Neurobiology of Ovarian Hormones: "It Ain't Just Sex Anymore!"

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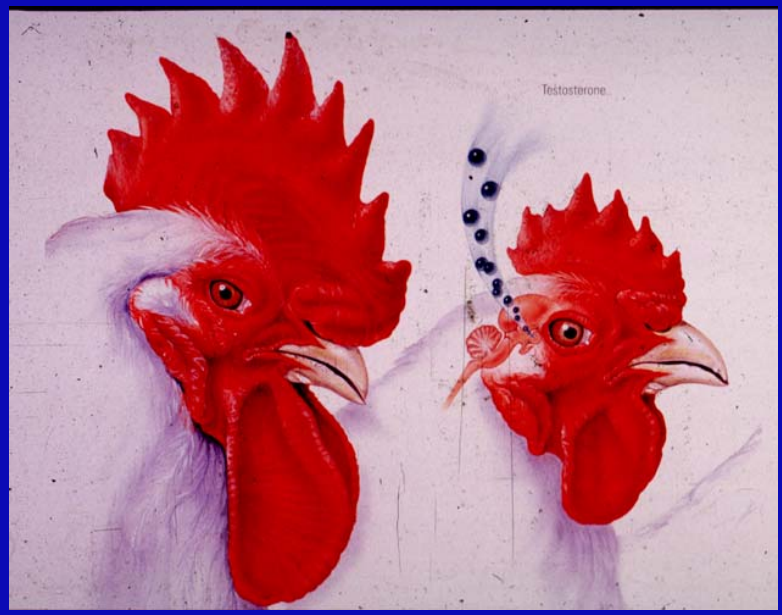
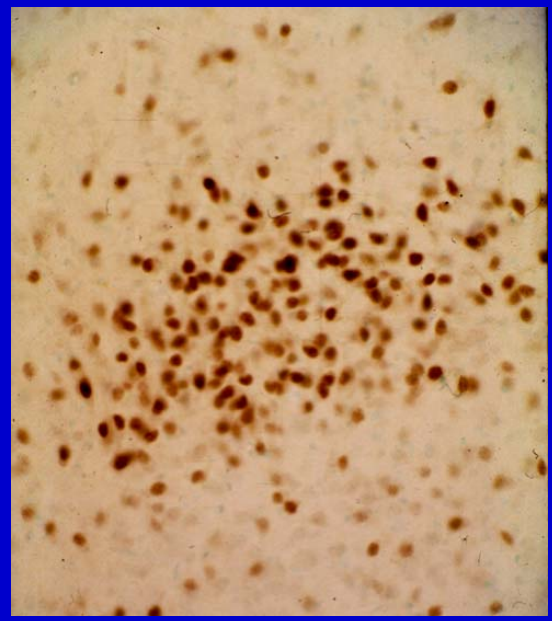


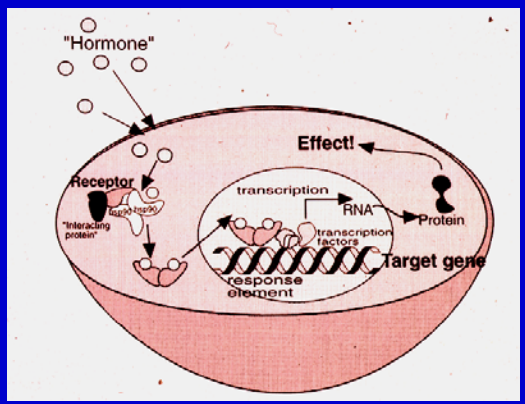
Berthold, 1849

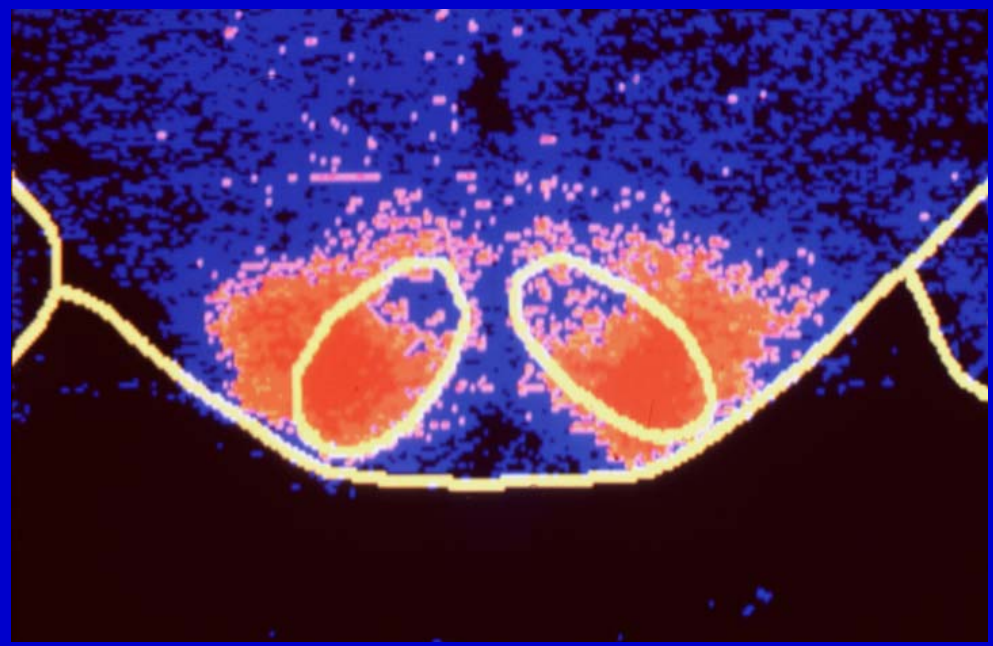
1. Hormone implants in brain - reprod function 1960's
2. Gender differences Raisman and Fields 1972
3. Testosterone metabolism -aromatization, DHT
4. Sex hormone actions - nuclear and non-nuclear
5. Implications for medicine - women's health programs

ER alpha in ventromedial nuclei of rat hypothalamus (VMN)



The VMN is the site of E regulation of female sexual behavior



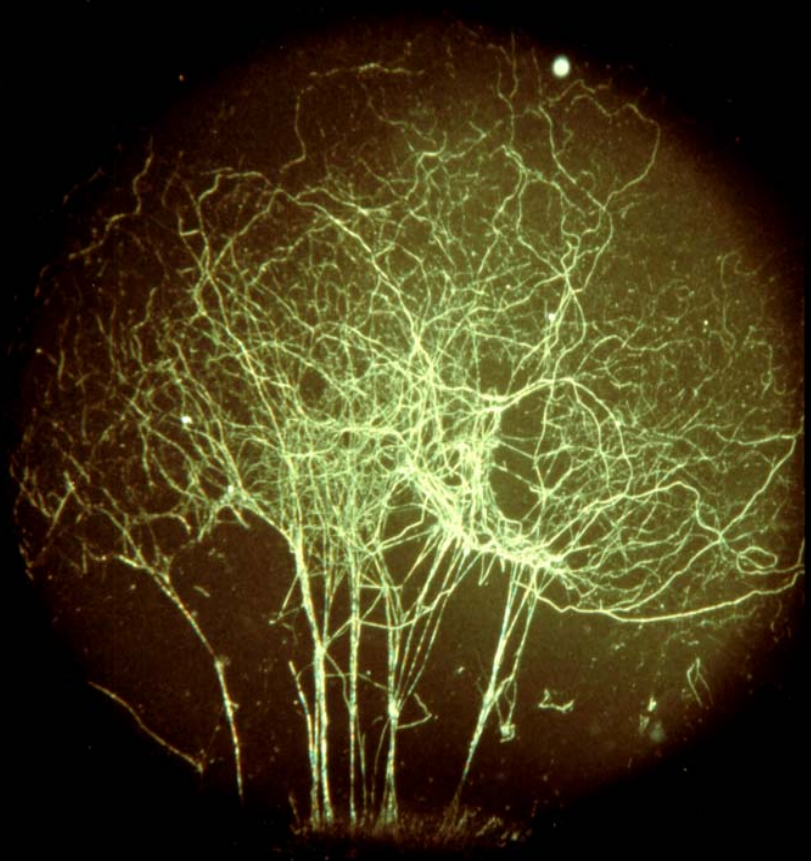


Estrogen induction of oxytocin receptors

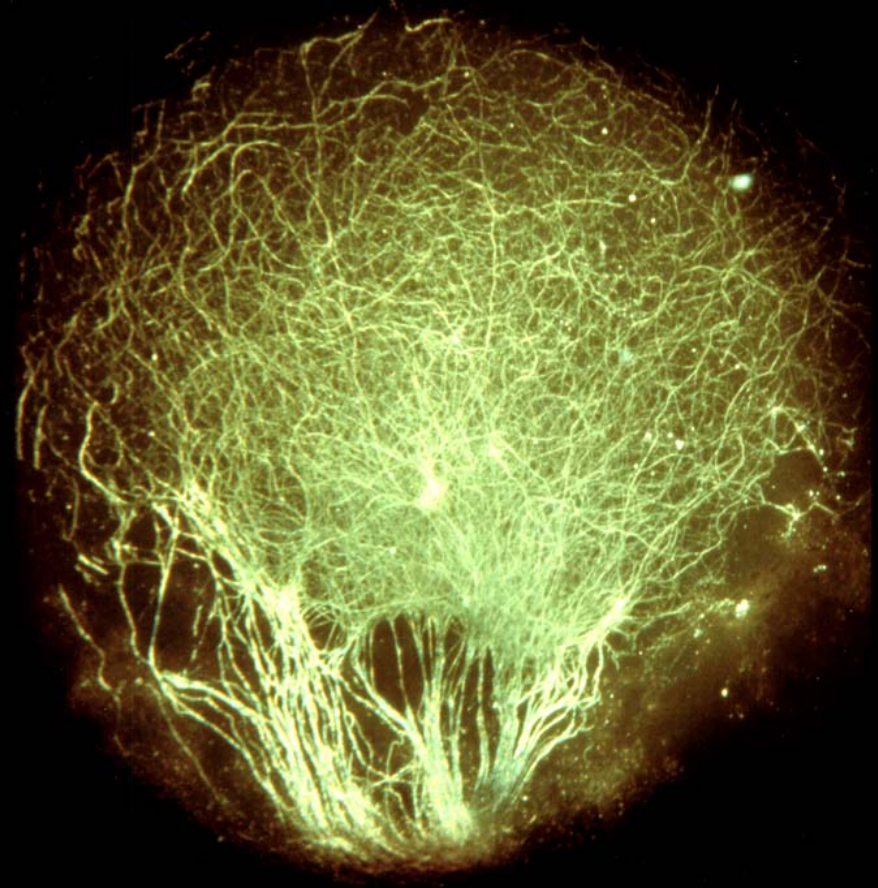
Developmental Effects of Estrogen in Brain

- Neurogenesis
- Neuronal survival
- Neuronal growth and differentiation

Dr. Dominique Toran-Allerand, Columbia University



Endogenous Estrogen



Exogenous Estrogen

dt-a

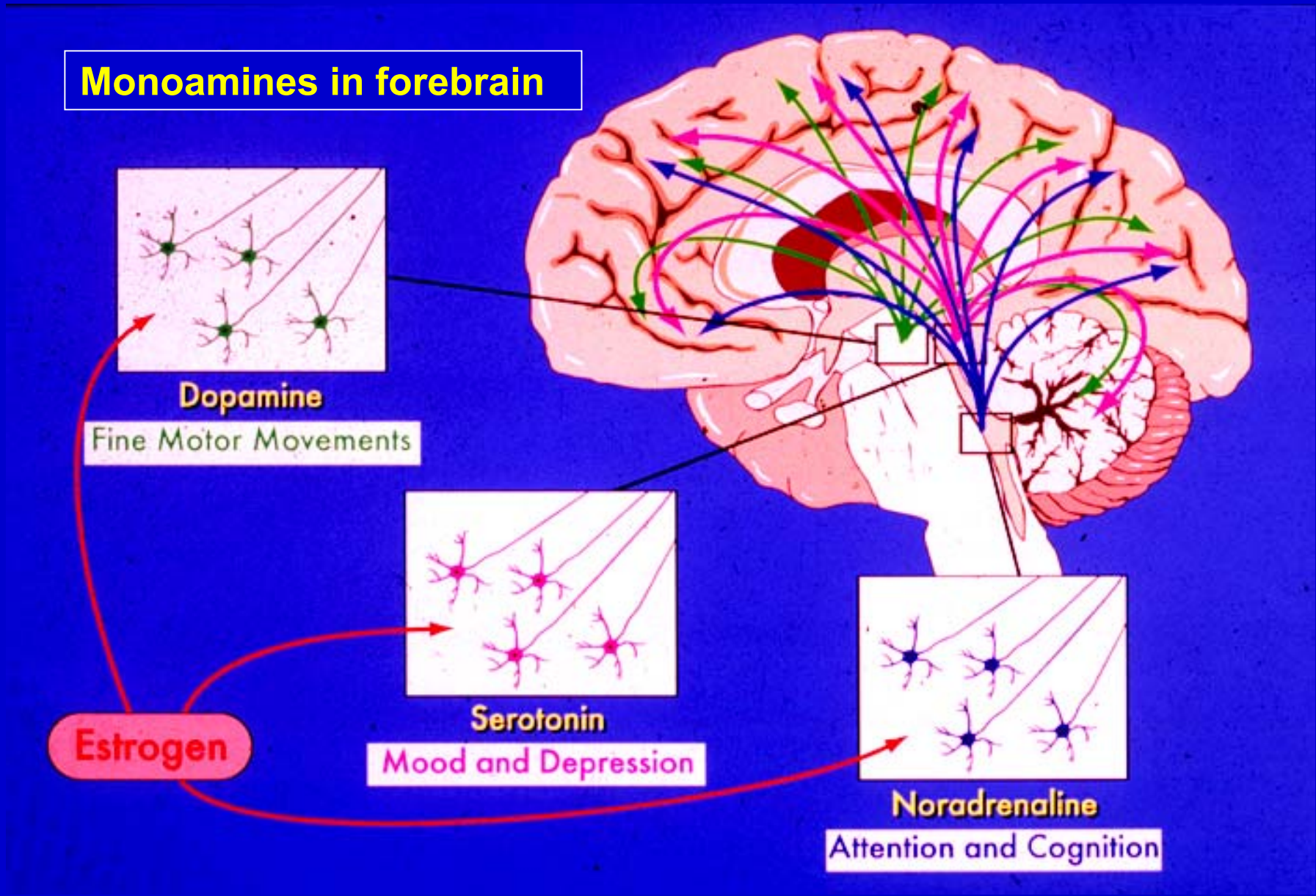
Ovarian Hormones - Non-Reproductive Actions

