Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 8196–8201, July 1997
Neurobiology

Disruption of hippocampal development *in vivo* by CR-50 mAb against Reelin

KAZUNORI NAKAJIMA*†, KATSUHIKO MIKOSHIBA*‡, TAKAKI MIYATA*‡§, CHIKAKO KUDO*, AND MASAHARU OGAWA§

*Molecular Neurobiology Laboratory, Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN), Tsukuba, Ibaraki 305, Japan; ‡Department of Molecular Neurobiology, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108, Japan; and §Department of Physiology, Kochi Medical School, Nankoku, Kochi 783, Japan

Communicated by Thomas S. Reese, National Institutes of Health, Bethesda, MD, May 13, 1997 (received for review January 10, 1997)

ABSTRACT **We previously generated a monoclonal alloantibody, CR-50, by immunizing *reeler* mutant mice with homogenates of normal embryonic brains. This antibody recently was shown to recognize a Reelin protein, which is coded by the recently identified candidate gene for the *reeler* mutation. However, it is still unclear whether Reelin, especially the CR-50 epitope region, is indeed responsible for the *reeler* phenotype *in vivo*. Here we show that Reelin is localized on Cajal–Retzius neurons in the hippocampus and that intraventricular injection of CR-50 at the embryonic stage disrupts the organized development of the hippocampus *in vivo*, converting it to a *reeler* pattern. Labeling experiments with 5-bromodeoxyuridine demonstrated that the labeled cells in the stratum pyramidale of the CR-50-treated mice were distributed in a pattern similar to that of *reeler*. Thus, Cajal–Retzius neurons play a crucial function in hippocampus development, and the CR-50 epitope on Reelin plays a central role in this function.**

The *reeler* mutant mouse provides a good model to study the molecular mechanisms of neuronal positioning. In these mice, neurons are generated normally, but they align abnormally in various cortical structures (1–7). We recently obtained alloantibodies by immunizing *reeler* mutants with homogenates of normal embryonic brains (8). The monoclonal alloantibody CR-50 specifically recognizes Cajal–Retzius neurons (9–11), one of the first types of cortical neurons to differentiate in the neocortex of normal embryos but not those of *reeler* embryos. By using dissociation-reaggregation cultures of neocortical cells, we showed that CR-50 can transform the normal histotypic aggregate pattern to the *reeler* pattern. Thus, occlusion of the CR-50 epitope was thought to be related to the *reeler* pattern of neocortex organization, at least *in vitro* (8). A cDNA of the candidate *reeler* gene recently was identified by insertional mutagenesis (12) or positional cloning (13, 14) and designated *reelin* (12). The predicted Reelin protein resembles extracellular matrix proteins, and thus is likely to be responsible for the *reeler* phenotype. However, it is not yet known if the *reelin* clone can complement the *reeler* defect. Very recently, the CR-50 was shown to recognize an epitope in the N-terminal region of Reelin (15). However, it is still unclear whether Reelin, and in particular the CR-50 epitope region, is indeed responsible for the *reeler* phenotype *in vivo*.

To address this question, we chose the hippocampus as a model. The events of hippocampal development have been described extensively (1, 2, 16–30). In the developing hippocampus, neurons migrate from the site of their origin near the ventricle to their final destination along a scaffold formed by radial fibers (27), where they are aligned in an organized cell layer. The layer is formed in an "inside-out" progression, in which the later generated neurons are aligned more superficially than the earlier generated neurons (1, 2, 19, 22, 23, 26). In the hippocampus of *reeler*, the pyramidal neurons are known to be less densely packed and, most prominently, the stratum pyramidale of the regio superior is interrupted by cell-poor rifts, resulting in irregular and fragmented cell laminae (3–7). In addition, the stratum pyramidale is formed in an inverted "outside-in" fashion (1, 2).

Here we show that the CR-50 epitope also is localized on Cajal–Retzius neurons in the hippocampus and that intraventricular injection of CR-50 disrupts the organized development of the hippocampus, resulting in a pattern similar to that found in *reeler*. Thus, the CR-50 epitope on the Reelin molecule plays an essential role in hippocampal development and is responsible for the *reeler* phenotype *in vivo*.

MATERIALS AND METHODS

Mice. Homozygous *reeler* mice used in this study were bred from heterozygous B6C3Fe-a/a-rl adults (The Jackson Laboratory) and maintained at the animal facilities of The Institute of Physical and Chemical Research (RIKEN), Ibaraki, Japan. ICR mice were obtained from CLEA Japan (Tokyo). The day at which a vaginal plug was detected was designated as embryonic day 0 (E0).

