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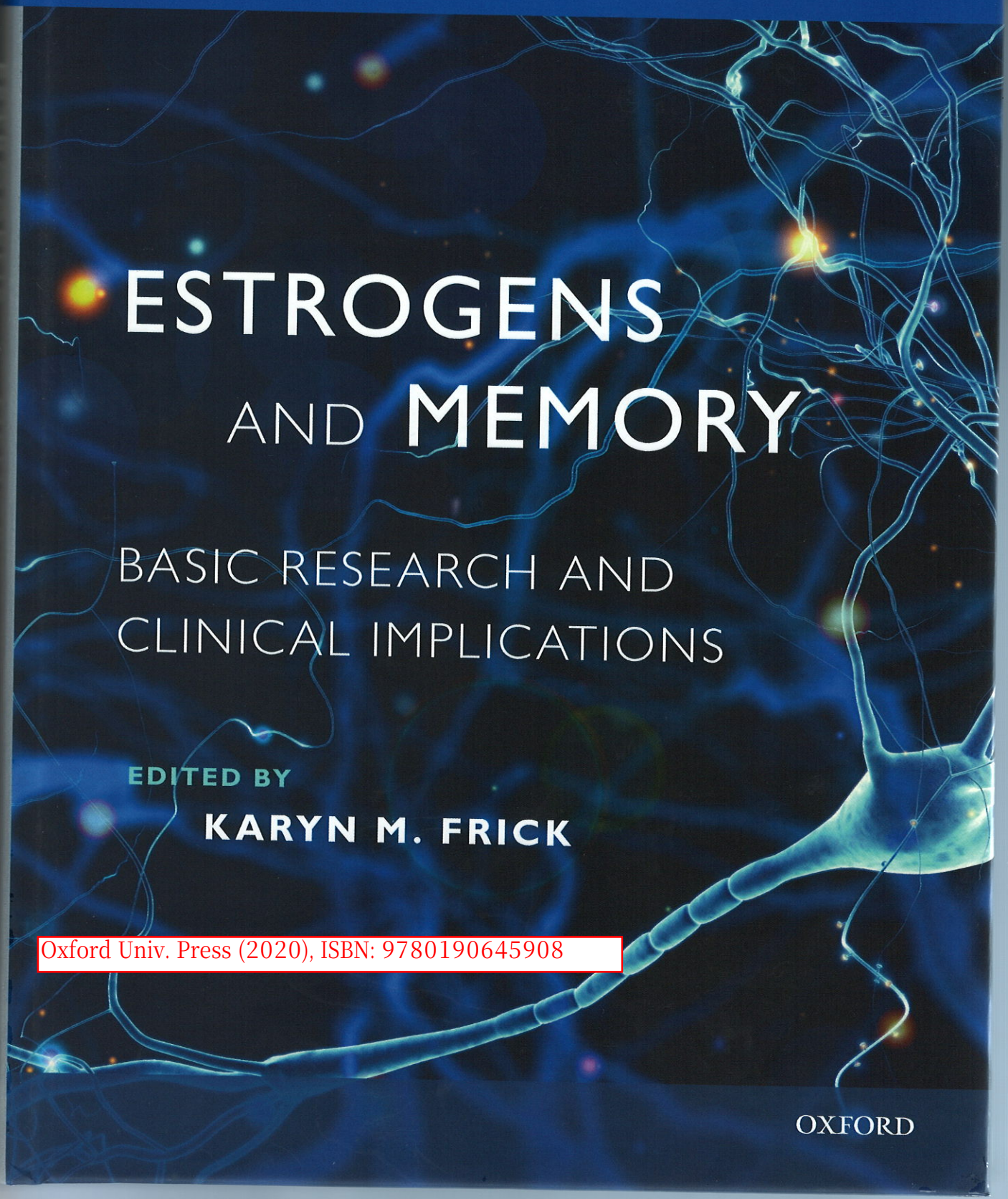

ESTROGENS AND MEMORY

BASIC RESEARCH AND CLINICAL IMPLICATIONS

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Rapid Effects of Estradiol on Dendritic Spines and Synaptic Plasticity in the Male and Female Hippocampus

ASAMI KATO, GEN MURAKAMI, YASUSHI HOJO, SIGEO HORIE, AND SUGURU KAWATO

INTRODUCTION

The physiological importance of rapid synaptic modulation by estrogens in the hippocampus may be supported by the neural activity-dependent local synthesis of estrogens and androgens (Kimoto et al., 2001; Kawato et al., 2002, 2003; Hojo et al., 2004, 2008; Kretz et al., 2004; Mukai et al., 2007, 2010; Okamoto et al., 2012; Kato et al., 2013). The in vivo level of adult hippocampal estradiol (E2) is higher (~8 nM for male, 0.5~4 nM female) than that of plasma E2 (~0.01 nM for male, 0.01~0.1 nM female), as determined by mass-spectrometric analysis (Hojo et al., 2009; Kato et al., 2013). Therefore, it is important to consider modulation effects by hippocampal E2 on synaptic plasticity.

