Non-genomic modulation of synapses by hippocampus-synthesized androgen, estrogen and stress steroids

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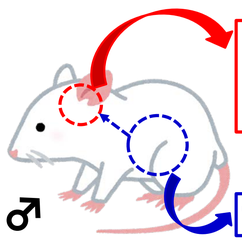
Abstract

(1) Adult male rat hippocampus synthesizes estrogen and androgen which rapidly modulate synapses via kinase signaling pathway. [Synthesis] Mass-spectrometric analysis demonstrated that exact levels of hippocampal estradiol (E2), testosterone (T), dihydrotestosterone (DHT) were 8 nM, 18 nM and 7 nM, respectively, which are much higher than their levels in plasma. [Synaptic Modulation] E2- and androgen-induced rapid non-genomic modulation (1- 2 h) was demonstrated by analysis of spinogenesis of adult male rat hippocampal slices (steroid-depleted slices after recovery incubation). Spine analysis was performed for pyramidal neurons in hippocampal slices. The density of spines and their head diameters were determined by mathematical software Spiso-3D which determines spine density and head diameter. E2 at 1 nM rapidly increased the density of small-head spines, in CA1 pyramidal neurons. DHT at 10 nM increased the density of middle-head spines and large-head spines. Signaling pathways are: synaptic membrane ERalpha or membrane AR→ LIMK, MAPK, PKA, PKC → cofilin or cortactin → actin polymerization→ new spine formation.
(2) Stress hormone (corticosterone) also induced rapid non-genomic spine increase, via membrane GR receptor and kinase signaling pathway. These results could explain mechanisms of "Fight-or-flight acute behavior against enemy".

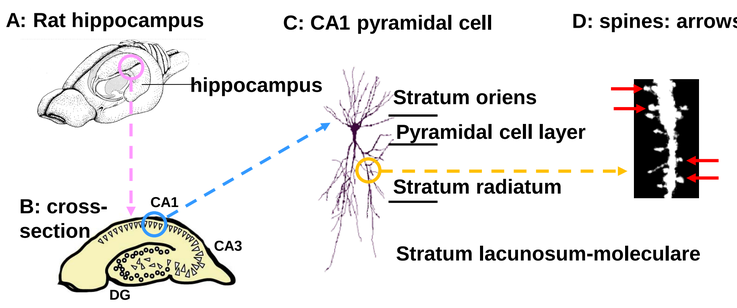


hippocampus-synthesized sex steroids
- higher level than plasma steroid level
- influx of plasma sex steroids into the brain

Testis : T supply to the brain

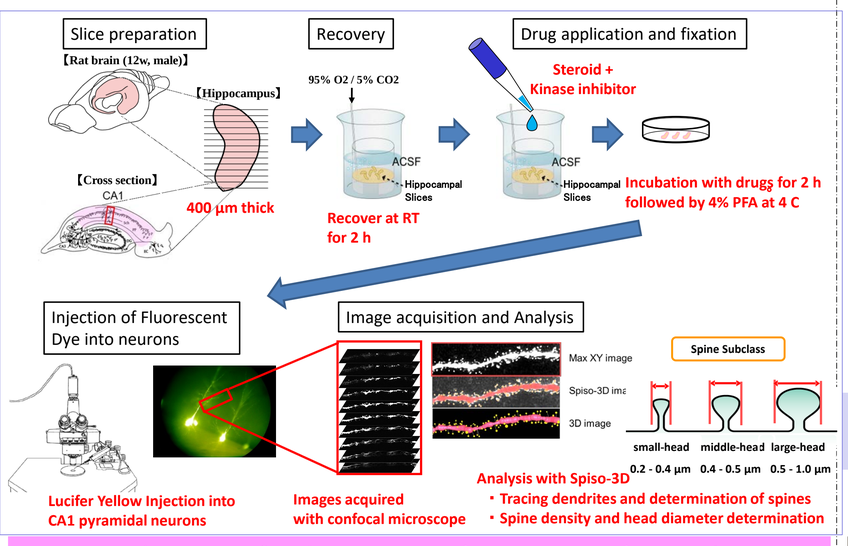
T, DHT, E2 supplementation in hippocampal slices recover the spine density which was once decreased by sex steroid-depletion in slices via incubation in ACSF.

