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Estradiol Promotes Spine Growth and Synapse Formation Without Affecting Pre-Established Networks

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ABSTRACT: Estrogens regulate dendritic spine density, but the mechanism and significance of this effect for brain networks remain unknown. We used repetitive imaging over several days to investigate how 17β-estradiol affected the turnover and long-term behavior of dendritic spines in CA1 cells of hippocampal slice cultures. We find that 17β-estradiol and serum in the culture medium tightly regulated spine density by promoting an increase in the rate of new spine formation and their transformation into synapses, without affecting spine elimination or stability. New spines formed during a transient 17β-estradiol application were preferentially eliminated upon removal of the hormone, in contrast with pre-existing spines that remained unaffected. Our results reveal that 17β-estradiol transiently regulates the complexity of hippocampal circuits without causing major alterations of pre-existing networks. © 2010 Wiley Periodicals, Inc.

KEY WORDS: 17β-estradiol; estrogen receptors; hippocampus; synaptogenesis; confocal imaging

Steroid hormones act as powerful modulators of the organization and function of brain networks (McEwen and Alves, 1999; McCarthy, 2008). In the hippocampus, but also in other brain regions, estradiol markedly affects the density of dendritic spines (Gould et al., 1990; Kretz et al., 2004; Mukai et al., 2007; Srivastava et al., 2008), which varies by about 30% during the estrous cycle in female rats (Woolley and McEwen, 1992; Prange-Kiel et al., 2008). In addition, estradiol has various acute effects on synaptic physiology and plasticity by modulating synaptic receptors and signaling pathways (Woolley, 2007; Liu et al., 2008; Kramar et al., 2009). How these actions of estrogen affect the dynamics of synaptic networks remains poorly understood. The growth-promoting effect of 17β-estradiol depends upon activation of estrogen receptors (Jelks et al., 2007), and evidence suggests that this leads to the formation of silent synapses and an increase in connectivity between neurons (Yankova et al., 2001; Srivastava et al., 2008). Although these new connections might enhance the complexity and processing power of existing circuits, they might also interfere with pre-established networks. We analyzed here this issue by assessing spine dynamics over several days in hippocampal slice cultures treated or not with estrogens.

As estradiol is present in serum, a component of the standard culture medium for slice cultures (Stoppi et al., 1991), we switched hippocampal slices to a serum free medium (SFM) after 8 days in vitro (DIV) and tested the effects 10 nM and 1 μM 17β-estradiol on the dynamics of spines on apical dendrites of EGFP transfected CA1 pyramidal neurons. Slice cultures were indifferently prepared from male and female neonates. Imaging was done using a repetitive protocol as previously described (De Roo et al., 2008), with one cell analyzed per slice culture. These experiments revealed that cultivation of slice cultures for 6 days in SFM, but not in serum, lead to a progressive, significant decline in spine number (Fig. 1; from 0.90 ± 0.04 spines/μm [n = 11] to 0.61 ± 0.07 spines/μm [n = 10]). Addition of 1 μM or 10 nM 17β-estradiol during 3 days of SFM (i.e., at 11 DIV) prevented further decrease and reversed spine density to control values by 14 DIV (Fig. 1E, 1 μM 17β-estradiol, 0.89 ± 0.07 spines/μm; 10 nM 17β-estradiol, 0.86 ± 0.08 spines/μm). This effect was estrogen receptor dependent as addition of the estrogen receptor antagonist ICI-182780 to serum reproduced the decline observed in SFM. Thus estrogen-like compounds present in the serum are likely to represent important modulators of spine density in slice cultures.

To examine the effect of estrogen on the dynamics of spines, we measured the number of new spines formed or lost during 24 h observation intervals in slices maintained in different culture conditions. In SFM, the rate of formation of new spines per 24 h (Fig. 2A, plus (+) signs; Fig. 2B; open circles; confidence interval: 11 ± 1%, n = 11) was almost twice lower than in serum containing medium (Fig. 2B; gray circles: confidence interval: 21 ± 2%; n = 5, P < 0.001). Interestingly, the rate of spine loss over the same period of observation (24 h) was not different in serum and SFM (Fig. 2A, minus (−) signs; Fig. 2C; mean values: 18 ± 2% of lost spines vs. 17 ± 1% of lost spines, respectively; P > 0.1). Accordingly, although formation and loss of spines counter-balanced each other in the presence of serum, neurons cultured in SFM lost more spines than they produced resulting in a net decrease of spine density (Fig. 1). Upon addition of 1 μM or 10 nM 17β-estradiol to cultures maintained in SFM, the number of new spines formed per 24 h increased markedly. The effect peaked during 2 days (Fig. 2B, black diamonds for 1 μM 17β-estradiol,
FIGURE 1. Serum and 17β-estradiol regulate spine density in hippocampal slice cultures. A: Illustration of CA1 pyramidal cells and (B) representative images of the same dendritic segments obtained at 11 DIV and 14 DIV of neurons maintained in serum containing medium (Serum); neurons maintained in serum free media (SFM) from DIV 8; neurons maintained in serum free media from 8 DIV and supplemented with 17β-estradiol (βE2; 1 μM) from 11 DIV to 14 DIV and neurons maintained in serum supplemented with ICI 180,782 (1 μM). C: Graphs summarizing the changes in spine density observed in serum, SFM and serum + ICI-182780 (an estrogen receptor antagonist; top graph) and SFM, SFM + 10 nM and SFM + 1 μM 17β-estradiol (βE2; bottom graph). Data are mean ± standard error of mean (SEM) of analyses made on 5, 10, 11, 10, 6, and 5 dendritic segments, respectively, and expressed as percent of the mean value obtained for slices maintained in serum. Scale bars: A: 50 μm; B: 1 μm; *P < 0.05; **P < 0.01; two-way analysis of variance (ANOVA).

