sigma). Confocal images were obtained using an LSM700 laser-scanning microscope system (Carl Zeiss, Oberkochen, Germany).

Quantitative analysis

In the sagittal sections, the RMS-OB route was divided at the line crossing the granule cell layer, and the anterior part was defined as 'OB' and posterior part was defined as 'RMS'. To quantify the

distribution of GFP/Dcx double-positive cells in the RMS and OB, images of the cells in 60 μ m thick sagittal serial sections were acquired using a BX51 fluorescence microscope (Olympus, Tokyo, Japan) or the LSM700 (Carl Zeiss), and the cells in the RMS and OB were counted. To prevent counting the same cells twice, we counted all the GFP⁺ cells for which the entire cell soma existed in the section. A neuron was considered to have an indentation when its soma was asymmetrically indented > 1.5 μ m.

RNA interference constructs

For the knockdown experiments, the targeting sequences of mouse Rac1 (Rac1 BLOCK-ITTM miR RNAi Select Mmi518256, Invitrogen) and LacZ (control) were cloned into a modified pCAGGS vector (Niwa *et al.* 1991) using the Gateway system (Invitrogen).

Electroporation *in vivo*

The electroporation was performed in P1 mice as described previously (Boutin *et al.* 2008; Hirota *et al.* 2012) with some modifications. Briefly, mice were anesthetized by isoflurane and fixed to the platform of a stereotaxic injection apparatus (David Kopf Instruments, Tujunga, CA, USA). The plasmid solution (1.5 μ L of a 4 μ g/ μ L solution) containing 0.01% Fast Green was injected into the lateral ventricles of each mouse, and electronic pulses (70 V, 50 msec, four times) were applied using an electroporator (CUY-21SC; Nepagene, Chiba, Japan) with a forceps-type electrode (CUY650P5).

Time-lapse analyses of new neurons migrating in brain slices

Organotypic brain slices were prepared from P4–5 mice (3 days after electroporation) as reported previously (Hirota *et al.* 2007, Kaneko *et al.* 2010, Murase & Horwitz 2002, Suzuki & Goldman 2003) with modifications. Dissected brains were cut into 150 μ m thick sagittal slices with a vibratome, then cultured on a Millicell Cell Culture Insert (Millipore, Billerica, MA, USA) submerged in Neurobasal medium (Invitrogen) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 2% B-27 (Invitrogen), and 50 U/mL penicillin–streptomycin. Before recording, the slices were cultured for 2 h in an incubator at 37°C, 5% CO₂ (Incubator XL Dark S1, Carl Zeiss). Time-lapse video recordings were obtained using an inverted light microscope (Axio-Observer, Carl Zeiss) equipped with the Colibri light emitting diode light system (Carl Zeiss), at low magnification using a 10 $\times$ dry objective lens. Every 6 min, images were obtained automatically, for 8–12 h. The migration speed was quantified using NIH ImageJ software (NIH, Bethesda, MD, USA).

FRET-based imaging of active Rac1

The Förster resonance energy transfer (FRET) probe for Rac1 (pRaichu-1011x) was kindly provided by Dr Michiyuki Matsuda (Aoki *et al.* 2004). The dissociated SVZ cells from P1–3 WT mice were transfected with pRaichu-1011x using an Amaxa Nucleofector II (Lonza, Basel, Switzerland). Time-lapse images were captured at 10-min intervals for 6 h using a Zeiss LSM 710 laser-scanning confocal microscope. The cyan fluorescent protein (CFP) channel was excited using a 458-nm argon line. The two emission channels were split using a 545-nm dichroic mirror, and a 475–525-nm bandpass filter for CFP and a 530-nm longpass filter for YFP (Chroma, Bellows Falls, VT, USA). The ratio image of FRET/CFP

was created with MetaMorph software (Molecular Devices, CA, USA) and used to represent the FRET ratio, which was calculated by the following equation: FRET ratio = intensity of FRET/CFP.

Statistical analysis

Data are shown as the mean $\pm$ SEM. Two groups were compared using an unpaired, two-tailed *t*-test. Multiple groups were compared by the Tukey–Kramer method. A value of $p < 0.05$ was considered statistically significant.