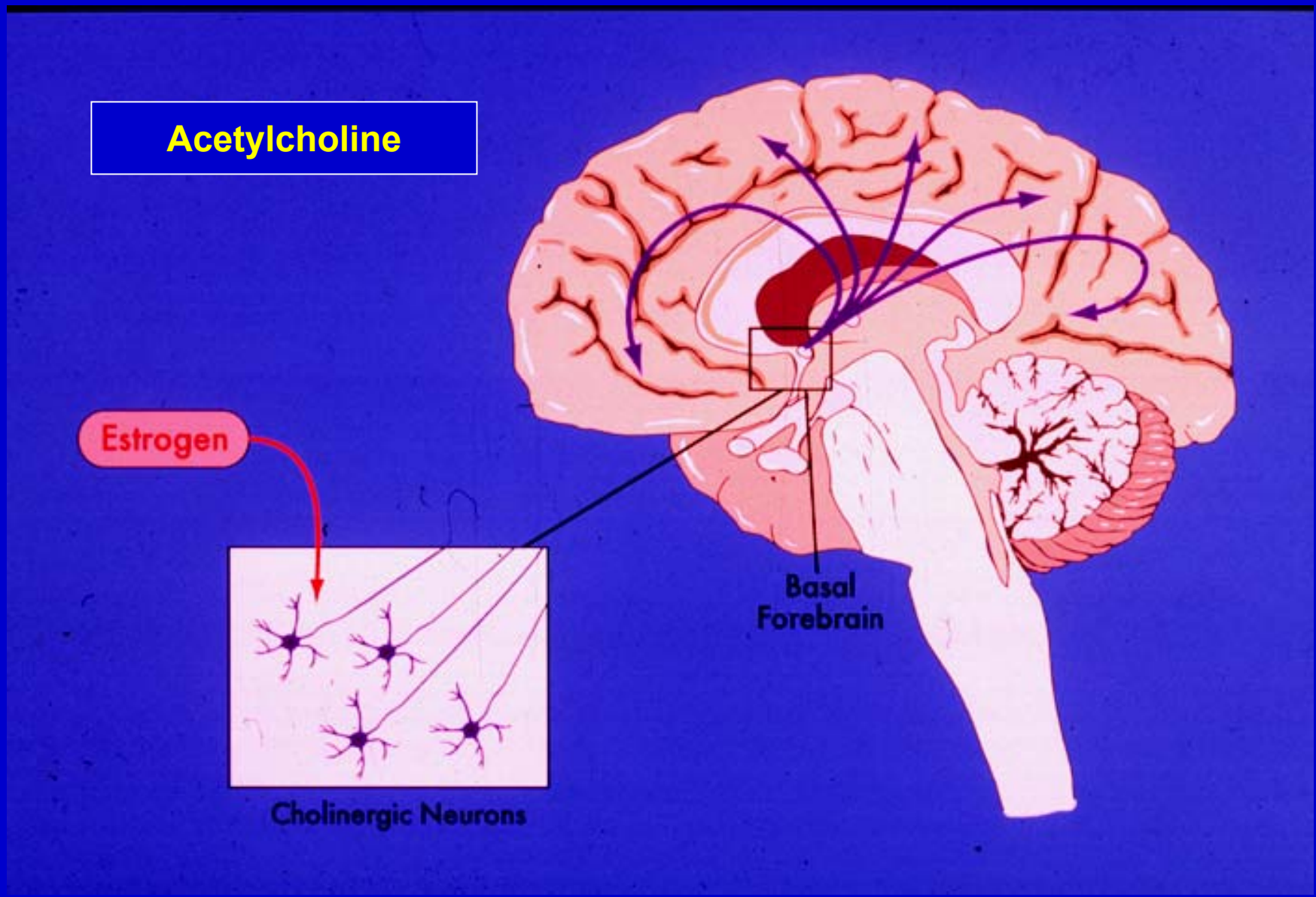
- Motor coordination.
- Epilepsy
- Premenstrual syndrome
- Depression
- Stroke
- Pain
- Cognitive function
- Dementia

Extra-hypothalamic brain systems affected by estrogens

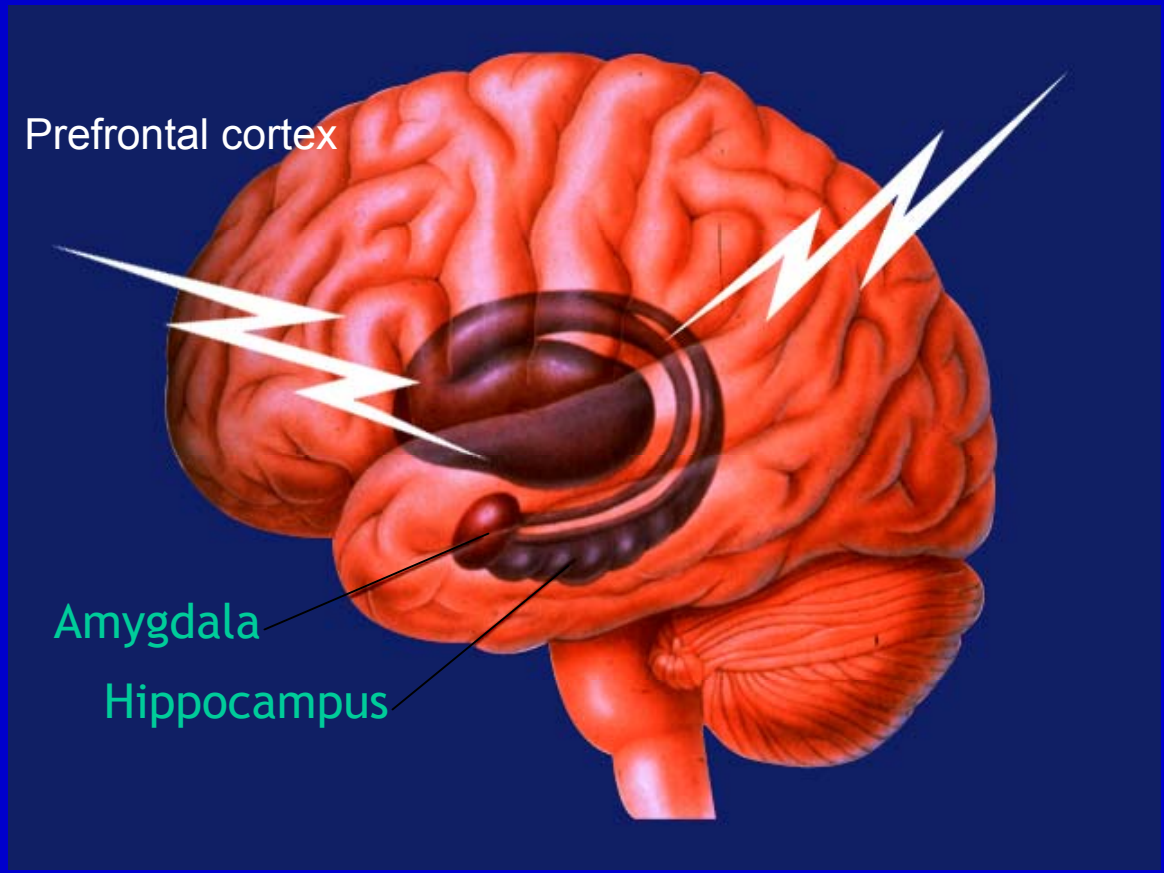
Basal forebrain cholinergic
Mesolimbic dopamine
Nigrostriatal dopamine
Brain stem noradrenergic
Midbrain serotonin
Cerebellum
Hippocampus
Cerebral cortex
Spinal cord

Monoamines in forebrain




Acetylcholine
Estrogen
Basal
Forebrain
Cholinergic Neurons

Memory and Synapse Formation



Spatial, episodic, declarative, contextual and working memory

"Add-Back" Estrogen Reverses Cognitive Deficits Induced by a Gonadotropin-Releasing Hormone Agonist in Women with Leiomyomata Uteri*

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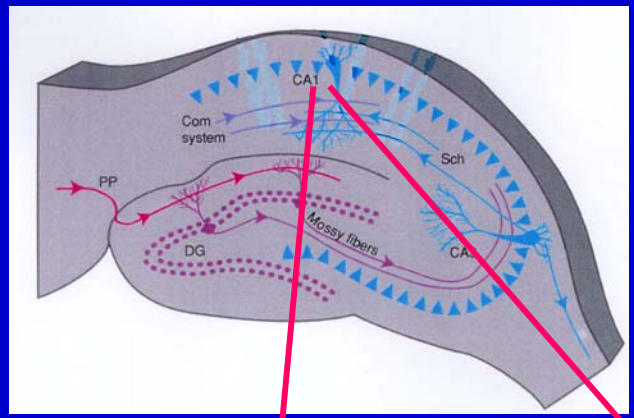
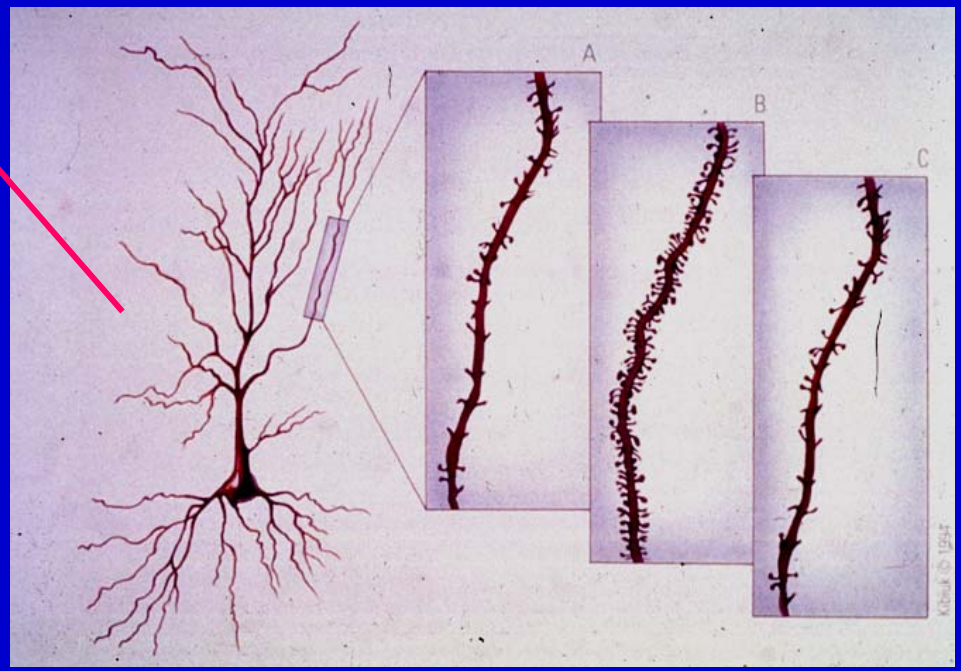
ABSTRACT

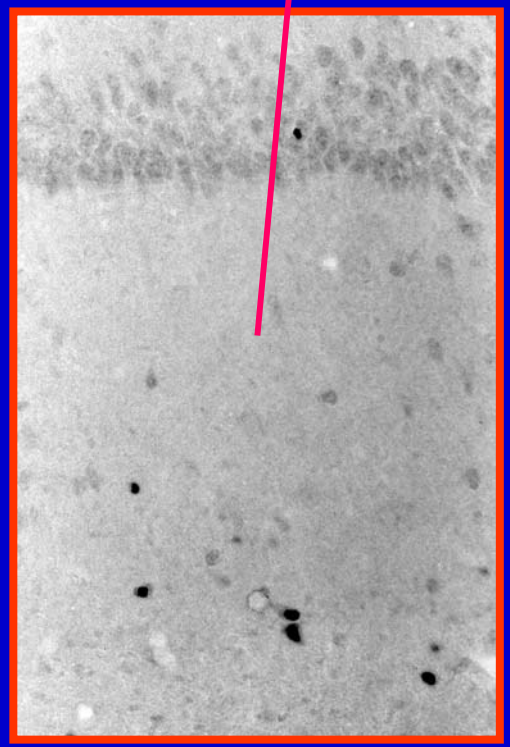
Treatment of women with uterine myomas with GnRH agonists results in symptoms of hypoestrogenism which can be prevented by concurrent "add-back" estrogen administration. We took advantage of these induced endocrine changes to investigate their effects on cognitive functioning in young women with myomas. Nineteen women with uterine myomas were tested before treatment. They all received the GnRH agonist, leuprolide acetate depot (LAD), every 4 weeks for 12 weeks and were then randomized to receive LAD plus estrogen or LAD plus placebo every 4 weeks for 8 additional weeks. Levels of all sex hormones decreased after 12 weeks of LAD treatment ($P < 0.01$), and only estradiol (E_2) levels increased ($P < 0.01$) following 8 weeks of subsequent treatment in the group that received LAD plus E_2. Scores on neuropsychological tests of verbal memory decreased from pretreatment to 12 weeks posttreatment with LAD ($P < 0.05$). These memory deficits were reversed in the group that received LAD plus E_2 for 8 weeks coincident with an increase in plasma E_2, whereas memory scores remained depressed in the group that received LAD plus placebo. These findings are consistent with those from studies on surgically menopausal women and strongly suggest that estrogen serves to maintain verbal memory in women. These results provide support for the efficacy of add-back estrogen regimens in women treated with GnRH agonists and also imply that estrogen may be important for maintaining memory in the postmenopause. (*J Clin Endocrinol Metab* **81:** 2545–2549, 1996)

Verbal memory improved by estrogen replacement



Dendritic spine density in stratum radiatum of CA1 fluctuates over the estrus cycle





ER α in interneuron
Cell nuclei

E-induction of spine synapses takes several days.

Progesterone causes rapid-down regulation within 12h.

NMDA receptor blockade prevents synapse formation.

E treatment enhances hippocampal- dependent memory in rodents and humans.

Estrogen Replacement Increases Spinophilin-immunoreactive Spine Number in the Prefrontal Cortex of Female Rhesus Monkeys

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While studies have shown that estrogen affects hippocampal spine density and function, behavioral studies in humans and nonhuman primates have also implicated the prefrontal cortex in the effects of estrogen on cognition. However, the potential for similar estrogen-induced increases in spines and synapses in the prefrontal cortex has not been investigated in primates. Moreover, it is not known if such an estrogen effect would be manifested throughout the neocortex or primarily in the regions involved in cognition. Therefore, we investigated the effects of estrogen on dendritic spines in the prefrontal and primary visual cortices of young rhesus monkeys. Young female monkeys were ovariectomized and administered either estradiol cypionate or vehicle by intramuscular injection. Using an antibody against the spine-associated protein, spinophilin, spine numbers were estimated in layer I of area 46 and in layer I of the opercular portion of area V1 (V1o). Spine numbers in layer I of area 46 were significantly increased (55%) in the ovariectomy + estrogen group compared to the ovariectomy + vehicle group, yet spine numbers in layer I of area V1o were equivalent across the two groups. The present results suggest that estrogen's effects on synaptic organization influence select neocortical layers and regions in a primate model, and provide a morphological basis for enhanced prefrontal cortical functions following estrogen replacement.

