

Cdk5 is required for multipolar-to-bipolar transition during radial neuronal migration and proper dendrite development of pyramidal neurons in the cerebral cortex

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The mammalian cerebral cortex consists of six layers that are generated via coordinated neuronal migration during the embryonic period. Recent studies identified specific phases of radial migration of cortical neurons. After the final division, neurons transform from a multipolar to a bipolar shape within the subventricular zone-intermediate zone (SVZ-IZ) and then migrate along radial glial fibres. Mice lacking Cdk5 exhibit abnormal corticogenesis owing to neuronal migration defects. When we introduced GFP into migrating neurons at E14.5 by in utero electroporation, we observed migrating neurons in wild-type but not in *Cdk5*^{-/-} embryos after 3-4 days. Introduction of the dominant-negative form of Cdk5 into the wild-type migrating neurons confirmed specific impairment of the multipolar-to-bipolar transition within the SVZ-IZ in a cell-autonomous manner. Cortex-specific Cdk5 conditional knockout mice showed inverted layering of the cerebral cortex and the layer V and callosal neurons, but not layer VI neurons, had severely impaired dendritic morphology. The amount of the dendritic protein Map2 was decreased in the cerebral cortex of Cdk5-deficient mice, and the axonal trajectory of cortical neurons within the cortex was also abnormal. These results indicate that Cdk5 is required for proper multipolar-to-bipolar transition, and a deficiency of Cdk5 results in abnormal morphology of pyramidal neurons. In addition, proper radial neuronal migration generates an inside-out pattern of cerebral cortex formation and normal axonal trajectories of cortical pyramidal neurons.

KEY WORDS: Neuronal migration, Cerebral cortex, Mouse, Cdk5, Map2 (Mtap2)

INTRODUCTION

In the mammalian brain, neurons settle into six layers within the cerebral cortex in an inside-out manner, with the earliest-generated neurons positioned in the deepest layers and later-generated neurons occupying the more superficial layers (Angevine and Sidman, 1961). This unique layering is achieved via coordinated radial migration of neurons from the ventricular zone (VZ) to their final destination. Proper positioning of neurons is particularly crucial for the formation of laminated cortical structures, such as the cerebral cortex, hippocampus and cerebellum. Recently, time-lapse imaging of migrating cerebral cortex neurons identified specific phases in radial neuronal migration (Nadarajah et al., 2001; Tabata and Nakajima, 2003; Noctor et al., 2004). After the final division, immature neurons transiently become multipolar, with multiple neurites within the subventricular zone (SVZ) and lower intermediate zone (IZ), which is also known as the premigratory zone. Then, neurons change their shape from multipolar to bipolar just before migrating from the premigratory zone to the cortical plate (CP). The molecular and cellular mechanisms that regulate this transition remain to be elucidated.

Cdk5 is a unique serine/threonine kinase with close homology to other Cdks but its kinase activity is mainly detected in postmitotic neurons (Dhavan and Tsai, 2001). Association of Cdk5 with a neuron-specific regulatory subunit, either p35 (Cdk5r1 – Mouse Genome Informatics) or its isoform p39, is crucial for kinase activity. Previous studies by us and other groups revealed that Cdk5 and its activating subunit p35 have crucial roles in the formation of cortical structures of the developing mouse brain, and this is brought about mainly by the regulation of neuronal migration (Ohshima et al., 1996; Gilmore et al., 1998; Chae et al., 1997; Ohshima et al., 2001). *Cdk5*^{-/-} mice exhibit substantial migratory deficits throughout the brain (Ohshima et al., 1996; Gilmore et al., 1998). In the cerebral cortex, neocortical *Cdk5*^{-/-} neurons destined for layers II to V stall below the subplate (Gilmore et al., 1998). A birth-date labelling study indicated that there are migration defects in *Cdk5*^{-/-} neurons (Gilmore et al., 1998), but these abnormalities need to be elucidated in detail.

Here we report our analysis of the migratory behaviour of *Cdk5*^{-/-} neurons in the developing cerebral cortex. We found that *Cdk5*^{-/-} neurons have severe impairment of the multipolar-to-bipolar transition during their radial migration. *Cdk5*^{-/-} mice die in the perinatal period before cerebral cortex layer formation is completed (Ohshima et al., 1996). To overcome this early lethality, we generated cortex-specific Cdk5-deficient mice by mating *Emx1Cre* mice (Iwasato et al., 2000) and *fCdk5/fCdk5* mice (Hirasawa et al., 2004). Using this mutant mouse line, we demonstrate that the inability of cortical neurons to transition from the multipolar phase of radial migration results in abnormal dendritic development and defective axonal trajectories of pyramidal neurons in the postnatal cerebral cortex. Further analysis of mutant mice indicated that Cdk5-dependent multipolar-bipolar transition of migrating neurons is a priori for proper corticogenesis in the developing mouse brain.

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MATERIALS AND METHODS

Mice

Cdk5^{-/-} mice were generated and maintained as described previously (Ohshima et al., 1996). *Cdk5 loxP*-flanked mice were generated as described (Hirasawa et al., 2004). The YFP-H mouse line (Feng et al., 2000) was obtained from Jackson Laboratory, MA. The *Emx1Cre* mouse line was generated by the knock-in strategy; Cre recombinase activity was restricted to the *Emx1* expression region (Iwasato et al., 2000) and detected as early as E10 as described (Cappello et al., 2006). *Emx1Cre* conditional *Cdk5* KO mice (*fCdk5*^{-/-}; *Emx1*^{Cre/+}; Cx*Cdk5*KO) were analysed along with littermate controls. *Reeler* mice (B6C3Fe-a/a-rl, Jackson Laboratory) were mated with YFP-H mice to obtain *reeler*;YFP-H mutant mice. Mice were fed ad libitum and provided with care as per RIKEN BSI guidelines.

In utero electroporation and time-lapse imaging

Female *Cdk5* heterozygotes were mated with *Cdk5* heterozygote males as well as ICR males (Japan SLC, Shizuoka, Japan) and, after becoming pregnant, were subjected to in utero electroporation as described previously (Tabata and Nakajima, 2001; Tabata and Nakajima, 2003). Tα1-EGFP was generated as described (Tabata and Nakajima, 2001). To generate CAG-EGFP and CAG-RFP, the cDNA of EGFP and DsRed2 from pEGFP-N1 and pDsRed2-N1 (Clontech, Palo Alto, CA) were inserted, respectively, into pCAGGS, which was kindly provided by Dr J. Miyazaki (Niwa et al., 1991). Tα1-EGFP efficiently labelled migrating neurons, and CAG-EGFP labelled migrating neurons as well as their progenitors (i.e. radial glial cells), as reported previously (Tabata and Nakajima, 2001). In order to express both *Cdk5*-DN and EGFP in the same cells, bi-expression vector CAG-GFP-CAG-*Cdk5*-DN (CGC-*Cdk5*-DN) was generated as follows. To generate CAG-EGFP-CAG (CGC), a 2.3 kb fragment containing the CAG promoter and the *Eco*RI site was amplified with pCAGGS by PCR, and inserted into the *Hind*III site downstream of the EGFP gene in CAG-EGFP. CAG-RFP-CAG was generated in the same way using CAG-RFP. *Cdk5*-DN (N144) was then inserted into CAG-EGFP-CAG and CAG-RFP-CAG to generate CGC-*Cdk5*-DN and CRC-*Cdk5*-DN, respectively. Time-lapse imaging of brain slices was conducted as described previously (Tabata and Nakajima, 2003). Briefly, coronal brain slices (200 μm) from the anterior one-third of the cerebral cortex were embedded in collagen gel on a permeable filter (Millicell-CM, 0.4 μm pore size, Millipore). The filter was placed on a glass-bottom dish with Neurobasal medium containing B27 (Invitrogen, San Diego, CA) and was incubated at 37°C in 5% CO₂ on a laser microscope (FV300, Olympus). Ten to twenty optical z-sections at 5 μm intervals were captured every 30 minutes and all focal planes were merged.