Results and discussion

Resting neurons undergo a change in cell morphology

Individual chain-migrating neurons show saltatory migration, executed by the repeated growth of the leading process (resting phase) followed by rapid advancement of the soma (migrating phase) (Bellion *et al.* 2005). In the chain, after a migrating neuron passes over a resting neuron, the resting neuron resumes its migration. Therefore, we hypothesized

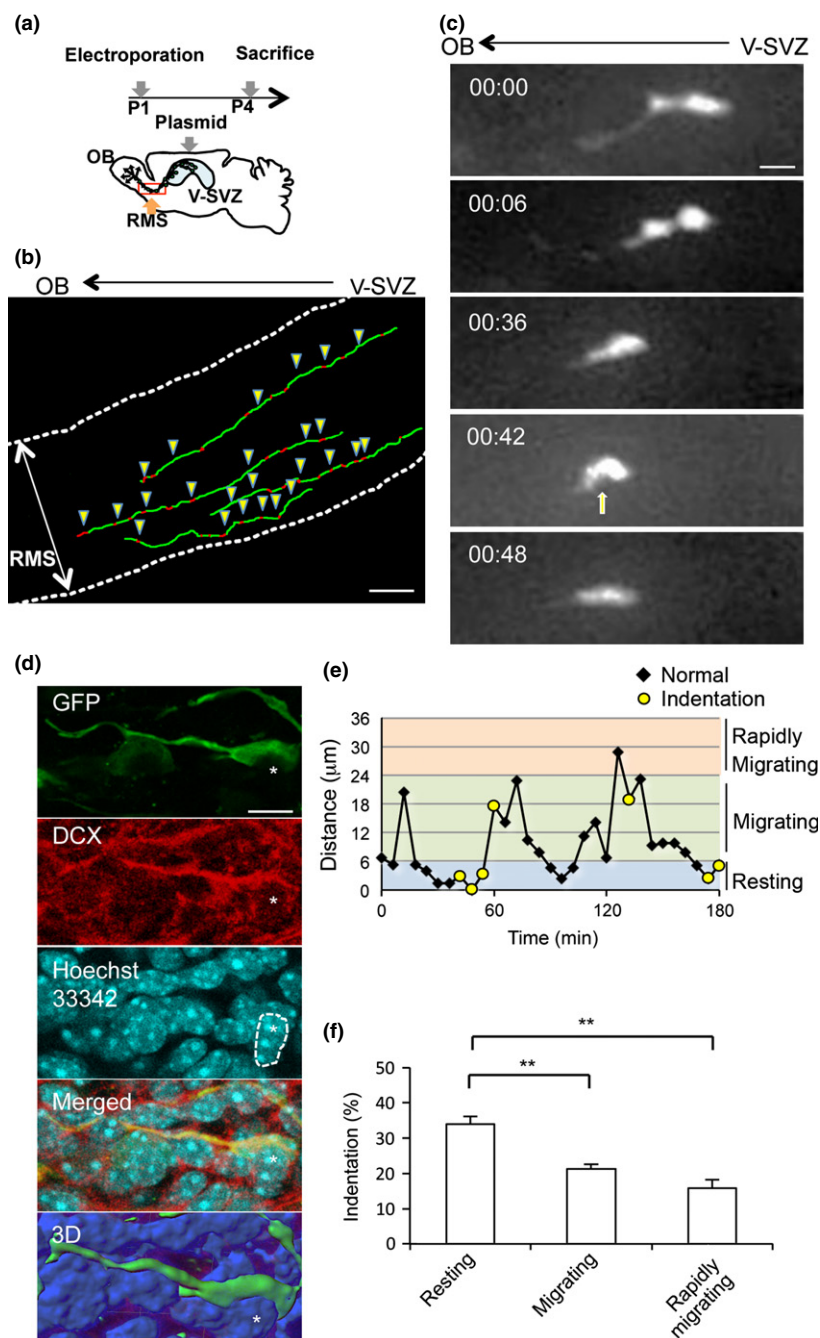


Fig. 1 Analysis of chain migration. (a) Schema of the experimental protocol. (b) Trajectory of migrating neurons in the rostral migratory stream (RMS) of P4 mice. Green lines: migrating phase; Red lines: resting phase; Arrowheads: Indentation. (c) Time-lapse sequence of migrating new neurons. Arrow indicates an indentation on a cell soma. (d) High-magnification images of an indented cell in the RMS. Asterisks: nucleus in the indentation. (e) Soma motility and indentation. The migration distance every 6 min was plotted. Yellow circles: indentation. (f) Quantification of the indentations formed during chain migration. The percentage of indented neurons in the resting, migrating, or rapidly migrating neurons is shown. Scale bars: 200 μ m (b), 10 μ m (c and d). ** $p < 0.01$.

that neurons in the migrating phase use neurons in the resting phase as a migratory scaffold. To analyze the movement of chain-migrating new neurons, we introduced an emerald green fluorescent protein-expressing vector by electroporation into the V-SVZ of P1 mice, and performed time-lapse imaging of the RMS (Fig. 1a). As expected, most of the neurons showed saltatory movement of the soma (migrating phase, green lines in Fig. 1b) and resting (resting phase, red lines in Fig. 1b). Quantitative analyses revealed that the neurons spent $44.8 \pm 6.0\%$ of their time in the migrating phase, and $55.2 \pm 6.0\%$ in the resting phase ($n = 60$ cells from three slices).