Keywords: cognition, dendritic spines, hormone replacement, macaque monkey, plasticity, steroids, visual cortex

Cerebral Cortex February 2004;14:215–223;

Cell Membrane and Nuclear Estrogen Receptors (ERs) Originate from a Single Transcript: Studies of ERα and ERβ Expressed in Chinese Hamster Ovary Cells

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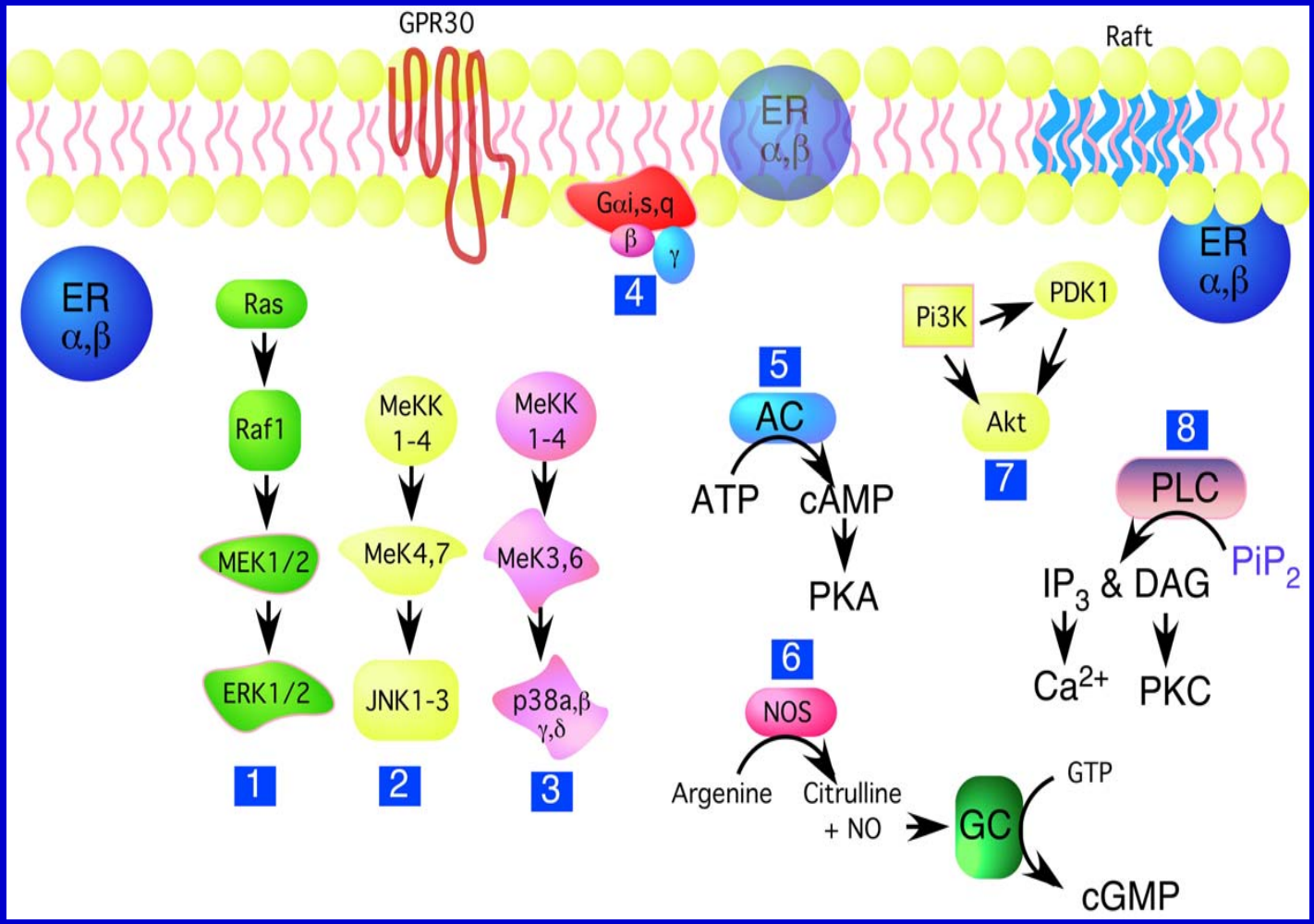
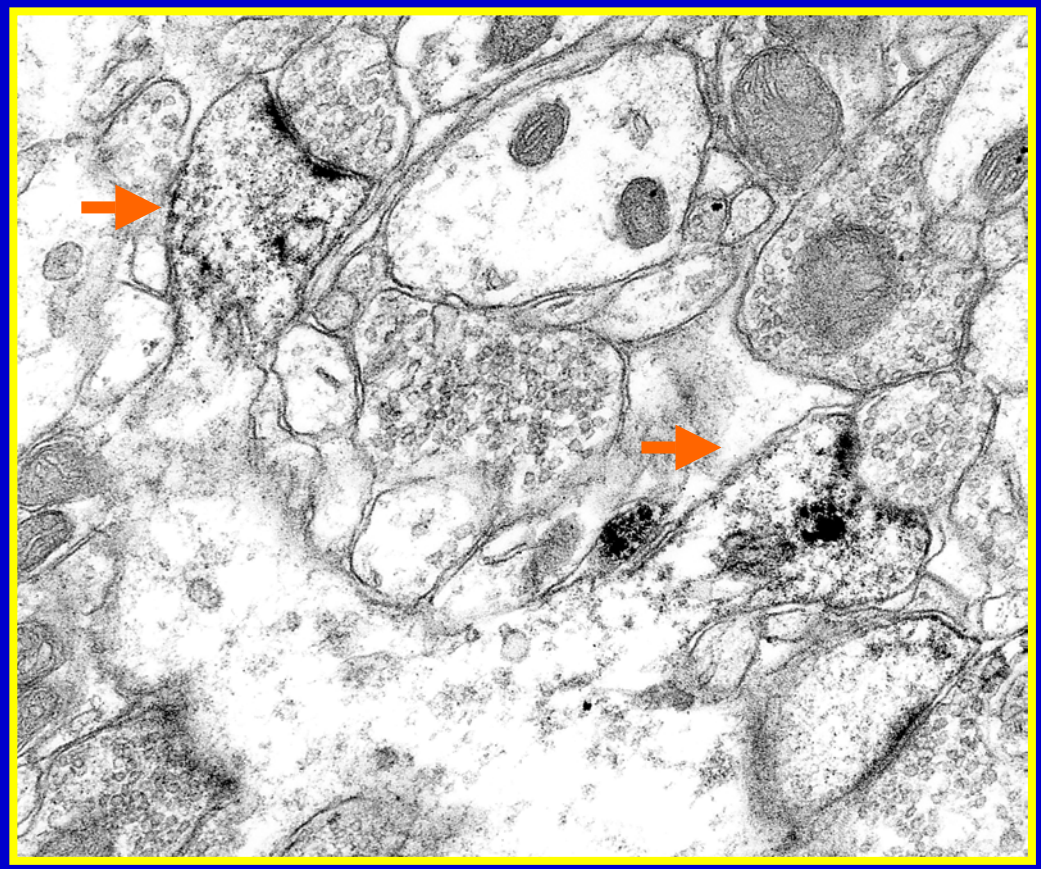
Molecular Endocrinology 13: 307-319, 1999

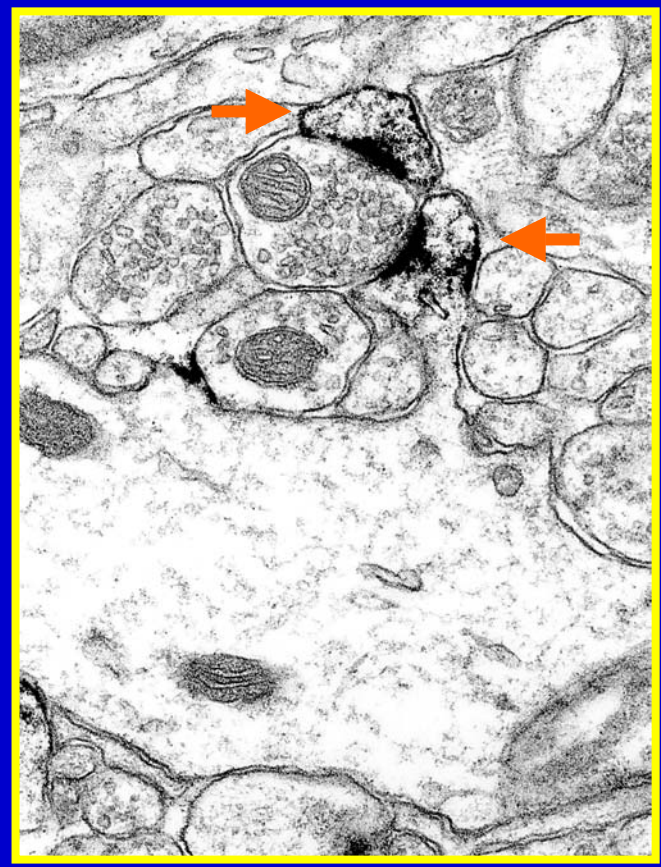
Possible Components of ER Rapid Signaling



Cartoon depicting basic components of estrogen receptor rapid signaling. Estrogen receptorα (ERα) and ERß are expressed in TG. ER that participate in rapid signaling may be present in the cytosol, membrane and/or associated with lipid rafts within the membrane. GPR30 is a seven transmembrane spanning receptor that has been shown to confer estrogen sensitivity to cells lacking the classical ER(□ or ß). Also presented are simplified diagrams of cellular signaling pathways that have been shown to be activated rapidly (< 15 min) by estrogen. For clarity, arrows interconnecting the signaling pathways with ER and with each other have been omitted (and in many cases are not known), as have the numerous accessory molecules and molecular isoforms. Numerals in the figure refer to the key below containing selected

Non-nuclear ER alpha in dendritic spines





By EM, ERα-I is detected in dendritic spines in the CA1 region of the hippocampus (Teri Milner, Weill College of Medicine, Cornell).

Estradiol increases input from different neurons on CA1 cells
Catherine Woolley PNAS 2001

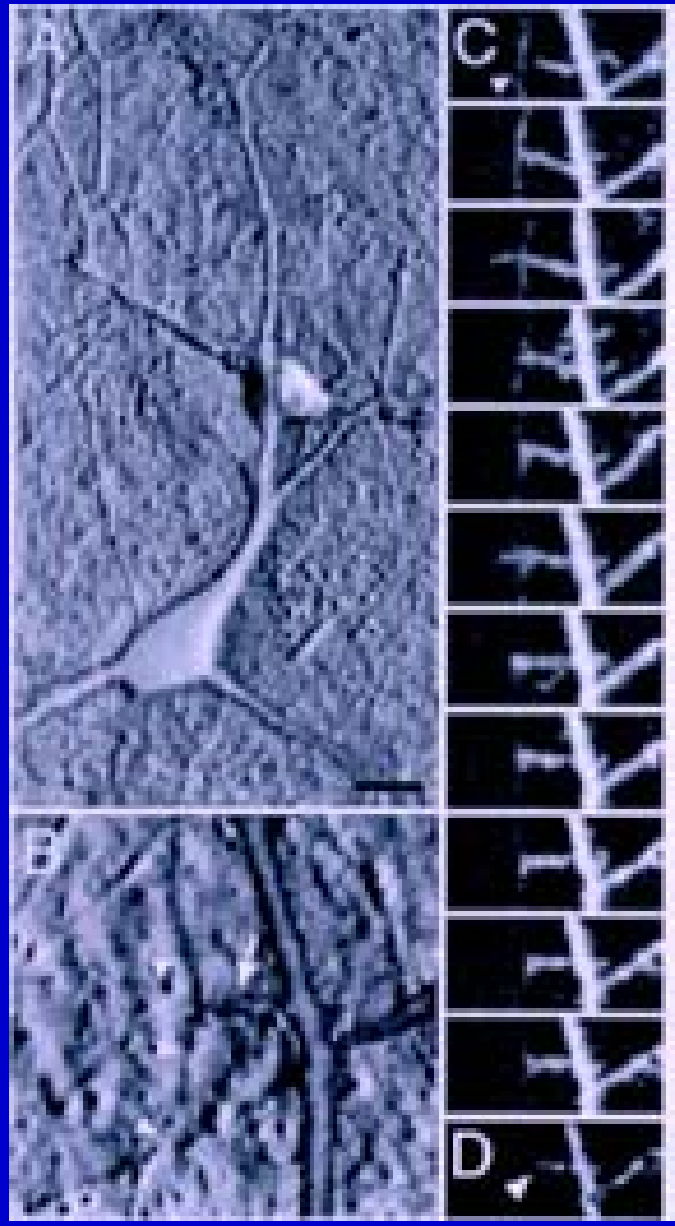


Figure 6. Dendritic Filopodia Can Initiate Synaptogenic Contacts with Nearby Axons

(A) A composite image of a FAST DiO-labeled neuron overlaid onto a DIC image of the same field.

(B) A high magnification of the region enclosed in a rectangle in (A). Note the axon (arrowheads) and the labeled filopodium (arrow) that spanned the gap between the dendritic shaft and the axon. Corresponds to the last timepoint in the time-lapse sequence shown in (C).

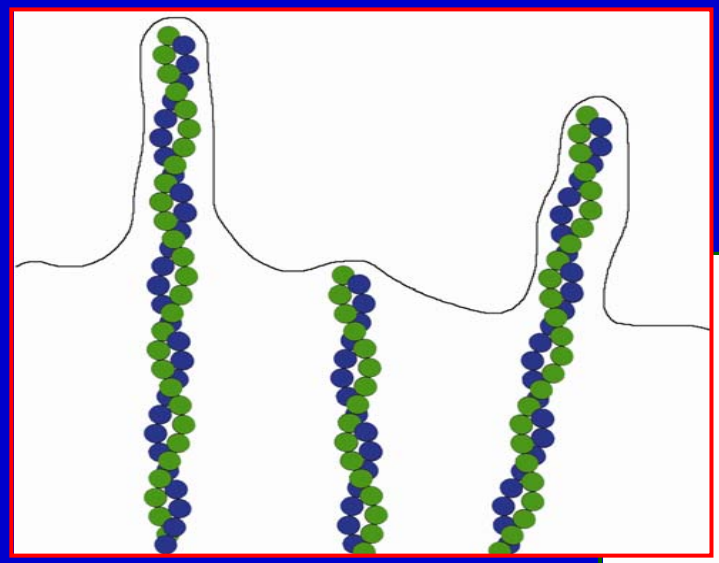
(C) Time-lapse sequence composed of 11 frames at approximately 9 min intervals of a subregion of (B). A filopodium extended from the dendritic shaft and established a contact with the axon (arrowhead in the upper panel). Once the contact was established, the filopodium stayed attached to the axon for the duration of the time-lapse sequence.

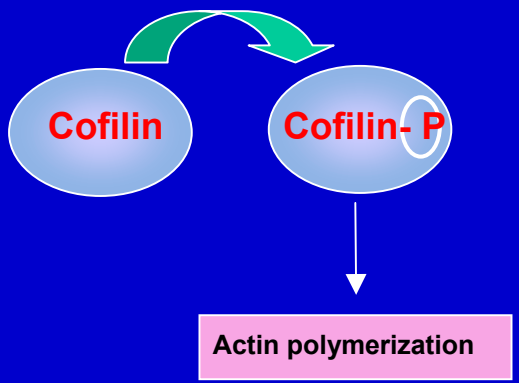
(D) A composite image of the FAST DiO-labeled dendrite (green) and FM 4-64-labeled presynaptic boutons (red). Note the bouton that had formed at the contact site between the axon and the dendrite (arrowhead). Scale bar, 10 μm (A).