Immunohistochemistry. Pregnant wild-type B6C3Fe mice were sacrificed at day 17 of gestation by an overdose of ether. Embryos were placed on ice for anesthesia and perfused transcardially with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). Fetal brains were removed and postfixed in the same fixative for 12 hr at 4°C. After the brains were replaced gradually with 30% sucrose in PBS, they were embedded in OCT compound (Miles) and frozen on dry ice. Cryostat coronal sections (10 μm) were mounted on glass slides. For immunostaining with CR-50, the sections were blocked with 5% normal goat serum in PBS containing 0.01% Triton X-100 (Sigma) and incubated with CR-50, followed by rhodamine-conjugated goat anti-mouse IgG (Organon Teknika–Cappel; 1:100). For double-labeling, rabbit polyclonal antibody against microtubule-associated protein 2 (MAP2) (1:1,000; kindly provided by M. Niinobe, Osaka University, Osaka, Japan) or rabbit anti-calretinin antibody (Chemicon; 1:500) was used with CR-50 and visualized with rhodamine-conjugated goat anti-rabbit IgG (Cappel; 1:100) and fluores-

Abbreviations: BrdUrd, 5-bromodeoxyuridine; E*n*, embryonic day *n*; FITC, fluorescein isothiocyanate; P*n*, postnatal day *n*; MAP2, microtubule-associated protein 2.

†To whom reprint requests should be sent at: Molecular Neurobiology Laboratory, The Institute of Physical and Chemical Research (RIKEN), 3–1-1 Koyadai, Tsukuba, Ibaraki 305, Japan. e-mail address: nakajima@rtc.riken.go.jp.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

cein isothiocyanate (FITC)-conjugated horse anti-mouse IgG (Vector Laboratories; 1:100).

5-Bromodeoxyuridine (BrdUrd) Labeling. Pregnant mice were injected intraperitoneally with BrdUrd (Sigma; 10 mg/ml in PBS) at 70 μg per gram of body weight. For immunostaining with anti-BrdUrd antibody, sections were treated with 5 M HCl and neutralized with 0.1 M sodium borate (pH 8.5). After the sections were blocked with 5% normal goat serum, they were incubated with mouse anti-BrdUrd (Becton Dickinson; 1:100) and then treated with FITC-conjugated horse anti-mouse IgG (Vector; 1:100). For double-staining, the sections were blocked, incubated with CR-50 and then biotinylated goat anti-mouse IgG (Vector; 1:200), followed by treatment with 5 M HCl. After neutralization with 0.1 M sodium borate, the sections were blocked again, incubated with sheep anti-BrdUrd (Fitzgerald, Concord, MA; 1:50), and treated with rhodamine-conjugated donkey anti-sheep IgG (Chemicon; 1:1,000) and FITC-avidin D (Vector; 1:200).

Hippocampal Culture. The hippocampus was dissected from E17 embryos and digested in 0.25% trypsin. Cells were washed, seeded onto polyethylenimine (Sigma)-coated culture dishes at 2×10^5 cells/cm^2, and grown at 37°C in a humidified atmosphere of 5% CO_2 in DMEM (Sigma) supplemented with 10% fetal calf serum.

Intraventricular Injection of Antibodies. Timed pregnant ICR mice were anesthetized with sodium pentobarbital (0.06 mg/g). After cesarean section, the uterine horns were exposed, and the lateral and third ventricles of the embryos were identified under transillumination. Two to four microliters of CR-50 ascites fluid (8 mg of IgG per ml), purified CR-50 (50 mg/ml in sterile saline), or Fab fragments of CR-50 (20 mg/ml in sterile saline) were injected into the ventricles using a glass capillary. Injected embryos were placed back into the abdominal cavity for spontaneous delivery. The CR-50 ascites fluid was obtained from BALB/c nude mice (CLEA Japan) injected with R3B9 hybridoma cell lines secreting CR-50. Purified CR-50 was prepared with protein G-Sepharose (Pharmacia) and concentrated with a Centriprep-30 (Amicon). Nonimmunized adult mouse IgG and mouse anti-fibronectin mAbs (Transduction Laboratories, Lexington, KY) were used as controls (50 mg/ml in sterile saline).

RESULTS

Immunohistochemical Analysis with CR-50 on Hippocampus. To examine the role of the Reelin protein in hippocampal development, we first performed an immunohistochemical analysis with CR-50. In the developing hippocampus (Fig. 1*A*), CR-50-positive cells were localized in the outer marginal zone (prospective stratum lacunosum-moleculare) of the hippocampus proper and in the dentate marginal zone (prospective molecular layer of the area dentata) (Fig. 1 *B* and *C*). The immunoreactivity decreased after birth and was barely detected at postnatal day 30 (P30) (data not shown), similar to the profile observed in the neocortex (8). Sections from *reeler* mutants never showed positive reactivity with CR-50 (data not shown). The CR-50-positive cells reside near the marginal ends of radial fibers, which are labeled with anti-nestin antibody (31, 32) (data not shown). These CR-50-positive cells exhibited a large cytoplasm with thick processes and also were labeled with an antibody against MAP2 (Fig. 2 *A-C*), which is a neuron-specific marker and is known to stain early generated neurons (33–35). Injection of BrdUrd into pregnant dams revealed that these cells arose on E9.5 to E11 (Fig. 2*D*), when most of the pyramidal neurons and granule neurons are not yet apparent (16). These data strongly suggest that the CR-50-positive cells in the hippocampus are equivalent to the Cajal–Retzius neurons in the neocortex.