Time-lapse images of resting new neurons showed that they formed a large indentation in the soma (Fig. 1c, 4th image). This indentation had disappeared in the next frame, 6 min later (Fig. 1c, 5th image), suggesting it was a transient event. We then immunostained fixed slices of brain tissue using an anti-GFP and anti-Dcx antibodies and Hoechst33342. This staining showed the nucleus/soma of one neuron within the indentation of a neighboring neuron, suggesting that the indentation was caused by intercellular interactions within the chain (Fig. 1d and Movie S1).

Next, we investigated the frequency of indented cells during the chain migration of new neurons in the RMS. The indentation was observed in both migrating- and resting-phase neurons (Fig. 1b and e); however, the percentage of resting-phase neurons with an indentation was significantly higher than that of migrating ones (resting: $34.1 \pm 2.0\%$; migrating: $21.4 \pm 1.3\%$; rapidly migrating: $15.8 \pm 2.5\%$; $n = 86$ cells from 3 slices; Fig. 1f). These results suggested that chain-migrating neurons undergo dynamic morphological changes not only in the migrating phase, but also in the resting phase.

Rac1 is required for the indentation of new neurons

We examined whether Rac1 regulates the cell-shape change in resting neurons. We confirmed that Rac1 was broadly expressed in the RMS cells, including new neurons (Bolis *et al.* 2003). Next, we investigated the effects of Rac1 knockdown on new neurons. An enhanced green fluorescent protein-Rac1 expression vector was transfected into COS7 cells with another vector encoding a knockdown sequence of Rac1 (miRac1) or a control sequence (miLacZ) and emerald green fluorescent protein. The Rac1-knockdown vector efficiently suppressed the expression of enhanced green fluorescent protein-Rac1 compared to the control (Fig. 2a). We introduced these plasmids into the lateral ventricles of P1 mice by electroporation. Four days later, the mice were killed, and sagittal brain sections were immunostained for GFP and Dcx (Fig. 2b and c). The percentage of GFP/Dcx double-positive cells that reached the OB was significantly greater than that found in the RMS in the control mice, but not in the miRac1-electroporated mice (distribution of GFP/Dcx double-positive cells with miLacZ: OB, $70 \pm 5.9\%$; RMS,

