Hippocampus- synthesized estrogen and androgen rapidly modulate dendritic spines and LTP in non-genomic manner

ADVANTAGES OF HIPPOCAMPUS-SYNTHESIZED ESTROGEN AND ANDROGEN

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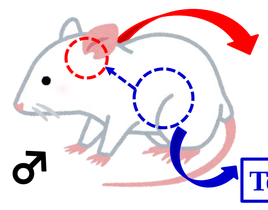
Abstract

Rapid non-genomic modulation by neurosteroids of synapses has advantages in regulation of learning and memory. Non-genomic rapid modulation of synapses can well be performed in combination with locally synthesized neurosteroids, not with circulating steroids whose concentration is changed slowly. [Local Synthesis in Hippocampal Neurons] We showed expression as well as neuronal/synaptic localization of essential enzymes (mRNA and protein) in the adult male rat hippocampus. Mass-spectrometric analysis demonstrated that hippocampal levels of estradiol (E2), testosterone (T), dihydrotestosterone (DHT) were 8 nM, 17 nM and 7 nM, respectively, which are significantly higher than their levels in plasma.

E2 synthesis is rapid (within 30 min) as observed by suppression of E2-LTP by application of letrozole, P450arom inhibitor, due to rapid decrease in E2 level.

[Rapid Dendritic Spine Modulation] E2 and androgen-induced rapid non-genomic modulations (within 2 h) are observed in spine increase and LTP-induction in adult male rat hippocampal slices. The density of spines and their head diameters were determined by 3-D mathematical analysis. E2 at 1 -10 nM rapidly (within 2 h) and selectively increased the density of small-head spines, in CA1 pyramidal neurons. T and DHT at 10 nM increased the density of small-head spines and large-head spines, respectively. [Rapid LTP Modulation] E2-induced rapid modulation (within 1 h) of LTP was demonstrated with adult male rat hippocampal slices. Upon weak theta-burst stimulation, 10 nM E2 perfusion (within 1 h) induced full-LTP (E2-assisted LTP), although without E2 this sub-threshold stimulation did not induce full-LTP. DHT weakens this E2-assisted LTP. [Synaptic Membrane Receptors] Contribution of classic ERalpha and AR to rapid modulations of spines and LTP was confirmed. Classic ERalpha and AR were expressed at postsynapses, as observed by immunoelectron microscopic analysis. ERalpha agonist PPT induced rapid spine increase and LTP-induction. ERalpha antagonist ICI blocked both rapid spine increase and LTP-induction . AR antagonist flutamide blocked rapid spine increase. Synaptic ERalpha and AR may be bound to the membrane via palmitoylation of ERalpha and AR. [Kinase Networks in Downstream of Membrane Receptors] Downstream mechanisms of spine-increase and LTP by E2, T and DHT were revealed by using selective kinase inhibitors. Kinase inhibitors of LIMK, MAPK, PKA, PKC, Src individually blocked E2-induced and (T, DHT)-induced spine-increase. Kinase inhibitors of LIMK, MAPK, PKA, PKC individually blocked E2-assisted LTP. [Mechanisms of Rapid Modulation] Signaling pathways are summarized as follows: (1) E2 or (T, DHT) → synaptic ERalpha or AR → activation of LIMK, MAPK, PKA, PKC, Src kinase → phosphorylation of cofilin (LIMK) or cortactin (other kinases) → actin polymerization→ new spine formation. (2) E2 → synaptic ERalpha → activation of MAPK, PKA, PKC → NMDA-R phosphorylation → elevated Ca influx→ AMPA-R phosphorylation → activation of CaMKII → LTP-induction.

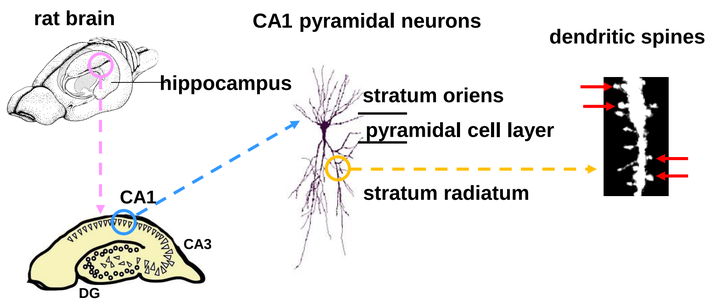
[Attention] For determination, E2, T, DHT must be extracted from freshly removed hippocampal tissues ! in order to obtain precise concentrations at 1 – 10 nM. If you use the freeze-thaw hippocampus, then you lose a lot of E2 molecules by oxidation of OH at C3-position during freezing, resulting in picomolar E2. However, you do not lose T and DHT (at nanomolar level), because their O at C3-position is not oxidized. Note that, during freezing and thawing of hippocampal tissues, antioxidants (e.g., glutathione and ascorbate) are released from cells, because water crystallization breaks cell membranes . Therefore, E2 oxidation of OH at C3-position occurs further.



