Modular patterning of structure and function of the striatum by retinoid receptor signaling

Wen-Lin Liao*, Hsiu-Chao Tsai*, Hsiao-Fang Wang*, Josephine Chang*, Kuan-Ming Lu*, Hsiao-Lin Wu*, Yi-Chao Lee†, Ting-Fen Tsai†, Hiroshi Takahashi†, Michael Wagner†, Norbert B. Gyselinck‡, Pierre Chambon*†, and Fu-Chin Liu**††

*Institute of Neuroscience, †Center of Neuroscience, ‡Department of Life Sciences, National Yang-Ming University, Taipei, Taiwan 112, Republic of China; 1Center for Gene Regulation and Signal Transduction Research, National Cheng Kung University, Tainan, Taiwan 701, Republic of China; 2Developmental Neurobiology Group, Mitsubishi Kagaku Institute of Life Sciences, Tokyo, 194-8511, Japan; 3Department of Anatomy and Cell Biology, College of Medicine, State University of New York Health Science Center, Brooklyn, NY 11203; and 4Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale/University Louis Pasteur, Colle`ge de France, 67404 Illkirch Cedex, CNRS, Illkirch, France.

Contributed by Pierre Chambon, March 2, 2008 (sent for review December 21, 2007)

Retinoid signaling plays a crucial role in patterning rhombomeres in the hindbrain and motor neurons in the spinal cord during development. A fundamentally interesting question is whether retinoids can pattern functional organization in the forebrain that generates a high order of cognitive behavior. The striatum contains a compartmental structure of striosome (or “patch”) and interstriosomal (i.e., “matrix”) compartments. How this highly complex mosaic design is patterned by retinoid receptor signaling in the developing striatum (11–13) is an important question. The present study is aimed at elucidating how retinoid receptor signaling in the developing striatum regulates neuronal differentiation, migration, and connectivity.

Results

Aberrant Compartmentation in the Striatum of RARβ−/− Mutant Mice. Striosomes express high levels of μ-opioid receptor (MOR1) and dynorphin but low levels of calbindin-D28K and met-enkephalin. In the wild-type striatum, MOR1 immunostaining is highly expressed in the striosomes and is absent in the interstriosomal matrix. The number of calbindin-positive neurons was, however, not significantly altered in mutant striatum (wild type, 1,315.5 ± 207.7; mutant, 1,297.0 ± 315.6/mm²; P = 0.887, n = 3). Immunostaining of met-enkephalin showed similar results to that of calbindin (Fig. 1 C, C′, E, and E′). Notably, in addition to the changes of striosomal markers, the striatal area was decreased in the rostral but not the caudal mutant striatum (Fig. 1G), suggesting a cytoarchitectural change. The perinatal strio-

www.pnas.org/cgi/doi/10.1073/pnas.0802109105

Author contributions: W.-L.L. and F.-C.L. designed research; W.-L.L., H.-C.T., H.-F.W., J.C., F.-C.L., and P.C. contributed new reagents/analytic tools; W.-L.L., H.-L.F., and F.-C.L. analyzed data; and W.-L.L. and F.-C.L. wrote the paper.

The authors declare no conflict of interest.

To whom correspondence should be addressed. E-mail: chambon@titus.u-strasbg.fr or fuchin@ym.edu.tw.

This article contains supporting information online at www.pnas.org/cgi/content/full/0802109105/DCSupplemental.

© 2008 by The National Academy of Sciences of the USA
Loss of Late-Born Striosomal Neurons in RARβ−/− mutant Mice. The concurrent decreases of several striosomal markers suggested that RARβ-null mutation might affect neurogenesis and/or survival of striosomal neurons. Striosomal cells (S cells) and matrix cells (M cells) finish their last mitosis at different time windows (15, 16). The majority of S and M cells can be pulse-labeled with BrdU at E11.5–E13.5 and after E16.5, respectively, in the mouse. Within the S cell population, most S cells in the caudal striatum and the subcallosal lateral streak are born at E11–E12.5, whereas most S cells in the rostral striatum are born later at E12.5–E13.5 in the mouse (17) (W-L.L. and F-C.L., unpublished observations).