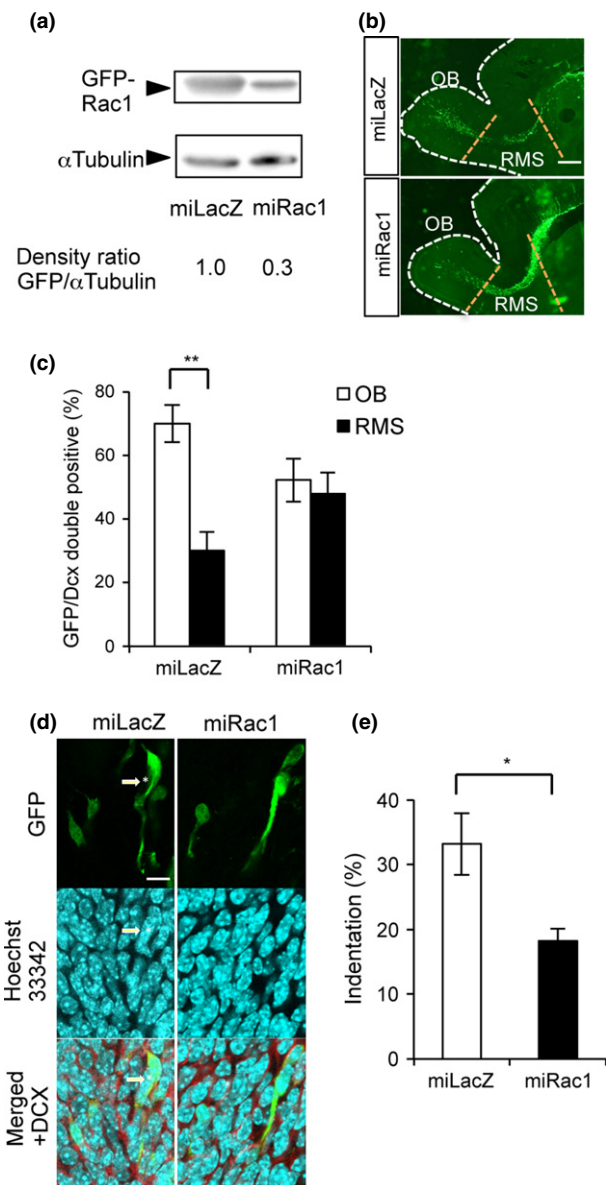


Fig. 2 Rac1 is required for indentation formation in new neurons. (a) Validation of Rac1 knockdown. COS7 cells were co-transfected with pEGFP-Rac1, and pCAGGS-emerald green fluorescent protein (EmGFP)-miLacZ (control) or pCAGGS-EmGFP-miRac1 using Lipofectamine LTX reagent (Invitrogen). Forty-eight hours after transfection, the cell lysates were analyzed by immunoblotting with an anti-GFP or anti-Tubulin antibody. (b) Low-magnification images of sagittal brain sections. Orange dashed-lines indicate the boundary between the olfactory bulb (OB) and rostral migratory stream (RMS). (c) Distribution of GFP/Doublecortin (Dcx)+ new neurons in the OB and RMS. (d and e) Effect of Rac1-knockdown on the indentations in GFP-positive neurons. White arrow: Indentation. Asterisk: Nucleus in the indentation. Scale bars: 500 μ m (b), 10 μ m (d). * $p < 0.05$, ** $p < 0.01$.

$30.0 \pm 5.9\%$, $p < 0.01$, $n = 4896$ cells from three mice; miRac1: OB, $52.2 \pm 6.8\%$; RMS, $47.8 \pm 6.8\%$, $p = 0.67$, $n = 3688$ from three mice, Fig. 2b and c) suggesting that the

inhibition of Rac1 signaling in new neurons impaired their migration toward the OB. Rac1 knockdown did not affect the number of cleaved caspase-3-positive cells in the RMS (percentage of GFP-cleaved caspase-3 double-positive cells/GFP positive cells: miLacZ: $0.00081 \pm 0.0001\%$, $n = 13,318$ cells from three mice; miRac1: $0.00084 \pm 0.0005\%$, $n = 12,902$ cells from three mice, $p = 0.96$), suggesting that the knockdown of Rac1 did not affect the cell survival of the V-SVZ-derived new neurons.

To investigate the effect of inhibiting Rac1 signaling on the indentation frequency, we compared the cell morphology between control and Rac1-knockdown cells in the OB and RMS. The percentage of indentation-forming neurons was significantly decreased in the Rac1-knockdown neurons compared with the control neurons (miLacZ: $33.2 \pm 4.8\%$, $n = 1153$ cells from three mice; miRac1: $18.2 \pm 1.9\%$, $n = 1251$ from three mice, $p < 0.05$; Fig. 2d and e). Consistent with these findings, the over-expression of a