In addition to slow/genomic (within days) functions of E2, rapid action of E2 has been demonstrated by a number of electrophysiological investigations in rats and mice, particularly concerning long-term potentiation (LTP; Bi et al., 2001; Ooishi, Kawato et al., 2012) and long-term depression (LTD; Vouimba et al., 2000). The rapid effect of E2 may be driven through estrogen receptors (ER) alpha (ERα) or beta (ERβ), localized at the synaptic membrane, as judged from many recent investigations (Mukai et al., 2007, 2010; Hasegawa et al., 2015).

E2 plays an essential role not only in female but also in male hippocampi, since the male rat hippocampus endogenously synthesizes several-fold more E2 than the female hippocampus (Kawato et al., 2002; Hojo et al., 2004, 2008; Kretz et al., 2004; Higo et al., 2009; Kato et al., 2013). As such, the majority of this review focuses on the effects of E2 on dendritic spine density in hippocampal slices prepared from gonadally intact male rats.

In this review, we first focus on kinase-dependent signaling mechanisms involved in rapid modulation by E2 of dendritic spinogenesis in male rodents. We then focus on the role of synaptic (membrane) estrogen receptors in these effects. We also consider dynamic changes of spine structures over time and sex differences in spine regulation. Finally, the importance of local hippocampal synthesis of E2 and androgens is also discussed.

RAPID MODULATION OF SPINES IN MALE RATS BY E2 VIA KINASE NETWORKS

Spinogenesis in Male CA1

To establish effects of E2 on spines in the male hippocampus, we investigated the effects of E2 on the modulation of dendritic spine density and morphology with single spine imaging of Lucifer Yellow-injected neurons in hippocampal slices prepared from gonadally intact adult male rats. We analyzed secondary branches of the apical dendrites of pyramidal cell bodies in the stratum radiatum of the CA1 region. One nM of E2 significantly increased the total spine density within 2 hours (Mukai et al., 2007; Hasegawa et al., 2015). This increase was observed when the E2 level was more than 1 nM, whereas 0.1 nM of E2 did not affect spine density, probably due to the basal level of E2 at ~0.5 nM (Hojo et al., 2011; Ooishi, Kawato et al., 2012).

Given the rapid nature of these effects, we reasoned that activation of cell-signaling cascades might be involved. We thus investigated the involvement of kinase networks in the E2-induced spine increase in young adult male rats using selective kinase inhibitors (Hasegawa et al., 2015). We discovered that many kinases were involved in E2-induced rapid spinogenesis in the CA1 region, including LIM domain kinase (LIMK),