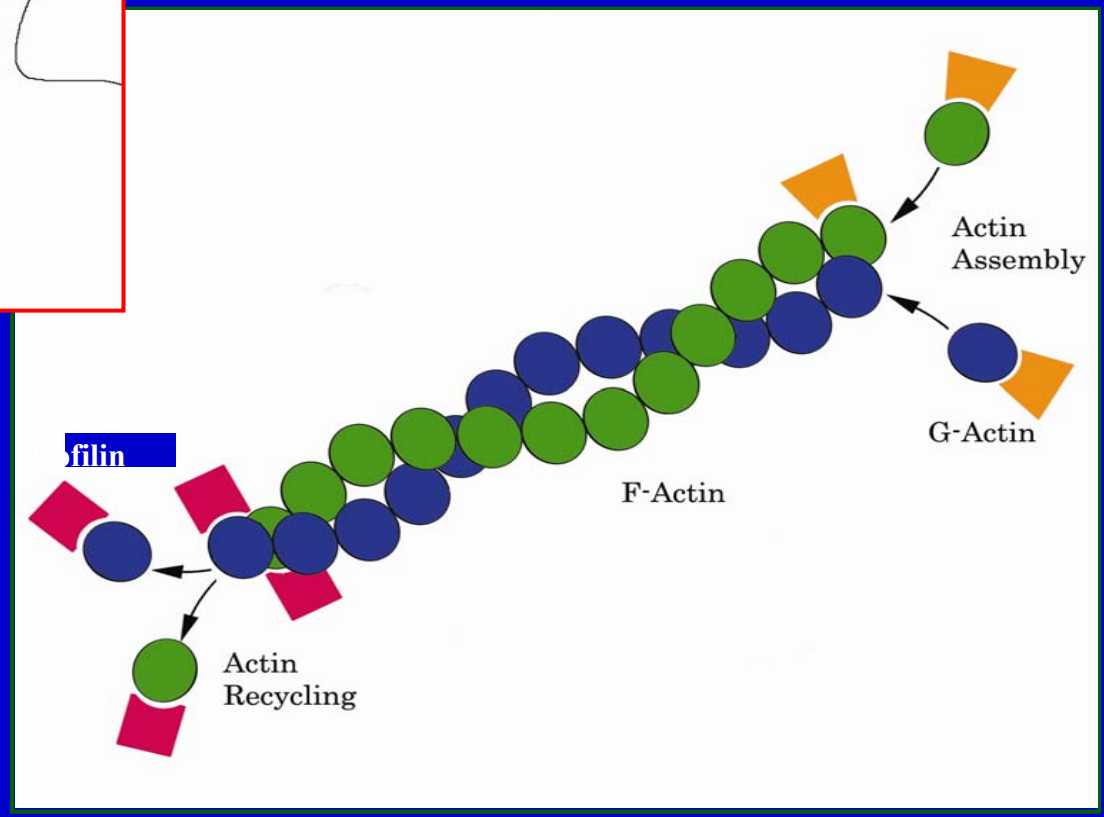
Dendritic filopodia initiate synaptic contacts with nearby axons.

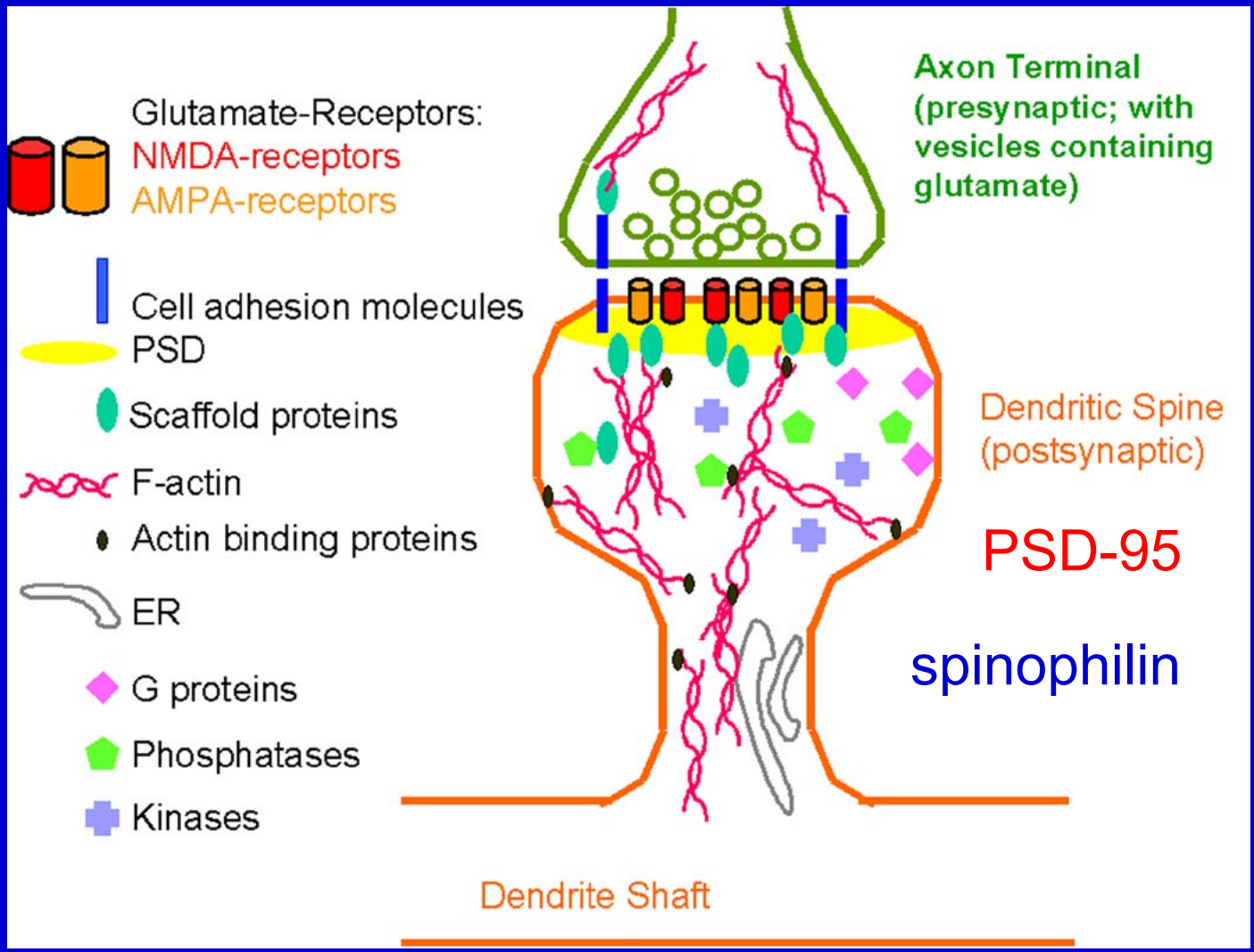
Dr. Steve Smith, Stanford

Cofilin, an actin depolymerizing factor



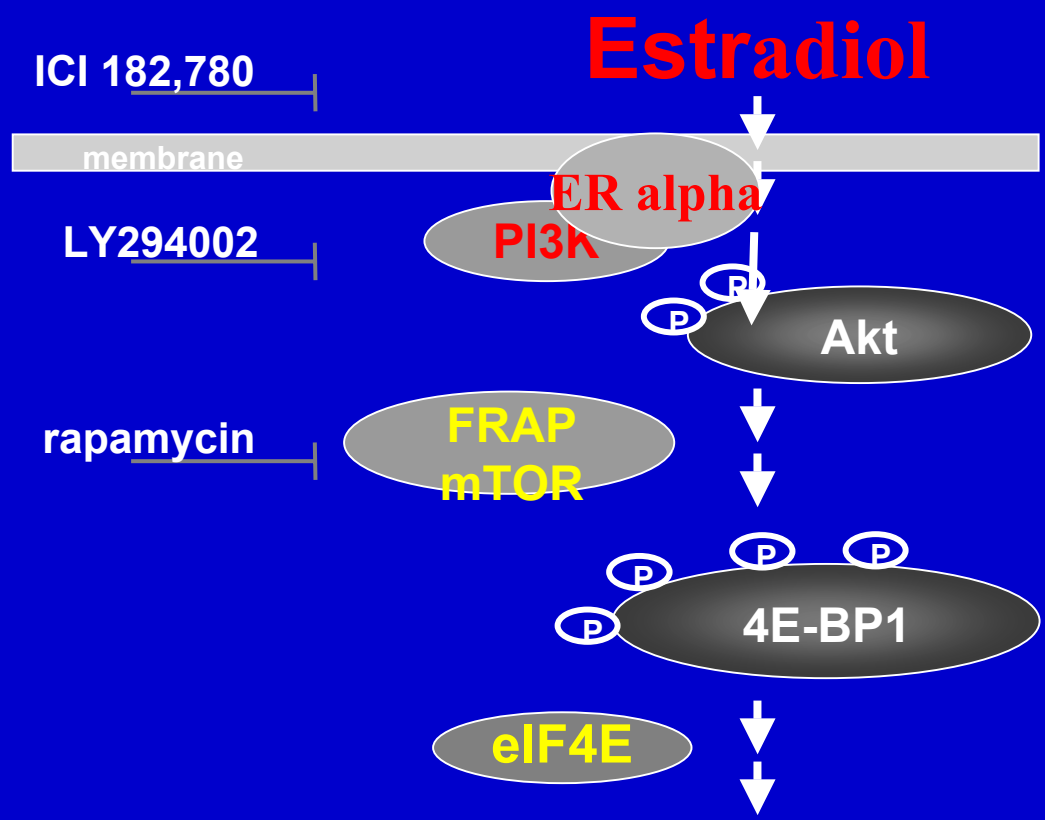






Dr. Shelley Halpain Scripps Institute

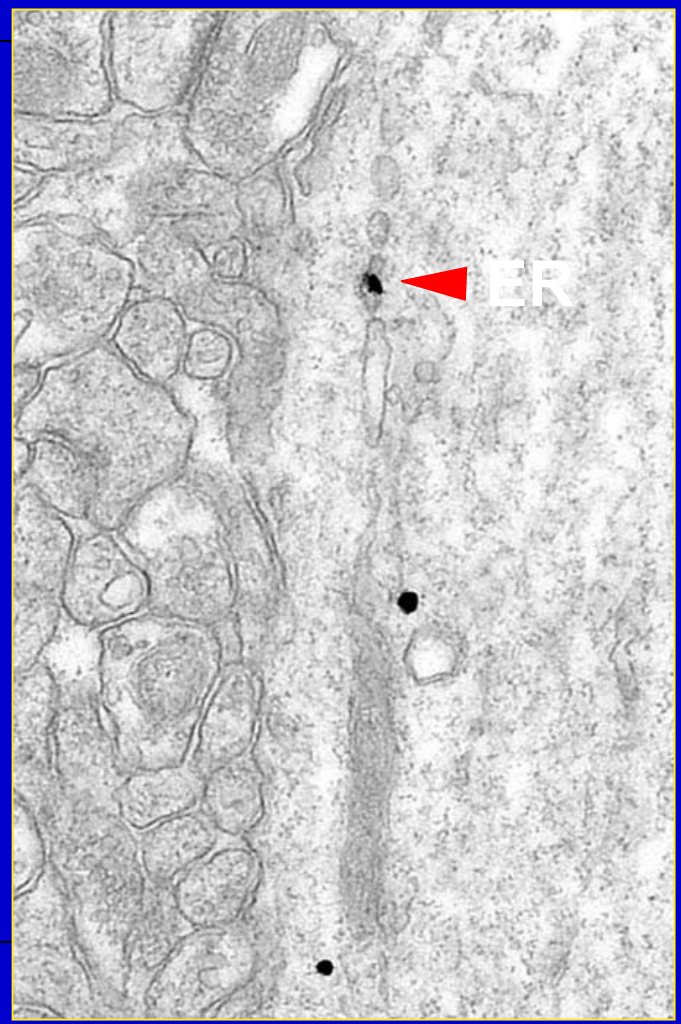
NG-108-15 cells



PSD-95 Protein Translation

Akama and McEwen J. Neurosci 2003

The number of pAKT immunogold particles on endoplasmic reticula and polyribosomes in dendritic shafts is higher in proestrus rats



Milner T, Znamensky V, Akama K, McEwen B

Summary: Distributed sites of estrogen actions

- 1-- Cell nuclear ER in GABA neurons; E stimulates removal of E inhibition
- 2-- Cell nuclei of CA1 - E activation of pCREB; regulation of spinophilin, other genes?
- 3-- E regulation of LIMK and cofilin - actin polymerization.
- 4--E induced translation of mRNA in dendrites via PI3 kinase and Akt.

Rapid estrogen actions

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Copyright © 2003 by The Endocrine Society
doi: 10.1210/en.2003-0081

Rapid Enhancement of Visual and Place Memory by Estrogens in Rats

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Department of Psychology, Hunter College of City University of New York (V.N.L.), Graduate Program in Psychology (L.F.J.), Graduate and University Center of City University of New York, and Center for Reproductive Sciences, Department of Obstetrics and Gynecology, Columbia University College of Physicians and Surgeons (N.J.M.), New York, New York 10021

Estrogenic effects on visual (object recognition) and place (object placement) memory were investigated. Ovariectomized (OVX) rats received acute sc injections 30 min before a sample trial (viewing objects), and 4 h later a recognition/retention trial was performed. During recognition/retention trials, discrimination between sample (old) and new objects (visual memory) or between objects in sample (old) and new locations (place memory) was tested. Subjects given 17α- or 17β-estradiol or diethylstilbestrol (DES) 30 min before sample trials discriminated between objects or locations during recognition/retention trials, whereas vehicle-treated, OVX rats did not. Estrogens were given a posttrial to investigate whether enhancements were due to effects on memory processes or psychological/performance parameters. Hormones were given immediately after or 2 h after sample trials (delayed injections), and recognition/retention were tested 4 h after the sample trial. Both object and place discriminations were enhanced when estrogens were given immediately after sample trials, but not when injections were delayed. These results provide evidence that estrogen rapidly enhances visual and place memory. Moreover, posttraining injections suggest effects on mnemonic processes, consolidation, or encoding, not on performance parameters. Place memory enhancements required higher estrogen doses, both pre- and posttrial. The rapid time course, stereospecificity of responses (α- and β-estradiol are effective), and efficacy of various estrogens suggest interactions at other than classic estrogen α- or β-receptors in mediating the effects. Thus, these results provide the first demonstration of rapid memory enhancements by estrogen and implicate nongenomic mechanisms, possibly an extranuclear receptor(s), in mediating the response. (*Endocrinology* 144: 2836–2844, 2003)

Some major issues

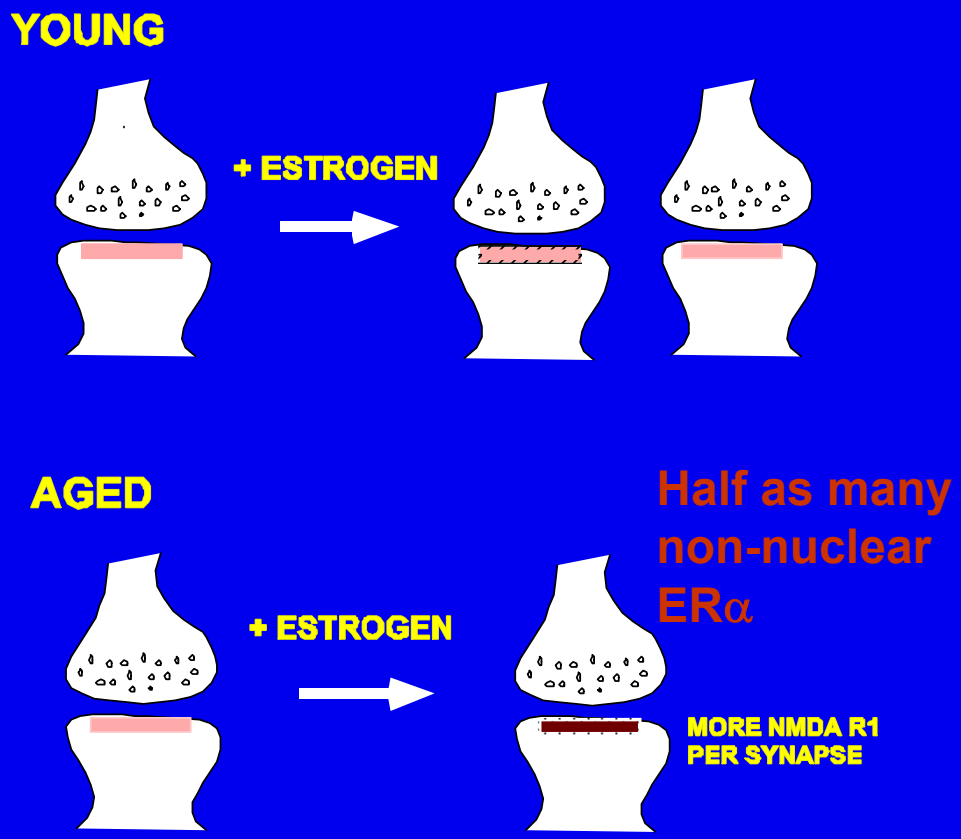
1. Aging

2. Hormone therapy: E and P

3.What about males?