28.2 ± 2.3% of new spines at DIV12–13, n = 10, P < 0.001, gray diamonds for 10 nM 17β-estradiol, 27.0 ± 4.1% of new spines, n = 6, P < 0.01), and then reached values comparable to those obtained under serum conditions. The new spines formed in the presence of 17β-estradiol did not however differ in terms of head width or length from those formed in serum or SFM (data not shown). In contrast, 17β-estradiol had no statistically significant effect on the rate of spine loss during the whole observation period (Fig. 2C; P > 0.1). All changes produced by estrogens on the rate of spine formation were antagonized by ICI-182780 (mean values 11–12 DIV: 17β-estradiol [1 μM] + ICI-182780 [1 μM]: 15 ± 2% of new spines, n = 6; 17β-estradiol [10 nM] + ICI-182780 (1 μM): 14 ± 2% of new spines, n = 7; vs. SFM: 11 ± 3%, P > 0.1). These results thus indicate that the main effect of estrogen on spine density is attributable to the hormone’s regulation of the growth of new protrusions, without any effect on the rate of spine disappearance.

We then examined whether these new spines formed synapses by analyzing whether they expressed a postsynaptic density. For this, cells were cotransfected with EGFP and PSD-95-DsRed2, and we assessed the proportion of new spines and total spines expressing PSD-95-DsRed2 puncta (see De Roo et al., 2008). Quantitative analyses showed that irrespective of the culture medium the same fraction of total spines expressed PSD-95-DsRed2 puncta (Fig. 2E), indicating that the same proportion of spines were indeed synapses. However, analysis of newly formed spines aged less than 24 h showed that a much larger fraction of them was PSD-95-DsRed2 positive in 17β-estradiol or serum containing medium than in SFM (Fig. 2F). Estrogen and serum thus not only promoted spine growth, but also their maturation and the formation of new synaptic contacts.

To understand the impact of these changes on synaptic networks, we then assessed the effects of 17β-estradiol on spine stability. For this, we compared the proportion of spines present in SFM at 11 DIV that were still present on subsequent days in SFM or after application of 17β-estradiol. As illustrated in Figure 3A, addition of 17β-estradiol (1 μM or 10 nM) to SFM did not change significantly the stability of dendritic spines present before the hormonal treatment (P > 0.1).

We then wondered how removal of 17β-estradiol would affect new and pre-established connections. For this, we applied 17β-estradiol transiently for 24 h in order to simulate the conditions of an estrogen peak, and determined using repetitive imaging how this affected (i) the new spines generated during the 24 h period that cells were in the presence of 17β-estradiol; (ii) the new spines generated during the 24 h period before application of 17β-estradiol; (iii) the pre-existing spines, present at the beginning of the experiment. The stability of these groups of spines was measured by analyzing the proportion of them persisting over 24 and 48 h. As shown in Figures 3B,C, new spines formed during 17β-estradiol application (n = 19 spines; 4 cells) were preferentially eliminated upon removal of the hormone, while in contrast, the new spines generated before application of 17β-estradiol; (iii) the pre-existing spines, present at the beginning of the experiment (n = 13 spines; 4 cells, P < 0.01) as well as the pre-existing spines present at the beginning of the experiment (n = 123 spines; 4 cells, P < 0.01) were considerably more stable and persistent. Thus, transient application of 17β-estradiol resulted in a consecutive formation and removal of a specific subpopulation of dendritic spines.