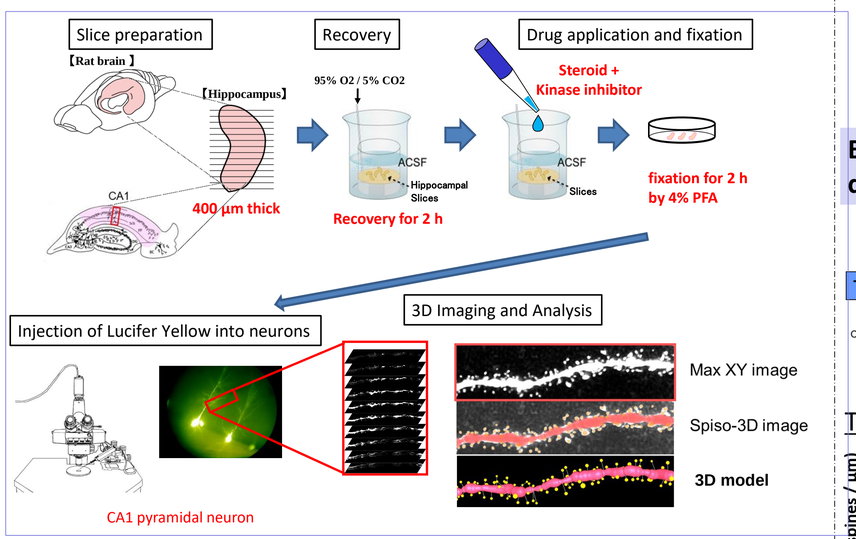
Hippocampus-synthesized sex steroids show higher levels than plasma steroid levels

Testis : T circulation

Methods

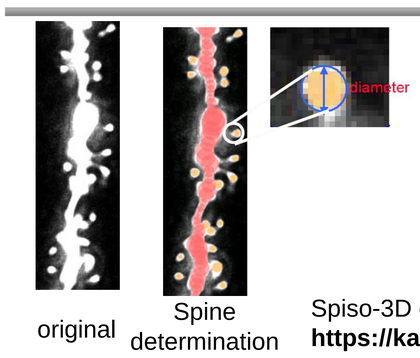


hippocampal slice preparation, confocal imaging, spine analysis



Attention> E2, T, DHT application to hippocampal slices recovers the spine density which was once decreased by depletion of steroids from acute slices during incubation in ACSF.

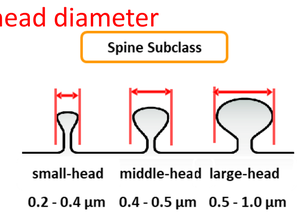
I Spiso-3D software determines spine density & head diameter



original

Spine determination

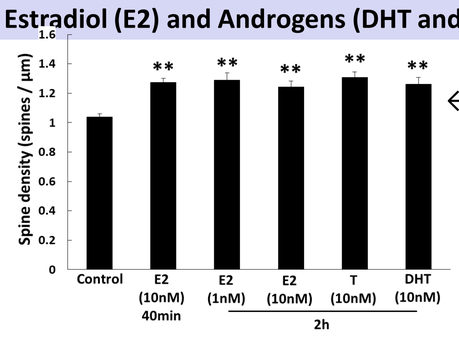
Tracing dendrite and determining head diameter



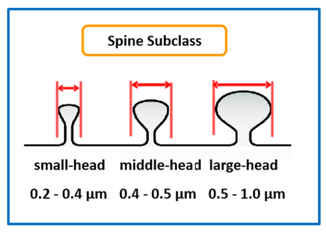
Spiso-3D can be downloaded from **https://kawato-glia.sakura.ne.jp/proj.html**