Because RARβ mutation-induced decreases of S markers primarily occurred in the rostral striatum, we pulse-labeled rostral S cells with two injections of BrdU at E12.75 and E13. Clusters of BrdU-labeled S cells were drastically reduced by 88.2% in the rostral RARβ−/− mutant striatum (Fig. 1H, H′, I, I′, and K). Similar findings were observed in the newborn mutant striatum (Fig. S3 A, A′, C, and C′). The subcallosal lateral streak, which comprised early-born S cells, remained largely unaffected in the rostral mutant striatum (Fig. 1J, J′, and K). Importantly, the loss of late-born S cells occurred at E12.75 before the segregation of S and M cells into compartments, because the reduction of BrdU-labeled cells was detected in the mutant lateral ganglionic eminence (LGE, striatal anlage) at 28 h postinjection of BrdU when the BrdU-labeled postmitotic cells had migrated into the differentiated mantle zone but were not yet organized into compartments (Fig. 2 A, A′, and C).

Defective Neurogenesis of Late-Born Striosomal Cells in Stratal Anlage of RARβ−/− mutant Embryos. The loss of late-born S cells in the mutant striatum might be due to aberrant cell death and/or defective neurogenesis induced by RARβ-null mutation. We assayed cell death by TUNEL staining. The number of apoptotic cells in the wild-type LGE did not significantly differ from that in the mutant LGE at E12.75, E13.5, E14.5, and E15.5 (Fig. S4), which ruled out cell death as the primary cause of cell loss.

In addition to the mantle zone, low levels of RARβ were expressed in the ventricular zone (VZ) of LGE as detected by RT-PCR and in situ hybridization (Fig. S5), which is consistent with the report that RARβ is expressed by proliferating progenitors in neurospheres derived from striatal anlage (18). To determine whether the reduction of S cells was due to defective proliferation of S progenitors in the germinal zones, we pulse-labeled late-born S progenitors with BrdU at E12.75 and E13.5 and examined the BrdU labeling in the newborn LGE. Because RARβ-null mutation-induced decreases of S markers primarily occurred in the rostral striatum, we pulse-labeled rostral S cells with two injections of BrdU at E12.75 and E13.5. BrdU-positive cells were reduced by 16.7% in the VZ of RARβ−/− mutant LGE (Fig. 2 B and B′, D, and E). The reduction was specific to the LGE, because it did not occur in the ventricular pallium of cortical anlage (Fig. 2 E). In parallel to the defective proliferation, cyclin E2 (Ccn2), a cell cycle protein regulating transition from G1 to S phase (19), was decreased by 16.1% in the mutant LGE (Fig. 2 F, F′, and H). Moreover, the proneural gene Mash1 was also reduced by 19.3% in the mutant LGE (Fig. 2 G, G′, and I). BrdU-positive cells tended to decrease in the mutant subventricular zone (SVZ), a secondary proliferative population in the germinal zones (86.2 ± 7.5% of wild type, P = 0.0718, n = 6).

The deficit in cell proliferation was specific to late-born S cells, because pulse labeling of early-born S cells at E11.5 or M cells at E16.5 with BrdU did not show significant differences of BrdU-positive cells in the LGE/developing striatum between the wild-type and RARβ−/− mutant embryos (Fig. 2 D). These results are in good accordance with the aforementioned findings that early-born S cells and M cells were spared in the adult mutant striatum (Fig. 1 F and K). Note that the loss of late-born S progenitors was not due to depletion of precursor pools by precocious differentiation, because no apparent increase of TuJ1-positive neurons was observed in E12.75 and E13.5 mutant LGE (data not shown).

