Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 351 (2006) 553–558
BBRC
www.elsevier.com/locate/ybbrc

Comparison between basal and apical dendritic spines in estrogen-induced rapid spinogenesis of CA1 principal neurons in the adult hippocampus

Gen Murakami [a,b,c,1], Tomokazu Tsurugizawa [a,1], Yusuke Hatanaka [a], Yoshimasa Komatsuzaki [a,d], Nobuaki Tanabe [a], Hideo Mukai [a,b,c], Yasushi Hojo [a,b,c], Shiro Kominami [e], Takeshi Yamazaki [e], Tetsuya Kimoto [a,b], Suguru Kawato [a,b,c,*]

[a] *Department of Biophysics and Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Komaba 3-8-1, Meguro, Tokyo 153, Japan*
[b] *Core Research for Evolutional Science and Technology Project of Japan Science and Technology Agency, Graduate School of Arts and Sciences, The University of Tokyo, Komaba 3-8-1, Meguro, Tokyo 153, Japan*
[c] *Bioinformatics Project of Japan Science and Technology Agency, Graduate School of Arts and Sciences, The University of Tokyo, Komaba 3-8-1, Meguro, Tokyo 153, Japan*
[d] *Department of Physics and Applied Physics, College of Humanities and Sciences, Nihon University, 3-25-40 Sakurajosui, Setagaya-ku, Tokyo 156-8550, Japan*
[e] *Graduate School of Integrated Arts and Sciences, Hiroshima University, Higashi-Hiroshima 739, Japan*

Received 7 October 2006
Available online 19 October 2006

Abstract

Modulation of hippocampal synaptic plasticity by estrogen has been attracting much attention. Here, we demonstrated the rapid effect of 17β-estradiol on the density and morphology of spines in the stratum oriens (s.o., basal side) and in the stratum lacunosum-moleculare (s.l.m., apical side) by imaging Lucifer Yellow-injected CA1 neurons in adult male rat hippocampal slices, because spines in s.o. and s.l.m. have been poorly understood as compared with spines in the stratum radiatum. The application of 1 nM estradiol-induced a rapid increase in the density of spines of pyramidal neurons within 2 h. This increase by estradiol was blocked by Erk MAP kinase inhibitor and estrogen receptor inhibitor in both regions. Effect of blockade by agonists of AMPA receptors and NMDA receptors was different between s.o. and s.l.m. In both regions, ERα agonist PPT induced the same enhancing effect of spinogenesis as that induced by estradiol.

Keywords: Estrogen; Estrogen receptor; Spine; CA1; Hippocampus; Synaptic plasticity

The hippocampus is essentially involved in learning and memory processes, and is known to be a target for the modulatory actions of estrogens and androgens produced in the gonads, reaching the brain via blood circulation [1–4]. Extensive studies have been performed to investigate their role in modulating hippocampal plasticity and function, slowly and genomically (over 1–4 days). For example, the density of dendritic spines of pyramidal neurons in the CA1 stratum radiatum (apical side) is modulated *in vivo* by supplement of estrogens in ovariectomized animals [1,3–6] and androgens in castrated animals [2], both showing increase/recover of the number of spines. *In vitro* investigations have also shown that spine density is increased in the CA1 stratum radiatum following several days' treatment of cultured hippocampal slices with estradiol [7,8].

On the other hand, little is known regarding estrogen effect on other subregions of CA1 pyramidal neurons. CA1 neurons consist of four regions, i.e., the stratum

* Corresponding author. Fax: +81 3 5454 6517.
E-mail address: kawato@phys.c.u-tokyo.ac.jp (S. Kawato).
[1] These authors contributed equally to the present work.

0006-291X/$ - see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.bbrc.2006.10.066

oriens, the cell body, the stratum radiatum, and the stratum lacunosum-moleculare. There is only one report showing that the dendritic spine density of CA1 neurons has been increased in stratum oriens and stratum lacunosum-moleculare *in vivo* by supplement of estrogens over 4 days in ovariectomized rats [1].