Dissociated neuronal culture and imaging analysis

E14.5 cortical neurons of *Cdk5*^{+/+} and *Cdk5*^{-/-} were cultured as described (Hayashi et al., 2002) with slight modification. Briefly, the cerebral cortex was excised, incubated with 0.25% trypsin in PBS for 10 minutes at 37°C, dissociated, and washed in culture medium which consisted of DMEM/Ham's F-12 mixture (Sigma, St Louis, MO) supplemented with N-2 supplement (Invitrogen), 5% fetal bovine serum, 5% calf serum, 4 ng/μl EGF, 8 ng/μl basic FGF, penicillin and streptomycin. The dissociated cells were cultured on fibronectin-coated dishes, on which an astrocyte feeder was cultured outside, at 3 × 10⁴ cells/cm² in culture medium for 3 days. After washing with PBS, cultured neurons were fixed with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer for 10 minutes at room temperature. After washing with PBS three times, cells were incubated with blocking buffer (1% bovine serum albumin and 0.2% Triton X-100 in PBS) at room temperature for 30 minutes. The cells were then incubated with anti-βIII-tubulin antibody (1:500 Tuj1, Covance, Denver, PA) at room temperature for 1 hour followed by Alexa-Fluor 488 (Molecular Probes, Eugene, OR). Cell images were acquired with a fluorescent microscope. The percentage of pyramidal neurons among Tuj1-positive cells was calculated in randomly selected fields. Pyramidal neurons were defined as cells that had a pyramidal cell body with one major dendrite that gradually tapered off from the cell body (Threadgill et al., 1997). Data are shown as means ± s.d.

Bromodeoxyuridine injection, immunohistochemical staining and in situ hybridisation

For bromodeoxyuridine (BrdU) experiments, pregnant dams were injected with BrdU (100 μg/g, intraperitoneal) at the indicated age and analysed at P10. Paraffin sections (8 μm) of fixed brain (4% PFA in 0.1 M phosphate buffer) were cut and stained with monoclonal anti-BrdU antibody as described (Gilmore et al., 1998). For the immunohistochemical analysis, frozen sections (15 μm) were cut and stained as described (Gilmore et al., 1998; Ohshima et al., 2001) using the following primary antibodies: polyclonal antibodies for GFP (MBL, Tokyo, Japan) and *Cdk5* (C-8, Santa Cruz, CA) and monoclonal antibody for Map2 (Chemicon). In situ hybridisation was performed as described (Ohshima et al., 2002) in the coronal sections from Cx*Cdk5*KO and control mice at P10 using *Foxp2* (Ferland et al., 2003), *Er81* (Arber et al., 2000) and *Cux2* (Nieto et al., 2004) as probes. For in situ hybridisation in the coronal sections from *Cdk5*^{+/+} and *Cdk5*^{-/-} embryos at E16.5 were stained using *Neurod1* and *Dcx* as probes. Mouse cDNAs for *Neurod1* and *Dcx* were the FANTOM3 (The FANTOM Consortium, 2005) and IMAGE clones, respectively.

1,1'-dioctadecyl-3-3'-3'-tetramethylindocarbocyanine perchlorate (DiI) tracing

DiI tracing was performed as described previously (Ohshima et al., 2002). Briefly, after intracardiac perfusion with 4% PFA in 0.1 M phosphate buffer (pH 7.4), brains were removed from the skulls. Several small crystals of DiI were inserted into the dorsal thalamus or cerebral cortex of *Cdk5*^{+/+} and *Cdk5*^{-/-} embryos at E18.5 or Cx*Cdk5*KO mice and their littermate controls at P10. The brains were kept in the same fixative for 3-4 weeks in the dark at 37°C. The brains were embedded in 2% agarose, cut into 100 μm sections with a vibratome and photographed with a rhodamine filter.

Western blot analysis

Protein extraction and western blotting were carried out as described (Ohshima et al., 2005) with the following primary antibodies: polyclonal antibody for *Cdk5* (C-8), Map2 (Chemicon), NF-M (NF-150, Chemicon) and actin (α-actin, Sigma). Data are shown as mean ± s.d. and analysed by Student's *t*-test. *P* < 0.05 was considered statistically significant.

Adenovirus preparation, infection and time-lapse imaging

The adenoviral vector construction was carried out basically as previously described (Hashimoto and Mikoshiba, 2004). The adenoviral vector Adex-CAG-Lyn-Venus expresses membrane-targeted improved YFP called Venus (Nagai et al., 2002) under control of the CAG promoter. Lyn-Venus was generated by fusing the sequence of the palmitoylation site of tyrosine kinase Lyn (amino acids 246-307) to the N-terminus of Venus. The detailed feature of Adex-CAG-Lyn-Venus will be described elsewhere (T.M., M.H. and M.O., unpublished). Pregnant mice were deeply anesthetised with sodium pentobarbitone at 50 μg/g body weight. Adenoviral vectors (2.0 × 10⁶ pfu total) were injected into the ventricle of the embryos at E12.5. The embryos were removed later (E14.5). Preparation and culture of brain slices were carried out as described above. A Leica ASMDW workstation was used for multidimensional live tissue imaging (Leica, Heidelberg, Germany). The microscope setup consisted of an inverted microscope (DMIRE2, Leica) and multipoint time-lapse stage controlled by ASMDW software (Leica). The deconvolution Deblur software (Leica) was used for deblurring the z-stacked images. Then, the deconvoluted images were processed to AVI movies by ASMDW and Premiere software (Adobe, San Jose, CA).

RESULTS

Cdk5-deficient cortical neurons at the embryonic stage

Morphological features of migrating *Cdk5*^{-/-} neurons

Our previous study of *Cdk5*^{-/-} mice indicated that early-born layer VI neurons migrate normally and split the preplate into the marginal zone and subplate, but later-born neurons that constitute layers II to V are positioned abnormally under the subplate in the mutant cortex (Gilmore et al., 1998). We first analysed the morphology of migrating later-born *Cdk5*^{-/-} neurons. To visualise

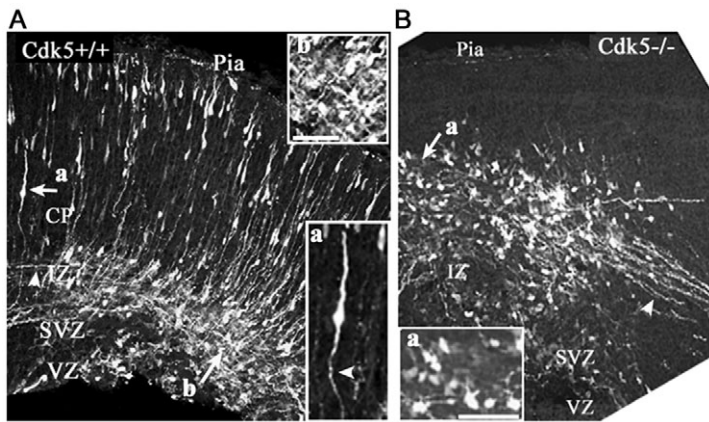


Fig. 1. Visualisation of migrating neurons in *Cdk5*^{+/+} and *Cdk5*^{-/-} mice by introduction of Tα1-EGFP plasmid into VZ cells by in utero electroporation. EGFP was introduced into (A) *Cdk5*^{+/+} and (B) *Cdk5*^{-/-} mouse embryos at E14.5, and brains were fixed at E17.5. Sections were stained with anti-GFP antibody. Insets show areas indicated by arrows (a,b) at higher magnification. (A) Over the period of 3 days, neurons migrated into the CP with the typical bipolar shape (a) in *Cdk5*^{+/+} mice. Migrating neurons in the CP extend their leading processes toward the pia and their axons (arrowhead) toward the VZ. In the SVZ-IZ, GFP-positive neurons of multipolar shape (b) are observed at this stage. (B) By contrast, in *Cdk5*^{-/-} mice neurons arrested in the SVZ-IZ in a multipolar shape (a), and their axons (arrowhead) run oblique within the SVZ-IZ. CP, cortical plate; SVZ, subventricular zone; IZ, intermediate zone; VZ, ventricular zone. Scale bar: 25 μm.

the morphology of migrating cortical neurons in the cerebral cortex, we introduced an enhanced GFP (EGFP) by in utero electroporation (Tabata and Nakajima, 2001; Tabata and Nakajima, 2003) at embryonic day 14.5 (E14.5). Embryos were fixed 2 (E16.5), 3 (E17.5) or 4 days (E18.5) after in utero electroporation, and the morphologies of GFP-labelled neurons examined by immunostaining with anti-GFP antibody. At E16.5, most GFP-positive cells were localised in the premigratory zone. GFP-labelled radial glial cells exhibited a bipolar morphology, and their fibres extended toward the pia in the cerebral cortex of *Cdk5*^{+/+} as well as *Cdk5*^{-/-} embryos (see Fig. S1 in the supplementary material). When we examined E17.5 embryos, GFP-labelled neurons exhibited either a multipolar morphology at the premigratory zone or a bipolar morphology at the upper IZ and CP in *Cdk5*^{+/+} embryos (Fig. 1A), indicating multipolar-to-bipolar transition during radial migration from the premigratory zone (SVZ and lower IZ) to the CP as previously reported (Tabata and Nakajima, 2003; Noctor et al., 2004). In the *Cdk5*^{+/+} brain, GFP-labelled neurons extended their axons towards the IZ and then turned sharply towards the midline (Fig. 1A). By contrast, GFP-labelled neurons in the *Cdk5*^{-/-} brain migrated upward, keeping their multipolar morphology; bipolar neurons were not observed in the premigratory zone or CP (Fig. 1B). Even 4 days after labelling (E18.5), no GFP-positive cells in a bipolar shape were detected in the cerebral cortex of *Cdk5*^{-/-} embryos (data not shown). Axonal trajectories of GFP-labelled *Cdk5*^{-/-} neurons ran

obliquely within the cortex (arrowhead in Fig. 1B). These results indicate a specific deficit of multipolar-to-bipolar transition during the migration from the premigratory zone to the CP in *Cdk5*^{-/-} neurons.