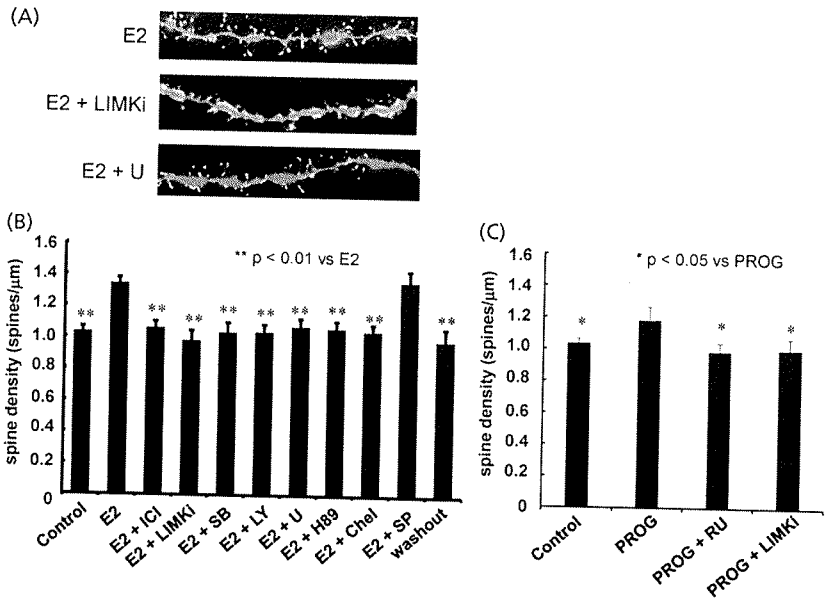

FIGURE 3.1. Involvement of kinase networks in rapid spinogenesis in the CA1 of adult male rat slices. (A) Confocal microscopic images of spines and dendrites in the stratum radiatum of hippocampal pyramidal neurons, analyzed with Spiso-3D. Isolated acute hippocampal slices were treated with 1 nM E2 for 2 hours. LIMKi is a LIMK inhibitor and U (U0126) is an ERK MAPK inhibitor. (B) Effect of 2 hours of treatment by 1 nM E2 and kinase blockers on total spine density. ICI is an estrogen receptor (ER) inhibitor. SB (SB203580) is a p38 MAPK inhibitor. LY (LY294002) is a PI3K inhibitor. H89 is a PKA inhibitor. Chel (Chelerythrine) is a PKC inhibitor. SP (SP600125) is a JNK inhibitor. Washout of E2 was performed by additional 2-hour incubation in artificial cerebrospinal fluid (ACSF). In control, slices were incubated in ACSF for 2 hours. (C) Effect of treatments by 100 nM PROG and blockers on total spine density. RU (RU486) is a PR inhibitor. Modified from Hasegawa et al. (2015) (See color plate).

extracellular signal-regulated mitogen-activated protein kinase (ERK MAPK), p38 MAPK, cAMP-dependent protein kinase A (PKA), protein kinase C (PKC), phosphoinositide 3-kinase (PI3K), and glycogen synthase kinase 3 (GSK-3β). Inhibitors of each kinase abolished the E2 effect and reversed the total spine density to control levels (Figure 3.1A, B). In contrast, blocking c-Jun N-terminal kinase (JNK) did not change total spine density. In addition to these kinases, calcineurin, a phosphatase, was also responsible for E2-induced rapid spinogenesis, suggesting a dynamic regulation of E2's effects in male rats by both kinases and phosphatases. These effects may generalize to other species as well, as MAPK involvement in E2-spinogenesis was also observed in fixed hippocampal slices from male mice (Murakami et al., 2015). In support, one recent in vivo study found that E2-induced spinogenesis in the CA1 region of ovariectomized mice depended on activation of ERK MAPK or mammalian target of rapamycin (mTOR) in the dorsal hippocampus (Tuscher et al., 2016). Moreover, other work in ovariectomized mice demonstrated that phosphorylation of ERK MAPK, PI3K, PKA, and mTOR in the dorsal hippocampus are necessary for E2 to enhance hippocampal memory consolidation (Fernandez et al., 2008; Lewis et al., 2008; Fortress et al., 2013), suggesting that E2-induced increases in spinogenesis may contribute to its beneficial effects on memory.

Head Diameter Analysis

Because total spine density does not provide sufficient information for understanding complex kinase effects, we have also analyzed changes in spine head diameter. We classified spines into three categories depending on their head diameters; in the case of rats, for example, diameters of 0.2 to 0.4 μm were categorized as small-head spines, 0.4 to 0.5 μm as middle-head spines, and 0.5 to 1.0 μm as large-head spines (Mukai et al., 2011), in which spine head size has a close relationship with the number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors as well as the efficiency in signal transduction. The number of AMPA receptors (including metabotropic glutamate

receptor [mGluR] 1 [GluR1] subunits) in the spine increases as the size of the postsynaptic density increases, whereas the number of *N*-methyl D-aspartate (NMDA) receptors (including NR2B subunits) may be relatively constant. Therefore, larger head spines may store memory more efficiently than smaller head spines.

Treatment with 1 nM E2 for 2 hours increased the density of small-head spines, whereas the density of middle- and large-head spines was not significantly altered. Blocking LIMK, ERK MAPK, p38 MAPK, PKA, PKC, PI3K, or GSK-3β abolished the effect of E2 on CA1 dendritic spine densities, reverting the density of the small-head spines to control levels (Hasegawa et al., 2015).

Mechanisms underlying Spine Increases in Males

Due to their key roles in regulating actin polymerization, LIMK and cofilin are important candidates for modulators of E2-induced actin reassembly leading to spinogenesis through the activation of the following sequence: LIMK → phosphorylation of cofilin → actin polymerization → spine increase (Aizawa et al., 2001; Liston et al., 2013; Hasegawa et al., 2015; Ikeda et al., 2015). In addition to cofilin, the ERK MAPK cascade may couple with PKA and PKC through the activation of PKC → Raf1→ MAPK or PKA → B-Raf → MAPK to induce phosphorylation of cortactin, actin polymerization, and an increase in spines. PI3K and phosphatase (calcineurin) are also involved.