4. Sex and stress

1. The aging hippocampus



Michelle Adams, John Morrison et.al.
Mt. Sinai Medical Center, New York

Adams M, Shah RA, Janssen WF, Morrison J
PNAS 98:8071-6, 2001

ELSEVIER

Neurobiology of Aging 21 (2000) 107–116

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Long-term treatment with estrogen and progesterone enhances acquisition of a spatial memory task by ovariectomized aged rats☆

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Received 14 October 1999; received in revised form 24 January 2000; accepted 24 January 2000

Abstract

Female Sprague–Dawley rats were ovariectomized at 13 months of age. Four groups received different regimens of estrogen or estrogen plus progesterone replacement beginning either immediately, 3 months, or 10 months after ovariectomy and were compared with non-hormone-treated controls. Eight to twelve months after ovariectomy, animals were trained on a delayed matching-to-position (DMP) spatial memory task. Long-term treatment with estrogen or estrogen plus progesterone significantly enhanced acquisition of the DMP task by aged animals after long-term loss of ovarian function. Weekly administration of estrogen and progesterone was at least as effective as, if not more effective than, continuous treatment with estrogen alone. In addition, treatment initiated 3 months, but not 10 months, after ovariectomy was as effective at enhancing DMP acquisition as continuous estrogen treatment initiated immediately after ovariectomy, suggesting a window of opportunity after the loss of ovarian function during which hormone replacement can effectively prevent the effects of aging and hormone deprivation on cognitive function. These findings suggest that repeated treatment with estrogen and progesterone initiated within a specific period of time after the loss of ovarian function may be effective at preventing specific negative effects of hormone deprivation on brain aging and cognitive decline.

Keywords: Hormone replacement; Aging; Alzheimer's disease; Learning

2. Hormone Therapy - Estrogen and "Progestin"

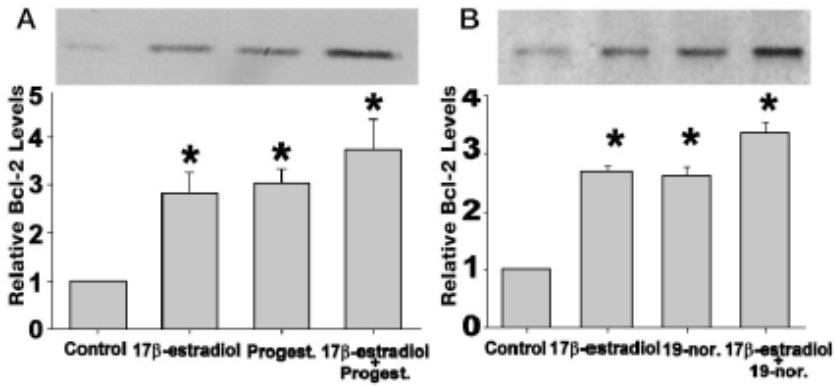

FIG. 5. Effect of 17β-E2 and progestin on Bcl-2 expression in hippocampal neurons. Primary hippocampal neuron cultures were treated with 17β-E2 (10 ng/ml) with or without the indicated progestins (10 ng/ml) or with vehicle as a negative control for 24 h. Whole-cell lysates were subjected to SDS-PAGE and probed with anti-Bcl-2 antibody. A, Progesterone; B, 19-norprogesterone; C, MPA. *Bars* represent mean ± SEM. +, $P < 0.05$, compared with control cultures; *, $P < 0.05$, compared with 17β-E2-treated cultures; n = 3 independent experiments.

Combined Therapy - MPA vs Progesterone

Ultralow-Dose Micronized 17β-Estradiol and Bone Density and Bone Metabolism in Older Women

A Randomized Controlled Trial

Karen M. Prestwood, MD
Anne M. Kenny, MD
Alison Kleppinger, MS
Martin Kulldorff, PhD

Context Estrogen therapy is known to prevent osteoporosis, but studies have shown that conventional doses increase adverse events. Whether lower doses, one quarter of standard treatment, prevent bone loss is not known.

Objective To examine the effect of 3 years of treatment with 0.25 mg/d of micronized 17β-estradiol on bone mineral density (BMD) and bone turnover in healthy older postmenopausal women.

Design, Setting, and Participants Randomized, double-blind, placebo-controlled trial conducted from July 24, 1998, through June 14, 2002, at a university general clinical research center in the United States. Healthy, community-dwelling women (N = 167) who were older than 65 years at enrollment.

Intervention Dosage of 0.25 mg/d of micronized 17β-estradiol (n = 83) or placebo (n = 84); all women who had not had a hysterectomy received 100 mg/d of oral micronized progesterone for 2-week periods every 6 months.

Main Outcome Measures The BMD of the hip, spine, wrist, and total body measured annually for 3 years. Serum and urine biochemical markers of bone resorption and formation and sex hormones were measured at baseline, 3 months, and during years 1 and 3 of treatment.

Results Mean BMD increased at all sites for participants taking low-dose estrogen (17β-estradiol) compared with placebo ($P<.001$). Compared with participants receiving placebo, participants taking low-dose estrogen had BMD increases of 2.6% for the femoral neck; 3.6%, total hip; 2.8%, spine; and 1.2%, total body. Markers of bone turnover, N-telopeptides of type 1 collagen, and bone alkaline phosphatase decreased significantly ($P<.001$) in participants taking low-dose estrogen compared with placebo. Estradiol, estrone, and sex hormone–binding globulin levels increased in the estrogen-treated group compared with placebo. The adverse effect profile was similar; specifically, there were no statistically significant differences in breast tenderness, changes in endometrial thickness or pathological effects, or annual mammographic results between the 2 groups. The number of abnormal mammograms over 3 years was 15 for the low-dose estrogen group and 10 for the placebo group (8 occurred at baseline) ($P = .26$). There were no reports of breast cancer during the study.

Conclusions In older women, a dosage of 0.25 mg/d of 17β-estradiol increased bone density of the hip, spine, and total body, and reduced bone turnover, with minimal adverse effects. Future studies evaluating the effect of low-dose estrogen on fractures are indicated.

JAMA. 2003;290:1042-1048 www.jama.com

OSTEOPOROSIS IS A MAJOR cause of disability and excess mortality in older women. Estrogen therapy has been used for treatment and prevention of osteoporosis; however, many older women are reluctant to use it because of adverse effects. Recent data from the Women's Health Initiative demonstrated that postmenopausal women who took hormone therapy for approximately 7 years had decreased hip fracture risk; however, the dose and preparation of hormone therapy used in the study also increased the risk of breast cancer, heart disease, stroke, and deep venous thrombosis.[1] Previous studies demonstrated that a conventional dose of estrogen therapy (1.0 mg/d of 17β-estradiol or 0.625 mg/d of conjugated equine estrogen [CEE]) reduced bone turnover and bone loss and was associated with reduced fracture incidence in older women.[2-4] Moreover, half the conventional hormone therapy dose (0.3 mg/d of CEE and 2.5 mg/d of medroxyprogesterone acetate [MPA] or 0.5 mg/d of 17β-estradiol) decreased bone turnover and increased bone mass in older women when taken with adequate doses of calcium and vitamin D.[5,6] We previously demonstrated that 0.25 mg/d of 17β-estradiol decreased markers of bone turnover to the same degree as 0.5 mg/d or 1.0 mg/d of 17β-estradiol with an adverse effect profile that was equivalent to placebo.[7] We designed this study to determine the long-term effects on bone and overall safety of treatment with 0.25 mg/d of 17β-estradiol in older women.

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Selective-Estrogen Response Modulator (SERM) Strategy

1. SERM's enhance cholinergic function but at least one SERM blocks E-induced synapse formation.

2. SERM's increase vasomotor symptoms (hot flashes).

3. Since SERM's suppress cancer and protect bone, one strategy is to use SERM's that do not enter the CNS and then give low dose E to aid the brain.

Estrogen receptor α, not β, is a critical link in estradiol-mediated protection against brain injury

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Edited by William H. Daughaday, University of California, Irvine, CA, and approved December 15, 2000 (received for review October 11, 2000)

Estradiol protects against brain injury, neurodegeneration, and cognitive decline. Our previous work demonstrates that physiological levels of estradiol protect against stroke injury and that this protection may be mediated through receptor-dependent alterations of gene expression. In this report, we tested the hypothesis that estrogen receptors play a pivotal role in mediating neuroprotective actions of estradiol and dissected the potential biological roles of each estrogen receptor (ER) subtype, ERα and ERβ, in the injured brain. To investigate and delineate these mechanisms, we used ERα-knockout (ERαKO) and ERβ-knockout (ERβKO) mice in an animal model of stroke. We performed our studies by using a controlled endocrine paradigm, because endogenous levels of estradiol differ dramatically among ERαKO, ERβKO, and wild-type mice. We ovariectomized ERαKO, ERβKO, and the respective wild-type mice and implanted them with capsules filled with oil (vehicle) or a dose of 17β-estradiol that produces physiological hormone levels in serum. One week later, mice underwent ischemia. Our results demonstrate that deletion of ERα completely abolishes the protective actions of estradiol in all regions of the brain; whereas the ability of estradiol to protect against brain injury is totally preserved in the absence of ERβ. Thus, our results clearly establish that the ERα subtype is a critical mechanistic link in mediating the protective effects of physiological levels of estradiol in brain injury. Our discovery that ERα mediates protection of the brain carries far-reaching implications for the selective targeting of ERs in the treatment and prevention of neural dysfunction associated with normal aging or brain injury.

Behavioral/Systems/Cognitive

Hippocampal Excitability Increases during the Estrous Cycle in the Rat: A Potential Role for Brain-Derived Neurotrophic Factor

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To test the hypothesis that induction of BDNF may contribute to changes in hippocampal excitability occurring during the female reproductive cycle, we examined the distribution of BDNF immunoreactivity and changes in CA1 and CA3 electrophysiology across the estrous cycle in rats. Hippocampal BDNF immunoreactivity increased on the day of proestrus as well as on the following morning (estrus), relative to metestrus or ovariectomized animals. Changes in immunoreactivity were clearest in mossy fiber axons of dentate gyrus granule cells, which contain the highest concentration of BDNF. Increased immunoreactivity was also apparent in the neuropil-containing dendrites of CA1 and CA3 neurons. Electrophysiological recordings in hippocampal slices showed robust cycle-dependent differences. Evoked responses of CA1 neurons to Schaffer collateral stimulation changed over the cycle, with larger maximum responses at both proestrus and estrus relative to metestrus. In area CA3, repetitive hilar stimuli frequently evoked multiple population spikes at proestrus and estrus but only rarely at other cycle stages, and never in slices of ovariectomized rats. Hyperexcitability in area CA3 at proestrus was blocked by exposure to the high-affinity neurotrophin receptor antagonist K252a, or an antagonist of the α7 nicotinic cholinergic receptor, whereas it was induced at metestrus by the addition of BDNF to hippocampal slices.

These studies suggest that hippocampal BDNF levels change across the estrous cycle, accompanied by neurophysiological responses that resemble the effects of BDNF treatment. An estrogen-induced interaction of BDNF and α7 nicotinic receptors on mossy fibers seems responsible for estrous cycle changes in area CA3. Periovulatory changes in hippocampal function may, thus, involve estrogen-induced increases in BDNF expression.

Key words: cholinergic; epilepsy; estrogen; neurotrophic; nicotinic; mossy fibers; progesterone; testosterone

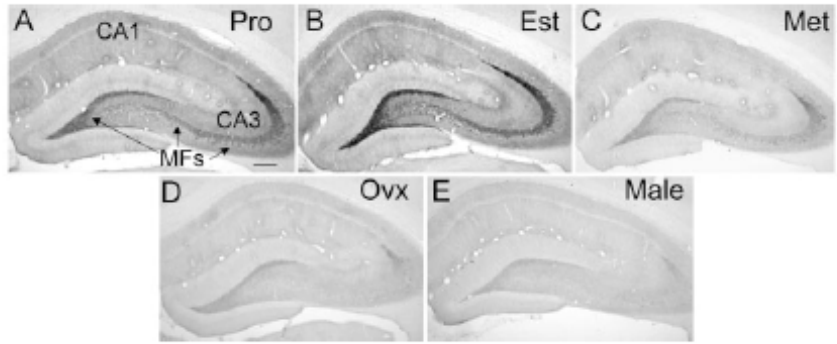


Figure 1. Changes in BDNF immunoreactivity during the estrous cycle relative to ovariectomized and intact male rats. BDNF immunoreactivity is shown for sections of the following animals: *A*, proestrous female (Pro); *B*, estrous female (Est); *C*, metestrous female (Met); *D*, ovariectomized female (Ovx); *E*, intact male (Male). The immunoreactivity of mossy fibers (MFs; *A*, arrows) is strong at proestrus and estrus. Scale bar: (in *A*), 200 μm.

Parkinson's story: a lesson in estrogen dose

1. Early, high dose E - increased Parkinson's symptoms; "anti-dopaminergic"

Eg. Bedard PJ, Lengelier P, Villeneuve A Estrogens and the extrapyramidal system. The Lancet 1977;2: 1367-8

2. Later, low dose E - enhances DA effects; "pro-dopaminerigic"

Eg. Becker JB Estrogen rapidly potentiates amphetamine-induced striatal DA release and Rotational behavior. Neurosci Lett 1990;118:169-171.