Morphological features of neurons expressing the dominant-negative form of Cdk5

Introduction of the dominant-negative form of Cdk5 (Cdk5-DN) into migrating neurons causes a delay in radial migration, but not impairment of the transformation from a multipolar to bipolar shape (Hatanaka et al., 2004). To examine the morphology and migratory behaviour of the neurons expressing Cdk5-DN, we utilised a dual CAG promoter vector, CAG-RFP-CAG-Cdk5-DN (CRC-Cdk5-DN), in which RFP and Cdk5-DN were transcribed via their own CAG promoter within a single plasmid. When we introduced only the CRC-Cdk5-DN plasmid (100% Cdk5-DN), the most RFP-positive neurons were positioned within the premigratory zone and had the multipolar shape (Fig. 2). Few neurons were bipolar and the intensity of RFP in these neurons was relatively low (Fig. 2), indicating a lower level of Cdk5-DN expression. When we applied CRC-Cdk5-DN in different concentrations by diluting with CAG-RFP, different degrees of migration defects were observed (Fig. 2). In the brains with a CAG-RFP dilution of 20% (1:4) or 50% (1:1), a delayed migration of neurons expressing Cdk5-DN was observed in the CP, but the majority of RFP-positive neurons transformed into the bipolar shape within the upper IZ. However, careful

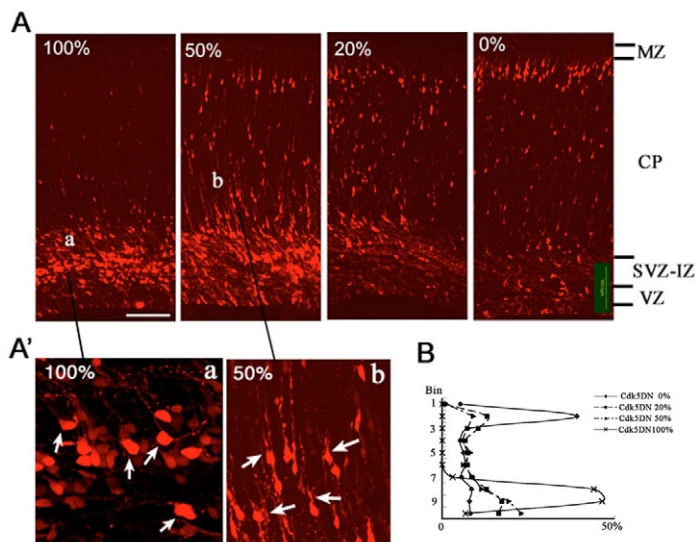


Fig. 2. Cell-autonomous impairment of the multipolar-to-bipolar transformation within the SVZ-IZ in neurons expressing Cdk5-DN. Mixtures of CRC-Cdk5-DN and CAG-RFP were used in different ratios for in utero electroporation of E14.5 mouse embryos. (A,A') The distribution and morphology of RFP-positive cells (red) were analysed in coronal sections at E18.5. Higher magnifications of the indicated areas of 100% (a) and 50% Cdk5-DN (b) are shown in A'. Dose-dependent disturbance of neuronal migration by CRC-Cdk5-DN was also cell-autonomous. When only Cdk5-DN was used (100%), RFP-positive neurons arrested within the SVZ-IZ in the multipolar shape (arrows in A'). This result matched the phenotype of *Cdk5*^{-/-} neurons, indicating that defective Cdk5 activity in migrating neurons caused migration arrest in a cell-autonomous manner. When Cdk5-DN was used at 50%, migration delay was observed. RFP-positive neurons have bipolar shape in CP but some of the RFP-positive neurons have branched leading processes (arrows in A'b). MZ, marginal zone; CP, cortical plate; SVZ-IZ, subventricular zone-intermediate zone; VZ, ventricular zone. Scale bar: 100 μm. (B) Bin distribution of RFP-positive neurons at each ratio of Cdk5-DN. The cortical mantle was divided into ten equally spaced bins and the percentage of cells in each bin calculated.

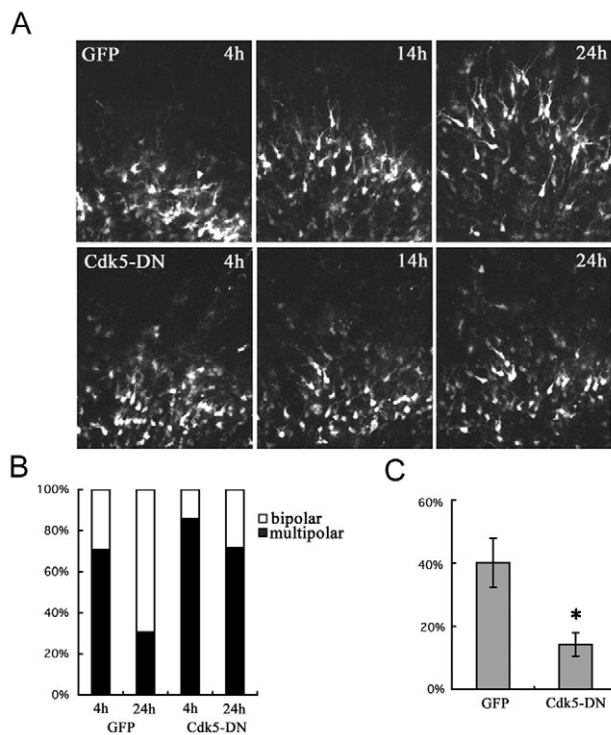


Fig. 3. Time-lapse imaging of migrating neurons expressing either CAG-EGFP or CGC-Cdk5-DN. (A) Representative results are shown for mouse brain slices electroporated with either CAG-EGFP (GFP) or CGC-Cdk5-DN (Cdk5-DN) at E14.5. (B) Based on their morphology and behaviour, GFP-positive neurons were divided into two categories: bipolar shape (bipolar) or multipolar shape (multipolar) when observed at 4 hours (4 h) and 24 hours (24 h) post-electroporation, and the percentage of each is shown for CAG-EGFP and CGC-Cdk5-DN brain slices. (C) The percentage of GFP-positive neurons that transformed from multipolar to bipolar during a 20-hour period was calculated for each slice and subjected to statistical comparison. Multipolar-to-bipolar transformation was impaired in neurons expressing Cdk5-DN (GFP, $40.15 \pm 14.25\%$; Cdk5-DN, $7.75 \pm 3.77\%$, $n=4$); *, $P < 0.001$.

observation at higher magnification revealed that some RFP-positive neurons in the CP had branched leading processes (Fig. 2A'b, arrows).