In stratum radiatum, Schaffer collaterals, originating from CA3 pyramidal neurons, project to dendrites of CA1 neurons. In the stratum oriens, recurrent collaterals project to dendrites of pyramidal neurons, and in the stratum lacunosum-moleculare, neurons from entorhinal cortex project to dendrites of CA1 pyramidal neurons.

In addition to slow effect, we demonstrated that 17β-estradiol rapidly (within 2 h) increased the spine density of CA1 neurons in the stratum radiatum [9]. Interestingly in the CA3, estradiol rapidly decreased the density of thorns of pyramidal neurons in the stratum lucidum [10]. These results suggest that estradiol-induced modulation of spines is region specific.

In the present study, we demonstrated the similarity and difference between the stratum oriens and the stratum lacunosum-moleculare concerning the rapid (within 2 h) modulation of density of spines of CA1 neurons in acute hippocampal slices by estradiol.

Materials and methods

Animals. Twelve-week-old adult male Wistar rats were purchased from Saitama Experimental Animal Supply. Male rats are particularly used in order to elucidate an essential function of endogenously synthesized estradiol in the male hippocampus [11]. All experiments using animals in this study were conducted according to the institutional guidelines.

Chemicals. Lucifer Yellow, 17β-estradiol, ICI 182,780, (propyl-pyrazole-trinyl)tris-phenol (PPT), (4-hydroxyphenyl)-propionitrile (DPN), MK-801, cyano-nitroquinoxaline-dione (CNQX), and PD98059 were purchased from Sigma (USA). Actinomycin D was purchased from Wako Pure Chemicals (Japan).

Imaging and analysis of dendritic spine morphology. Spine imaging and analysis with confocal microscopy was performed essentially as described previously [9,10,12]. Briefly, the brains were removed from rats and placed in artificial cerebrospinal fluid (ACSF) at 4 °C. The hippocampal slices were prepared with a vibratome (Dosaka, Japan) with 400 μm thickness. ACSF consisted of (mM): 124 NaCl, 5.0 KCl, 1.25 NaH_2PO_4, 2.0 $MgSO_4$, 2.0 $CaCl_2$, 22 $NaHCO_3$, and 10 glucose, and was equilibrated with 95% O_2/5% CO_2. Hippocampal slices were incubated in ACSF held at 25 °C for 2 h. Slices were then incubated with 0 or 1 nM estradiol or other drugs such as 50 μM MK-801, 20 μM CNQX, 100 nM PPT, 100 nM DPN, or 50 μM PD98059. Slices were prefixed with 4% paraformaldehyde. Neurons within slices were visualized by an injection of Lucifer Yellow under E600FN microscope (Nikon, Japan) equipped with an infrared camera C2400-79 H (Hamamatsu Photonics, Japan).

The imaging was performed from sequential *z*-series scans with MRC-1024 confocal microscope (Bio-Rad, USA) [12]. For analysis of spines, three-dimensional images were constructed from approximately 40 sequential *z*-series sections of neurons scanned every 0.5 μm with a 60× oil immersion lens.

The density of spines was analyzed by tracing neurons with Neurolucida software (MicroBrightField, USA). Spine shapes were classified into four categories as follows [13]. (1) A mushroom spine, which has a large distinguishable head and neck. The head diameter (D) is greater than 0.6 μm. (2) A thin spine, which has a small distinguishable head and an elongated spine neck, $D < 0.6$ μm. (3) A filopodium, which is a long filamentous protrusion with no distinguishable head. The total spine length (L) is much longer than the neck diameter (d). (4) A stubby spine, which does not have a distinguishable head, has an L value similar to d.

Results

The Lucifer Yellow-injected neurons of the CA1 region were imaged using confocal laser microscopy. Fig. 1 shows the maximal intensity projections onto XY plane.

We here focus on the stratum oriens (basal side) and stratum lacunosum-moleculare (apical side) of CA1 neurons for analysis of spinogenesis. Note that, we already reported the analysis on estradiol-induced rapid spinogenesis in stratum radiatum elsewhere [9]. Following a 2-h treatment with 17β-estradiol, treated dendrites have significantly more spines than control dendrites (i.e., with no estradiol). Because a 2-h treatment with 1 nM estradiol was most effective in spinogenesis from time-dependence and dose-dependence experiments [9], these incubation conditions were used in the current investigations unless specified.