Moreover, basal Ca^{2+} concentration in spines via spontaneous Ca^{2+} influx/efflux through NMDA receptors is necessary for E2-induced rapid spinogenesis, because the NMDA receptor blocker MK-801 abolished the effects of E2 (Mukai et al., 2007). In hippocampal slices, spontaneous Ca^{2+} influx/efflux through NMDA receptors occurs due to spontaneous open/close of NMDA receptors (spontaneous firing).

CA3 and Dentate Gyrus Spinogenesis in Males

Although much attention has been focused on the CA1 region of the hippocampus, E2 also rapidly effects postsynaptic morphology in the dentate gyrus (DG) and CA3, the other major regions of the hippocampus. E2's effects on the DG (spines) and CA3 (thorns) are mediated via ER and kinase networks (Tsurugizawa et al., 2005; Hojo et al., 2015).

In the stratum radiatum of the DG, incubation with 1 nM of E2 for 2 hours increased not only small-head but also middle-head spines of granule cells. ERs and several kinases, including ERK MAPK, PKA, and PKC, were involved in this E2-induced effect (Hojo et al., 2015), similar to E2's effects in CA1. GSK-3β was not involved in the E2-induced spinogenesis in the DG, although GSK-3β played an important role in rapid E2 signaling in CA1. Poor responsiveness of DG granule neuron spinogenesis was reported during the estrous cycle (Gould et al., 1990). Weak responsiveness to E2 was also found in long-term E2 exposure (>several months) to ovariectomized female rats (Miranda et al., 1999). Thus, it is curious why we observed high E2-responsiveness in our studies. This discrepancy may be related to the fact that our E2-depleted DG neurons (E2 was deprived by recovery incubation of slices) were exposed to E2 only briefly (Hojo et al., 2015).

In the stratum lucidum of CA3, 1 nM E2 decreased, but did not increased, postsynaptic thorns within 2 hours (Tsurugizawa et al., 2005). Thorns are postsynaptic structures that have multiple heads, forming synapses with mossy fibers projecting from the DG. Blockade of ERK MAPK abolished the E2-induced thorn decrease (Tsurugizawa et al., 2005). The mechanism of the regional difference in responsiveness to E2 and involvement of kinases is currently unknown.

ROLE OF SYNAPTIC ESTROGEN RECEPTORS IN MEDIATING EFFECTS OF E2 ON SPINES IN MALES

The synaptic receptor(s) underlying the effects of E2 must be identified to explain the mechanism of rapid (1–2 hours) modulation of synaptic plasticity in the hippocampus.

Blocking ERs with ICI 182, 780 (ICI), an antagonist of ERα and ERβ, completely abrogated the enhancing effect of E2 on spine density in multiple regions of the hippocampus, suggesting that the rapid effect of E2 on spinogenesis is mediated by intracellular ERs (Tsurugizawa et al., 2005; Mukai et al., 2007; Hasegawa et al., 2015; Hojo et al., 2015). Involvement of ERα and ERβ in rapid spinogenesis was further demonstrated using ER agonists (Mukai et al., 2007; Hasegawa et al., 2015; Tsurugizawa et al., 2005). The ERα agonist (propyl-pyrazole-trinyl) tris-phenol (PPT), but not ERβ agonist (4-hydroxyphenyl)-propionitrile (DPN), rapidly (within 2 hours) increased

dendritic spine density on male rat CA1 pyramidal neurons (Hasegawa et al., 2015). These results support the involvement of ERα, but not ERβ, in rapid CA1 spinogenesis (Mukai et al., 2007). A study using ER knockout mice further confirmed the involvement of ERα in rapid CA1 spinogenesis (Murakami et al., 2015). In this study, treatment with E2 for 2 hours increased the density of middle-head spines in wild-type male mouse hippocampal slices. The E2-induced increase in middle-head spines was also observed in ERβKO mice (which express ERα), but not in ERαKO. These results indicate that ERα, but not ERβ, is necessary for estrogen-induced spinogenesis on CA1 pyramidal neurons in male mice.