Time-lapse imaging of migratory behaviour of neurons expressing Cdk5-DN

To study the migratory behaviour of neurons expressing Cdk5-DN, we conducted time-lapse observation of brain tissue slices. Brains were electroporated in utero at E14.5 with either the CAG-EGFP plasmid, in which EGFP was used for the visualisation, or the CAG-EGFP-CAG-Cdk5-DN (CGC-Cdk5-DN) plasmid, and slices were prepared at E16.5 and imaged at 30-minute intervals for 24 hours from 2 days after electroporation, as previously described (Tabata and Nakajima, 2003). Based on the morphology and behaviour of GFP-positive neurons at 4 and 24 hours after onset of observation, each neuron was classified as either 'multipolar', in which a neuron has extended multiple (more than three) neurites, or 'bipolar', in which a neuron has one or two neurites (also identified as leading and tailing processes), as described (Tabata and Nakajima, 2003; Noctor et al., 2004). The morphology and behaviour of GFP-positive neurons were already different at 4 hours between brain slices with CAG-EGFP and those with CGC-Cdk5-DN. These differences were

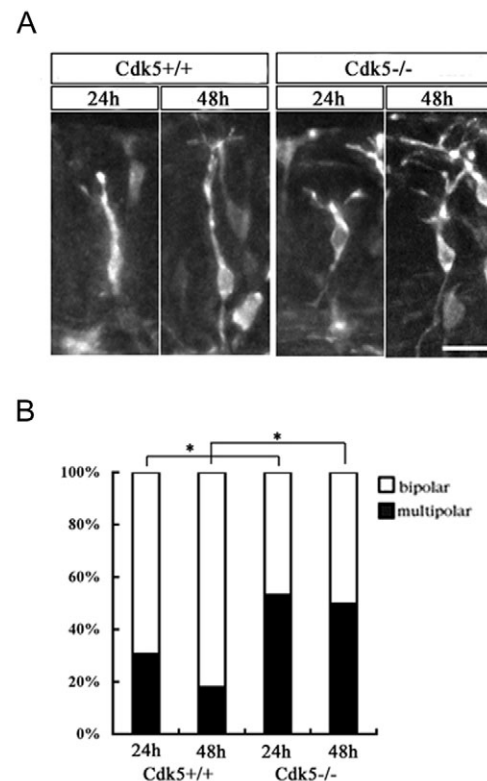


Fig. 4. Time-lapse analysis of migratory behaviour of *Cdk5*^{-/-} neurons. (A) *Cdk5*^{+/+} and *Cdk5*^{-/-} mouse embryos were infected with Adex-CAG-Lyn-Venus at E12.5, and brain slice cultures were started at E14.5. Representative images are shown of migrating neurons after 24 hours (24 h) and 48 hours (48 h) of culture. Scale bar 10 μ m. (B) GFP-positive neurons were classified as multipolar or bipolar by their morphology and behaviour at 24 and 48 hours, and the percentage is shown for each genotype. *, $P < 0.01$.

enhanced at 24 hours (Fig. 3A,B; see Movie 1 in the supplementary material). Within 20 hours, 40.15% (s.d. 14.25) of multipolar GFP-positive neurons became bipolar in the brain slices with CAG-EGFP, whereas only 7.75% (s.d. 3.77) of multipolar neurons became bipolar in those with CGC-Cdk5-DN (Fig. 3C). These results clearly indicate that neurons specifically require Cdk5 for multipolar-to-bipolar transition in the premigratory zone.

Time-lapse imaging of migratory behaviour of *Cdk5*^{-/-} neurons

We carried out time-lapse recordings of migratory behaviour in *Cdk5*^{-/-} brain slices from different stages of corticogenesis. *Cdk5*^{-/-} brain slices were particularly sensitive to mechanical stress in the advanced stages of corticogenesis. Therefore, we focused on the analysis of migratory behaviours of *Cdk5*^{-/-} neurons at an early stage. We introduced CAG-Lyn-Venus at E12.5 using the adenoviral infection system and recorded migratory behaviour for 48 hours from 2 days after adenoviral infection. On this schedule, adenoviral infection occurred at the dorsal telencephalon and labelled mostly layer V neurons as described previously (Hashimoto and Mikoshiba, 2004). In the control slices, we observed both multipolar and bipolar shapes in the premigratory zones and in the CP, which just starts to form at this stage. However, in *Cdk5*^{-/-} slices, a higher percentage of GFP-positive cells remained in multipolar shapes at 48 hours (Fig. 4; see Movie 2 in the supplementary material). Time-lapse imaging

of early-stage *Cdk5*^{-/-} embryos revealed impaired transition of neurons from multipolar to bipolar shapes even at the early stage of neuronal migration.

Because there are morphological defects in pyramidal neurons during radial neuronal migration in *Cdk5*^{-/-} embryos, it is possible that Cdk5 is required for normal development of pyramidal neurons. To test this, we cultured cortical neurons from *Cdk5*^{-/-} embryonic brains and compared the pyramidal neurons with those from *Cdk5*^{+/+} brains. Threadgill et al. (Threadgill et al., 1997) defined a pyramidal neuron as a neuron with a single dendrite that gradually tapers off from the cell body; with this definition, they found that about 45% of cultured cortical neurons from rat cerebral cortex exhibit the morphology of pyramidal neurons at 3 DIV. Using the same definition, we conducted dissociated cultures of cortical cells isolated from the cerebral cortex of E14.5 brains and observed no difference in the percentage of pyramidal neurons generated from *Cdk5*^{-/-} embryos (45.8%, s.d. 5.7) compared with those from *Cdk5*^{+/+} embryos (46.2%, s.d. 5.2) at 3 DIV (see Fig. S2 in the supplementary material). These results indicate that defective morphology is a unique phenomenon observed in migrating *Cdk5*^{-/-} neurons in vivo.

Neuronal differentiation of *Cdk5*^{-/-} neurons in vivo

A recent study suggested the coupling of neuronal migration with neurogenesis and neuronal differentiation is induced by proneural bHLH genes that upregulate the expression of doublecortin (*Dcx*) and *p35* mRNAs in migrating neurons (Ge et al., 2006). Because Cdk5 is involved in neuronal differentiation (Dahvan and Tsai, 2001), it is possible that the defects in radial migration and cellular polarities of cerebral cortical neurons described here are secondary consequences of defective neuronal differentiation. To evaluate neuronal differentiation in migrating *Cdk5*^{-/-} neurons, we performed in situ hybridisation for the expression of *Neurod1*, *Dcx* and *p35* mRNAs in *Cdk5*^{+/+} and *Cdk5*^{-/-} embryos at E16.5. *Neurod1* and *Dcx* mRNA expression levels in *Cdk5*^{-/-} neurons in the SVZ and IZ were comparable to those in *Cdk5*^{+/+} neurons (Fig. 5). Therefore, initial differentiation of migrating *Cdk5*^{-/-} neurons occurs normally, and migration defects in Cdk5-deficient cortical neurons are not caused by defective neuronal differentiation.

Cdk5-deficient cortex in the postnatal stage

Loss of Cdk5 in the developing cerebral cortex of cortex-specific *Cdk5* conditional knockout mice

We found that multipolar-to-bipolar transition of cortical neurons in *Cdk5*^{-/-} mice was impaired and this might affect the morphology of cortical pyramidal neurons in the postnatal cerebral cortex. However, we cannot examine the postnatal morphology of pyramidal neurons in *Cdk5*^{-/-} mice because they die perinatally (Ohshima et al., 1996). In order to investigate the Cdk5 loss-of-function phenotype in the cortex of the postnatal mouse brain, we used the Cre/loxP conditional knockout system. To delete the *Cdk5* gene in a cortex-specific manner, we utilised the *Emx1*Cre mouse, in which Cre recombinase is expressed in the cerebral and hippocampal cortices as early as E10 (Cappello et al., 2006). Cortex-specific *Cdk5* conditional KO (CxCdk5KO) mice were generated as *Emx1*^{Cre/+};*fCdk5*^{-/-} by mating *Emx1*^{Cre/+};*Cdk5*^{+/+} with *fCdk5*^{-/-} mice.

To confirm the loss of Cdk5 expression in the cortex of CxCdk5KO mice, we determined the levels of Cdk5 protein at E15.5, postnatal day 2 (P2) and P10. The cerebral cortex was dissected out from the brain, and homogenised brain samples were subjected to western blot analysis (Fig. 6). A substantial decrease in Cdk5 protein was observed in the cerebral cortex from CxCdk5KO

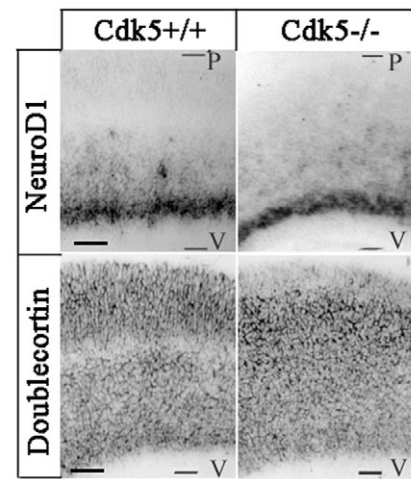


Fig. 5. In situ hybridisation in the coronal sections from *Cdk5*^{+/+} and *Cdk5*^{-/-} mouse embryos at E16.5 using *Neurod1* and doublecortin probes. The expression of these genes in the premigratory zone of *Cdk5*^{-/-} embryos was comparable to that in *Cdk5*^{+/+} embryos, indicating that initial neuronal differentiation is not disturbed in *Cdk5*^{-/-} mice. P, pia; V, ventricle. Scale bars: 100 μ m.