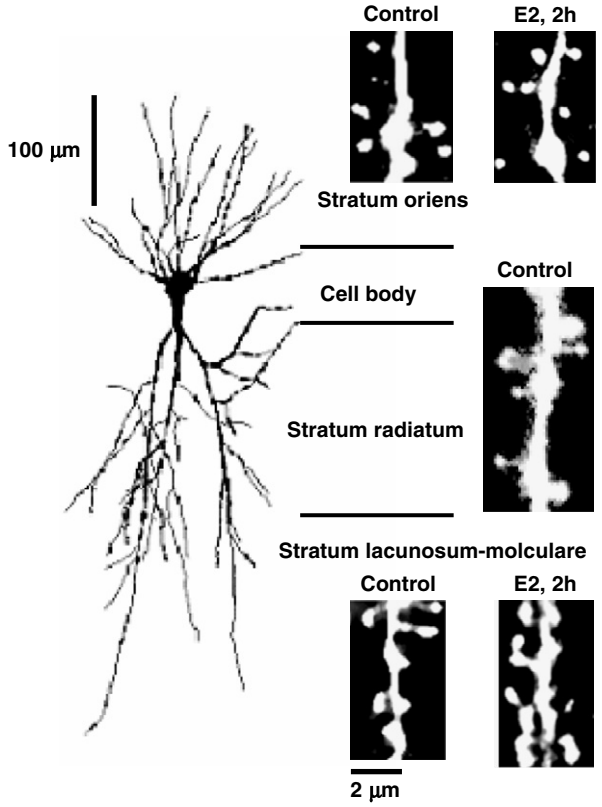

Fig. 1. Confocal micrographs showing spines along the secondary dendrites of hippocampal CA1 pyramidal neurons. (Left) A whole image of Lucifer Yellow-injected CA1 neuron with vertical bar 100 μm. (Right) Maximal intensity projections onto XY plane from *z*-series confocal micrographs, showing spines along the dendrites of CA1 pyramidal neurons. From top to bottom, distribution of spines along the basal dendrite in stratum oriens (Control and E2), distribution of spines along the apical dendrite in stratum radiatum (Control), and the distribution of spines along the apical dendrite in stratum lacunosum-moleculare (Control and E2), horizontal bar 2 μm. Slices were treated in ACSF for 2 h without drugs (Control) or with 1 nM E2 (E2).

A 2-h treatment increased the total spine density from 0.74 spines/μm (control, 0 nM estradiol) to 1.01 spines/μm (1 nM estradiol) in the stratum oriens (by 1.36 times), and from 0.84 spines/μm (control, 0 nM estradiol) to 1.30 spines/μm (1 nM estradiol) in the stratum lacunosum-moleculare (by 1.55 times) (Fig. 2A). PPT at 100 nM induced a significant enhancement of the spine density to 1.04 spines/μm in the stratum oriens, and to 1.26 spines/μm in the stratum lacunosum-moleculare (Fig. 2A). However, ERβ agonist DPN [14] at 100 nM did not increase the spine density.

Blocking of ERα by 1 μM ICI 182,780 suppressed the enhancing effect of estradiol on the spine density significantly in both the stratum oriens (0.85 spines/μm) and the stratum lacunosum-moleculare (0.70 spines/μm). Blocking of AMPA receptors by 20 μM CNQX prevented estradiol enhancing effect completely in the stratum oriens (0.74 spines/μm), but only partially in the stratum lacunosum-moleculare (1.17 spines/μm). Blocking of NMDA receptors by MK-801 prevented estradiol enhancing effect partially in the stratum oriens (0.89 spines/μm), and completely in the stratum lacunosum-moleculare (0.69 spines/μm). Blocking of phosphorylation of Erk MAP kinase by