Relationship Between the Reelin-Positive Cells and the Calretinin-Positive Cells. The existence of Cajal–Retzius neurons in the hippocampus recently was proposed (36–38). Because these cells were identified with an anti-calretinin antibody (37, 38), we double-stained sections with CR-50 and anti-calretinin. In the marginal zones of the hippocampus proper and the area dentata, there were some calretinin-positive cells in the population stained with CR-50, but there were also CR-50-positive, calretinin-negative cells (Fig. 2 *E-G*). Cultured cells from the hippocampus also revealed the existence of both calretinin-positive cells and calretinin-negative cells among the CR-50-positive population (Fig. 2 *H-J*). These results suggest that the Cajal–Retzius neurons identified with CR-50 include the previously described calretinin-positive cells in the marginal zone and an additional population of CR-50-positive, calretinin-negative cells.

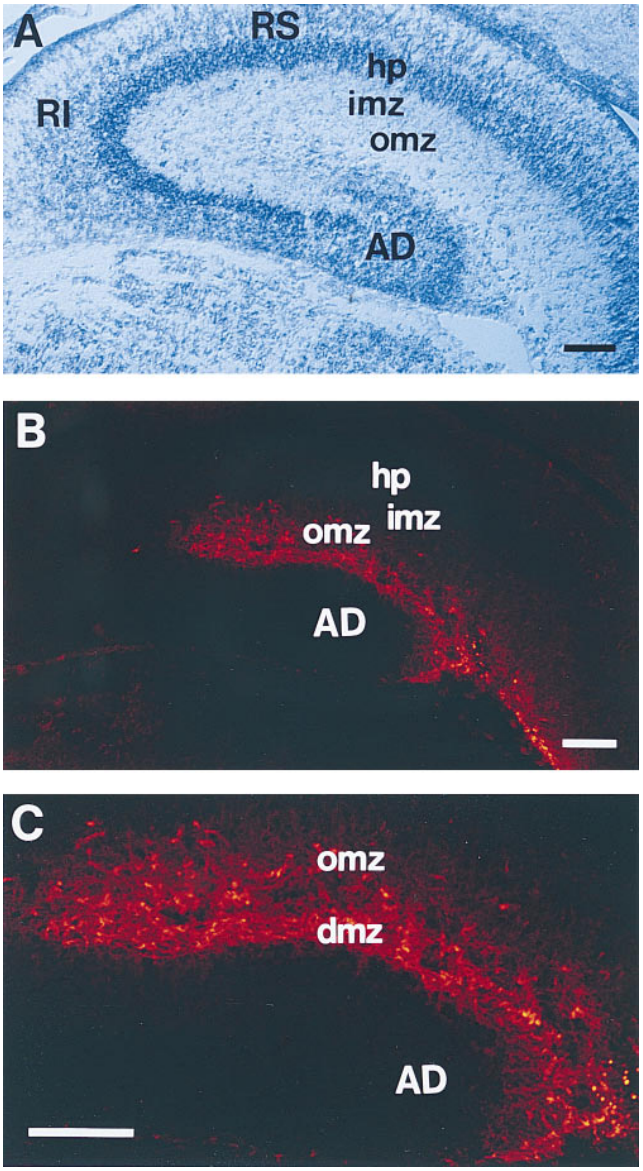

FIG. 1. Immunolocalization of the CR-50 epitope in the developing hippocampus. (*A*) Toluidine blue staining of wild-type mouse hippocampus at E17. (*B* and *C*) Immunohistochemical staining of E17 hippocampus with CR-50, showing localization of its epitope in the outer marginal zone of the hippocampus proper and in the dentate marginal zone. AD, area dentata; dmz, dentate marginal zone; hp, hippocampal plate; imz, inner marginal zone; omz, outer marginal zone; RI, regio inferior; RS, regio superior. (Scale bars, 100 μm.)