Methods



↑ **glutamatergic neurons (▽) in the hippocampus**
C: CA1 stratum radiatum neurons receives information from Schaffer collateral fibers from CA3 region.
D: Single dendrite has many spines (postsynapse).

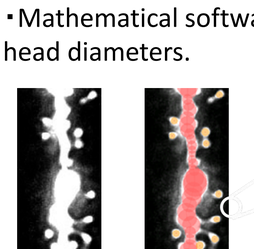
hippocampal slice preparation, confocal imaging, spine analysis



Super-resolution confocal microscope (Zeiss LSM880) is used in Airy Scan mode with 32 honeycomb detectors.
Deconvolution of Image function I(x) is performed with Airy Scan software, resulting in resolution of nearly 120 nm. Image brightness is increased by 4-fold.

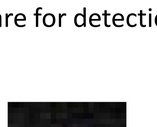
I Spiso-3D mathematical analysis determines differences in hormone effects on spines

Spiso-3D is achieved through JST Bioinformatics Project.
・Mathematical software for detection of spines, calculating spine head diameters.



original

after Spiso determination



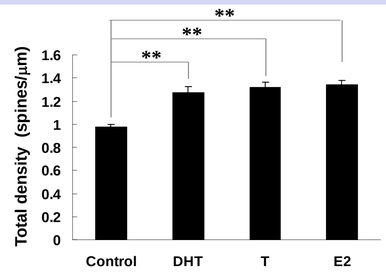
Spine is determined as negative eigenvalues of 2nd derivative tensors H of image function I(x)

$$I(\mathbf{x}+\varepsilon\mathbf{u}) = I(\mathbf{x}) + \varepsilon I'(\mathbf{u}) + \frac{1}{2}\varepsilon^2 I''(\mathbf{u}) + \cdots$$
$$= I(\mathbf{x}) + \varepsilon \cdot gradI \cdot \mathbf{u} + \frac{1}{2}\varepsilon^2 \mathbf{u}^t \mathbf{H} \mathbf{u}$$
$$= I(\mathbf{x}+\varepsilon\mathbf{u}) = I(\mathbf{x}) + \varepsilon(g_+ u_+ + g_- u_-) + \frac{1}{2}\varepsilon^2(\lambda_+ u_+^2 + \lambda_- u_-^2)$$

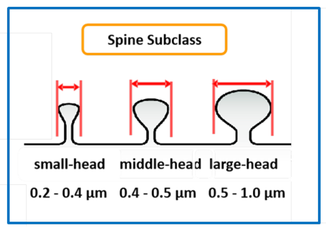
Spiso-3D on Kawato Lab's homepage
http://kawato-glia.sakura.ne.jp
can be downloaded.

II Androgen and estrogen rapidly increase spines (recovery effects)

Androgens (DHT and T) and estradiol (E2) acutely increase spines.



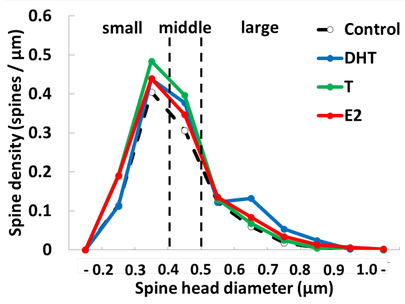
← Total spine density

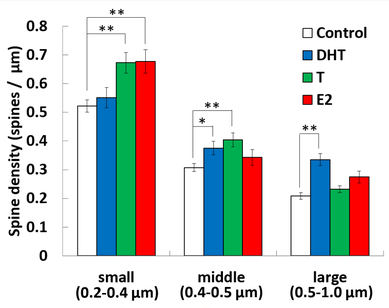


Further analysis of head diameter distribution

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

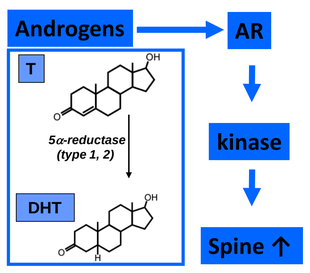
E2 and T mainly increases small-head spines (0.2 －0.4 μm) whereas DHT mainly increases large-head spines (0.5 －1.0 μm).