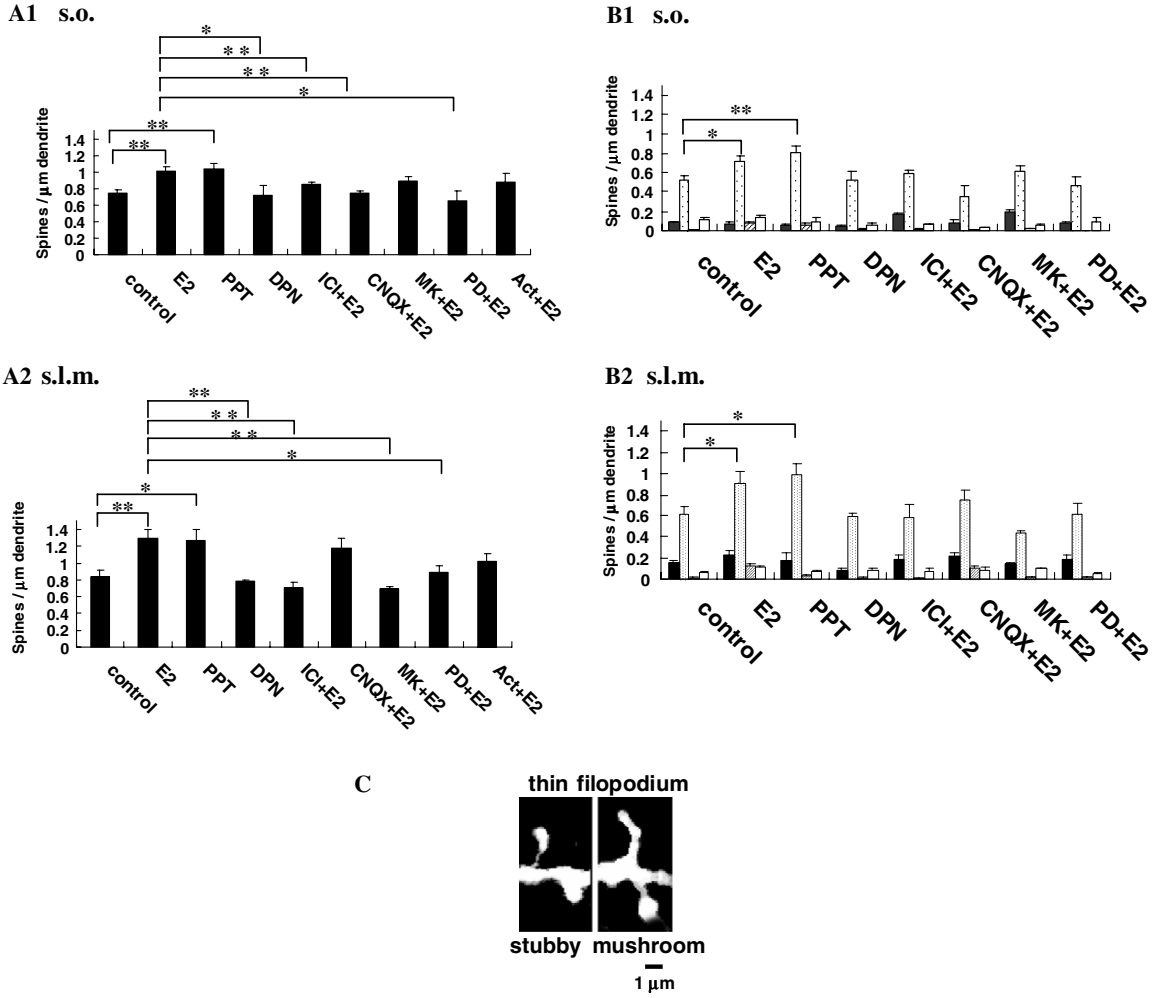

Fig. 2. Changes in the density and morphology of spines upon 2-h treatments of 17β-estradiol (E2) and drugs in hippocampal slices. Spines were analyzed along the secondary dendrites of pyramidal neurons in the stratum oriens (s.o.) and the stratum lacunosum-moleculare (s.l.m.) of CA1 neurons. (A) Effect of drug treatments on the total spine density in CA1 neurons. (A1) The stratum oriens and (A2) the stratum lacunosum-moleculare. Vertical axis is the average number of spines per 1 μm. A 2-h treatment in ACSF without drugs (Control), with 1 nM E2 (E2), with 100 nM PPT (PPT), with 100 nM DPN (DPN), with 1 nM E2 and 1 μM ICI 182,780 (ICI + E2), with 1 nM E2 and 20 μM CNQX (CNQX + E2), with 1 nM E2 and 50 μM MK-801 (MK + E2), with 1 nM E2 and 50 μM PD98059 (PD + E2), with 1 nM E2 and 4 μM Actinomycin D (Act + E2). (B) Density of four subtypes of spines in (B1) the stratum oriens and (B2) the stratum lacunosum-moleculare. Vertical axis is the average number of spines per 1 μm of dendrite. A 2-h treatment in ACSF without drugs (Control), with 1 nM E2 (E2), with 100 nM PPT (PPT), with 100 nM DPN (DPN), with 1 nM E2 and 1 μM ICI 182,780 (ICI + E2), with 1 nM E2 and 20 μM CNQX (CNQX + E2), with 1 nM E2 and 50 μM MK-801 (MK + E2), with 1 nM E2 and 50 μM PD98059 (PD + E2). In each group, from left to right, (a) mushroom (black column), (b) thin (dotted column), (c) filopodium (hatched column), and (d) stubby (open column). (C) Four different spine subtypes are indicated as: mushroom type, thin type, filopodium type, and stubby type. Bar 1 μm. In (A) and (B) results are reported as mean ± SEM from $N = 4$–9 different neurons, and $n = 300$–1000 total spines were analyzed. The significance of the drug effect was examined using Tukey–Kramer post hoc multiple comparisons test when one way ANOVA tests yielded $^{*}P < 0.05$ or $^{**}P < 0.01$.

50 μM PD98059 completely prevented the estradiol-induced spinogenesis in both the stratum oriens and the stratum lacunosum-moleculare.

Blocking by Actinomycin D did not have a significant effect in both s.o. and s.l.m., implying that genomic processes do not contribute significantly to the estradiol-induced spinogenesis.