II Estrogen and Androgen rapidly increase spines in CA1

Estradiol (E2) and Androgens (DHT and T) acutely increase spines



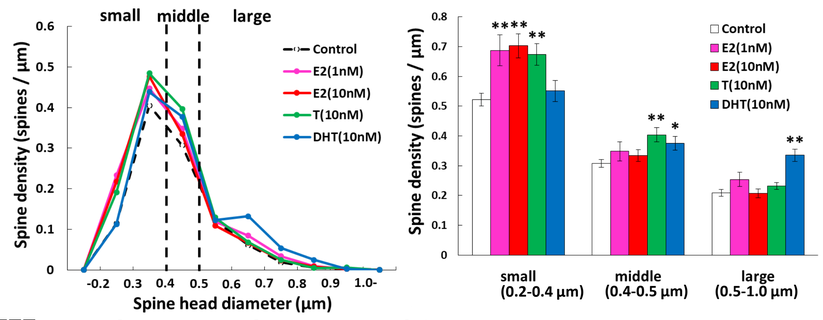
← Total spine density



Further analysis of head diameter distribution

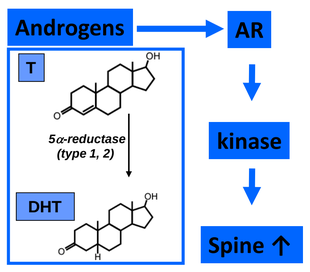
Large-head spines have more AMPA receptors and memory capacity than small-head spines.

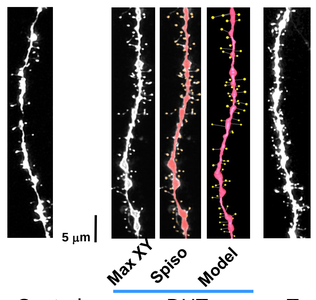
E2 and T mainly increases small-head spines (0.2 —0.4 μm) whereas DHT mainly increases large-head spines (0.5 —1.0 μm)



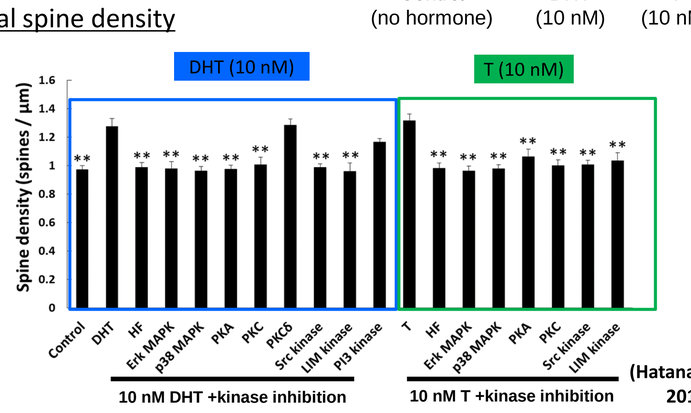
III Androgen and estrogen drive wide variety of kinases in spinogenesis in CA1

Androgens (DHT and T, 10 nM) increase spines via androgen receptor (AR) and various kinases in CA1



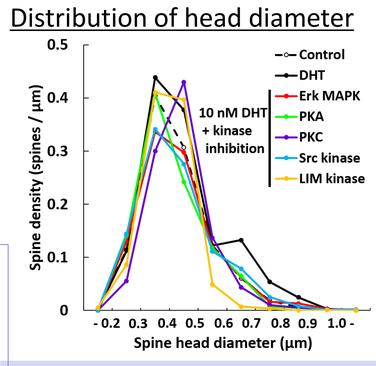


Total spine density

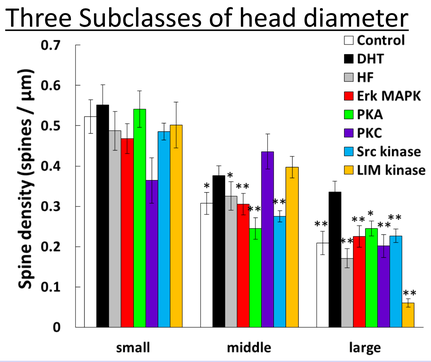


(Hatanaka et al., 2015 *Brain Res.*)