3. Some evidence that E is neuroprotective in MPTP models of Parkinson's disease.

Eg. Dluzen DE Neuroprotective effects of estrogen on the nigrostriatal DA system. J. Neurocytology 2000;29:387-99.

Callier S, Morissette M, Grandbois M, Di Paolo T Stereospecific prevention by estradiol 17β of MPTP-induced DA depletion in mice. Synapse 2000;37:245-51.

Oligodendrocytes express ER alpha and ER beta

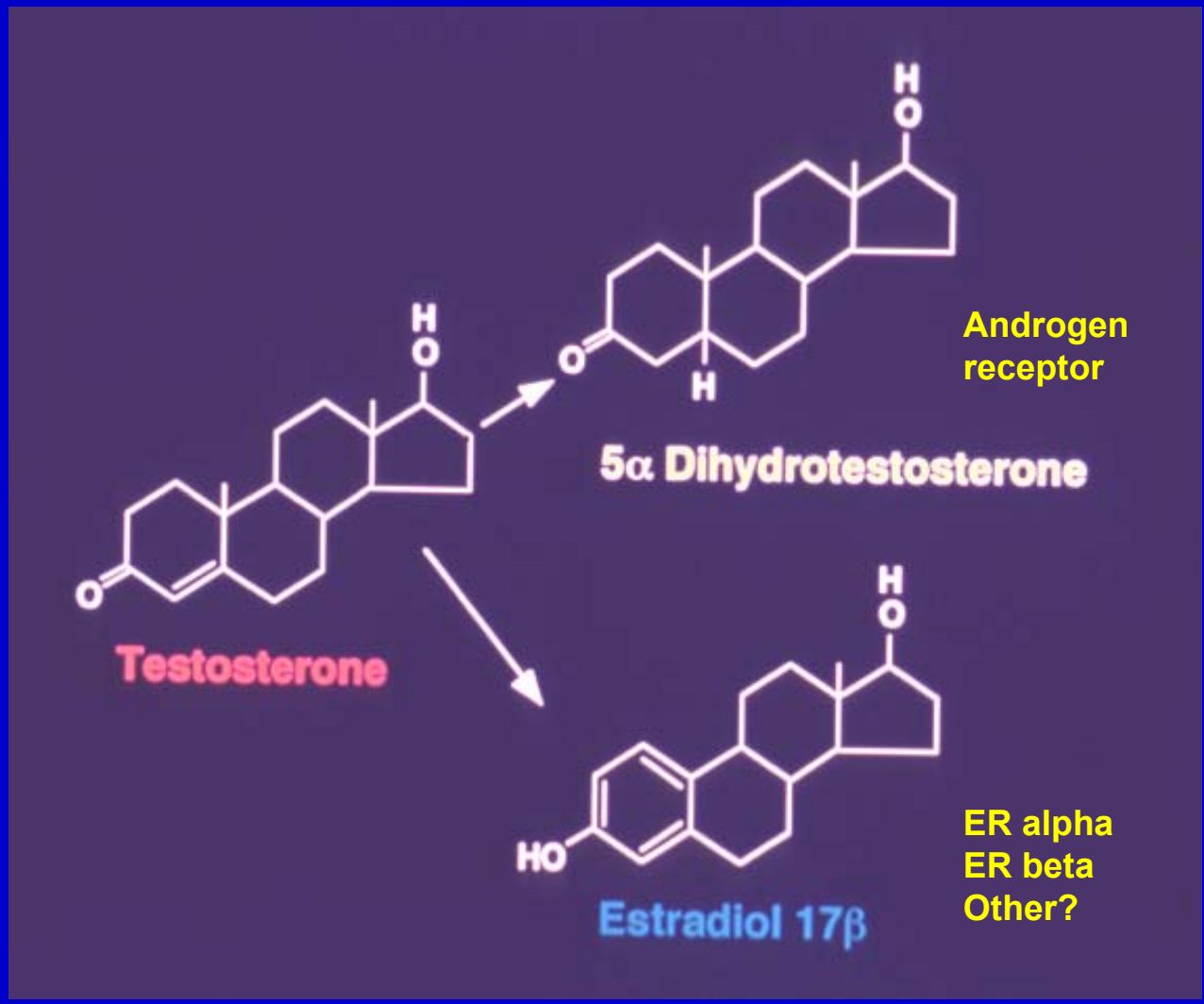
Estradiol 17 beta protects them against cytotoxicity-induced cell death.

Blocked by ICI 182 780

Takao T, Flint N, Lee L, Ying X, Merrill J, Chandross KJ
J. Neurochem 89: 660-673 (2004)

Microglial cells and astrocytes also respond to E

Aromatization of testosterone



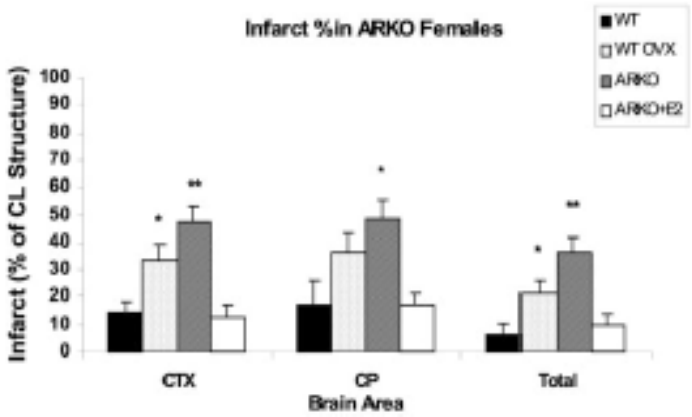


Figure 2. Total and regional infarction (percentage of contralateral (CL) structure) in female mice. ArKO mice had significantly greater ischemic damage in both the cortex (CTX) and caudate-putamen (CP) compared with ovary-intact WT mice. ArKO females also had significantly more total and cortical damage than ovariectomized WT mice. Supplementation of ArKO females with E2 ameliorated ischemic damage to levels seen in intact WT mice. $^{*}p < 0.05$; $^{**}p < 0.01$).

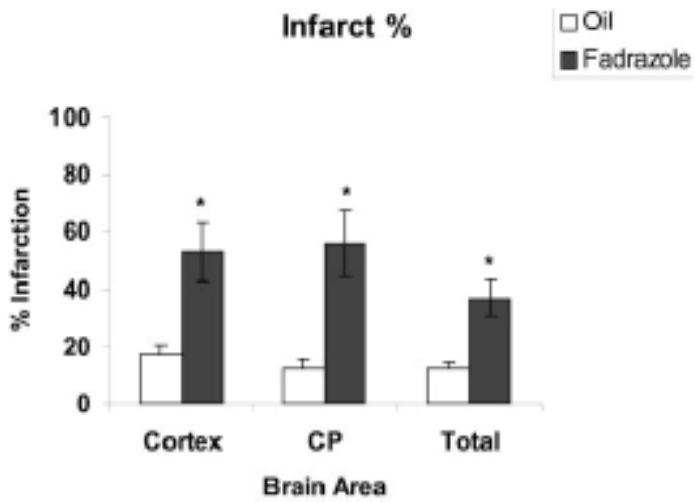
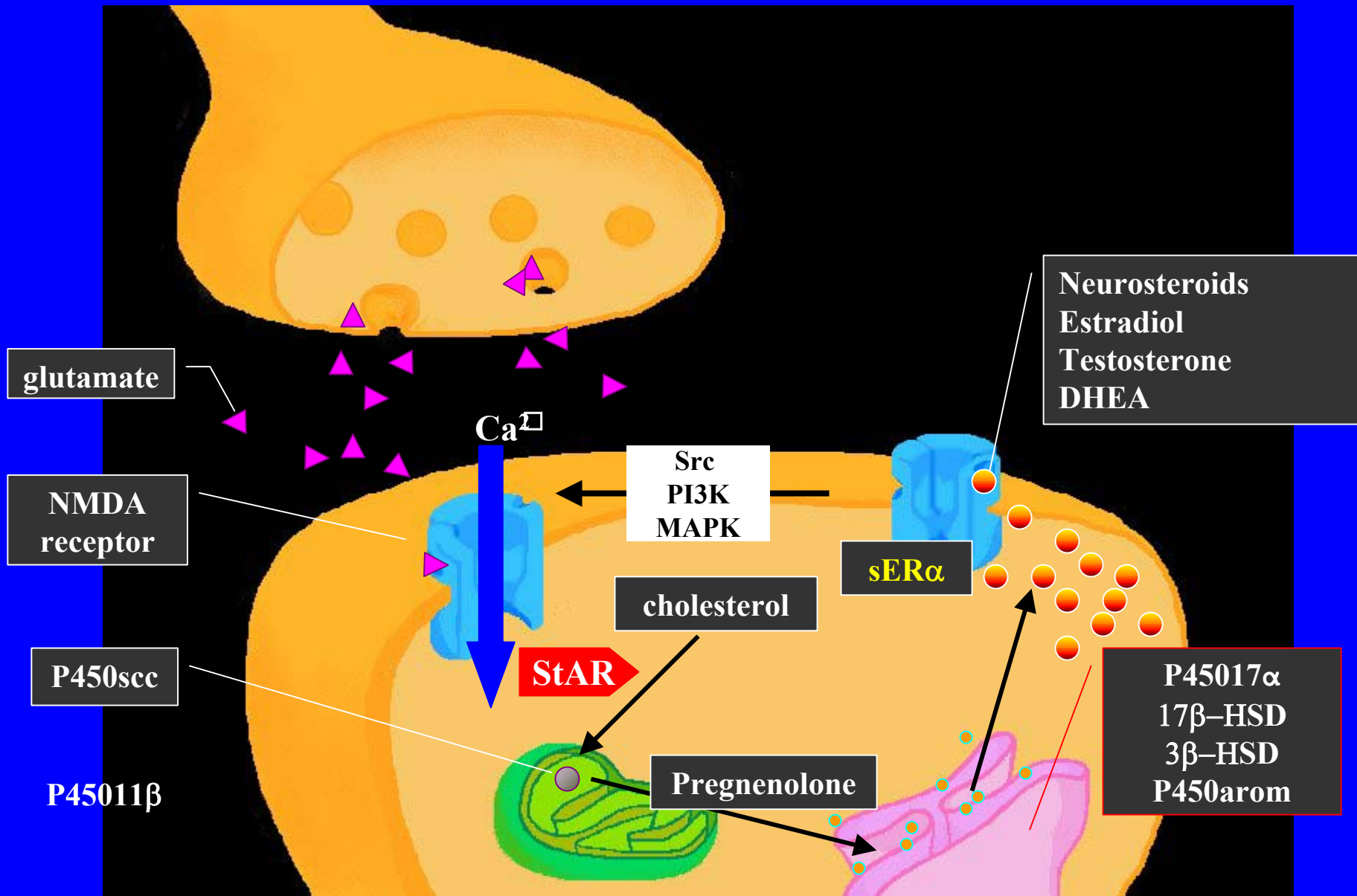


Figure 3. WT female mice treated with the aromatase inhibitor fadrozole had significantly larger infarcts (as measured as a percentage of the contralateral structure) compared with oil-treated mice in both the cortex and caudate-putamen (CP). $^{*}p < 0.01$.

Courtesy of Prof. Kawato

Model: Synthesis of Neurosteroids



Estradiol - Cerebellum

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Dendritic Growth and Spine Formation in Response to Estrogen in the Developing Purkinje Cell

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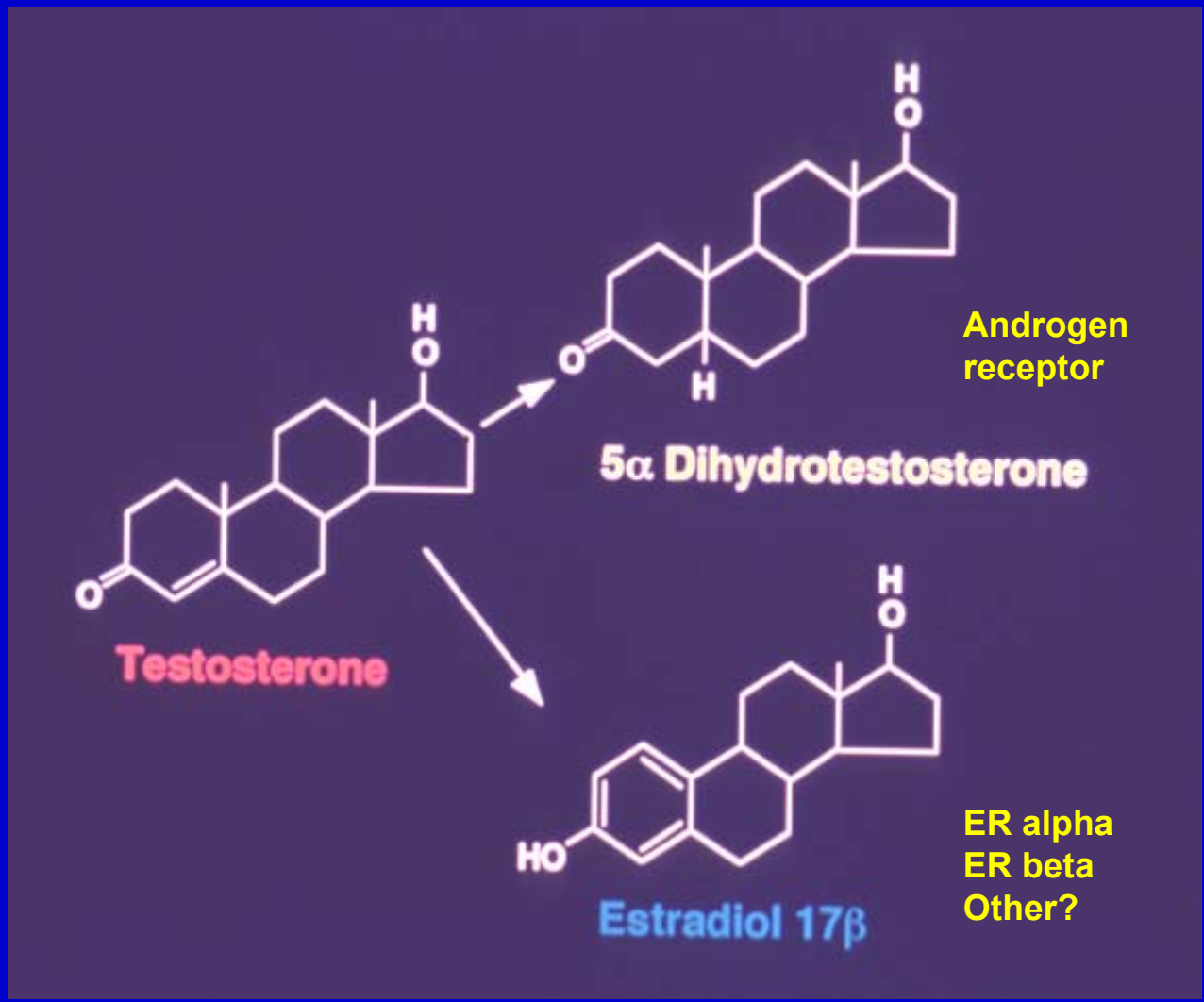
Neurosteroids are synthesized *de novo* in the brain, and the cerebellar Purkinje cell is a major site for neurosteroid formation. We have demonstrated that the Purkinje cell possesses intracellular receptor for progesterone and actively produces progesterone *de novo* from cholesterol only during rat neonatal life, when cerebellar cortical formation occurs dramatically. We have further demonstrated that progesterone promotes dendritic growth, spinogenesis, and synaptogenesis via its receptor in this neuron in the neonate. On the other hand, estrogen may also play an important role in the process of cerebellar cortical formation, because the neonatal rat Purkinje cell possesses estrogen receptor (ER)β. However, estrogen formation in the neonatal cerebellum is still unclear. In this study, we therefore analyzed the biosynthesis and action of estrogen in Purkinje cells during neonatal life. RT-PCR-Southern and *in situ* hybridization analyses showed that Purkinje cells expressed the key enzyme of estrogen formation, cytochrome P450 aromatase, in neonatal rats. A specific enzyme immunoassay for estradiol further indicated that cerebellar estradiol concentrations in the neonate were significantly higher than those in the prepuberty and adult. Both *in vitro* and *in vivo* studies with newborn rats showed that estradiol promoted dose-dependent dendritic growth of Purkinje cells. Estradiol also increased the density of Purkinje dendritic spines. These effects were inhibited by the ER antagonist tamoxifen. These results suggest that estradiol in the developing Purkinje cell promotes dendritic growth and spinogenesis via ERβ in this neuron. Estradiol as well as progesterone may contribute to the growth of Purkinje cells during the cerebellar cortical formation. (*Endocrinology* 144: 4466–4477, 2003)