The morphological changes in spines induced by 2-h drug-treatments were quantitatively assessed (Fig. 2B1, B2, and C). In control slices (0 nM estradiol), the relative population of spines was approximately 13% and 18% for mushroom spine, 72% and 73% for thin spine, 1% and 2% for filopodium, and 15% and 8% for stubby spine, in the stratum oriens and the stratum lacunosum-moleculare, respectively. Upon estradiol treatment, the density of thin spine was increased significantly, from 0.53 to 0.71 spines/μm in the stratum oriens, and from 0.61 to 0.90 spines/μm in the stratum lacunosum-moleculare (Fig. 2B1 and B2). The density of filopodium was also increased significantly upon estradiol treatment, from 0.01 to 0.08 spines/μm in the stratum oriens, and from 0.01 to 0.13 spines/μm in the stratum lacunosum-moleculare. On the other hand, the densities of mushroom spine (from 0.09 to 0.07 spines/μm in the stratum oriens, and from 0.15 to 0.23 spines/μm in the stratum lacunosum-moleculare) and stubby spine (from 0.11 to 0.14 spines/μm in the stratum oriens, and from 0.06 to 0.11 spines/μm in the stratum lacunosum-moleculare) were not significantly altered within experimental error (Fig. 2B1 and B2). PPT induced nearly an identical change to that obtained by estradiol. Blocking by PD98059, ICI significantly suppressed the enhancing effect of estradiol on the density of thin spines (Fig. 2B1 and B2).

Discussion

Until the current investigations, the stratum oriens and the stratum lacunosum-moleculare have not been extensively investigated concerning the rapid estradiol effect on spinogenesis and spine morphology. We selected acute hippocampal slices as most intact *in vitro* adult neuron networks, suitable for investigations of rapid effect of estradiol, free from indirect effects of estradiol on glutamatergic neurons via serotonergic/cholinergic neurons, projecting to the hippocampus [2,5].

We recently reported that in the CA1 stratum radiatum the total density of spine having contacts with Schaffer collateral terminals originated from CA3, increased dramatically to 154% upon 2 h application of 1 nM estradiol (see Fig. 3) [9]. PPT induced the same enhancing effect as estradiol on spinogenesis but DPN did not enhance spinogenesis. In stratum radiatum this estradiol-induced enhancement of spinogenesis was suppressed completely by ICI, MK-801, and PD98059 but only partially by CNQX. From the morphological investigations, estradiol treatments selectively increased the density of thin spine from 0.57 to 0.97 spines/μm.