Disruption of Hippocampal Development *in Vivo* by CR-50 Antibody. To investigate whether the CR-50-positive Cajal–Retzius neurons play a role in the alignment of the pyramidal

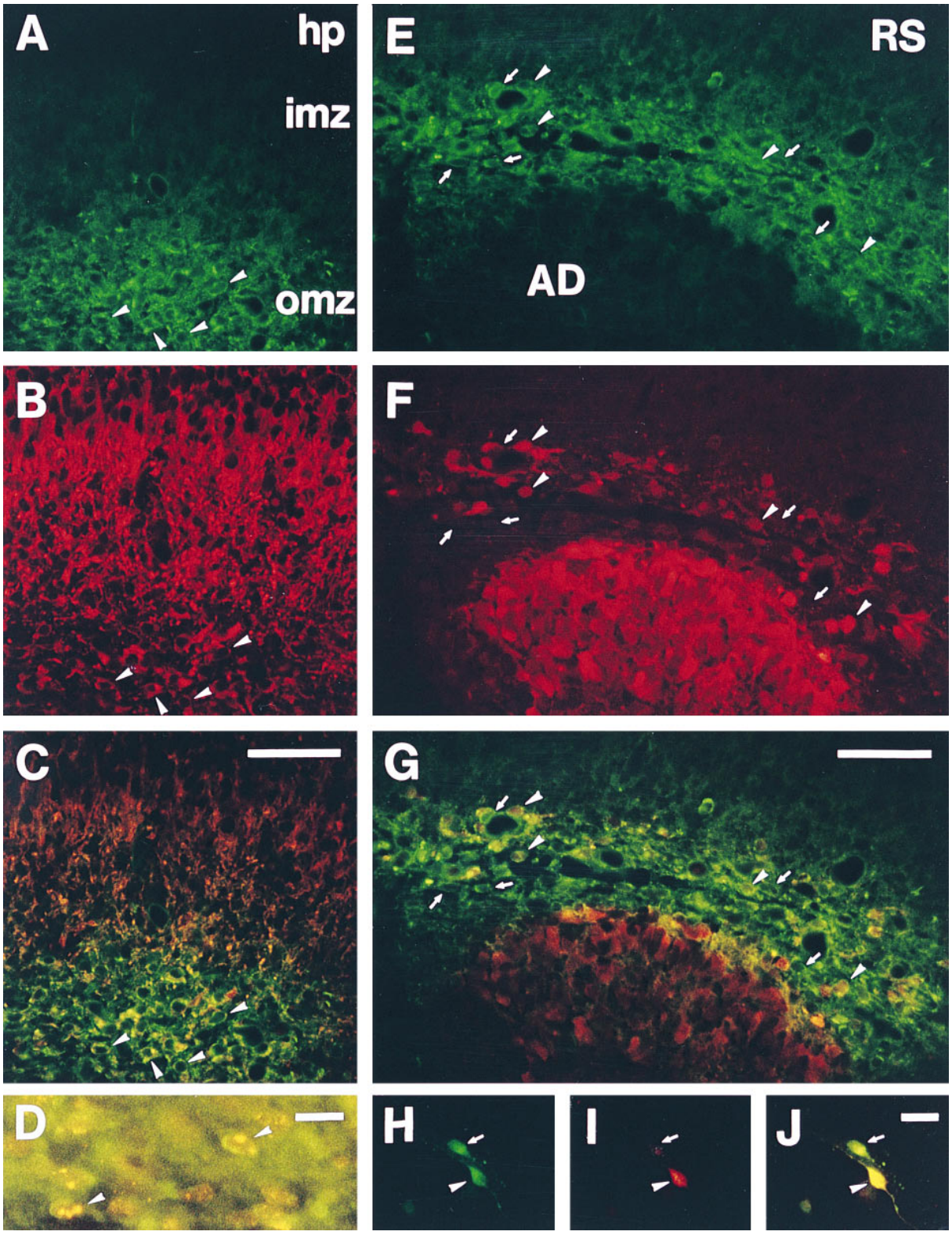

FIG. 2. Characterization of the CR-50-positive cells. (*A–C*) Double-staining with CR-50 and anti-MAP2 on wild-type mouse hippocampus at E17. CR-50-positive cells (*A*) are also positive for anti-MAP2 (*B*) (arrowheads). A double-exposed photomicrograph of *A* and *B* is shown in *C*. (Scale bar in *C*, 50 μm.) (*D*) BrdUrd injected at E9.5 was detected with anti-BrdUrd antibody (red) at E17, showing that the CR-50-positive cells (green) arose at this stage (arrowheads). Some other CR-50-positive cells were labeled at E10 or E11 (not shown). (Scale bar, 10 μm.) (*E–G*) Double-staining with CR-50 (green) (*E*) and anti-calretinin (red) (*F*) on E17 mouse hippocampus revealed the existence of both calretinin-positive cells (arrowheads) and calretinin-negative cells (arrows) among the CR-50-positive population in the marginal zones. A double-exposed photomicrograph is shown in *G*. Early granule neurons in the area dentata are also calretinin-positive, but negative for CR-50. (Scale bar in *G*, 50 μm.) (*H–J*) Embryonic hippocampus was cultured at E17 and fixed the next day. The cells were double-stained with CR-50 (green) (*H*) and anti-calretinin (red) (*I*). A double-exposed photomicrograph is shown in *J*. There were both calretinin-positive cells (arrowheads) and calretinin-negative cells (arrows) among the CR-50-positive population. (Scale bar in *J*, 20 μm.)