3. What about males…and androgens?



Brief Communication

Gonadal Hormones Affect Spine Synaptic Density in the CA1 Hippocampal Subfield of Male Rats

Csaba Leranth,[1,2] Ors Petnehazy,[1] and Neil J. MacLusky[3]
Departments of [1]Obstetrics and Gynecology and [2]Neurobiology, Yale University School of Medicine, New Haven, Connecticut 06520, and [3]Center for Reproductive Sciences, Columbia University Medical School, New York, New York 10032

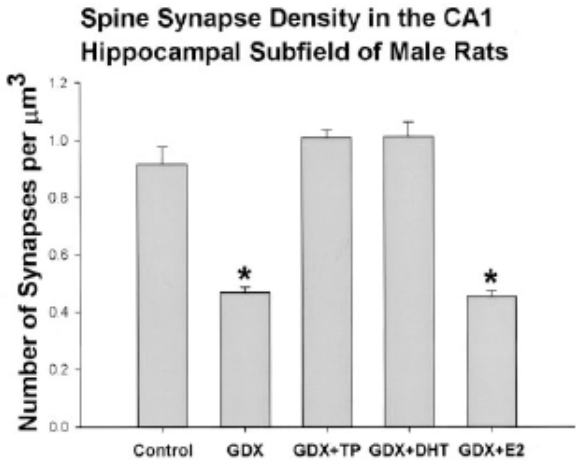


Figure 2. Bar graph shows the result of the unbiased stereological calculation of spine synapse density in the stratum radiatum of the CA1 subfield of control, gonadectomized (*GDX*), gonadectomized plus testosterone-treated (*GDX+T*), gonadectomized plus dihydrotesterone-treated (*GDX+DHT*), and gonadectomized plus estrogen-treated (*GDX+E2*) male rats. There is no significant difference between the density values of spine synapses between the *Control*, *GDX+T*, and *GDX+DHT* animals. However, the spine synapse density of the *GDX* and *GDX+E2* rats is significantly ($p < 0.001$) lower (48%) than that of control animals.

4. Gender and stress

Sex differences in stress effects on classical conditioning point other brain regions than the hippocampus - eg. cerebellum

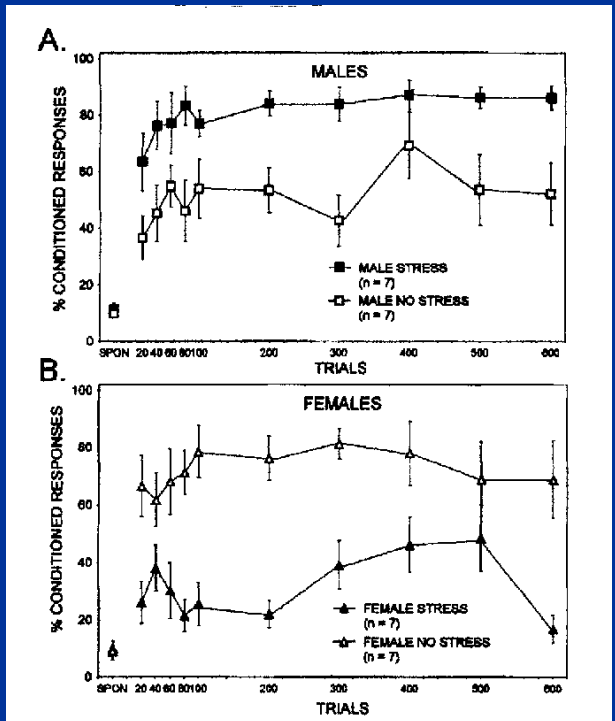


FIG. 1. Effects of sex and stress on acquisition of the conditioned eyeblink response. (*A*) In males, exposure to the stressor facilitated acquisition of the CR 24 h after stressor cessation. (*B*) In females, exposure to the same stressor impaired acquisition of the CR 24 h after stressor cessation. Unstressed females elicited more CRs than unstressed males during the first day of training (1–300 trials), but were not significantly different from each other by the second day of training (301–600 trials). Spontaneous (SPON) eyeblink activity was not affected by sex.

Wood and Shors PNAS, US 95: 4066, 1998

Testosterone *in utero* and at birth dictates how stressful experience will affect learning in adulthood

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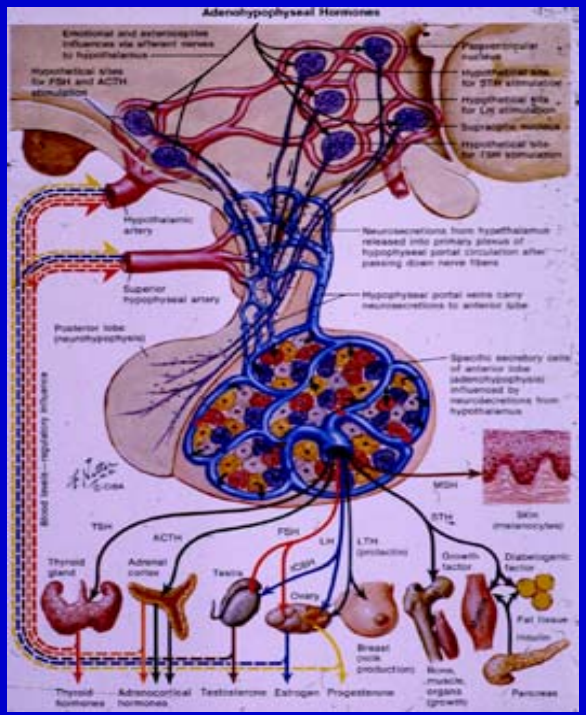
Edited by Bruce S. McEwen, The Rockefeller University, New York, NY, and approved July 31, 2002 (received for review April 4, 2002)

Exposure to an acute stressful event can enhance learning in male rats, whereas exposure to the same event dramatically impairs performance in females. Here we tested whether the presence of sex hormones during early development organizes these opposite effects of stress on learning in males vs. females. In the first experiment, males were castrated at birth whereas females were injected with testosterone. Rats were trained as adults on the hippocampal-dependent learning task of trace eyeblink conditioning. Performance in adult males that had been castrated at birth was still enhanced by exposure to an acute stressful experience. However, adult females injected with testosterone at birth responded in the opposite direction, i.e., exposure to the stressor that typically reduces performance instead enhanced their levels of conditioning. In the second experiment, exposure to testosterone was manipulated *in utero* by injecting pregnant females with a testosterone antagonist. After foster rearing, adult offspring were exposed to the stressor and trained on the hippocampal-dependent learning task of trace conditioning. Although performance in adult females was unaffected by antagonizing testosterone *in utero*, i.e., stress still reduced performance, the enhancement of conditioning after stress in adult males was prevented. Thus, the presence of sex hormones during gestation and development organizes whether and how acute stressful experience will affect the ability to acquire new information in adulthood. As with many sexual behaviors, these cognitive responses to stress appear to be masculinized by exposure to testosterone and feminized by its absence during very early development.

Testosterone reverses females.

Prenatal cyproterone acetate reverses males.

PNAS 99: 13955, 2002



Prolactin and Oxytocin

1. Anxiolytic effect of prolactin
2. “Anti-stress” effect of oxytocin
3. Role of oxytocin in maternal care.

The Journal of Neuroscience, May 1, 2001, 21(9):3207–3214

Anxiolytic and Anti-Stress Effects of Brain Prolactin: Improved Efficacy of Antisense Targeting of the Prolactin Receptor by Molecular Modeling

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We provide the first evidence that prolactin is a neuromodulator of behavioral and neuroendocrine stress coping in the rat. In virgin female and male rats, intracerebral infusion of ovine prolactin (oPRL) into the lateral cerebral ventricle (intracerebroventricular) exerted an anxiolytic effect on the elevated plus-maze in a dose-dependent manner (0.1 and 1.0 μg/5 μl; $p < 0.01$). In contrast, downregulation of the expression of the long form of brain prolactin receptors by chronic intracerebroventricular infusion of an antisense oligodeoxynucleotide (ODN) (osmotic minipump, 0.5 $\mu g \cdot 0.5\ \mu l^{-1} \cdot hr^{-1}$; 5 d) increased anxiety-related behavior on the plus-maze compared with mixed bases-treated and vehicle-treated rats ($p < 0.01$), again demonstrating an anxiolytic effect of PRL acting at brain level. Furthermore, in jugular vein-catheterized female rats, the stress-induced increase of corticotropin secretion was decreased after chronic intracerebroventricular infusion of oPRL (osmotic minipump, 1.0 $\mu g \cdot 0.5\ \mu l^{-1} \cdot hr^{-1}$; $p < 0.05$) and, in contrast, was further elevated by antisense targeting of the brain prolactin receptors ($p < 0.01$). This provides evidence for a receptor-mediated attenuation of the responsiveness of the hypothalamo-pituitary-adrenal (HPA) axis by prolactin. The antisense ODN sequence was selected on the basis of secondary structure molecular modeling of the target mRNA to improve antisense ODN–mRNA hybridization. Receptor autoradiography confirmed the expected improvement in the efficacy of downregulation of prolactin receptor expression [empirically designed antisense, 30%; $p > 0.05$, not significant; adjustment of target position after mRNA modeling, 72%; $p < 0.05$). Taken together, prolactin acting at brain level has to be considered as a novel regulator of both emotionality and HPA axis reactivity.

Key words: ACTH; anxiety; choroid plexus; plus-maze; HPA axis; mRNA secondary structure

Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors

Frances Champagne, Josie Diorio, Shakti Sharma, and Michael J. Meaney*

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Edited by Bruce S. McEwen, The Rockefeller University, New York, NY, and approved August 15, 2001 (received for review May 7, 2001)

Naturally occurring variations in maternal licking/grooming influence neural development and are transmitted from mother to female offspring. We found that the induction of maternal behavior in virgin females through constant exposure to pups (pup sensitization) was significantly shorter in the offspring of High compared with Low licking/grooming mothers, suggesting differences in maternal responsivity. In randomly selected females screened for individual differences in maternal responsivity and subsequently mated, there was a significant and negative correlation ($r = -0.73$) between the latency to exhibit maternal behavior in the pup sensitization paradigm and the frequency of pup licking/grooming during lactation. Females that were more maternally responsive to pups and that showed increased levels of pup licking/grooming also showed significantly higher oxytocin receptor levels in the medial preoptic area, the lateral septum, the central nucleus (n.) of the amygdala, the paraventricular n. of the hypothalamus, and the bed n. of the stria terminalis. Intracerebroventricular administration of an oxytocin receptor antagonist to mothers on postpartum day 3 completely eliminated the differences in pup licking/grooming, suggesting that differences in oxytocin receptor levels are functionally related to maternal behavior. Finally, estrogen treatment of virgin females significantly increased oxytocin receptor binding in the medial preoptic area and lateral septum of female offspring of High, but not Low, licking/grooming mothers. These findings suggest that maternal licking/grooming influences the development of estrogen sensitivity in brain regions that regulate maternal behavior, providing a potential mechanism for the intergenerational transmission of individual differences in maternal behavior.

PNAS 23: 12736-41, 2001

Pergamon

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BRAIN OXYTOCIN: DIFFERENTIAL INHIBITION OF NEUROENDOCRINE STRESS RESPONSES AND ANXIETY-RELATED BEHAVIOUR IN VIRGIN, PREGNANT AND LACTATING RATS

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Max Planck Institute of Psychiatry, Kraepelinstr. 2, 80804 Munich, Germany

Abstract—The involvement of brain oxytocin in the attenuated responsiveness of the hypothalamo-pituitary–adrenal axis and the oxytocin systems to external stressors found in pregnant and lactating rats has been studied, including both neuroendocrine and behavioural aspects. Intracerebroventricular infusion of an oxytocin receptor antagonist (0.75 μg/5 μl), but not of vehicle, elevated basal corticotropin and corticosterone secretion into blood of virgin female, but not of late pregnant or lactating rats. Oxytocin antagonist treatment further elevated the stress-induced (exposure to the elevated plus-maze or forced swimming) secretion of both corticotropin and corticosterone, but only in virgin and not in pregnant or lactating rats. Thus, corticotropin and corticosterone plasma concentrations remained attenuated in antagonist-treated pregnant and lactating animals. In contrast, infusion of the oxytocin antagonist significantly elevated the stress-induced secretion of oxytocin into blood in pregnant and lactating, but not in virgin, animals, indicating an autoinhibitory influence of intracerebral oxytocin on neurohypophysial oxytocin secretion induced by non-reproduction-related stimuli. Treatment with oxytocin antagonist 10 min prior to behavioural testing on the elevated plus-maze significantly reduced the anxiety-related behaviour in both pregnant and lactating rats, without exerting similar effects in virgin female rats.

The results demonstrate a tonic inhibitory effect of endogenous oxytocin on corticotropin and, consequently, corticosterone secretion in virgin female rats, an effect which is absent in the peripartum period. In contrast, an anxiolytic action of endogenous oxytocin was detectable exclusively in pregnant and lactating rats. Therefore, we conclude that the actions of intracerebral oxytocin include independent effects on the responses of the hypothalamo-pituitary–adrenal axis and oxytocin systems to stressors and the anxiety-related behaviour which are modulated by the reproductive state of the animals. © 1999 IBRO. Published by Elsevier Science Ltd.