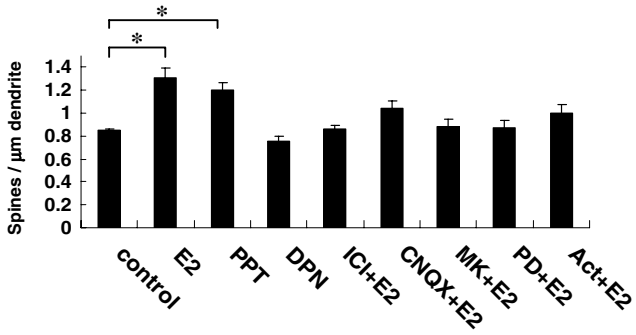

Fig. 3. Changes in the density and morphology of spines of CA1 neurons in the stratum radiatum upon treatments of 17β-estradiol (E2) and drugs in hippocampal slices. Effect of drug treatments on the total spine density in CA1 neurons. Vertical axis is the average number of spines per 1 μm. A 2-h treatment in ACSF without drugs (Control), with 1 nM E2 (E2), with 100 nM PPT (PPT), with 100 nM DPN (DPN), with 1 nM E2 and 1 μM ICI 182,780 (ICI + E2), with 1 nM E2 and 20 μM CNQX (CNQX + E2), with 1 nM E2 and 50 μM MK-801 (MK + E2), with 1 nM E2 and 50 μM PD98059 (PD + E2), with 1 nM E2 and 4 μM Actinomycin D (Act + E2).

Our results showed that in all three subregions (the stratum oriens, the stratum radiatum, and the stratum lacunosum-moleculare) of CA1, the density and the spine morphology was demonstrated to be significantly affected by short (2 h) estradiol treatments in adult male hippocampus. The density of thin spines was dramatically increased, although the density of mushroom and stubby spines was not significantly altered. This enhancement of the total spine density by estradiol was nearly the same between the stratum oriens (1.36-fold), the stratum lacunosum-moleculare (1.38-fold), and the stratum radiatum (1.54-fold) (Fig. 3). Such a selective modulation of thin spines is essential for synaptic plasticity controlled by estradiol.

We demonstrated the rapid effects of estradiol were mediated by a signal transduction pathway via Erk MAP kinase, as judged from inhibitor analysis (Fig. 2A). Application of PD98059 suppressed the estradiol-induced increase in the density of spines in all three subregions of CA1, indicating that the increase was triggered by Erk MAP kinase pathway. In addition, previously we reported that in CA3 pyramidal neurons of the adult male rat hippocampus, application of PD98059 also suppressed the effect of estradiol-induced rapid decrease of thorns of thorny excrescences [10].

Blockade by CNQX and MK-801 resulted in different effects depending on subregions. CNQX did not inhibit the estradiol-induced increase in the spine density in the stratum lacunosum-moleculare as well as the stratum radiatum, however, CNQX completely suppressed the increase in the spine density in the stratum oriens. MK-801 completely suppressed the increase in the spine density in the stratum lacunosum-moleculare as well as the stratum radiatum, however, MK-801 only partially suppressed the increase in the spine density in the stratum oriens. These results suggest that estradiol-induced spinogenesis requires spontaneous postsynaptic Ca^{2+} influx via NMDA receptors of the apical dendritic spines (in the stratum lacuno-

sum-moleculare and the stratum radiatum). On the other hand, in basal dendritic spines (in the stratum oriens), estradiol-induced spinogenesis might depend on postsynaptic Ca^{2+} influx via different Ca^{2+} channels other than NMDA receptors, for example, voltage activated calcium channels which participated in the estradiol-induced decrease of the density of thorns in the CA3 pyramidal neurons.

Interestingly, the spine density is not always increased by the estradiol-treatments. In CA3 pyramidal neurons, total density of thorns of thorny excrescences (spine-like postsynaptic structures in CA3), having contacts with mossy fiber terminals originated from granule cells, decreased dramatically to approximately 70% [10]. Therefore, the estradiol-induced spinogenesis is highly region specific and heterogeneous.