somesth express high levels of MOR1, dopamine- and cyclic adenosine 3‘:5’-monophosphate-regulated phosphoprotein (DARP32), and tyrosine hydroxylase (TH). These three markers were also reduced primarily in the rostral part of newborn mutant striatum (Fig. S2 A–J). Ebf-1, a marker for developing matrix (14), was increased in the rostral but not in the caudal part of newborn mutant striatum (Fig. S2 K–M). Defective striosomes also occurred prematurely at E16.5. At E16.5, DARP32-positive neurons were not yet organized into striosomal pattern in the wild-type striatum, but DARP32-positive neurons were significantly reduced in the mutant striatum (Fig. S3 B and B′), which indicated that the reduction of striosomes occurred before compartmental formation.
RA Increased Cell Proliferation of Striatal Progenitors. It remained a puzzle within the S cell population why RARβ mutation primarily targeted the late-born S cells, whereas it relatively spared the early-born S cells. A possible account for this differential effect is that the early-born S cells might be incompetent to transduce RA signals. To test this hypothesis, wild-type mouse embryos were maternally treated with all-trans RA (5 mg/kg) every 12 h from E10.5 to E11.5 and then pulse-labeled with BrdU for 1 h before embryo culling. The RA treatments resulted in increases of BrdU-positive cells in the VZ of LGE at rostral, middle, and caudal levels (Fig. 3B, B’, and E) and decreases of TuJ1-positive areas in the mantle zone (Fig. 3B and B’). These results indicated that early-born S progenitors were in fact competent to transduce RA signals by expanding its population.

To further test whether the RA-enhanced cell proliferation was mediated through RARβ signaling, the same set of experiments were performed in RARβ−/− mutant embryos and their wild-type littersmates. RA treatments did not effectively increase proliferation of early-born S cells in RARβ−/− mutant LGE at rostral and middle levels (Fig. 3F), which indicated that the lack of RARβ largely prevented RA from increasing proliferation of early-born S cells.

We also tested whether exogenous RA could alter proliferation of late-born S cells by maternally treating wild-type mouse embryos with RA every 12 h from E12.5 to E13.5 and then pulse-labeling with BrdU for 1 h before culling. The RA treatments did not alter cell proliferation in the rostral and middle parts of E13.5 LGE (Fig. 3G). We surmised that this might be due to high levels of endogenous RA present in the rostral/middle LGE at E12.5–E13.5, which could limit the effect of exogenous RA (see below, Fig. 4B, E, and H). Accordingly, RA-induced cell proliferation should occur in the region where endogenous RA was low. Indeed, exogenous RA increased BrdU-positive cells in the VZ of caudal part of E13.5 LGE (Fig. 3D, D’, and G), where a low level of endogenous RA was present (see below, Fig. 4C, F, and I).

Chronic RA Treatments Resulted in Enlarged Caudal Striosomes. We further assayed the effects of chronic RA treatments during the entire neurogenesis of S and M cells. The embryos treated with RA every 12 h from E11.5 to E17.5 had enlarged DARPP-32- and GluR1-positive striosomes in the caudal striatum (Fig. S6). No significant effect was observed in rostral striosomes, which contained late-born S cells, nor did the population of M cells appear to be affected by the RA treatments (data not shown). Because striosomes in the caudal striatum were made up of early-born S cells, these results were consistent with the findings that exogenous RA mainly increased proliferation of early-born S cells (Fig. 3B, B’, and E) and further suggest that as a consequence, more early-born S cells are recruited to caudal striosomes.

RA Directly Regulated Proliferation of Striatal Progenitor-Derived Cells in Vitro. To further determine whether RA could directly regulate proliferation of striatal progenitors, we used the ST14A cell line, which was immortalized from rat striatal progenitors during the neurogenesis time window of S cells at E14 (20). The proliferating ST14A cells expressed RARβ and RXRβ transcripts (Fig. S7D). All-trans RA (1 μM) increased proliferation of ST14A cells, because BrdU-labeled cells were significantly increased by 142.3% with RA treatment (Fig. S7B and C). RA also increased Ccne2 and decreased the differentiation marker of microtubule-associated protein 2 (Fig. S7D).