Together, this study reveals important new features regarding the mechanisms through which 17β-estradiol regulates synaptic networks in the hippocampus. First and in line with previous
FIGURE 2. Estradiol regulates spine formation, but not spine loss, and promotes synapse formation. A: Illustration of spine turnover over a 24 h interval between 12 DIV and 13 DIV on dendritic segments of cultures maintained in the presence of serum (Serum), serum free media (SFM), or SFM + 17β-estradiol (βE2; 1 μM). Plus and minus signs illustrate new and lost spines, respectively. Scale bar: 1 μm. B: Number of new spines detected in 24 h intervals and expressed as percent of pre-existing spines. Data are mean ± SEM of analyses made on 10, 11, 6, and 5 cells in respectively SFM, SFM + 10 nM 17β-estradiol (βE2; 10 nM), and serum (Serum). 17β-estradiol was added after the observation at 11 DIV (black bar); *P < 0.05; **P < 0.01; 2-way ANOVA). C: Same as in B, but for lost spines. D: Dendritic segment of a CA1 pyramidal cell cultured in the presence of 1 μM 17β-estradiol cotransfected with EGFP and PSD-95-DsRed2 showing the formation of a new spine 24 h later (plus sign, 2nd panel) and the presence in this new spine of a PSD-95-DsRed2 puncta (arrow head, 3rd panel). The 4th panel shows the merged image. Scale bar, 1 μm. E: Proportion of total spines expressing a PSD-95-DsRed2 puncta in SFM, SFM + 1 μM 17β-estradiol (βE2), and serum. Data are mean ± SEM of respectively 9, 4, and 5 cells analyzed. F: Proportion of newly formed spines aged less than 24 h that expressed PSD-95-DsRed2 puncta. Data are mean ± SEM (SFM: n = 9 new spines/4 cells; SFM + βE2: n = 21 new spines/4 cells; serum: n = 23 new spines/5 cells; **P < 0.01, one-way ANOVA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
FIGURE 3. Estradiol transiently increases spine density without perturbing pre-existing networks. A: Stability of spines present at the beginning of the experiment and assessed as the percentage of spines still present 24, 48, and 72 h later under SFM (n = 7 cells) or SFM + 17β-estradiol (βE2, n = 7; *P < 0.01). B: Experiment illustrating a new spine formed before (*) and another during (arrow head) a 24 h application of 17β-estradiol (βE2; between DIV12–13). C: Stability of new spines formed within a 24 h interval either before (born in SFM, open circles, n = 13) or during a 24 h treatment with 17β-estradiol (born in βE2, black circles, n = 19) and compared with spines present at the beginning of the experiments (gray circles, n = 123). For each category, stability was assessed as the proportion of spines still present 24 and 48 h later. Spines formed during estrogenic treatment are preferentially eliminated upon removal of estradiol (*P < 0.05; **P < 0.01; two-way ANOVA).

By analyzing spine dynamics, our data show, in agreement with previous studies (Jelks et al., 2007; Srivastava et al., 2008), that the increase in spine density produced by 17β-estradiol essentially results from an increase in spine growth. However, interestingly, this is done without alterations in the number of disappearing spines. Thus clearly, formation and elimination of spines are regulated by different mechanisms (De Roo et al., 2009). A second effect of 17β-estradiol was to promote the transformation of new spines into synapses through the expression of a PSD. This might be linked to some of the acute activity-dependent effects of 17β-estradiol on plasticity (Jelks et al., 2007; Srivastava et al., 2008; Kramar et al., 2009), but might also reflect a role of 17β-estradiol in regulating the expression of synaptic proteins (Fester et al., 2009b).

The third and probably most interesting finding of the this study is that 17β-estradiol, while regulating spine density, does not seem to affect the stability and behavior of pre-existing circuits. The number of disappearing spines remained unaffected upon 17β-estradiol, and the stability of spines present before 17β-estradiol treatment was not affected by the hormone. Furthermore, transient application of 17β-estradiol lead to an increased spine density followed, upon removal of the hormone, by a preferential disappearance of those spines formed during estrogen treatment, but without effects on pre-existing connections. These results thus strongly suggest that 17β-estradiol promotes the formation of a pool of transient, highly dynamic spines that could enhance the processing functionality of the hippocampal network by increasing the number and divergence of synapses (Holtmaat et al., 2005). This is consistent with the observation that estrogen stimulates contact formation between previously unconnected neurons (Yankova et al., 2001). However, the important information provided here is that this change is potentially transient, that most of the new connections formed in response to 17β-estradiol disappear upon removal of the hormone without altering the structural characteristics of the existing network. This conclusion is of interest for understanding the significance of the variations in spine density reported during the estrous cycle. A greater sensitivity and/or capacity for adaptation might be required during the estrous period to allow for specific behaviors, but without interfering with previously acquired information. Variations in estrogen levels could therefore transiently boost the integration properties of the network, thus promoting learning and memory mechanisms (Li et al., 2004; Liu et al., 2008), while at the same time preserving pre-existing circuits, thus without
significantly modifying information acquired prior to hormone application.

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REFERENCES