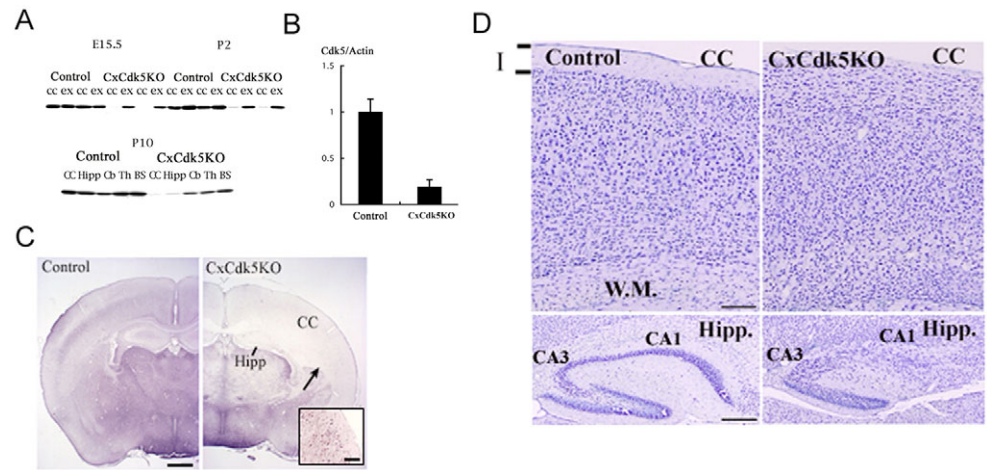
mice at E15.5 and P2 (Fig. 6A). We also detected low levels of Cdk5 protein in the hippocampus as well as in the cerebral cortex of CxCdk5KO mice at P10 [Fig. 6B; Cdk5/actin control with mean of 1 (s.d. 0.14); CxCdk5KO with a mean of 0.189 (s.d. 0.08), $n=4$, $P<0.01$]. Immunohistochemical analysis of the CxCdk5KO brain revealed a loss of Cdk5 immunoreactivity in the specific brain areas expressing Cre recombinase, including the cerebral and hippocampal cortices. Throughout these areas, we detected Cdk5-positive interneurons that derived from the ganglionic eminence, from which Cdk5 was not removed in CxCdk5KO mice (Fig. 6C, inset). Most CxCdk5KO mice survived their perinatal period and no difference in body weight was evident between CxCdk5KO mice and littermate controls at P0 (i.e. at day of birth; control, 1.32 ± 0.84 ; CxCdk5KO, 1.38 ± 0.04 , $n=5$). However, CxCdk5KO mice had retarded growth, with their body weight being significantly lower in the early postnatal days (see Fig. S3A in the supplementary material), and died between two and three weeks of age.

Inverted cortex in CxCdk5KO mice

Nissl staining revealed a defective layer structure of the cerebral cortex in CxCdk5KO mice, although the cerebral wall thickness was not changed (Fig. 6C,D) and no significant loss of neocortical neurons was observed (data not shown). Limited posterior extension of the cerebral cortex was also observed in the CxCdk5KO mice (see Fig. S3B,C in the supplementary material). In the hippocampus, neurons that normally form pyramidal cell layers of CA1 to CA3 were scattered throughout the hippocampus without forming layer structures (Fig. 6D). To analyse the positioning of cortical neurons according to their birth date, we performed serial birth-dating by BrdU (Fig. 7A). BrdU was injected into pregnant females carrying embryos of E12.5 to E16.5 and analysed at P10. In the control, neurons at each birth date segregated and formed a distribution peak in an 'inside-out' manner (Fig. 7A). However, the distribution of neurons with the same birth date was completely inverted ('outside-in') in the cerebral cortex of CxCdk5KO mice (Fig. 7A). To confirm the inverted nature of the cortex of CxCdk5KO mice, we conducted in situ hybridisation with layer markers *Foxp2*, *Er81* (*Etv1* – Mouse

Fig. 6. Cortex-specific *Cdk5*

knockout mice. (A) Loss of *Cdk5* in the cerebral cortex and hippocampus was confirmed by western blot analysis. Samples from each brain region were dissected from embryonic or postnatal mouse brains at the indicated stages and subjected to western blot analysis for *Cdk5* proteins. *Cdk5* protein levels in the cerebral cortex (cc) were low compared with those in other brain regions (ex) at E15.5 and P2. At P10, brains were separated into the cerebral cortex (CC), hippocampus (Hipp), thalamus (Th), and brain stem (BS) and analysed for *Cdk5* and actin by western blot. **(B)** Reduction in the *Cdk5* protein level (*Cdk5*/actin) was confirmed in cerebral cortex at P10. Mean \pm s.d.; $n=6$; *, $P<0.01$. **(C)** Immunostaining of the coronal section of the cerebral cortex from control and Cx*Cdk5*KO mice at P10 with anti-*Cdk5* antibody. *Cdk5* staining was observed in all regions of the control mouse brain at P10. This staining pattern was missing in the cerebral cortex and hippocampus from Cx*Cdk5*KO mice. Higher magnification of the area indicated by the arrow in the right-hand panel reveals the presence of *Cdk5*-positive interneurons, which derive from the ganglionic eminence where Cre recombinase is not expressed; these are also GABA positive (data not shown). Scale bar: 200 μ m; inset, 100 μ m. **(D)** Comparison of cerebral cortex in Nissl-stained sagittal sections revealed abnormal laminar structure in Cx*Cdk5*KO mice. In the cerebral cortex, cell-sparse structures of layer I (I) and white matter (W.M.) observed in the control were not evident in the cerebral cortex in Cx*Cdk5*KO mice. In the hippocampus, pyramidal neurons failed to form a confined layer in Cx*Cdk5*KO mice. Scale bar: 100 μ m.



Genome Informatics) and *Cux2* (*Cutl2* – Mouse Genome Informatics). In agreement with previous reports (Ferland et al., 2003; Arber et al., 2000; Nieto et al., 2004), *Foxp2*-positive neurons were distributed in layer VI, the deepest layer, whereas *Er81*-positive neurons were positioned in layer V, and *Cux2*-positive neurons were in superficial layers II and III in the cerebral cortex of control mice (Fig. 7B). However, in the Cx*Cdk5*KO cerebral cortex,

Foxp2-positive neurons occupied a superficial position and *Cux2* occupied the deeper position. These results confirmed an inversion of the cerebral cortex layer structure in Cx*Cdk5*KO mice.

Using in utero electroporation and GFP immunostaining, we analysed the position and morphology of neurons in the cerebral cortex of Cx*Cdk5*KO mice. The CAG-EGFP plasmid was introduced at E14.5, and fixed sections were examined at E17.5, P1 and P3. At E17.5, GFP-positive neurons in Cx*Cdk5*KO embryos had