Spatiotemporal Regulation of Endogenous Levels of RA in Striatal Anlage. Given that early-born S cells are capable of responding to RA signals, why is this cell population less affected by RARβ mutation? We postulated that the concentration of RA might be very low in E10.5–E11.5 LGE when the early-born S cells undergo neurogenesis. During early telencephalic development, a major RA source for the LGE is synthesized by retinaldehyde dehydrogenase 3 (Raldh3) (10, 21), although Raldh1 is present in the mesostratial afferents (22). We found that few cells expressing Raldh3 mRNA were present in E11.5 LGE (Fig. 4A). In contrast, when the late-born S cells undergo neurogenesis at E12.5–E13.5, many Raldh3-positive cells were present in the rostral/middle LGE but...
not in the caudal LGE (Fig. 4C) (21). We further determined the endogenous RA level in the LGE with a coculture assay. Using the Sili-15 RA reporter cells, RA produced by explant tissue could be detected by activation of the β-galactosidase reporter gene (9, 23). Few X-gal-positive cells were found when cocultured with E11.5 LGE (Fig. 4D and G). In contrast, many X-gal-positive cells were detected when cocultured with the rostral (Fig. 4E and H) but not the caudal part of E13.5 LGE (Fig. 4F and I). These results confirmed that endogenous RA in the LGE was very low during the neurogenesis time window of early-born S cells, whereas a substantial level of RA was present during the neurogenesis time window of late-born S cells in the rostral LGE.

**Altersations of Dopamine Agonist-Induced Stereotypic Motor Behaviors in RARβ−/− Mutant Mice.** The selective loss of rostral striosomes in RARβ−/− mutant mice provided a unique opportunity to look into the functional significance of S compartments at the behavioral level. We treated the mice with apomorphine, a stereotypy-eliciting dopamine agonist, and examined the stereotypic motor behaviors, including head bobbing, rearing, and grooming (Fig. 5A–C; data not shown). Apomorphine (3 mg/kg) increased the locomotor activity in wild-type and RARβ−/− mutant mice to similar degrees (Fig. 5A). Apomorphine at the dosage of 3 mg/kg was ineffective in inducing repeated head bobbing in wild-type mice, but it drastically induced stereotypic head-bobbing movement in RARβ−/− mutant mice at 20 and 50 min after injection (P < 0.001, two-way ANOVA; Fig. 5B), which suggested the head-bobbing behavior was exaggerated in RARβ−/− mutant mice. Apomorphine at 3 mg/kg was also ineffective in altering the rearing activity in wild-type mice (Fig. 5C), but the rearing activity was completely lost in RARβ−/− mutant mice at 20 min after apomorphine injection (P < 0.001, two-way ANOVA; Fig. 5C). The grooming activity tended to decrease in apomorphine-treated RARβ−/− mutant mice, but the decrease did not reach statistical significance (data not shown).

**Discussion**

Our study provides previously undescribed genetic evidence that retinoid receptor signaling plays a crucial role in patterning the functional organization in the striatum that is involved in generation...
of psychomotor behavior. Our experiments demonstrate heterogeneity of S cell populations along the rostrocaudal axis in terms of RARβ signaling. The population of late-born S cells, which is preferentially expanded by high levels of RA through RARβ signaling to form a large S compartment in the rostral striatum, may engage in modulating neural circuits of psychomotor function.

**Regulation of Proliferation of S Progenitors by RARβ Signaling.** RARβ mutation resulted in defective proliferation of late-born S cells. In addition to the differentiated mantle zone, RARβ is expressed at low levels in the progenitor domains of LGE (Fig. S5) and by progenitor cells in neurephores derived from striatal anlage (18). The expression of RARβ in striatal progenitors suggests a cell-autonomous effect of RARβ in regulating proliferation of striatal progenitors, which is corroborated by our finding that RA increased proliferation of striatal progenitors-derived ST14A cells. A previous cell lineage study has suggested a heterogeneity of S and M progenitors within the proliferative VZ (24). It is likely that both the late-born S progenitors and the M progenitors were concurrently labeled by the 1-hour pulse of BrdU at E12.75 LGE. Because the population of M cells is the dominant population in the striatum (25), the population sizes of S compartments along the rostrocaudal axis should presumably have topographically homogenous significance. The enlarged S compartment by RARβ signaling plays a crucial role in setting up the population sizes of S compartments along the rostrocaudal axis (Fig. 6). Under physiological conditions, the early-born S cells, due to deficiency of RA at their neurogenesis at E10.5–E11.5, develop into a small S compartment in the caudal striatum. Subsequently, elevated concentration of RA at E12.5–E13.5 LGE may enable full activation of RARβ signaling in late-born S cells, which leads to preferential expansion of the late-born S cell population. The late-born S cell population eventually evolves to form a fully blown S compartment in the rostral striatum.