The rapid effects of estradiol are probably mediated by a signal transduction pathway via postsynaptic ERα as judged from agonist or antagonist analysis in all regions of CA1 (Figs. 2A and 3). We demonstrated that the increase in the spine density was suppressed by ICI, ERα antagonist. In addition, ERα agonist PPT induced the increase in the spine density, however ERβ agonist DPN did not modulate spine density. The significant localization of ERα in spines was demonstrated in CA1 pyramidal neurons in addition to nuclei using immunogold electron microscopy and purified ERα antibody, RC-19 [9,10,15]. Because both ERα and Erk MAP kinase were demonstrated to be present in the postsynaptic density of spines, therefore an efficient coupling between these two proteins can occur at spines [9].

Chronic and genomic effect of estradiol on spinogenesis

In previous observations, a number of *in vivo* studies have been performed about the effect of estrogen supplement on the density of spines of CA1 pyramidal neurons in the stratum radiatum, showing the increase of the density of CA1 spines [16,17]. It should be noted that the mechanisms of estradiol action may be different between *in vivo* and *in vitro* experiments, because indirect effects of estradiol may also occur *in vivo* via cholinergic or serotonergic neurons, projecting to the hippocampus, in addition to direct influence of estradiol on glutamatergic neurons [2,17–20]. In stratum oriens and stratum lacunosum-moleculare there is only one report showing that dendritic spine density of CA1 neurons has been increased *in vivo* by supplement of estrogens over 4 days in ovariectomized female rats [1]. Note that detailed investigations with agonists, antagonists, or other inhibitors have not been performed in *in vivo* studies.

The current investigations demonstrated that the signaling of estradiol-driving spinogenesis is subregion specific even within the CA1. Careful attention should be paid to explain the estradiol-induced spinogenesis of primary cultured hippocampal neurons [7], because dispersed primary cultures are mixture of CA1, CA3, and dentate gyrus neurons.

In addition to endocrine-derived hormones, we recently demonstrated endogenous *de novo* synthesis of estrogens in male adult rat hippocampal neurons [11,15,21,22]. These results imply that hippocampal neurons are exposed to locally synthesized estradiol which might modulate synaptic plasticity rapidly. The observation of synaptic localization of ERα in glutamatergic principal neurons is in good accordance with the rapid modulation on the density of spines by ERα specific agonist PPT as well as estradiol.