How might ERs regulate CA1 spine density? Expression of ERα in glutamatergic pyramidal neurons is clearly demonstrated in rat and mouse hippocampus by immunostaining with purified antibody RC-19 (Mukai et al., 2007). ERα is localized not only in nuclei/cytoplasm but also in presynaptic and postsynaptic terminals, as revealed by immunogold electron-microscopic analysis (Mukai et al., 2007). Expression of ERβ in presynaptic and postsynaptic terminals was also observed by immunogold electron-microscopic analysis (Milner et al., 2005). Association of ERα with postsynaptic densities (PSDs) was observed by Western blot of PSD fractions (Mukai et al., 2007), implying synaptic membrane binding of ERα. As such, ERα and ERβ are well positioned to mediate the rapid effects of E2 on dendritic spinogenesis. In cultured cells of peripheral origin, some populations of ERα and ERβ are plasma membrane bound, and they are anchored via caveolin (Razandi et al., 2002; Pedram et al., 2006). Therefore, membrane binding for ERα or ERβ might also occur in neurons, including raft membranes (Milner et al., 2005; Mukai et al., 2007, 2010; Hojo et al., 2008). In cultured hippocampal neurons, ERα and ERβ complex with mGluRs and caveolin proteins to regulate phosphorylation of the transcription factor cAMP response element binding protein (CREB), suggesting a membrane-associated signalling mechanism through which intracellular ERs may regulate rapid spinogenesis (Boulware et al., 2005, 2007).

Another candidate to mediate synaptic ER effects is the G-protein coupled estrogen receptor (GPER) localized within cellular membranes. GPER is expressed in the membrane of the endoplasmic reticulum but not in the plasma membrane (Revankar et al., 2005). However, GPER may not participate in spine modulation, because of the low binding affinity of GPER with E2 and lack of rapid E2 signaling in SKBR-3 cells (ER negative, GPER positive; Pedram et al., 2006; Otto et al., 2009; Kim et al., 2016). Different from the activation of ERα and ERβ, the GPER agonist G-1 did not activate ERK MAPK in the dorsal hippocampus of ovariectomized female mice, but instead activated JNK and enhanced object recognition and spatial memory performance (Kim et al., 2016). Therefore, the cell-signaling mechanisms triggered by GPER activation seem different from those triggered by ERα and ERβ.

PROGESTERONE-INDUCED RAPID SPINOGENESIS IN MALES

Progesterone (PROG) is another important sex steroid hormone that has been shown to regulate hippocampal spinogenesis in ovariectomized rats (Woolley and McEwen, 1993). Thus, in addition to E2, we also investigated rapid spinogenesis by PROG in males. Spine density was increased upon incubation of 100 nM PROG with hippocampal slices for 2 hours (Figure 3.1C). The applied PROG concentration was chosen to match endogenous concentrations in the hippocampus (Kato et al., 2013; Hojo et al., 2014; Hasegawa et al., 2015). The PROG-induced spine increase in males was suppressed by inhibitors of nuclear PROG receptors (PRs) and LIMK, indicating that PRs may work as extranuclear/synaptic receptors (Waters et al., 2008) and that LIMK is involved in PROG-induced nongenomic spinogenesis (Fig. 1C). This possibility is supported by recent data showing that the rapid memory-enhancing effects of PROG in ovariectomized mice are blocked by ERK MAPK inhibition (Fortress et al., 2015).

SPINES IN THE CYCLING FEMALE HIPPOCAMPUS

Spine Fluctuation across the Estrous Cycle in Female CA1

Cyclic changes in dendritic spine density across the estrous cycle in gonadally intact female rodents have been extensively studied (Gould et al., 1990; Woolley and McEwen, 1992), and this fluctuation is an essential difference between females and males. The estrous cycle consists of four approximately day-long stages, that is, proestrus (Pro), estrus (Est), diestrus 1 (D1), and diestrus 2 (D2).

Because previous studies combined D1 and D2 into single category called Diestrus (D),

we recently examined spine density in D1 and D2 separately using CA1 hippocampal slices after perfusion-fixation in vivo (Kato et al., 2013). Total spine density peaked during Pro, decreased from Pro to Est, increased from Est to D1, decreased from D1 to D2, and increased from D2 to Pro (Figure 3.2). These cyclic spine density changes might be dependent on alterations in kinase activity, since phosphorylation levels of several kinases, which are involved in spinogenesis, change across the estrous cycle. Phosphorylation of ERK MAPK, Akt, and LIMK in the hippocampus are highest during Pro, and then decrease to Est, at which point levels are at their nadir (Spencer et al., 2008). These data mirror the increased phosphorylation observed for these kinases in the hippocampus after exogenous E2 administration in ovariectomized females (Fernandez et al., 2008; Yildirim et al., 2008; Fan et al., 2010). At Diestrus (D; mixture of D1 and D2), phosphorylation of Akt is intermediate between Est and Pro, but that of LIMK is comparable to Est (Bi et al., 2001; Znamensky et al., 2003; Spencer et al., 2008). As mentioned earlier, however, it can be difficult to draw conclusions from studies that do not separate D1 and D2.