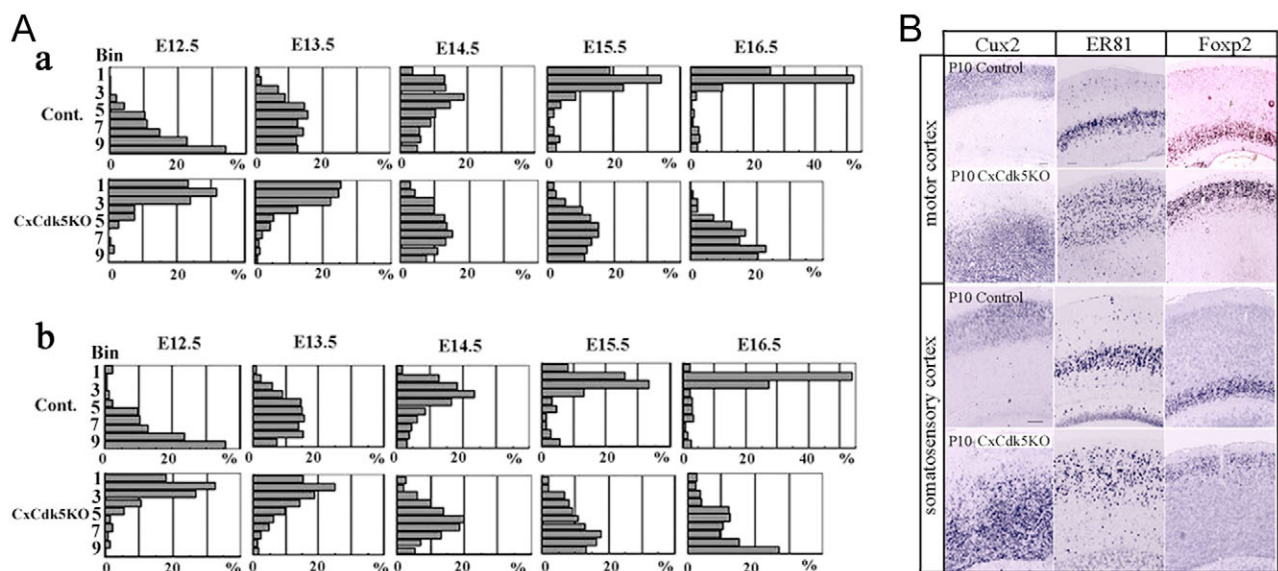


Fig. 7. Inverted cortex in Cx*Cdk5*KO mice. (A) Bin distributions of BrdU-positive cells shown as mean percentage of three mice for control (Cont.) and Cx*Cdk5*KO mice (Cx*Cdk5*KO) at indicated time points. The cortical mantle was divided into ten equally spaced bins, and the percentage of BrdU-positive cells in each bin is shown. Birth-dating BrdU labelling from E12.5 to E16.5 revealed a typical inside-out pattern of generation of the laminar structure in the cerebral cortex of control mice at P10. This pattern was inverted, resulting in an outside-in pattern in the motor cortex (a) and somatosensory cortex (b) of Cx*Cdk5*KO mice. **(B)** In situ hybridisation study with layer markers *Cux2*, *Er81* and *Foxp2* at P10. In the control, *Cux2*-positive, *Er81*-positive and *Foxp2*-positive neurons accumulated in layer II/III, layer V and layer VI, respectively. This pattern was inverted in the Cx*Cdk5*KO cortex. Scale bar: 100 μ m.

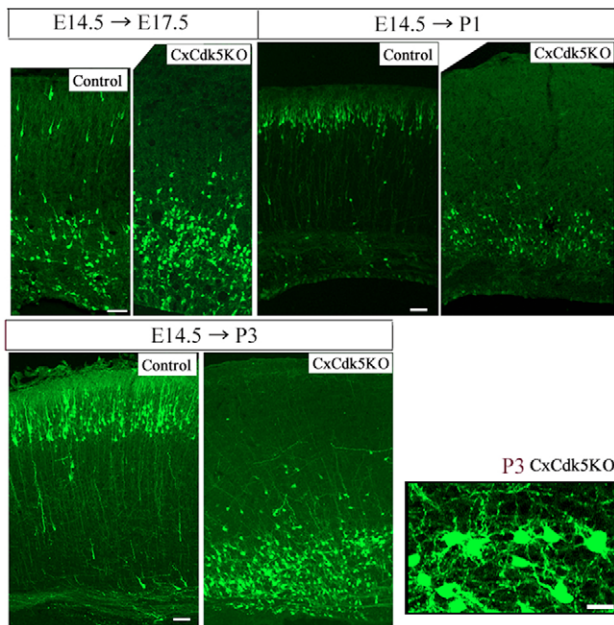


Fig. 8. Morphologies of GFP-labelled neurons at E14.5 in the cerebral cortex of control and CxSdk5KO mice.

GFP immunostaining of coronal sections from CxSdk5KO mice and their littermate controls which were electroporated at E14.5 with CAG-EGFP plasmid. Brains were fixed at E17.5, P1 or P3 and stained with anti-GFP antibody (green). GFP-positive neurons in CxSdk5KO mice were positioned deep down and had multipolar morphology. Scale bars: 100 μ m. The bottom-right panel is a higher magnification image of GFP-positive cells in the cerebral cortex of CxSdk5KO mice at P3 obtained with a confocal microscope. Scale bar: 20 μ m.

identical abnormalities to those in *Cdk5*^{-/-} mice as described above (Fig. 1). At P1 and P3, GFP-positive neurons in the sections from the littermate controls were positioned at layer II/III with typical pyramidal morphology. By contrast, GFP-positive neurons in CxSdk5KO mice were localised at the bottom of the cortex and had the multipolar morphology (Fig. 8). These results indicate that the inability of neurons to acquire the bipolar shape during radial migration resulted in defective pyramidal morphology, particularly of the dendritic structures.

Lack of Cdk5 results in abnormal dendrites of layer V neurons

Pyramidal neurons, typically large pyramidal neurons in layer V, have a bipolar shape with a single apical dendrite that extends towards the pia surface and a single axon that extends towards the ventricle side of the cortex. We previously generated *p35*^{-/-};YFP-H double-transgenic mice to visualise the detailed morphology of hippocampal pyramidal neurons (Ohshima et al., 2005). Because the pyramidal neurons of a subset of layer V neurons in the cerebral cortex, as well as those in the hippocampus, are YFP-positive in the YFP-H line after P10 (Feng et al., 2000), we could observe dendrite, soma and axon morphologies of these neurons in detail. We applied this strategy to analyse the morphological features of Cdk5-deficient pyramidal neurons in the postnatal cortex of CxSdk5KO mice. In the control mice, a typical pyramidal morphology of layer V neurons was observed: a single apical dendrite extending toward the pia with

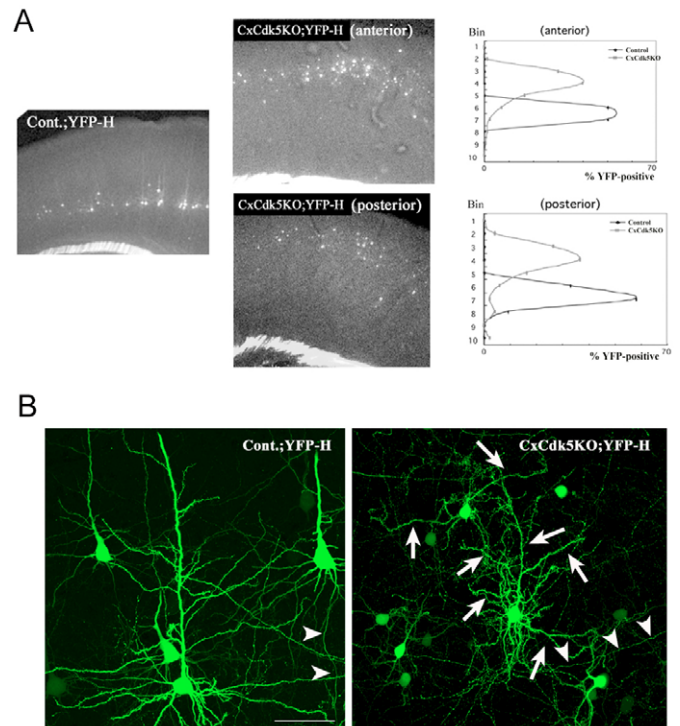


Fig. 9. Defective dendritic development in CxSdk5KO mice.

(A) Position and (B) detailed morphologies of layer V neurons in CxSdk5KO;YFP-H double-transgenic mice. (A) Typical examples of the cerebral cortex in coronal sections from control and CxSdk5KO mice at P14. Bin distributions of YFP-positive layer V neurons are shown (control, black; CxSdk5KO, grey). (B) Confocal images of the cerebral cortex of control (Cont.;YFP-H) and CxSdk5KO (CxSdk5KO;YFP-H) mice at P14. Abnormal dendritic structures (arrows) and axonal trajectories (arrowheads) are present in CxSdk5KO mice. Scale bar: 50 μ m.

secondary branches, basal dendrites and a single axon exiting from the ventricular side and running straight down (Fig. 9A). However, in CxSdk5KO mice, the dendrite morphology of layer V neurons was largely abnormal, in addition to their inverted position in the cortex (Fig. 9B). In the majority of layer V neurons, the apical dendrite was difficult to identify; instead, multiple dendrites extended directly from the soma (Fig. 9B). Furthermore, an abnormal axonal trajectory was also observed in the cortex of CxSdk5KO mice; each neuron had a single axon, but the exit site of the axon was not consistent and axons ran obliquely within the cortex (Fig. 9B). To elucidate the relationship between dendritic abnormalities and the ectopic position of layer V pyramidal neurons, we analysed the morphologies of layer V neurons in *reeler*;YFP-H mice. We observed an abnormal orientation of apical dendrites of layer V neurons, similar to previous reports (Terashima et al., 1983). Importantly, layer V pyramidal neurons preserved pyramidal morphology in the cerebral cortex of *reeler* mice, despite their ectopic localisation (see Fig. S4 in the supplementary material). This result indicates that abnormal dendritic structures in the CxSdk5KO mice are not a secondary consequence of ectopic localisation of layer V neurons.