References

[1] E. Gould, C.S. Woolley, M. Frankfurt, B.S. McEwen, Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood, J. Neurosci. 10 (1990) 1286–1291.
[2] C. Leranth, O. Petnehazy, N.J. MacLusky, Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats, J. Neurosci. 23 (2003) 1588–1592.
[3] C.S. Woolley, E. Gould, M. Frankfurt, B.S. McEwen, Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons, J. Neurosci. 10 (1990) 4035–4039.
[4] C.S. Woolley, B.S. McEwen, Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat, J. Neurosci. 12 (1992) 2549–2554.
[5] C. Leranth, M. Shanabrough, T.L. Horvath, Hormonal regulation of hippocampal spine synapse density involves subcortical mediation, Neuroscience 101 (2000) 349–356.
[6] C. Leranth, M. Shanabrough, D.E. Redmond Jr., Gonadal hormones are responsible for maintaining the integrity of spine synapses in the CA1 hippocampal subfield of female nonhuman primates, J. Comp. Neurol. 447 (2002) 34–42.
[7] D.D. Murphy, M. Segal, Regulation of dendritic spine density in cultured rat hippocampal neurons by steroid hormones, J. Neurosci. 16 (1996) 4059–4068.
[8] L.D. Pozzo-Miller, T. Inoue, D.D. Murphy, Estradiol increases spine density and NMDA-dependent Ca^{2+} transients in spines of CA1 pyramidal neurons from hippocampal slices, J. Neurophysiol. 81 (1999) 1404–1411.
[9] H. Mukai, T. Tsurugizawa, G. Murakami, S. Kominami, M. Ogiue-Ikeda, N. Takata, N. Tanabe, A. Furukawa, Y. Hojo, J.H. Morrison, W.G.M. Janssen, J.A. Rose, P. Chambon, S. Kato, T.Yamazaki, T. Kimoto, S. Kawato, Rapid modulation of long-term depression and spinogenesis depending on synaptic estrogen receptors in principal neurons of hippocampus, J. Neurochem., in press.
[10] T. Tsurugizawa, H. Mukai, N. Tanabe, G. Murakami, Y. Hojo, S. Kominami, K. Mitsuhashi, Y. Komatsuzaki, J.H. Morrison, W.G. Janssen, T. Kimoto, S. Kawato, Estrogen induces rapid decrease in dendritic thorns of CA3 pyramidal neurons in adult male rat hippocampus, Biochem. Biophys. Res. Commun. 337 (2005) 1345–1352.
[11] Y. Hojo, T.A. Hattori, T. Enami, A. Furukawa, K. Suzuki, H.T. Ishii, H. Mukai, J.H. Morrison, W.G. Janssen, S. Kominami, N. Harada, T. Kimoto, S. Kawato, Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes P45017{alpha} and P450 aromatase localized in neurons, Proc. Natl. Acad. Sci. USA 101 (2004) 865–870.
[12] Y. Komatsuzaki, G. Murakami, T. Tsurugizawa, H. Mukai, N. Tanabe, K. Mitsuhashi, M. Kawata, T. Kimoto, Y. Ooishi, S. Kawato, Rapid spinogenesis of pyramidal neurons induced by activation of glucocorticoid receptors in adult male rat hippocampus, Biochem. Biophys. Res. Commun. 335 (2005) 1002–1007.
[13] K.E. Sorra, K.M. Harris, Overview on the structure, composition, function, development, and plasticity of hippocampal dendritic spines, Hippocampus 10 (2000) 501–511.
[14] W.R. Harrington, S. Sheng, D.H. Barnett, L.N. Petz, J.A. Katzenellenbogen, B.S. Katzenellenbogen, Activities of estrogen receptor

alpha- and beta-selective ligands at diverse estrogen responsive gene sites mediating transactivation or transrepression, Mol. Cell Endocrinol. 206 (2003) 13–22.
[15] H. Mukai, N. Takata, H. Ishii, N. Tanabe, Y. Hojo, A. Furukawa, T. Kimoto, S. Kawato, Hippocampal synthesis of estrogens and androgens which are paracrine modulators of synaptic plasticity: Synaptocrinology, Neuroscience 138 (2006) 757–764.
[16] C.S. Woolley, B.S. McEwen, Estradiol regulates hippocampal dendritic spine density via an *N*-methyl-D-aspartate receptor-dependent mechanism, J. Neurosci. 14 (1994) 7680–7687.
[17] C.S. Woolley, N.G. Weiland, B.S. McEwen, P.A. Schwartzkroin, Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density, J. Neurosci. 17 (1997) 1848–1859.
[18] N.J. MacLusky, V.N. Luine, T. Hajszan, C. Leranth, The 17alpha and 17beta isomers of estradiol both induce rapid spine synapse formation in the CA1 hippocampal subfield of ovariectomized female rats, Endocrinology 146 (2005) 287–293.
[19] C.N. Rudick, C.S. Woolley, Estradiol induces a phasic Fos response in the hippocampal CA1 and CA3 regions of adult female rats, Hippocampus 10 (2000) 274–283.
[20] C.N. Rudick, C.S. Woolley, Selective estrogen receptor modulators regulate phasic activation of hippocampal CA1 pyramidal cells by estrogen, Endocrinology 144 (2003) 179–187.
[21] K. Shibuya, N. Takata, Y. Hojo, A. Furukawa, N. Yasumatsu, T. Kimoto, T. Enami, K. Suzuki, N. Tanabe, H. Ishii, H. Mukai, T. Takahashi, T.A. Hattori, S. Kawato, Hippocampal cytochrome P450s synthesize brain neurosteroids which are paracrine neuromodulators of synaptic signal transduction, Biochim. Biophys. Acta 1619 (2003) 301–316.
[22] S. Kawato, Y. Hojo, T. Kimoto, Histological and metabolism analysis of p450 expression in the brain, Methods Enzymol. 357 (2002) 241–249.