Aside from plasma-derived E2, the cyclic changes in CA1 spine density may also be caused by cyclic changes in the levels of E2 and PROG within the hippocampus that comprise the "hippocampal estrous cycle." Within the hippocampus, the level of E2 was highest (~4 nM) at Pro and that of PROG (~ 90 nM) was highest at D1; moreover, the greatest CA1 spine density coincided with these highest levels of E2 and PROG (Figure 3.2; Kato et al., 2013). Analysis of head diameter distribution allowed us to distinguish Pro spines from D1 spines, although they had almost the same total spine density. At the Pro stage, large-head spines were dominant, whereas middle-head spines were dominant at D1. Surprisingly, no significant change was observed across the estrous cycle in expression levels of mRNAs for sex steroidogenic enzymes and steroid receptors in the female hippocampus. Therefore, the cyclic changes in hippocampal steroid levels cannot be explained by mRNA levels of sex steroidogenic enzymes. However, the addition of fluctuating plasma PROG (20–50 nM with a peak at D1) to the constant level of hippocampus-synthesized PROG (~30 nM) can substantially raise the baseline cyclic fluctuation of total hippocampal PROG levels (40–90 nM). As a result, the cyclic fluctuation of hippocampal E2 (0.5–4 nM) could be elevated via the conversion of fluctuating PROG to E2, through conversion to androstenedione and estrone by hippocampal steroidogenic systems.

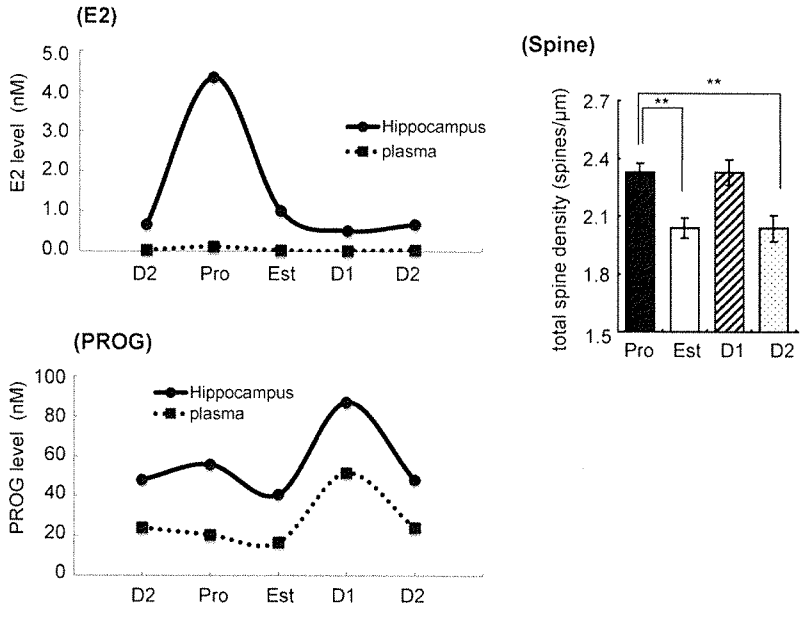

FIGURE 3.2. Cyclic fluctuation of E2 levels, PROG levels, and total spine density across estrous cycle in adult female rat CA1. Modified from Kato et al. (2013).