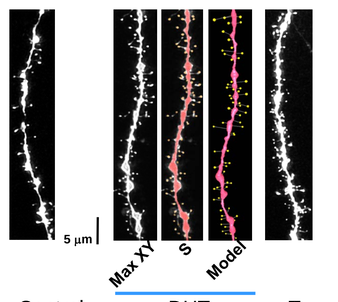




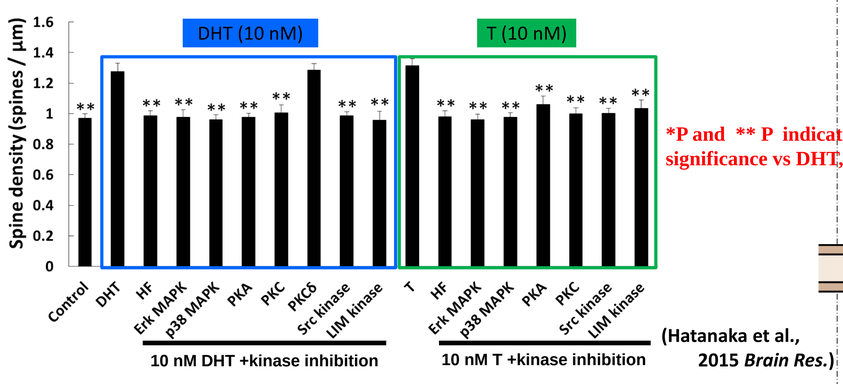
III Androgen and estrogen use the same kinases in spinogenesis, except for PI3K

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





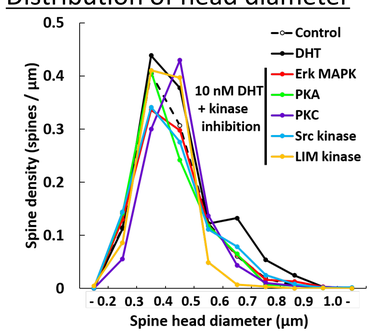
Total spine density



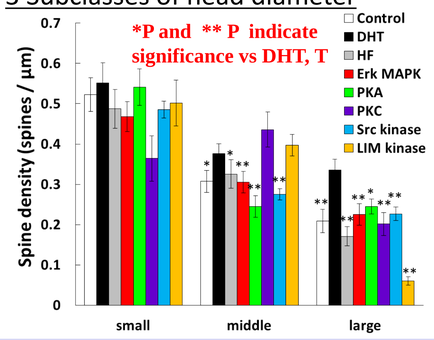
*P and ** P indicate significance vs DHT, T

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

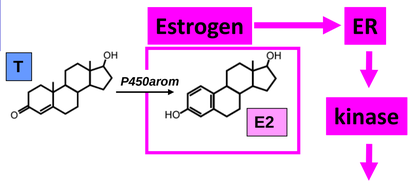
Distribution of head diameter



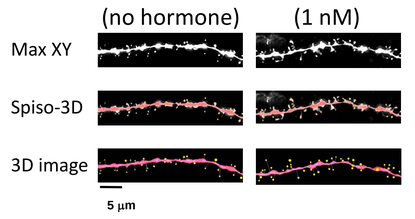
3 Subclasses of head diameter



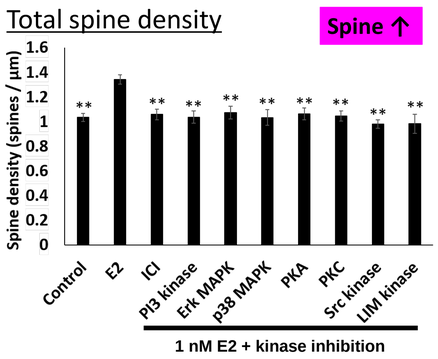
Estradiol (E2, 1 nM) binds estrogen receptor (ER) which activates downstream kinase networks, resulting in spine increase.