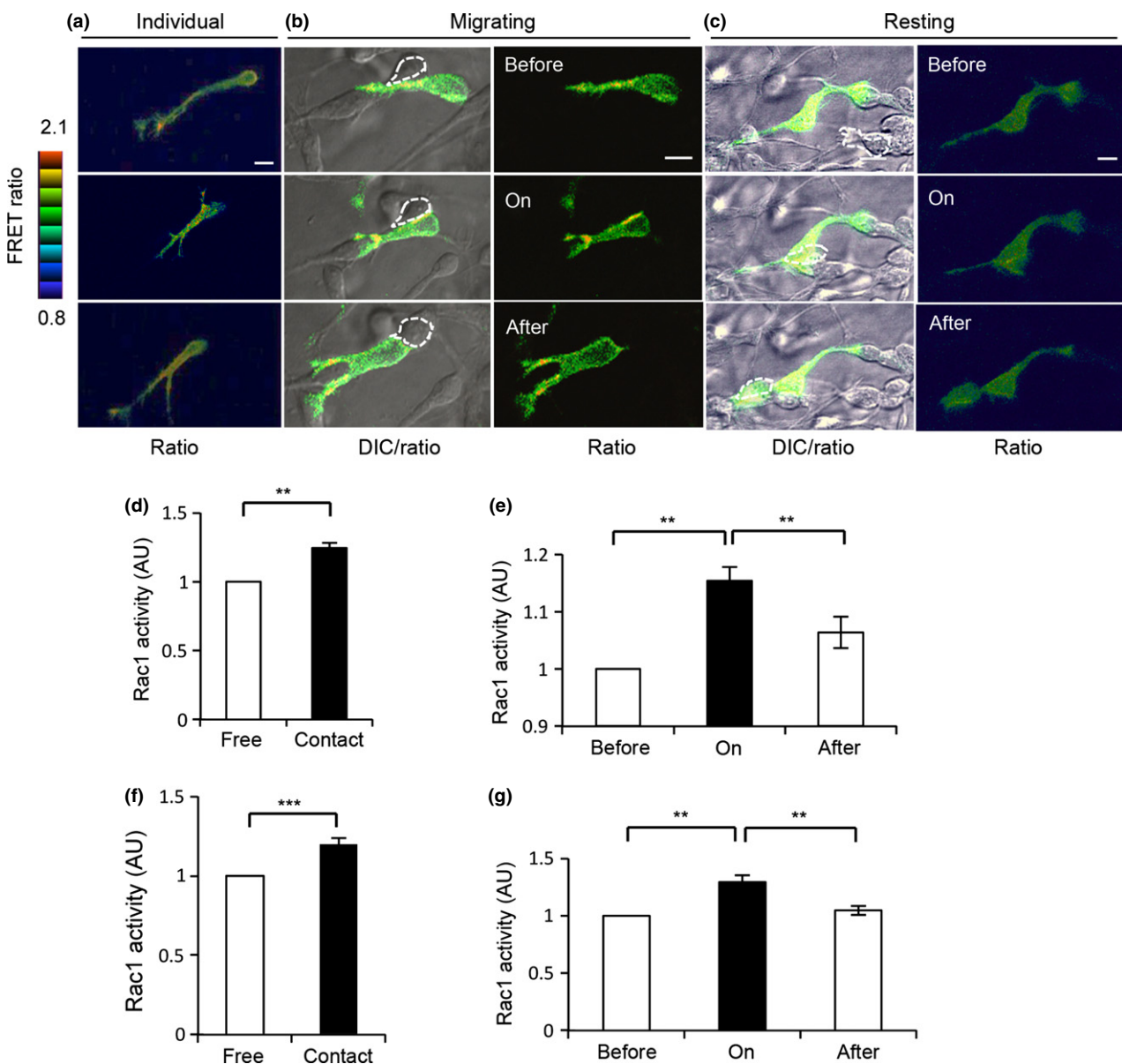


Fig. 3 Spatiotemporal activation of Rac1 in migrating new neurons. The calculated Förster resonance energy transfer (FRET) efficiency is differentiated by color in the intensity-modulated display (IMD) mode shown at the side. (a–c) Spatiotemporal activation of Rac1 in individually migrating and chain-forming neurons. Dashed lines

indicate the soma of a resting (b) or migrating (c) neuron, respectively. (d and f) Rac1 activation at free and cell–cell contact sites of chain-forming neurons. (e and g) Sequential quantification of the FRET/CFP ratio at the contact points of neuron membranes before, during (on), and after chain migration. Scale bar: 10 μm . ** $p < 0.01$, *** $p < 0.001$.

dominant-negative form of Rac1 (Rac1N17) also decreased the percentage of new neurons reaching the OB and the frequency of indentation (data not shown). Taken together, these data suggested that Rac1 is required for the efficient migration and indentation of new neurons.

Spatiotemporal activation of Rac1 in migrating new neurons

To visualize the spatiotemporal activation pattern of Rac1 in the migrating V-SVZ neurons, we performed an *in vitro* study using primary cultured new neurons in Matrigel, in which the neurons show both individual and chain migration (Wichterle *et al.* 1997). We introduced a FRET biosensor for Rac1, Raichu-1011x (Aoki *et al.* 2004), into the new neurons. Neurons expressing Raichu-1011x migrated individually or in chains in Matrigel, similar to non-transfected neurons.

First, we focused on the Rac1 activity of the migrating neurons. In individually migrating neurons, Rac1 activity was observed at the tip and the proximal part of the leading process, and/or at the rear part of the cell soma, depending on the step of migration (Fig. 3a). Neurons migrating in chains showed a similar Rac1 activation pattern (Fig. 3b). In addition, Rac1 activation was observed at the cell–cell contact sites of the migrating neurons in chains, when the nucleus of a migrating neuron apposed the soma of a resting one (FRET ratio: 1.24 ± 0.04 -fold increase at contact sites compared to free sites, $p < 0.01$, $n = 10$ cells; Fig. 3b and d; significant difference between before and during, $p < 0.01$; during and after, $p < 0.01$ by the Tukey–Kramer method, $n = 10$ cells, Fig. 3e, and Movie S2).

Next, we focused on the resting neurons in chains. Rac1 activation was observed at the cell–cell contact sites, and was dependent on the neuron's contact with migrating neurons (FRET ratio: 1.2 ± 0.1 -fold increase at contact sites compared to free sites, $p < 0.001$, $n = 10$ cells; Fig. 3c and f; significant difference between before and during, $p < 0.01$; during and after, $p < 0.01$ by the Tukey–Kramer method, $n = 10$ cells, Fig. 3g, and Movie S2).