Table 1. Proportion of embryos exhibiting cell-poor rifts in the stratum pyramidale of the regio superior

Treatment	Stage of injection	Proportion of embryos exhibiting rifts
CR-50 ascites	E12 and E14	11/18 (61%)
CR-50 ascites	E12 and E15	5/7 (71%)
CR-50 ascites	E13 and E15	17/27 (63%)
Purified CR-50	E12 and E14	4/5 (80%)
Purified CR-50	E13 and E15	15/20 (75%)
CR-50 Fab	E13 and E15	10/16 (63%)
Control mouse IgG	E12 and E14	0/6
Control mouse IgG	E12 and E15	0/6
Control mouse IgG	E13 and E15	0/5
Anti-fibronectin	E13 and E15	0/9

Embryos were injected at various embryonic stages with CR-50 ascites fluid, purified CR-50, Fab fragments of CR-50, control mouse IgG, or mouse anti-fibronectin mAb, and fixed at P0-P1. Because definite estimation of the cell alignment was difficult, only those exhibiting cell-poor rifts in the regio superior were counted as positive in these experiments.

neurons, and to determine whether occlusion of the CR-50 epitope results in a *reeler* phenotype *in vivo*, we injected CR-50 into the lateral and third ventricles at the embryonic stage *in utero*. A single injection of antibody at the embryonic stage (E11 to E14) seldom resulted in structural changes in the hippocampus (data not shown). A series of two injections at the embryonic stage was performed subsequently (as shown in Table 1), and the embryos were allowed to develop until P0 to P1. Binding of CR-50 to its epitope *in vivo* was confirmed by immunostaining with FITC-conjugated secondary antibody alone (Fig. 3*A*). The punctate staining pattern is similar to the pattern of *in vitro* CR-50 staining of living neocortex we observed previously (8). Between 61% and 80% of the injected embryos exhibited cell-poor rifts in the stratum pyramidale of the regio superior of the hippocampus (Fig. 3 *D–G*; Table 1), resembling the hippocampus structure in *reeler* mutants (Fig. 3*B*). In contrast, control mice injected with control mouse IgG (Fig. 3*C*) or monoclonal anti-fibronectin antibody had hippocampal development that was indistinguishable from normal mice (Table 1). Labeling experiments with BrdUrd at E13, when the generation of the pyramidal neurons is in its early stage, showed that, in control IgG-injected mice, the labeled cells were mainly distributed in the inner half of the stratum pyramidale (81% [$n = 200$]; Fig. 3*K*), comparable to normal