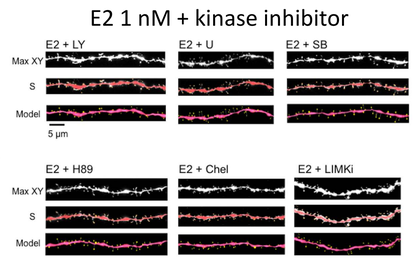
Trace Images analyzed with Spiso-3D



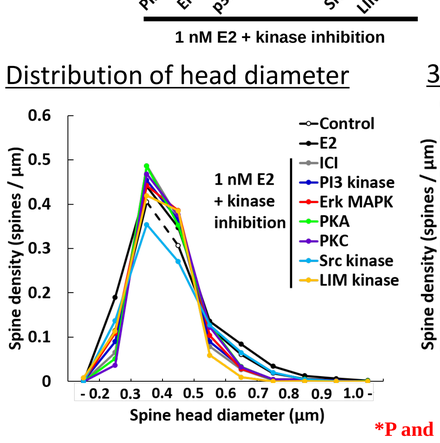
Total spine density



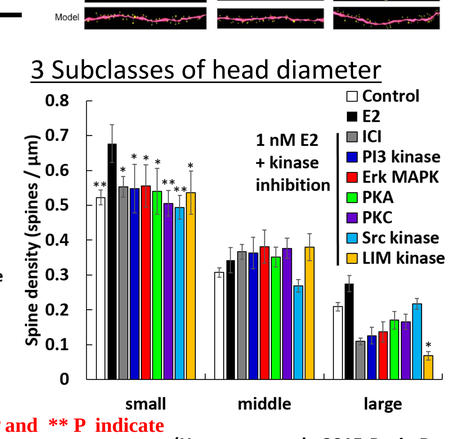
E2 1 nM + kinase inhibitor



Distribution of head diameter



3 Subclasses of head diameter

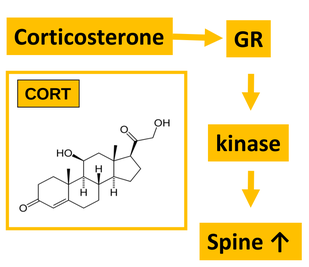


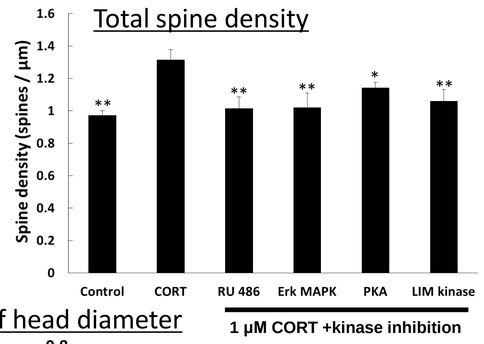
*P and ** P indicate significance vs E2

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

IV Stress hormone uses the same kinases as androgen and estrogen in rapid spinogenesis

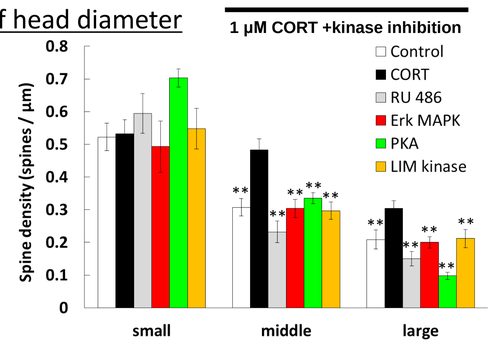
Corticosterone (CORT, 1 μM) increases spines via glucocorticoid receptor (GR) and various kinases.