These observations indicate that during chain migration, Rac1 is activated at the cell–cell contact sites in both the migrating and resting neurons. Taken together, these results indicate that the Rac1 activity at the contact site of resting neurons causes indentation.

Role of Rac1 activation in chain migration

To investigate Rac1's specific roles in chain migration, Rac1 knockdown or control (miLacZ) vector was introduced into V-SVZ cells dissociated from P1-three mice. After a 72-hr incubation, time-lapse imaging of the chain migration was performed. The percentage of resting neurons that formed an indentation in their soma was significantly decreased by Rac1 knockdown compared with the control (percentage of indent-forming cells during cell–cell contact: miLacZ, $81 \pm 8.8\%$; miRac1, $40 \pm 11.2\%$, $p < 0.01$, $n = 20$ cells;

Fig. 4a–c and Movie S3), suggesting that Rac1 was required for the indentation to form, and that contact between the migrating neurons caused both the Rac1 activation and the indentation.

Finally, to investigate the role of the Rac1-regulated indentations in the resting new neurons in migratory chains, the speed of chain-migrating neurons was measured. The knockdown of Rac1 in resting neurons significantly increased the time the migrating neurons took to move along them (miLacZ: 47.4 ± 6 min; miRac1: 93.9 ± 12.2 min,

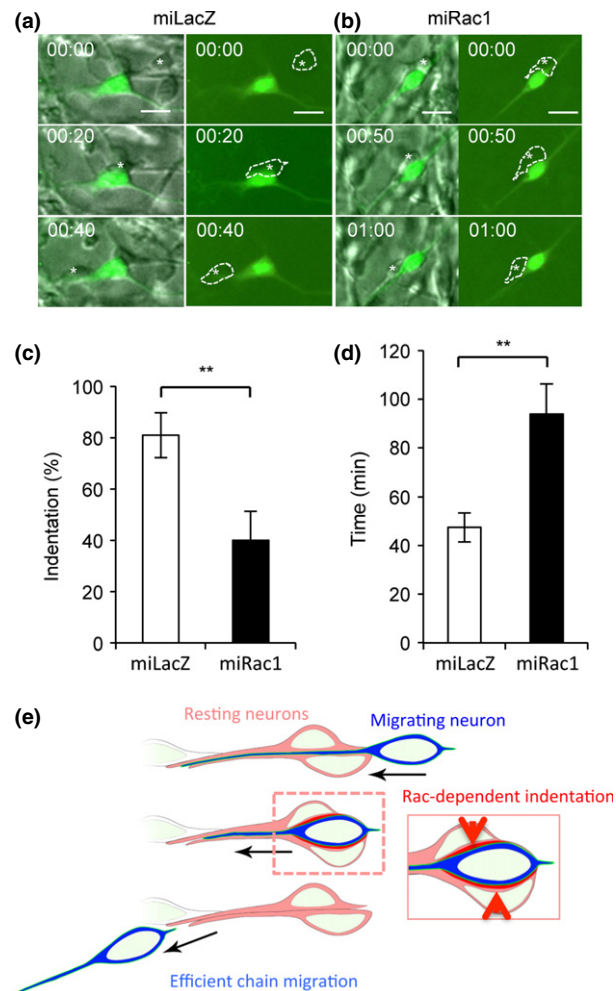


Fig. 4 Role of Rac1 activation in chain migration. (a, b) pCAGGS-emerald green fluorescent protein (EmGFP)-miLacZ (control) or pCAGGS-EmGFP-miRac1 were transferred into ventricular-subventricular zone (V-SVZ) cells by electroporation. Dashed lines indicate the soma of a migrating neuron. Scale bar: 10 μ m. (c) Frequency of indentation of resting neurons. (d) Time for a migrating neuron to move over a resting neuron. (e) Working model. During chain migration, a migrating neuron (blue) moves along resting neurons (pink). When the soma of a migrating neuron pushes between resting neurons, the resting neurons activate Rac1 at the cell–cell contact site (red) and form an indentation, thus creating a path for the migrating neuron. $**p < 0.01$.

$p < 0.01$, $n = 20$ cells; Fig. 4a–d and Movie S3). These results supported the idea that Rac1 activity in the resting neurons causes the indentation to form, and the indentation provides a scaffold for the efficient somatic translocation of migrating neurons.