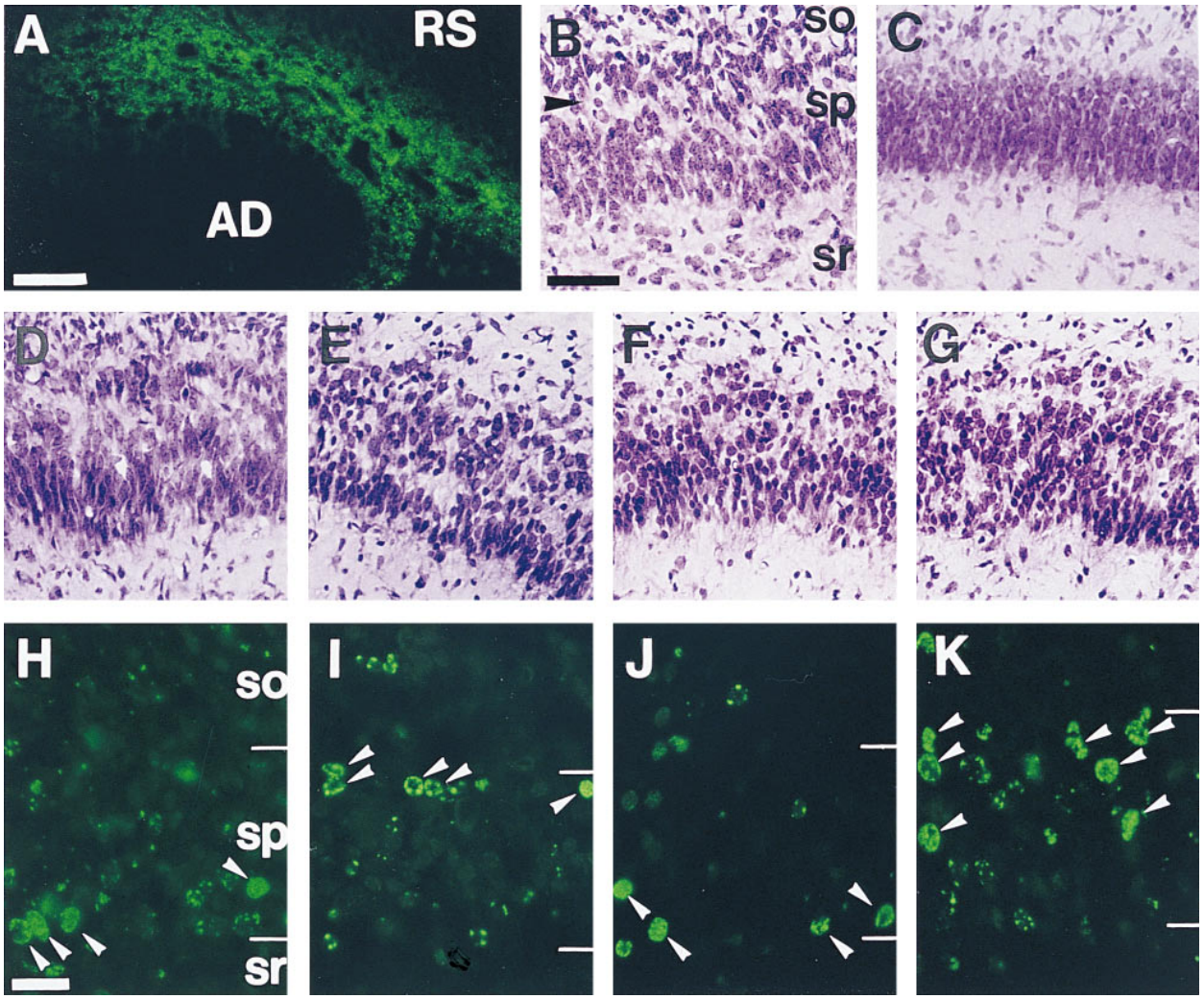

FIG. 3. Disruption of the organized cell layering in the stratum pyramidale of the regio superior and conversion to the *reeler* pattern with CR-50. (*A*) Binding of CR-50 to its epitope *in vivo* visualized by FITC-conjugated secondary antibody alone. (Scale bar, 50 μm.) (*B*) Cresyl violet staining of the regio superior of a P1 *reeler* mutant. Cell-poor rifts are shown with an arrowhead. (Scale bar, 50 μm.) (*C*) Wild-type P1 mouse that was injected with control mouse IgG at E12 and E14. (*D–G*) Wild-type P1 mice that were injected with CR-50 at E12 and E14 (*D*) or at E13 and E15 (*E–G*). Note the presence of cell-poor rifts in the stratum pyramidale and that the cells are rather loosely packed. (*H–K*) Dividing cells were labeled with BrdUrd at E13 and immunostained with anti-BrdUrd at P1. (*H*) Noninjected *reeler*, (*I*) noninjected wild-type mouse, (*J*) CR-50-injected wild-type mouse, and (*K*) control mouse IgG-injected wild-type mouse. Intensely labeled cells are shown with arrowheads. (Scale bar in *H*, 20 μm.) so, stratum oriens; sp, stratum pyramidale; sr, stratum radiatum.

mice (83% [n = 200]; Fig. 3*I*). However, in CR-50-injected mice, there were many intensely labeled cells localized in the outer half of the stratum pyramidale at P1 (71% [n = 200]; Fig. 3*J*), which is similar to the pattern in the *reeler* mouse (73% [n = 200]; Fig. 3*H*). These results demonstrate that the CR-50 epitope region on Reelin is crucial for the organized cell alignment of pyramidal neurons and that occlusion of the CR-50 epitope can lead to development of the *reeler* phenotype of the hippocampus *in vivo*.

DISCUSSION

In the embryonic hippocampus, CR-50 labeled Cajal–Retzius-like cells in the marginal zone of hippocampus proper and area dentata. From their position, morphological characteristics, developmental profile, MAP2 staining, and the birth-dating experiments, we conclude that these cells are equivalent to the Cajal–Retzius neurons in the neocortex. Although the Cajal–Retzius-like cells in the hippocampal region were reported to be positive for calretinin (37, 38), we found that some CR-50-positive cells are calretinin-negative. This discrepancy might be partially explained by the fact that the antibody recognition of calretinin depends on the calcium-binding status of the protein (39). Another possibility is that these CR-50-positive, calretinin-negative cells might become calretinin-positive in later stages of development. Further study is required to resolve this question. The present study suggests that, although most of the Cajal–Retzius neurons are indeed calretinin-positive, there might be another population that is negative for calretinin.