TIME-DEPENDENT SPINE DYNAMICS IN THE MALE HIPPOCAMPUS

Monitoring of time dependent changes in the density and morphology of the same spines reveal another aspect of rapid E2 effects. Time-lapse imaging of spines from mice expressing yellow fluorescent protein (YFP) showed the dynamic modulation of spine density and morphology by E2 in the CA1 region of the adult male mouse hippocampus by tracing the same spines every 30 minutes (Figure 3.3; Murakami et al., 2015). In time-lapse imaging of control slices without drug stimulation, spines were observed to both appear and disappear. This spine fluctuation is due to thermal fluctuation, which might induce fluctuation of phosphorylation/dephosphorylation of cofilin or cortactin by kinases. In steady-state conditions, the rate of appearance and disappearance of spines is identical, resulting in an equilibrium state in the absence of any stimulation. In the presence of E2, however, the equilibrium state is shifted toward an increased level of spinogenesis, resulting in a net increase in spine density (Murakami et al., 2015). Interestingly, we found that not only small-head spines but also middle- and large-head spines dramatically changed their head structures and head diameters in adult slices within 1 hour, suggesting that spine stability may not be always proportional to spine head sizes. These observations might raise doubts about a typical hypothesis of sequential spine development, for example, from small head spines (unstable), via middle head spines, to large head spines (very stable). Our results rather support that the appearance and disappearance of spines may not strongly depend on spine head size. These results, obtained from time-lapse imaging, are consistent with the results from fixed slices (fixed after 2 hours E2 treatments shown in Figure 3.1; Murakami et al., 2015). Although both time-lapse and fixed slice experiments indicate that E2 increases hippocampal spine density, the fixed slice preparation is less conclusive due to its statistical comparison between different slices. In contrast, time-lapse imaging has the advantage of direct temporal comparison of the same spines. Although the density and shapes of spines dynamically fluctuate naturally in live slices (Yuste and Bonhoeffer, 2004; Segal, 2005), the average spine density does not change significantly during slice incubation over 1 to 5 hours without drugs (Mukai et al., 2007). As such, the time-lapse preparation appears to be more sensitive to changes in average spine density than the fixed slice method.

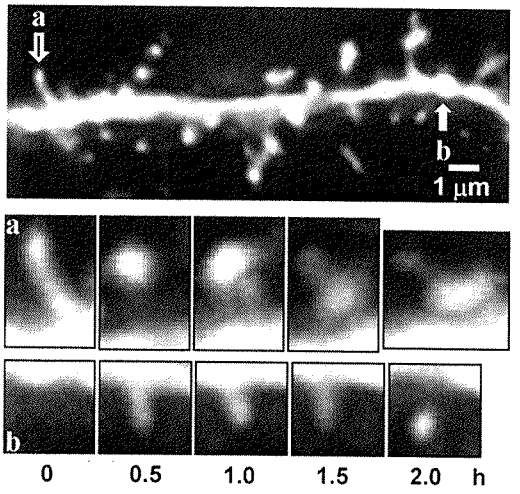

FIGURE 3.3. Time-lapse imaging of E2-induced spinogenesis in live hippocampal slices from adult male yellow fluorescent protein (YFP) mice. Confocal microscopic images were taken at t = 0, 0.5, 1, 1.5 and 2 hours after the initiation of 10 nM E2 perfusion. Spines at a and b positions dynamically change their shapes within 2 hours. Modified from Murakami et al. (2015).

HIPPOCAMPAL SYNTHESIS OF ESTROGENS AND ANDROGENS

The rapid modulation of cell signaling and spines associated with memory formation processes suggests that locally synthesized hippocampal steroids may play a greater role than circulating gonadal hormones, which travel a long distance before reaching the brain. Direct measurements of local E2 levels in hippocampal slices is very important because spine density is strongly dependent on levels of E2 and other steroids, including androgens (testosterone [T] and dihydrotestosterone [DHT]). Normally in isolated acute slices prepared by the dissection and vibratome-slicing of the hippocampus, all steroids are depleted after 2 hours of incubation, resulting in levels of less than 0.5 nM (Hojo et al., 2011; Ooishi, Mukai et al., 2012). In this case, the concentration of exogenously applied E2 (>1 nM) is above the local E2 concentration (<0.5 nM). On the other hand, the in vivo male hippocampus contains high levels of local E2 (~7 nM) and androgens (~18 nM T, 6 nM DHT; Hojo et al., 2009), and, therefore, application of inhibitors against steroid synthesis (e.g., letrozole or finasteride) may be an effective way to alter spines in male rats. On the contrary,

E2 levels in the in vivo female hippocampus are much lower (<1 nM on average) than in males (Kato et al., 2013), and, therefore, the application of steroidogenic inhibitors to female rats may not be as effective as in males.

It should be noted that the expression of steroidogenic enzymes in the adult male rat hippocampus, including cytochrome P450 (17α), P450arom and 17β-hydroxysteroid dehydrogenase (17β-HSD), was demonstrated at the level of mRNA and protein (Kimoto et al., 2001; Hojo et al., 2004, 2009; Higo et al., 2011). Furthermore, immunohistochemical analysis combined with immunogold electron microscopic analysis show that these enzymes are expressed in pyramidal neurons in the CA1–CA3 regions, as well as in the granule cells in the DG, and are localized at postsynapses and presynapses. The production of E2, T, and DHT was demonstrated by metabolism of radioactive substrates. These results show that hippocampal neurons are equipped with a full set of enzymes to catalyze the synthesis of E2 from cholesterol.