Recent studies in other systems have indicated that collective cell migration is dependent on a subpopulation with distinct motility characteristics, referred to as ‘leading cells,’ for example, the first cells in a migrating sheet or the tip cells in the sprouting and branching model. On the other hand, neural crest cells and V-SVZ new neurons show ‘stream’ migration, in which the cells migrate together but in a loose arrangement (Rorth 2009). Since the saltatory movement of the individual V-SVZ new neurons in chains is not synchronized, it was possible that the resting neurons serve as a scaffold for the actively migrating ones. Here, we found that migrating new neurons form an indentation during the resting phase (Fig. 1c). The neurons in the RMS are densely packed and tightly ensheathed by astrocytic tubes (Jankovski and Sotelo 1996; Whitman *et al.* 2009; Kaneko *et al.* 2010), suggesting that it should be difficult for the migrating neurons to pass between the resting ones. On the basis of our present results, we propose that Rac1-mediated morphological changes in the resting neurons create a path for the migrating neurons (Fig. 4e).

Our findings suggested that a physical path formed by the indentation in resting neurons promotes the efficient saltatory movement of the migrating neurons’ soma during chain migration in the RMS. In conclusion, this study demonstrated a novel non-cell-autonomous role for Rac1 in regulating the cell–cell interactions of new neurons migrating in the post-natal brain, and provides new insight into the mechanism of collective cell migration.

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Movie S1. 3D reconstruction of indentation-forming new neurons.

Movie S2. FRET imaging of Rac1 in chain-migrating new neurons.

Movie S3. Effect of Rac1 KD on indentation of resting neurons.

References

- Altman J. (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J. Comp. Neurol.* **137**, 433–457.
- Aoki K., Nakamura T. and Matsuda M. (2004) Spatio-temporal regulation of Rac1 and Cdc42 activity during nerve growth factor-induced neurite outgrowth in PC12 cells. *J. Biol. Chem.* **279**, 713–719.
- Battista D. and Rutishauser U. (2010) Removal of polysialic acid triggers dispersion of subventricularly derived neuroblasts into surrounding CNS tissues. *J. Neurosci.* **30**, 3995–4003.
- Bellion A., Baudoin J. P., Alvarez C., Bornens M. and Metin C. (2005) Nucleokinesis in tangentially migrating neurons comprises two alternating phases: forward migration of the Golgi/centrosome associated with centrosome splitting and myosin contraction at the rear. *J. Neurosci.* **25**, 5691–5699.
- Bolis A., Corbetta S., Cioce A. and de Curtis I. (2003) Differential distribution of Rac1 and Rac3 GTPases in the developing mouse brain: implications for a role of Rac3 in Purkinje cell differentiation. *Eur. J. Neurosci.* **18**, 2417–2424.
- Boutin C., Diestel S., Desoeuvre A., Tiveron M. C. and Cremer H. (2008) Efficient *in vivo* electroporation of the postnatal rodent forebrain. *PLoS ONE* **3**, e1883.
- Chazal G., Durbec P., Jankovski A., Rougon G. and Cremer H. (2000) Consequences of neural cell adhesion molecule deficiency on cell migration in the rostral migratory stream of the mouse. *J. Neurosci.* **20**, 1446–1457.
- Dumortier J. G., Martin S., Meyer D., Rosa F. M. and David N. B. (2012) Collective mesendoderm migration relies on an intrinsic directionality signal transmitted through cell contacts. *Proc. Natl Acad. Sci. USA* **109**, 16945–16950.
- Ehrlich J. S., Hansen M. D. and Nelson W. J. (2002) Spatio-temporal regulation of Rac1 localization and lamellipodia dynamics during epithelial cell-cell adhesion. *Dev. Cell* **3**, 259–270.
- Fukata M., Nakagawa M. and Kaibuchi K. (2003) Roles of Rho-family GTPases in cell polarisation and directional migration. *Curr. Opin. Cell Biol.* **15**, 590–597.
- Ghashghaei H. T., Lai C. and Anton E. S. (2007) Neuronal migration in the adult brain: are we there yet? *Nat. Rev. Neurosci.* **8**, 141–151.
- Govek E. E., Newey S. E. and Van Aelst L. (2005) The role of the Rho GTPases in neuronal development. *Genes Dev.* **19**, 1–49.
- Hatten M. E. (2002) *New directions in neuronal migration.* *Science* **297**, 1660–1663.
- Hirota Y., Ohshima T., Kaneko N., Ikeda M., Iwasato T., Kulkarni A. B., Mikoshiba K., Okano H. and Sawamoto K. (2007) Cyclin-dependent kinase 5 is required for control of neuroblast migration in the postnatal subventricular zone. *J. Neurosci.* **27**, 12829–12838.
- Hirota Y., Sawada M., Kida Y. S., Huang S. H., Yamada O., Sakaguchi M., Ogura T., Okano H. and Sawamoto K. (2012) Roles of planar cell polarity signaling in maturation of neuronal precursor cells in the postnatal mouse olfactory bulb. *Stem Cells* **30**, 1726–1733.
- Jaffe A. B. and Hall A. (2005) Rho GTPases: biochemistry and biology. *Annu. Rev. Cell Dev. Biol.* **21**, 247–269.
- Jankovski A. and Sotelo C. (1996) Subventricular zone-olfactory bulb migratory pathway in the adult mouse: cellular composition and specificity as determined by heterochronic and heterotopic transplantation. *J. Comp. Neurol.* **371**, 376–396.
- Kaneko N., Marin O., Koike M. *et al.* (2010) New neurons clear the path of astrocytic processes for their rapid migration in the adult brain. *Neuron* **67**, 213–223.