In the hippocampus, Reelin is essential for target-layer selection of afferent fibers, which recently was shown by incubating entorhinohippocampal cocultures with CR-50 (40). The present study indicates that Reelin also regulates the laminar organization of hippocampus. When CR-50 was added to the hippocampal cultures of newborn mice, no abnormal change of the cytoarchitectonics was observed in the hippocampus proper after 12 days *in vitro*, although the entorhinohippocampal pathway was altered (40). This and the present *in vivo* study indicate that the critical step of hippocampus cell layering takes place in embryonic stages. One possible mechanism could be through a direct interaction between Reelin and the pyramidal cells. At early stages of hippocampal development (E13–E14), a primitive plexiform layer is present (37), which is composed of earliest generated marginal zone cells, including Cajal–Retzius and prospective subplate neurons. This layer is then split (E15) into a superficial marginal zone and a deep subplate by the insertion of later generated pyramidal neurons that form a hippocampal plate (Fig. 1*A*). Because the CR-50-positive region is always attached to the MAP2-positive structure of pyramidal neurons (Fig. 2*C*), it is conceivable that the migrating neuroblasts might terminate migration and leave radial fibers when they encounter Reelin at the leading edge. This could explain why the hippocampal plate (future stratum pyramidale) is formed in an inside-out progression in a normal mouse. In *reeler*, because the migrating cells are not influenced by Reelin, they might not leave radial fibers properly and so the hippocampal plate might be formed in an outside-in progression. In addition, it is also conceivable that Reelin "repels" the subplate neurons from the Cajal–Retzius neurons and makes room for the later-generated pyramidal neurons, as previously suggested for neocortical formation (8, 15). This hypothesis is supported by the reports that the first abnormal phenotype in *reeler* neocortex is the failure of the preplate to split (41) and that early generated cortical neurons in *reeler* are more adhesive than normal *in vitro* (42). Because we failed to convert normal hippocampus to the *reeler* pattern by injecting CR-50 after the splitting of the primitive plexiform layer (E15; data not shown), the "repulsion" hypothesis also might apply in the hippocampus. Another possible mechanism is that the Cajal–Retzius neurons may affect migrating neuroblasts indirectly through the radial fibers, because CR-50-positive cells were found to reside near the marginal ends of the radial fibers, where Reelin is overlapped with the ends of the fibers (data not shown). The identification of the Reelin receptor and the cells that express it will solve this question and further our understanding of the molecular mechanisms of Reelin regulation in hippocampal development.

The cerebral cortex and the cerebellum also were examined briefly after the antibody injection. However, they apparently were not affected. One possible interpretation is that the injection site of the antibody (lateral and third ventricles) was too far from the Reelin-expressing regions [the marginal zone in the cerebral cortex (8) and the external granular layer or the nuclear transitory zone in the cerebellum (43)] and the antibody could not reach its epitope *in vivo*.

Although the processes of neocortical and hippocampal development have been reported to differ in some aspects (27–30), the present study shows that Cajal–Retzius neurons have an essential function in organized cell alignment in the hippocampus and in the neocortex, and that the CR-50 epitope is the key domain for this function *in vivo*. Taken together with the recent demonstration that CR-50 recognizes Reelin protein (15), this study indicates that the CR-50 epitope region on Reelin has a critical role for the *in vivo* actions of Reelin. Because the injected CR-50 labeled its epitope in the living hippocampus clearly (Fig. 3*A*), it is likely that the Reelin protein would be secreted from Cajal–Retzius neurons to the extracellular space and interact with other molecules through the CR-50 epitope region. Another possibility is that a significant conformational change of Reelin might be induced by the binding of CR-50, which would interfere with its interaction with other molecules. Thus, while Reelin is a large protein composed of 3,461 amino acids, the CR-50 epitope region near its N terminus (15) might be a key with which to dissect the molecular mechanism of Reelin functions. Further precise mapping and analysis of the CR-50 epitope region will give us some important information to formulate a hypothesis on the mode of interaction between Reelin and other molecules.

We thank M. Niinobe for anti-MAP2, E. Soriano, T. Curran, G. D'Arcangelo, and M. Sheldon for valuable comments on the manuscript, and members of respective animal facilities for their help maintaining mice. We also appreciate various technical support by K. Takahashi and M. Suenaga. This work was supported by the Science and Technology Agency of the Japanese Government and the Ministry of Education, Science, and Culture of Japan.

1. Caviness, V. J. (1973) *J. Comp. Neurol.* **151,** 113–120.
2. Stanfield, B. B. & Cowan, W. M. (1979) *J. Comp. Neurol.* **185,** 423–459.
3. Falconer, D. S. (1951) *J. Genet.* **50,** 192–201.
4. Meier, H. & Hoag, W. G. (1962) *J. Neuropathol. Exp. Neurol.* **21,** 649.
5. Caviness, V. S. & Sidman, R. L. (1973) *J. Comp. Neurol.* **147,** 235–254.
6. Stanfield, B. B. & Cowan, W. M. (1979) *J. Comp. Neurol.* **185,** 423–459.
7. Caviness, V. S., Crandall, J. E. & Edwards, M. A. (1988) in *Cerebral Cortex*, eds. Peters, A. & Jones, E. G. (Plenum, New York), Vol. 7, pp. 59–89.
8. Ogawa, M., Miyata, T., Nakajima, K., Yagyu, K., Seike, M., Ikenaka, K., Yamamoto, H. & Mikoshiba, K. (1995) *Neuron* **14,** 899–912.
9. Cajal, S. R. y (1890) *Gac. med. Catalana* **13,** 737–739.
10. Retzius, G. (1891) *Biol. Unters. Neue Folge* **3,** 90–102.
11. Marin-Padilla, M. (1984) in *Cerebral Cortex*, eds. Peters, A. & Jones, E. G. (Plenum, New York), Vol. 1, pp. 447–478.
12. D'Arcangelo, G., Miao, G. G., Chen, S. C., Soares, H. D., Morgan, J. I. & Curran, T. (1995) *Nature (London)* **374,** 719–723.
13. Hirotsune, S., Takahara, T., Sasaki, N., Hirose, K., Yoshiki, A., Ohashi, T., Kusakabe, M., Murakami, Y., Muramatsu, M., Wa-