**Potential Involvement of the Rostral S Compartment in Neural Circuits of Psychomotor Function.** The enlarged S compartment by RARβ signaling in the rostral striatum should presumably have topographically functional significance. Challenging the RARβ−/− mutant mice with apomorphine revealed a differentially responsive profile of various stereotypic behaviors, including exaggeration of head-bobbing movement and reduction of rearing activity. These behavioral phenotypes were unlikely due to changes of dopamine receptors in the mutant striatum, because D1 and D2 receptors are not altered in RARβ−/− mutant mice (12). The behavioral phenotypes instead imply that neurons in the rostral S compartment may modulate complex neural circuits of psychomotor control. It has been proposed that unbalanced activity shifting toward increased neuronal activity in S compartment is correlated with motor stereotypic behavior (30, 31), although another study has argued that motor stereotype does not require enhanced activity in S compartment.
neurons (32). The S compartment receives limbic system-associated inputs from the cerebral cortex and projects to dopaminergic neurons of the pars compacta of the substantia nigra, which may provide feedback control of dopaminergic inputs to striatal circuits (2, 3). The loss of rostral S compartment in RARβ−/− mutant striatum would conceivably result in aberrant neural circuits and perhaps change of dopamine tone within the basal ganglia, which in turn leads to altered drug-induced repetitive motor behavior. We cannot, however, rule out the possibility that there may be some uncharacterized defects in other brain regions that contribute to the behavioral phenotype of RARβ−/− mutant mice.

Aberrant RA Signaling and the Pathogenesis of Psychomotor Disorders. Clinical relevance of RA signaling in regulation of stereotypic motor behavior has been reported in patients with obsessive-compulsive disorder (OCD), because the stereotypic motor symptoms of OCD patients are alleviated with RA treatments (33). Autism, a neurodevelopmental disorder characterized by aberrant repetitive behavior, is linked to defective corticobasal ganglia circuits (34). A recent MRI study has reported a decrease in the volume of the caudate nucleus in patients with autism (35), and the etiology of autism may be associated with abnormal RA signaling and MOR deficiency (36, 37). Clinical case reports also implicate potential RA treatments for autism (38). Taken together, given that MOR1 is selectively expressed in striatal S compartments, our findings of RARβ mutation-induced aberrant S compartments and associated psychomotor dysfunction may have clinical implication for understanding the pathogenesis of autism and other psychomotor disorders.

Experimental Procedures

Detailed experimental procedures are described in SI Text.

Mutant Mice. RARβ mutant mice were generated (39). See Experimental Procedures in SI Text.

Immunohistochemistry. Immunohistochemistry was performed as described (40) with the following primary antibodies: rabbit MOR1 [1:10,000, gift of R. P. Elde (University of Minnesota, Minneapolis)] mouse calbindin (1:500, Sigma), rabbit met-enkephalin (1:2,000, gift of R. P. Elde), rabbit DARPP-32 (1:1,100, Cell Signaling), rabbit tyrosine hydroxylase (1:2,000, EugenTech), rabbit K167 (1:200, NovoCastra), rabbit jill-tubulin (Tu1, 1:1,000, Covance), sheep BrdU (1:200, Biodetection) and rabbit Glur1 (1:200, Upstate Biotechnology).

In Situ Hybridization. In situ hybridization was performed as described (13). See Experimental Procedures in SI Text.

Cell Culture. The cultivation of explant tissue, SIL-15 RA reporter cells, and ST14 cells was performed as described (9, 20, 23). See Experimental Procedures in SI Text.

Behavioral Tests. See Experimental Procedures in SI Text.

ACKNOWLEDGMENTS. We thank R. P. Elde and E. Cattaneo (University of Milan, Milan) for reagents, D.-Y. Chen for advice on statistics, and C. P. Hung for providing advice on statistics. This work was supported by National Health Research Institutes (Grants EX94, EX95, EX96, EX97-9402NII), National Research Program for Genomic Medicine (Grants NSC95-3112-B-010-014, NSC96-3112-B-010-007), and Ministry of Education (Grant for Top University Grant, 96A-D-T171) in Taiwan.

3. Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization. Trends Neurosci 15:133–139.