Necessity of rapid local E2 synthesis for the induction of LTP is recently found from the suppression of LTP by blocking E2 synthesis with P450arom inhibitors (Grassi et al., 2011).

SYNAPTIC PLASTICITY: LTP AND LTD

E2 exerts a rapid (e.g., 1 hour) influence on the synaptic plasticity of rat hippocampal glutamatergic neurons in slices, as has been demonstrated by a number of electrophysiological investigations in CA1 of rats and mice concerning LTP (Bi et al., 2001) and LTD (Vouimba et al., 2000; Mukai et al., 2007). We demonstrated the induction of LTP by the presence of E2 upon weak theta burst stimulation (weak TBS) in the CA1 region of the adult male hippocampus (Hasegawa et al., 2015). Because only weak TBS did not induce full-LTP, weak TBS was used as subthreshold stimulation. Upon stimulation with weak-TBS, we observed LTP induction by the presence of E2 (E2-LTP) after incubation of hippocampal slices with 10 nM E2 for 30 minutes. This E2-LTP was blocked by ICI and inhibitors of ERK MAPK, PKA, PKC, PI3K, NR2B, and CaMKII activation, individually. Taken together, our data suggest the following sequence of events through which E2 may lead to enhanced LTP: E2 binds to synaptic ERα or ERβ → activation of ERK MAPK, PKC, and PKA → activation of NMDA receptor by phosphorylation of NR2B → increase of Ca^{2+} influx though NMDA receptors during weak TBS → activation of CaMKII → phosphorylation of AMPA receptors → enhancement of LTP magnitude. Interestingly, perfusion with an mGluR1 inhibitor does not block E2-LTP in males, suggesting no involvement of mGluR1 (Hasegawa et al., 2015), although hippocampal mGluR1 signaling is necessary for female ERα- or ERβ-dependent object and spatial memory facilitation induced by E2 (Boulware et al., 2013). This discrepancy suggests a sex differences in the neural mechanisms through which E2 may enhance synaptic plasticity that should be further investigated.

In female hippocampal slices, the magnitude of LTP is larger during the Pro stage than during the D stage (measured without E2 application). Because these differences in LTP magnitude are associated with increased phosphorylation of ERK MAPK and NR2B subunits (Bi et al., 2001), these results imply that phosphorylated MAPK and NMDA receptors were saved during LTP measurements, even after recovery incubation of slices with artificial cerebrospinal fluid (ACSF). These results also imply that LTP is regulated by E2-induced kinase signaling in the female hippocampus.

LTD may be a mechanism used to "correct" wrong memories formed by initial LTP processes, which stores not only correct information but also incorrect information (Migaud et al., 1998). We analyzed the effect of E2 on chemical LTD induced by NMDA perfusion in male rat hippocampus. A 30-minute perfusion of 10 nM E2 significantly enhanced LTD (Mukai et al., 2007). The ERα agonist PPT, but not the ERβ agonist DPN, induces rapid enhancement of the NMDA-induced LTD, indicating that ERα drives the enhancement of LTD (Mukai et al., 2007). E2 did not enhance NMDA-induced LTD in ERαKO mice, also suggesting the involvement of ERα (Murakami et al., 2015).

CONCLUSION

In summary, E2-induced rapid spinogenesis is carried out by kinase-dependent nongenomic signaling, including LIMK, MAPK, PKA, PKC, and PI3K. Kinases regulate actin-binding proteins, such as cofilin and cortactin, leading to actin polymerization, resulting in spine increases. In both males and females, local hippocampal E2 synthesis supports this rapid E2 function. Interestingly, androgen (DHT, T)-induced rapid spinogenesis is also executed via similar kinase-dependent nongenomic signaling (Hatanaka et

al., 2009, 2015). To our surprise, corticosterone-induced rapid spinogenesis is also carried out via kinase-dependent non-genomic signaling (Komatsuzaki et al., 2012; Ikeda et al., 2015). It is interesting that synaptically localized classical steroid receptors (ER, PR, AR, glucocorticoid receptor GR) can activate kinase networks, and these receptors may be membrane bound. Therefore, steroids may share these common mechanisms for rapid regulation of spinogenesis.

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