We further analysed the morphology of callosal commissural (CC) neurons in CxSdk5KO mice at P10 using retrograde DiI labelling. In the control, mainly layer II/III pyramidal neurons were labelled by DiI, and these neurons had typical pyramidal morphology as described above (data not shown). However, labelled CC neurons had an

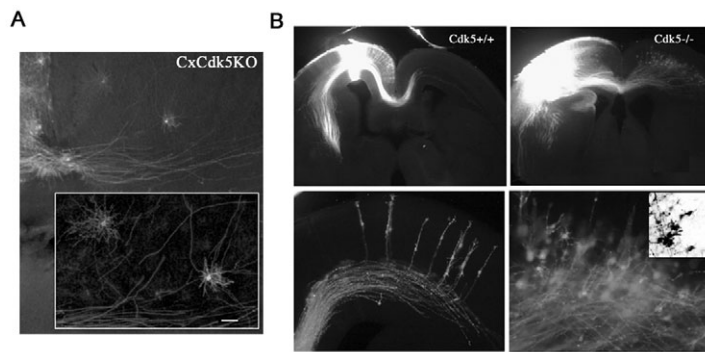


Fig. 10. DiI labelling of commissural neurons in P10 CxSdk5KO and *Cdk5*^{-/-} mice. DiI crystals were placed in the cerebral cortex of fixed brains of (A) P10 CxSdk5KO and (B) E18.5 *Cdk5*^{-/-} mice and littermate controls. Vibratome sections (100 μ m) were imaged using a fluorescent (A,B) or confocal (inset in B) microscope. (A) Abnormal dendrite development of commissural neurons in CxSdk5KO mice. Scale bar: 25 μ m. (B) In *Cdk5*^{+/+} mice, cortical pyramidal neurons extended their apical dendrites toward the pia. By contrast, *Cdk5*^{-/-} neurons extended their dendrites in multiple directions. Inset, confocal image of abnormal dendrite structures of *Cdk5*^{-/-} commissural neurons.

abnormal morphology in CxSdk5KO mice, similar to layer V neurons in CxSdk5KO;YFP-H mice (Fig. 10A). These results indicate that the abnormal morphology of pyramidal neurons occurred throughout the cortex in CxSdk5KO mice. By DiI labelling, we also found an abnormal dendritic morphology and axonal trajectory in the contralateral cerebral cortex in *Cdk5*^{-/-} mice at E18.5 (Fig. 10B).

We also studied the level of the Map2 (Mtap2 – Mouse Genome Informatics) protein, a dendrite marker, in the cerebral cortex of CxSdk5KO mice (Fig. 11A,B), as reduced expression of Map2 has been reported in *Cdk5*^{-/-} embryos at E16.5 and in cultured *Cdk5*^{-/-} neurons (Cicero and Herrup, 2005). Overall, staining of Map2 decreased in the cerebral cortex of CxSdk5KO mice at P10 (Fig. 11A), consistent with the decreased protein level of Map2 [Fig. 11B; Map2/actin control with a mean of 1 (s.d. 0.10) versus CxSdk5KO with a mean of 0.04 (s.d. 0.16), $n=4$, $P<0.01$]. Because the protein level of NF-M (neurofilament, medium polypeptide; also known as Nefm – Mouse Genome Informatics) was not changed [NF-M/actin control with mean of 1 (s.d. 0.12) versus CxSdk5KO with mean of 0.78 (s.d. 0.06), $n=4$, $P=0.055$], the specific decrease in Map2 might reflect the developmental deficit of the dendritic structure of pyramidal neurons, i.e. that defects in the dendrite structures of pyramidal neurons are associated with a lower level of Map2 in the cerebral cortex of CxSdk5KO mice. Map2-positive apical dendrites were evident in the cerebral cortex of the controls (Fig. 11A). Such radial staining of Map2 was missing in the cerebral cortex of CxSdk5KO mice. Instead, Map2-positive staining was detected in the superficial layer, where layer VI neurons existed in CxSdk5KO mice (Fig. 11A). The morphology of layer VI neurons, which extend

their axons toward the dorsal thalamus, was examined by retrograde labelling with DiI. As in our previous report (Gilmore et al., 1998), we found that layer VI neurons, although placed in the superficial layer, preserved the pyramidal morphology in the *Cdk5*^{-/-} embryonic brain (Fig. 11C). We also found that layer VI neurons had typical pyramidal morphology in the cerebral cortex in CxSdk5KO mice at P10 (Fig. 11D).

DISCUSSION

We have previously shown that Cdk5 is required for the proper neuronal migration of cortical neurons in the cerebral cortex (Ohshima et al., 1996; Gilmore et al., 1998). In the present study, we report that Cdk5 is mainly involved in the transitional phase of neurons from the multipolar to bipolar during radial neuronal migration. When we suppressed Cdk5 using Cdk5-DN, we observed a severe impairment of multipolar-to-bipolar transition in neurons expressing Cdk5-DN in the time-lapse imaging study (Fig. 3), as well as in vivo (Fig. 2). This result is consistent with our observation of the behaviour and morphology of migrating *Cdk5*^{-/-} neurons (Figs 1, 4). Our observation of neurons expressing Cdk5-DN differs from a previous study that found a substantial delay in the migration of neurons expressing Cdk5-DN after they became bipolar (Hatanaka et al., 2004). However, Hatanaka et al. used an equal ratio of EGFP and Cdk5-DN plasmid. When we used a 50% (1:1) dilution of Cdk5-DN, some neurons became bipolar, but their migration was delayed within the CP (Fig. 2). Interestingly, some of these neurons had branched leading processes (Fig. 2A' b, and milder morphological abnormalities that have been observed in *p35*^{-/-} neurons (Gupta et al., 2003) (T.O., T.A. and K.M.,

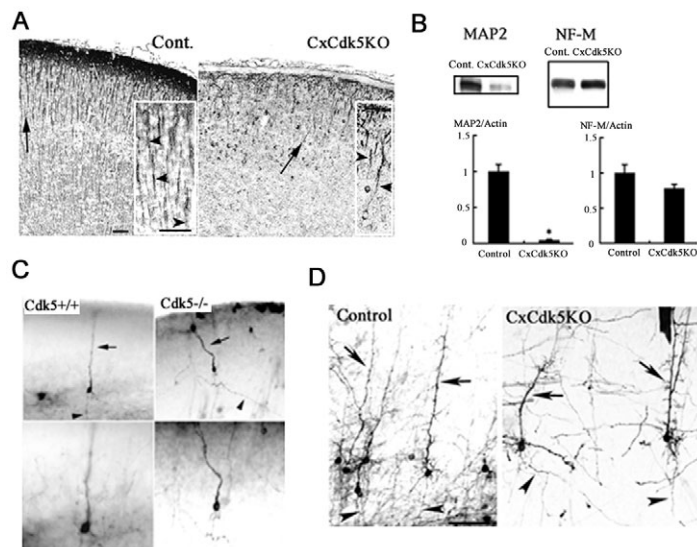


Fig. 11. Reduced Map2 expression in the CxSdk5KO cortex, and unaltered pyramidal morphology of layer VI neurons in *Cdk5*-deficient brains. (A) Map2 immunostaining revealed defective dendrite structures of pyramidal neurons in CxSdk5KO mice. Apical dendrites of pyramidal cortical neurons were typically Map2-positive in the control (inset, arrowheads). This pattern was missing, with only a few Map2-positive radial dendrites observed in the superficial layer of the CxSdk5KO cortex (inset, arrowheads). Insets are higher magnifications of the areas indicated by the arrows. Overall, staining of Map2 immunoreactivity was reduced in the CxSdk5KO cortex. (B) Protein levels for Map2, NF-M and actin were examined in the dissected cerebral cortices from control and CxSdk5KO mice at P10. The reduction in Map2/actin in CxSdk5KO mice was also confirmed by western blotting. *, $P<0.01$. The level of NF-M/actin was not significantly altered in CxSdk5KO mice. (C,D) Cortico-thalamic neurons, corresponding to layer VI neurons, extended their apical dendrite toward the pia in *Cdk5*^{-/-} (C) and CxSdk5KO (D) mice. Arrows indicate apical dendrites and arrowheads indicate their axons. Scale bars: 100 μ m.