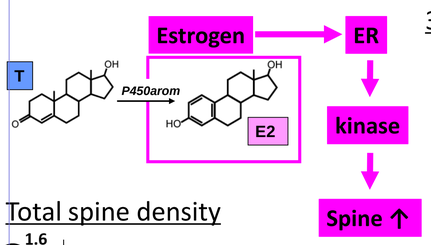
Distribution of head diameter



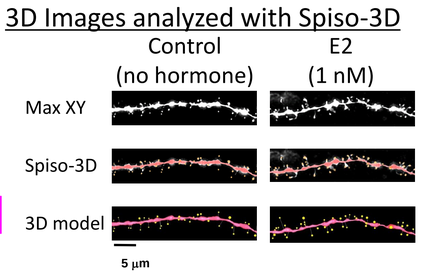
Three Subclasses of head diameter



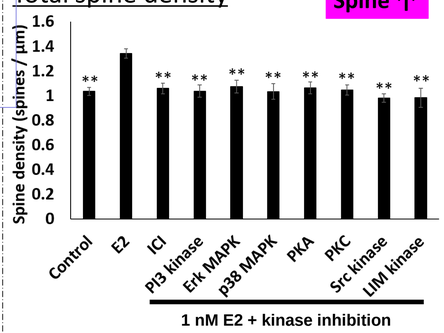
Estradiol (E2, 1 nM) binds estrogen receptor (ER) which activates downstream kinase networks, resulting in spine increase in CA1



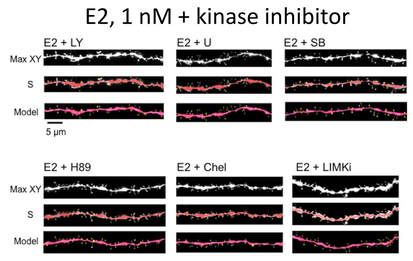
3D Images analyzed with Spiso-3D



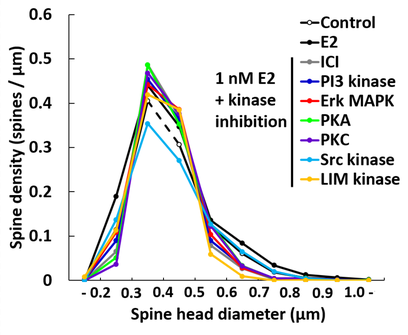
Total spine density



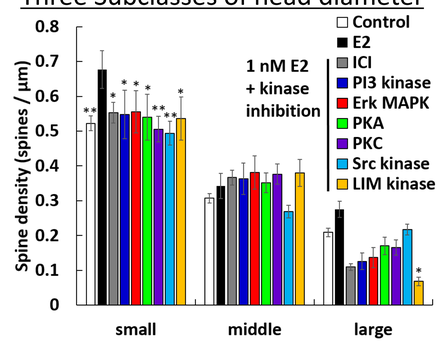
E2, 1 nM + kinase inhibitor



Distribution of head diameter

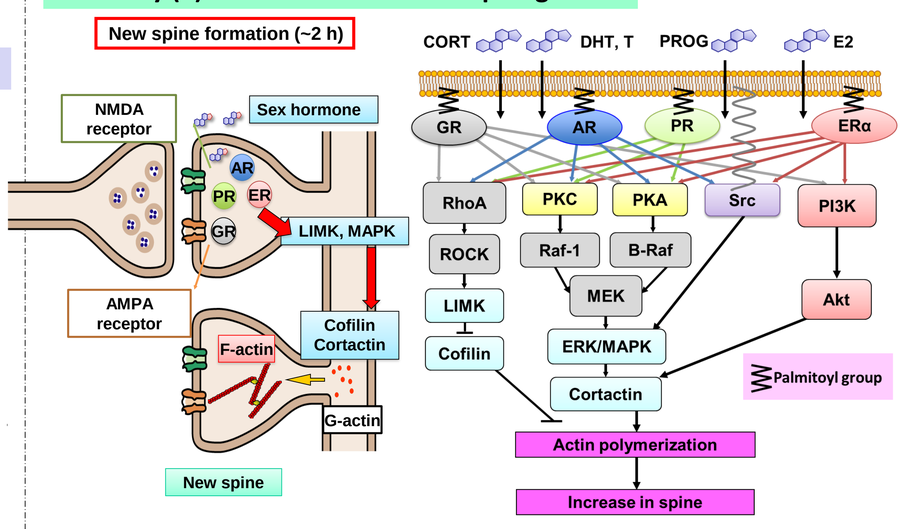


Three Subclasses of head diameter



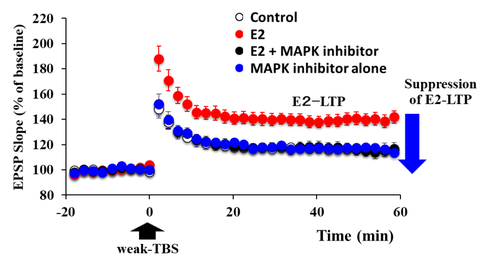
(Hasegawa et al., 2015 *Brain Res.*)