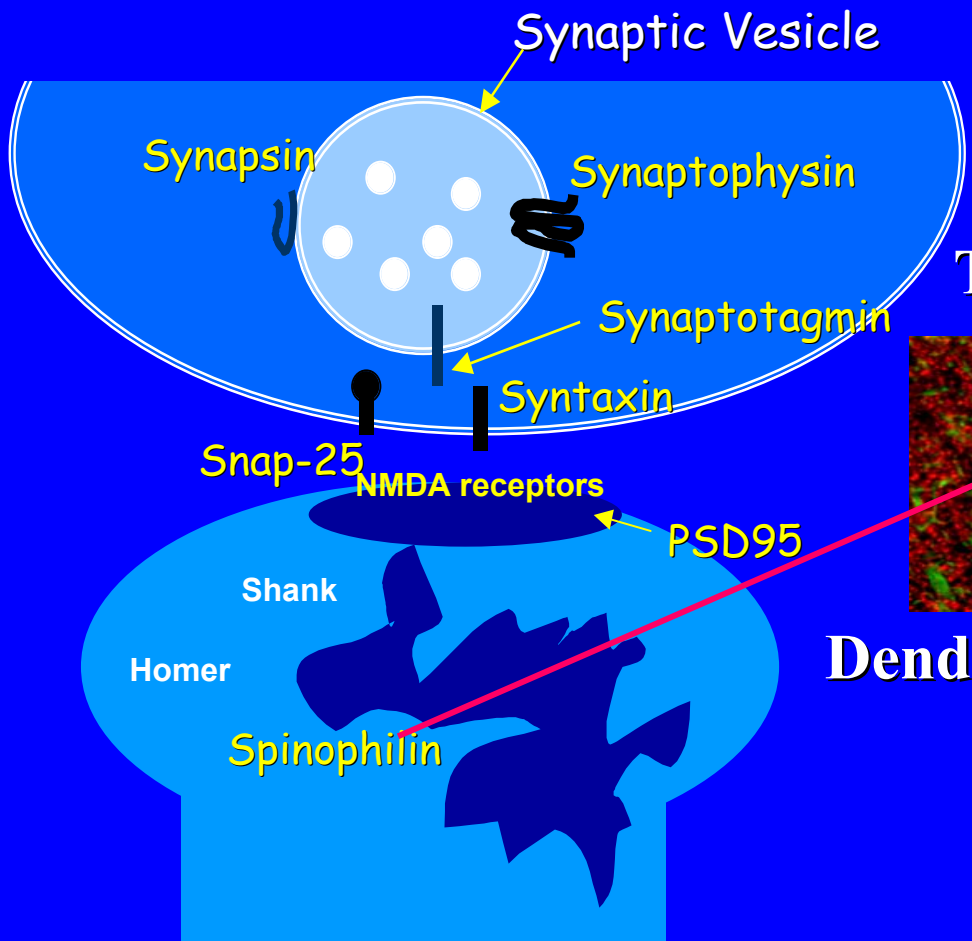
Key words: HPA axis, ACTH, corticosterone, elevated plus-maze, oxytocin, swimming.

It Ain't Just Sex Anymore!

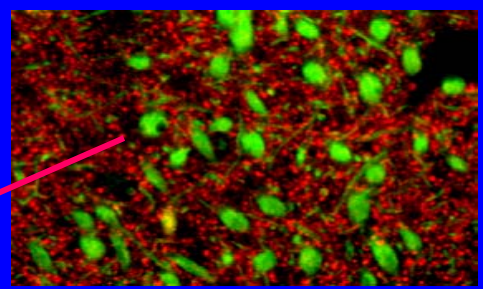
1. Gonadal hormones - widespread influences on brain
2. Many non-reproductive functions affected
3. Neuroprotection - eg stroke and aging
4. Estrogens, progesterone and progestins
5. Genomic and non-genomic mechanisms
6. Widespread role for aromatization of testosterone?
7. Developmentally-programmed gender differences
8. Sex-based differences in response to stressors
9. Important roles for pituitary hormones - prolactin, oxytocin in anxiety and stress responsiveness.

Radioimmunocytochemistry (RICC)

Examining Pre- and Post-Synaptic Proteins

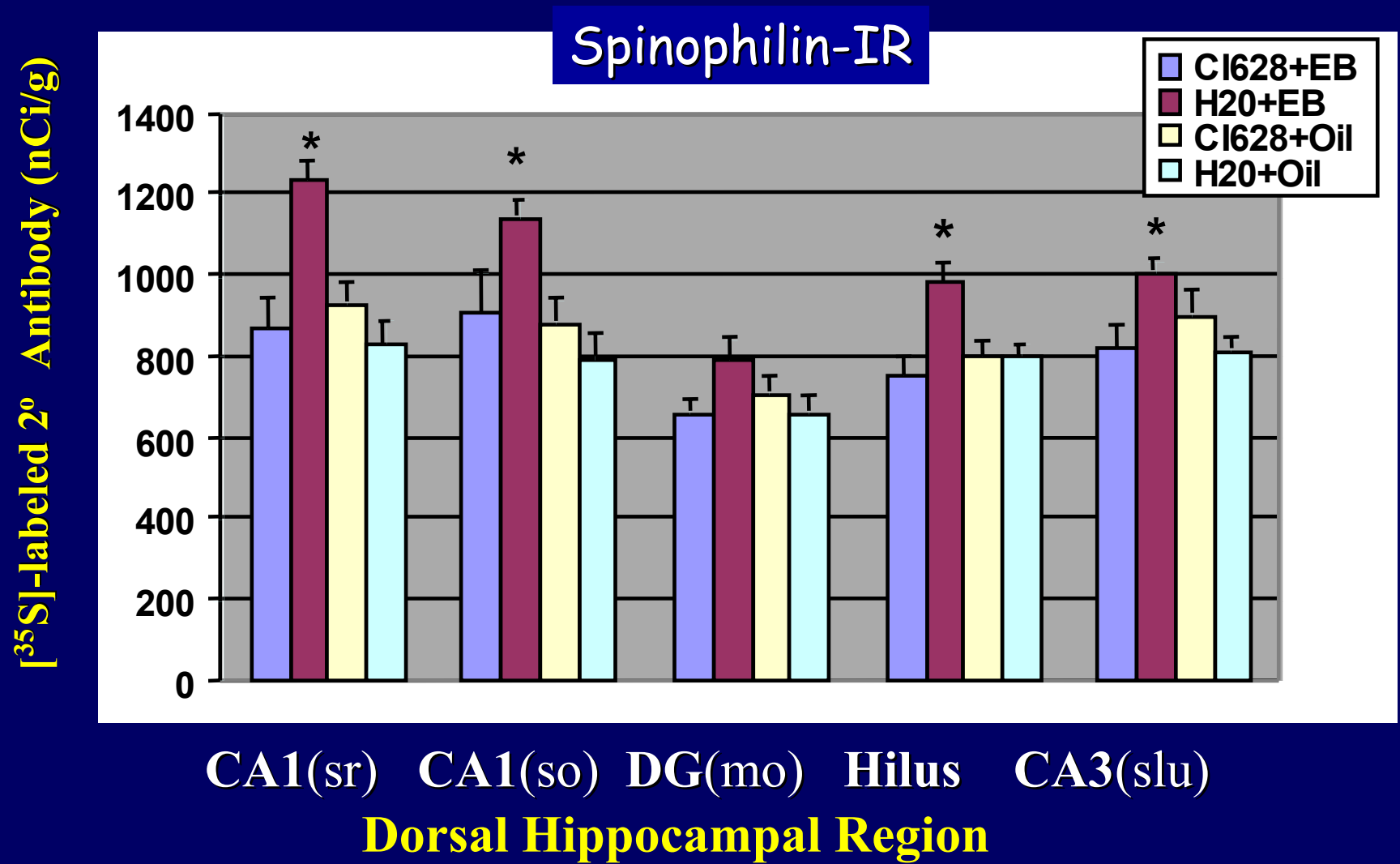


Terminal Bouton

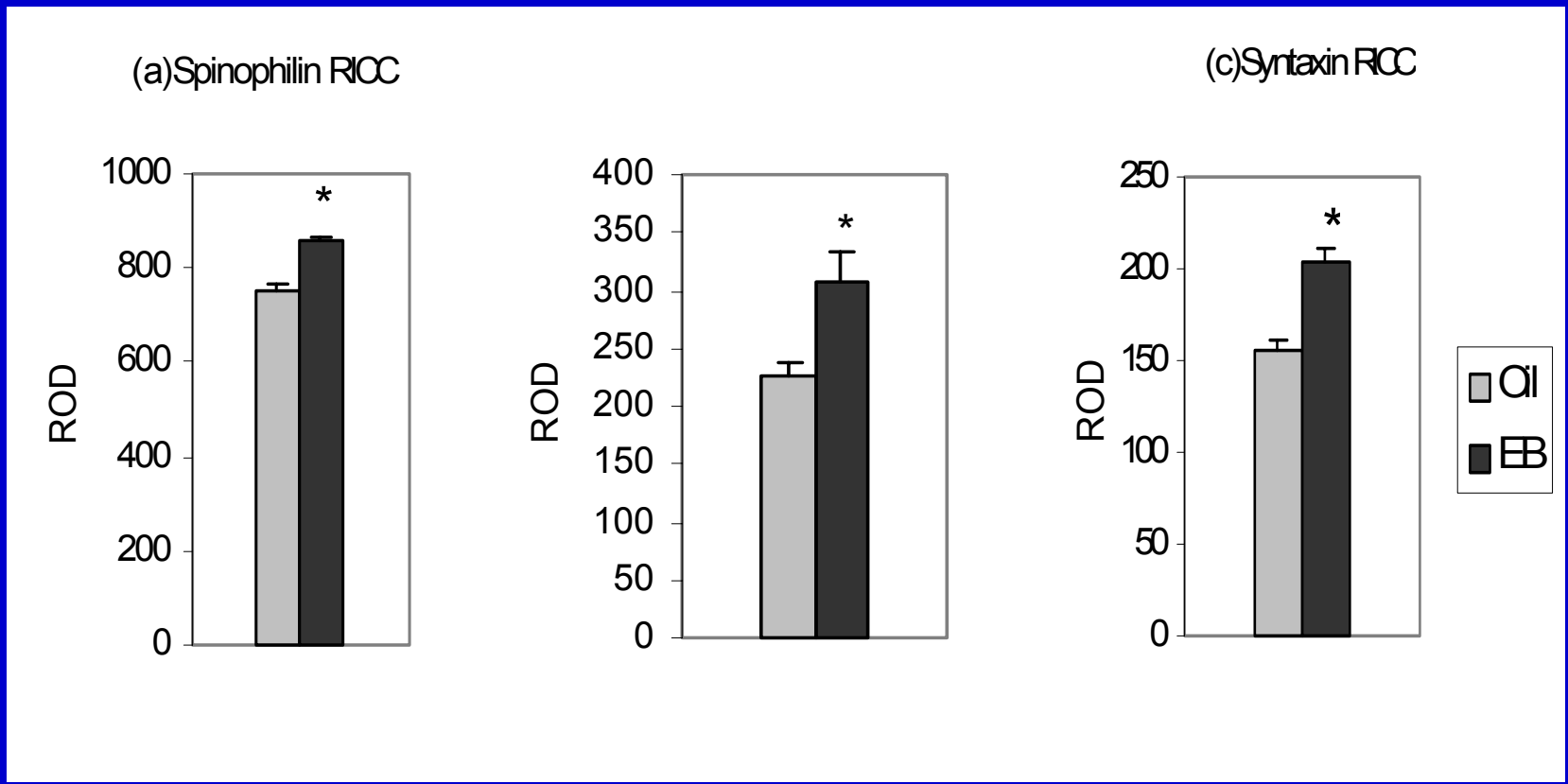


Dendritic Spine

Estrogen treatment of OVX rats induces spinophilin Immunoreactivity - blocked by a SERM, CI-628



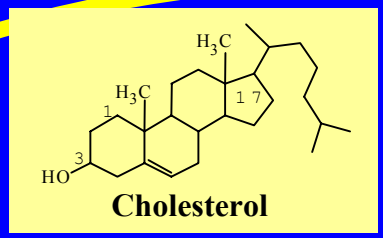
E effects on Spinophilin, PSD95, and Syntaxin RICC



C57 mouse

1. Synthesis: pathway of brain neurosteroid synthesis

Biophys. Biochim. Acta (2003) 1619
Shibuya…Kawato



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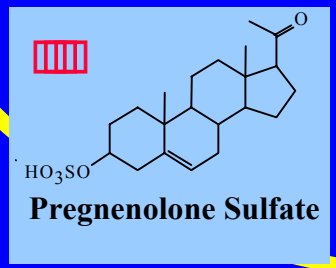
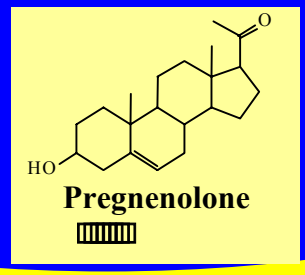
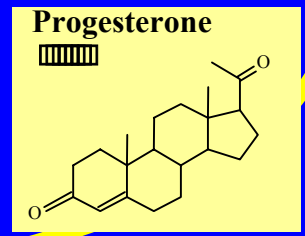


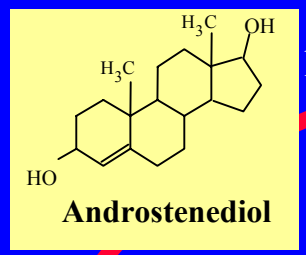


3β–HSD

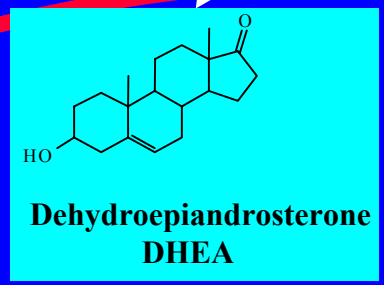


P45017α

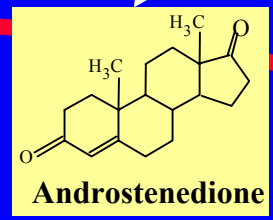
P45017α



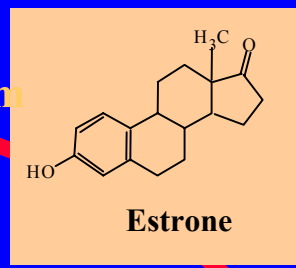
17β–HSD3



3β–HSD



P450arom



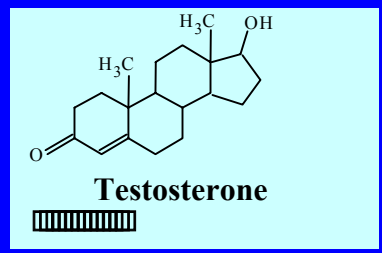
17β–HSD1

3β–HSD

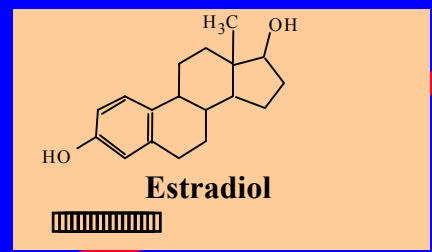
17β–HSD3

Dihydrotestosterone

5α–Reductase



P450arom



3a,5a-Androstandiol ??