unpublished). These results indicate that strong suppression of Cdk5 activity causes impaired multipolar-to-bipolar transition, just like that seen in the *Cdk5^{-/-}* mice, and moderate suppression of Cdk5 delays radial migration within the CP after acquisition of the bipolar shape, although these bipolar neurons sometimes have branched leading processes. These observations also suggest that the multiple morphologies we observed in *Cdk5^{-/-}* migrating neurons were not simply the result of migration arrest at the multipolar stage of radial migration. When we cultured the *Cdk5^{-/-}* neurons from the cerebral cortex, however, *Cdk5^{-/-}* neurons were able to acquire the pyramidal morphology (see Fig. S2 in the supplementary material), indicating that loss of Cdk5 does not severely affect the ability of cortical neurons to acquire the pyramidal morphology. Morphological change during transition from multipolar to bipolar might require dynamic rearrangement of the cytoskeleton of migrating neurons and might require Cdk5 activity. Recent studies indicate that this transitional process is considered a stage of neocortical development vulnerable to disruption, as RNAi for migration-related proteins including Lis1 (Pafah1b1 – Mouse Genome Informatics) and Dcx disrupts this transition as well (reviewed by Bai et al., 2003; Tsai et al., 2005; LoTurco and Bai, 2006).

The most interesting finding is the abnormal morphology of pyramidal neurons in the cerebral cortex of Cx*Cdk5*KO mice. These neurons failed to develop an apical dendrite structure and extended multiple dendrites. Because this defect was observed in *Cdk5^{-/-}* mice at E18.5 (Fig. 10B), as well as in Cx*Cdk5*KO mice postnatally (Figs 8, 9), it is likely that this abnormality occurs as a consequence of the inability of neurons to acquire a bipolar morphology during their radial migration. Our analysis with DiI labelling indicated that abnormal pyramidal morphology occurred throughout the cortical neurons corresponding to layer II/III and layer V (Fig. 10A). Our preliminary results from Golgi staining of the Cx*Cdk5*KO mouse brain are consistent with these results (T.O., T.A. and K.M., unpublished). It is known that intrinsic afferent input plays an important role in the sculpting of the mature neuronal dendritic tree. Therefore, we cannot exclude the possibility that the ectopic position of Cdk5-deficient pyramidal neurons might be at least partially responsible for the abnormalities of dendrite structures described above. However, our analysis of *reeler* mice indicates that the typical pyramidal morphology, with a single apical dendrite, is preserved in layer V neurons even in their ectopic positions in this mutant (see Fig. S4 in the supplementary material). Another possible explanation for abnormal dendrite structure in Cx*Cdk5*KO mice is that the absence of Cdk5 causes neuronal apoptosis (Li et al., 2002), and the remaining neurons exhibit dendrite abnormalities. However, we did not detect any significant loss of cortical neurons in postnatal-stage Cx*Cdk5*KO mice (data not shown). Therefore, we consider that the morphological abnormality in Cx*Cdk5*KO mice is cell-autonomous and tightly related to the transitional defect from multipolar to bipolar during neuronal migration.

Why does Cdk5 deficiency cause substantial defects in the radial migration of cortical neurons? Recent studies identified Cdk5 substrates that are related to neuronal migration, including Dcx, Nudel (Ndel1 – Mouse Genome Informatics) and Fak1 (Ptk2 – Mouse Genome Informatics) (Tanaka et al., 2004b; Sasaki et al., 2000; Niethammer et al., 2000; Xie et al., 2003). Phosphorylation of Nudel, Dcx and Fak1 at Cdk5-phosphorylation sites is considered important to organise the link between centrosome and nucleus through their association with microtubules (Tanaka et al., 2004a). siRNA of Dcx causes premature arrest of migrating multipolar neurons in the premigratory zone (Bai et al., 2003). Inhibition of Rac1 arrests the migration of cerebral cortical neurons in the multipolar morphology

(Kawauchi et al., 2003); therefore, improper regulation of RhoGTPase in migrating neurons might impair their transformation from multipolar to bipolar. Other studies suggest that RhoA activity is inhibited within the IZ for proper radial neuronal migration (Hand et al., 2005; Ge et al., 2006). Interestingly, Cdk5 phosphorylates p27 (Cdkn1b – Mouse Genome Informatics) at Ser10 and stabilises p27 in the cytoplasm of migrating neurons (Kawauchi et al., 2006). Because p27 inhibits RhoA activity (Besson et al., 2004), Cdk5-mediated phosphorylation of p27 regulates RhoA activity during radial neuronal migration (Kawauchi et al., 2006). Cdk5 also phosphorylates the actin-binding protein filamin A, and a decrease in the expression of filamin A results in the accumulation of multipolar cells in the premigratory zone (Nagano et al., 2004). Therefore, it is clear that Cdk5 phosphorylates multiple substrates and regulates the dynamics of microtubules and of the cytoskeletal protein actin in the transitional phase from multipolar to bipolar. Cdk5 also regulates nucleokinesis, which maintains a proper distance between the nucleus and centrosome (Tanaka et al., 2004a). Recent studies indicate involvement of non-muscle type myosin II in the nucleokinesis at the rear of migrating neurons (Schaar and McConnell, 2005; Bellion et al., 2005) and the possible involvement of Cdk5 in this process remains to be elucidated.

A previous study demonstrated that early-born neurons, which correspond to layer VI, migrate by somal translocation and that this migration is Cdk5-independent (Nadarajah et al., 2001). Our results from in situ hybridisation (Fig. 7B) and DiI labelling experiments (Fig. 11C,D) suggest that layer VI neurons migrate properly and obtain a pyramidal morphology. Our explanation for why Cdk5 deficiency has little impact on layer VI neurons is that layer VI neurons use somal translocation as their migration mode and skip the transitional process from the multipolar to bipolar morphology (Noctor et al., 2004). Thus, they can migrate properly and obtain a pyramidal morphology in the absence of Cdk5. In this sense, our current study supports the proposed idea that early- and later-born neurons use distinct molecular and cellular mechanisms for their radial migration (Nadarajah et al., 2001; Hatanaka et al., 2004). An alternate explanation is that neurons need to migrate a relatively short distance in the early stages of cortical development; therefore, *Cdk5^{-/-}* neurons would be able to reach the pia even in the multipolar morphology or in the incompletely transformed bipolar shape, such as the branched shape, previously reported in *p35^{-/-}* neurons (Gupta et al., 2003).

Although there is as yet no direct evidence in migrating neurons for a transition from the leading process to apical dendrite, the bipolar shape in migrating neurons is likely to be a fundamental requirement for the pyramidal neurons to obtain their correct morphology (Hatanaka and Murakami, 2002). Our results provide evidence to support this view, because the inability to acquire a bipolar morphology during migration resulted in abnormal dendrite development in Cdk5-deficient pyramidal neurons. In addition, recent observations suggest that migrating neurons already extend their axons (Noctor et al., 2004; Hatanaka and Murakami, 2002). In the present study, we also observed that extensions of axons from migrating neurons (Fig. 1A) and axonal trajectories are impaired in Cdk5-deficient neurons (Fig. 1B; Fig. 9), probably because of their defective migration. Based on our findings in the Cdk5-deficient mouse models, we propose that the transition of migrating neurons into a bipolar shape is essential to form the pyramidal morphology in which neurons extend their single apical dendrite toward the pia, and extend single axons towards the bottom of the cortex, and that this radial axis is brought about by the active radial migration of neurons in a Cdk5-dependent manner.

We thank Drs T. Jessell and C. Walsh for *in situ* probes; Dr J. Miyazaki for the CAG-GS plasmid; and Elias Utreras and Harry Grant for critical reading of this paper. This work was supported by Grants-in-Aid for Scientific Research on Priority Area Molecular Brain Science from NEXT of Japan (18022050 to T.O.), and by NEXT, the Japan Society for the Promotion Science, the Japan Brain Foundation and the Tokyo Biochemical Research Foundation (to K.N.).

Supplementary material

Supplementary material for this article is available at <http://dev.biologists.org/cgi/content/full/134/12/2273/DC1>

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