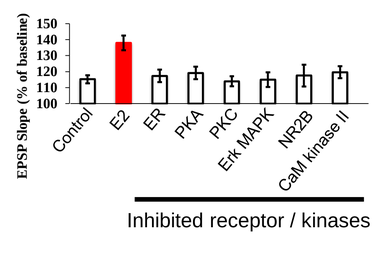
Summary (1) model illustration of spinogenesis



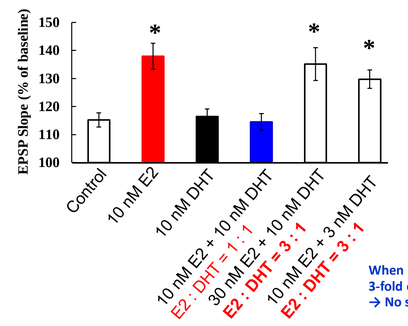
III Electrophysiology: E2 enhances LTP, DHT suppresses E2-LTP

1. Estrogen (E2) acutely induces long-term potentiation (E2-LTP). Kinases are involved in signaling of E2-LTP. (Hasegawa et al., 2015 *Brain Res.*)

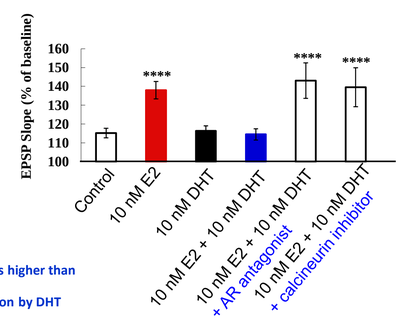




2. DHT vs E2 : androgen (DHT) may weaken E2-induced hyper excitation of LTP. DHT drives calcineurin (phosphatase).

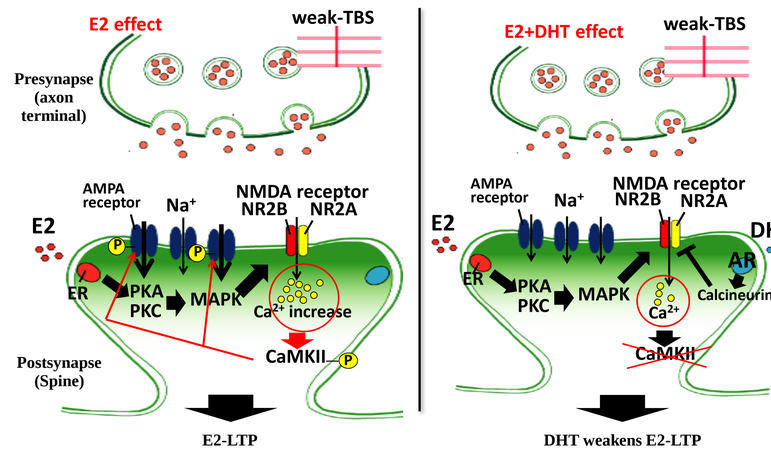


When E2 level is higher than 3-fold of DHT → No suppression by DHT

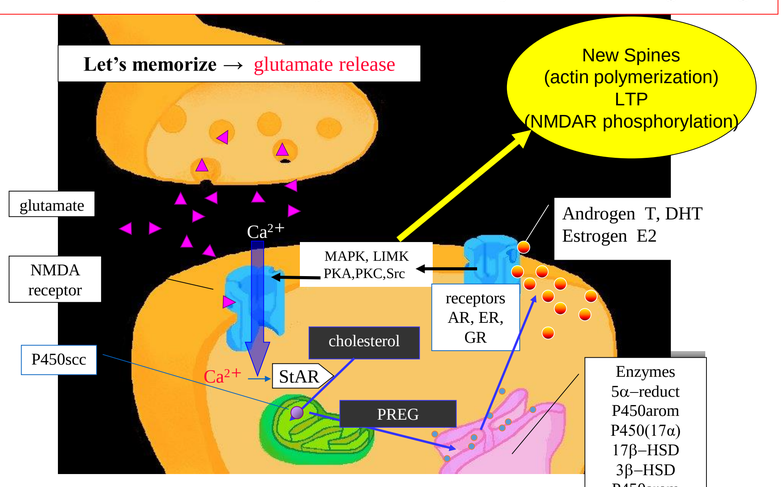


Summary (2) mechanisms of LTP modulation

- Estrogen induces E2-LTP (activation of NMDA-R) with kinases.
- DHT may weaken E2-LTP via suppression of NMDA receptor.



Rapid synthesis and action of neuro-estrogen and androgen. Membrane receptors modulate synaptic plasticity via kinase signaling.



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