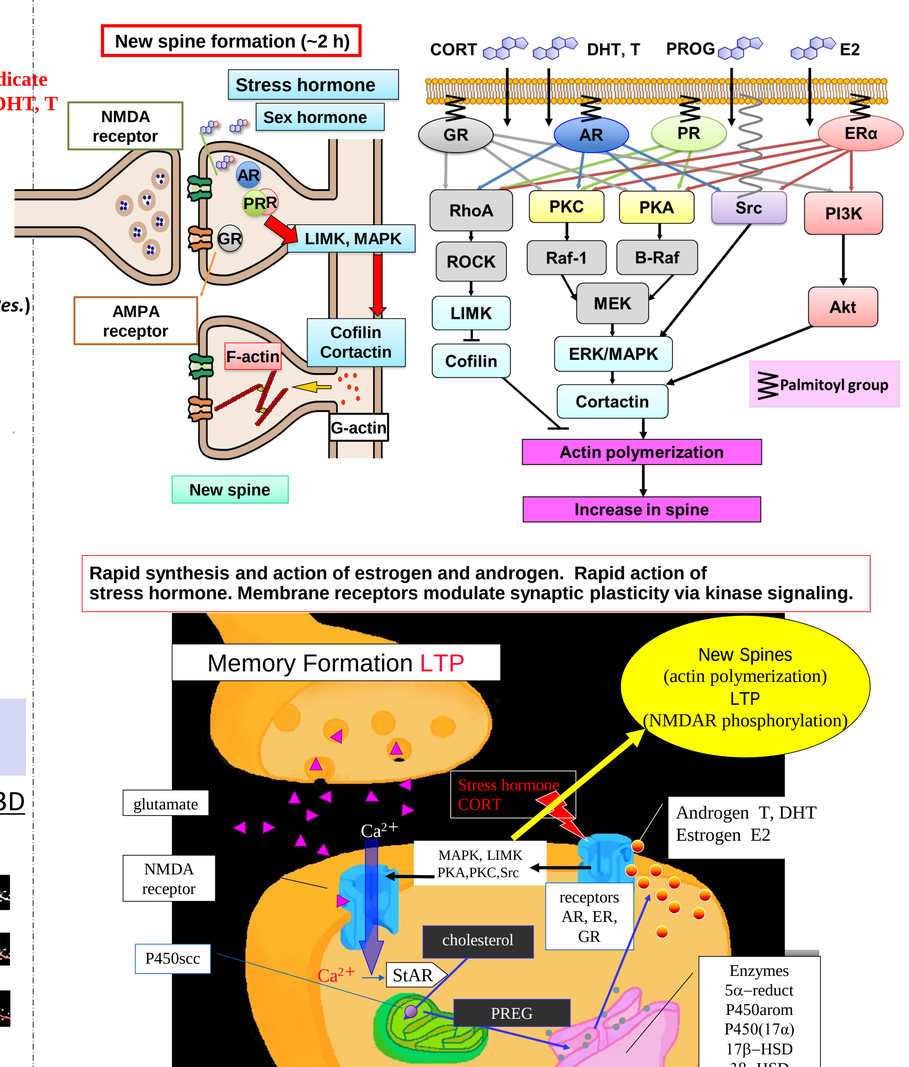
3 Subclasses of head diameter

*P and ** P indicate significance vs CORT



Summary and Discussion : signaling of spine increase

- DHT, T, E2（and PROG) drive MAPK (→ cortactin) and LIMK (→cofilin) which are most important kinases.
- PKA, PKC and Src kinase are also driven by DHT, T and E2.
- E2 drives PI3K, but DHT and T do not drive PI3K.
- Not nonspecific effects, because some kinases are not involved (JunK).
- membrane AR, ER, GR, PR are classic receptors but they are membrane anchored via palmitoylation. ・membrane GPR30 probably not contributes.
- Both non-genomic and genomic effects are necessary for complete neural functions.
- *In vivo* intrahippocampal infusion of E2 rapidly (0.5 h －2 h) improves cognitive function of OVX rats and mice.
- DHT-injection to castrated rats shows anti-anxiety behavior, but only a few report are present about cognitive improvement.



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

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