- Khodosevich K. and Monyer H. (2010) Signaling involved in neurite outgrowth of postnatally born subventricular zone neurons *in vitro*. *BMC Neurosci* **11**, 18.
- Leone D. P., Srinivasan K., Brakebusch C. and McConnell S. K. (2010) The rho GTPase Rac1 is required for proliferation and survival of progenitors in the developing forebrain. *Dev Neurobiol* **70**, 659–678.
- Lois C. and Alvarez-Buylla A. (1994) Long-distance neuronal migration in the adult mammalian brain. *Science* **264**, 1145–1148.
- Luo L. (2000) Rho GTPases in neuronal morphogenesis. *Nat. Rev. Neurosci.* **1**, 173–180.
- Marin O. and Rubenstein J. L. (2003) Cell migration in the forebrain. *Annu. Rev. Neurosci.* **26**, 441–483.
- Migeotte I., Omelchenko T., Hall A. and Anderson K. V. (2010) Rac1-dependent collective cell migration is required for specification of the anterior-posterior body axis of the mouse. *PLoS Biol.* **8**, e1000442.
- Murase S. and Horwitz A. F. (2002) Deleted in colorectal carcinoma and differentially expressed integrins mediate the directional migration of neural precursors in the rostral migratory stream. *J. Neurosci.* **22**, 3568–3579.
- Niwa H., Yamamura K. and Miyazaki J. (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* **108**, 193–199.
- Ono K., Tomasiewicz H., Magnuson T. and Rutishauser U. (1994) N-CAM mutation inhibits tangential neuronal migration and is phenocopied by enzymatic removal of polysialic acid. *Neuron* **13**, 595–609.
- Pankov R., Endo Y., Even-Ram S., Araki M., Clark K., Cukierman E., Matsumoto K. and Yamada K. M. (2005) A Rac switch regulates random versus directionally persistent cell migration. *J. Cell Biol.* **170**, 793–802.
- Ramel D., Wang X., Laflamme C., Montell D. J. and Emery G. (2013) Rab11 regulates cell-cell communication during collective cell movements. *Nat. Cell Biol.* **15**, 317–324.
- Ridley A. J., Paterson H. F., Johnston C. L., Diekmann D. and Hall A. (1992) The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell* **70**, 401–410.
- Rorth P. (2009) Collective cell migration. *Annu. Rev. Cell Dev. Biol.* **25**, 407–429.
- Sawada M., Huang S.-h., Hirota Y., Kaneko N. and Sawamoto K. (2011) Neuronal Migration in the Adult Brain, in *Neurogenesis in the Adult Brain II* (Seki T., Sawamoto K., Parent J. M. and Alvarez-Buylla A. eds.), pp. 319–336. Springer, Berlin, Heidelberg, Germany.
- Suzuki S. O. and Goldman J. E. (2003) Multiple cell populations in the early postnatal subventricular zone take distinct migratory pathways: a dynamic study of glial and neuronal progenitor migration. *J. Neurosci.* **23**, 4240–4250.
- Wang X., He L., Wu Y. I., Hahn K. M. and Montell D. J. (2010) Light-mediated activation reveals a key role for Rac in collective guidance of cell movement *in vivo*. *Nat. Cell Biol.* **12**, 591–597.
- Whitman M. C., Fan W., Relat L., Rodriguez-Gil D. J. and Greer C. A. (2009) Blood vessels form a migratory scaffold in the rostral migratory stream. *J. Comp. Neurol.* **516**, 94–104.
- Wichterle H., Garcia-Verdugo J. M. and Alvarez-Buylla A. (1997) Direct evidence for homotypic, glia-independent neuronal migration. *Neuron* **18**, 779–791.
- Woo S., Housley M. P., Weiner O. D. and Stainier D. Y. (2012) Nodal signaling regulates endodermal cell motility and actin dynamics via Rac1 and Prex1. *J. Cell Biol.* **198**, 941–952.