tanabe, S., Nakao, K., Katsuki, M. & Hayashizaki, Y. (1995) *Nat. Genet.* **10,** 77–83.
14. Bar, I., Lambert de Rouvroit, C., Krizman, D. B., Royaux, I., Dernoncourt, C., Ruelle, D., Beckers, M. C. & Goffinet, A. M. (1995) *Genomics* **26,** 543–549.
15. D'Arcangelo, G., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K. & Curran, T. (1997) *J. Neurosci.* **17,** 23–31.
16. Angevine, J. B. (1965) *Exp. Neurol. Suppl.* **2,** 1–70.
17. Stensaas, L. J. (1967) *J. Comp. Neurol.* **129,** 59–70.
18. Rakic, P. (1972) *J. Comp. Neurol.* **145,** 61–84.
19. Bayer, S. A. (1980) *J. Comp. Neurol.* **190,** 87–114.
20. Bayer, S. A. (1980) *J. Comp. Neurol.* **190,** 115–134.
21. Levitt, P. & Rakic, P. (1980) *J. Comp. Neurol.* **193,** 815–840.
22. Rakic, P. & Nowakowski, R. S. (1981) *J. Comp. Neurol.* **196,** 99–128.
23. Stanfield, B. B. & Cowan, W. M. (1988) in *Cerebral Cortex*, eds. Peters, A. & Jones, E. G. (Plenum, New York), Vol. 7, pp. 91–131.
24. Altman, J. & Bayer, S. A. (1990) *J. Comp. Neurol.* **301,** 325–342.
25. Altman, J. & Bayer, S. A. (1990) *J. Comp. Neurol.* **301,** 365–381.
26. Reznikov, K. Y. (1991) *Adv. Anat. Embryol. Cell Biol.* **122,** 1–81.
27. Nowakowski, R. S. & Rakic, P. (1979) *J. Neurocytol.* **8,** 697–718.
28. Nowakowski, R. S. & Rakic, P. (1981) *J. Comp. Neurol.* **196,** 129–154.
29. Smart, I. H. (1982) *J. Anat.* **135,** 763–93.
30. Altman, J. & Bayer, S. A. (1990) *J. Comp. Neurol.* **301,** 343–364.
31. Hockfield, S. & McKay, R. D. (1985) *J. Neurosci.* **5,** 3310–3328.
32. Lendahl, U., Zimmerman, L. B. & McKay, R. D. (1990) *Cell* **60,** 585–595.
33. Crandall, J. E., Jacobson, M. & Kosik, K. S. (1986) *Dev. Brain Res.* **28,** 127–133.
34. Niinobe, M., Maeda, N., Ino, H. & Mikoshiba, K. (1988) *J. Neurochem.* **51,** 1132–1139.
35. Chun, J. J. M. & Shatz, C. J. (1989) *J. Neurosci.* **9,** 1648–1667.
36. von Haebler, D., Stabel, J., Draguhn, A. & Heinemann, U. (1993) *Exp. Brain Res.* **94,** 33–42.
37. Soriano, E., del Rio, J. A., Martinez, A. & Super, H. (1994) *J. Comp. Neurol.* **342,** 571–595.
38. del Rio, J. A., Martinez, A., Fonseca, M., Auladell, C. & Soriano, E. (1995) *Cereb. Cortex* **5,** 13–21.
39. Winsky, L. & Kuznicki, J. (1996) *J. Neurochem.* **66,** 764–771.
40. del Rio, J. A., Heimrich, B., Borrell, V., Forster, E., Drakew, A., Alcantara, S., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K., Derer, P., Frotscher, M. & Soriano, E. (1997) *Nature (London)* **385,** 70–74.
41. Goffinet, A. M. (1979) *Anat. Embryol.* **157,** 205–216.
42. Hoffarth, R. M., Johnston, J. G., Krushel, L. A. & van der Kooy, D. (1995) *J. Neurosci.* **15,** 4838–4850.
43. Miyata, T., Nakajima, K., Aruga, J., Takahashi, S., Ikenaka, K., Mikoshiba, K. & Ogawa, M. (1996) *J. Comp. Neurol.* **372,** 215–228.