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Estrogen and Hippocampal Synaptic Plasticity

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REVIEW ARTICLE

Estrogen and hippocampal synaptic plasticity

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During the past several years, there has been increasing interest in the effects of estrogen on neural function. This enthusiasm is driven, in part, by the results of early clinical studies suggesting that estrogen therapy given after menopause may prevent, or at least delay, the onset of Alzheimer's disease in older women. However, later clinical trials of women with probable Alzheimer's disease had contrary results. Much of the current research related to estrogen and brain function is focused in two directions. One involves clinical studies that examine the potential of estrogen in protecting against cognitive decline during normal aging and against Alzheimer's disease (neuroprotection). The other direction, which is the primary focus of this review, involves laboratory studies that examine the mechanisms by which estrogen can modify the structure of nerve cells and alter the way neurons communicate with other cells in the brain (neuroplasticity). In this review, we examine recent evidence from experimental and clinical research on the rapid effects of estrogen on several mechanisms that involve synaptic plasticity in the nervous system, including hippocampal excitability, long-term potentiation and depression related to sex and aging differences, cellular neuroprotection and probable molecular mechanisms of the action of estrogen in brain tissue.

Keywords: Estrogen, hippocampus, plasticity, aging, glutamate

Long-term potentiation

Long-term potentiation (LTP) of synaptic transmission in hippocampus and neocortex is considered to be a cellular model of memory trace formation in the brain, at least for certain forms of memory (Landfield and Deadwyler, 1988; Bliss and Collingridge, 1993; Baudry *et al.*, 2000). The large body of work on the molecular and synaptic mechanisms underlying LTP (Bear and Malenka, 1994; Geinisman, 2000) is matched by a growing number of studies suggesting the critical role of LTP in behavioral learning and memory (Shors and Matzel, 1997; Morris *et al.*, 2003). Regardless of whether LTP is the substrate of the synaptic modifications that occur during learning in forebrain structures of vertebrates, studies of its mechanisms have revealed several processes that undoubtedly play crucial roles in memory formation (Bi and Poo, 2001). In area CA1 of the hippocampus, the most widely studied form of LTP requires glutamate NMDA receptor activation for its induction, and an increase in AMPA receptor function for its expression and maintenance. In addition, Teyler and associates have demonstrated a second form of tetanus-induced LTP in CA1 that is independent of NMDA receptors, and involves voltage-dependent calcium channels (Grover and Teyler, 1990).

Estrogen and cognition

A small, but growing animal literature indicates that 17β-estradiol, the most potent biologically relevant estrogen, facilitates some forms of learning and memory, particularly for hippocampal-dependent tasks. A post-training injection of 17β-estradiol facilitates retention in the Morris water maze (Singh *et al.*, 1994), and a cholinergic agonist enhanced this effect (Packard and Teather, 1997). In another series of studies, the effects of 17β-estradiol and raloxifene (a selective estrogen-receptor modulator) have been evaluated on the acquisition of a delayed matching to position in a T-maze task and on hippocampal acetylcholine release in ovariectomized rats. The results show that 17β-estradiol, but not raloxifene, enhances the T-maze-task performance, and that 17β-estradiol and a high dose of raloxifene increase potassium-stimulated acetylcholine release in the hippocampus (Gibbs *et al.*, 1994). By contrast, some studies find little or no effect of the estrous cycle (and thereby of endogenous levels of 17β-estradiol) on tasks involving spatial memory (Galea *et al.*, 1995; Berry *et al.*, 1997; Warren and Juraska, 1997; Woolley, 1998).

In humans, the depletion of estrogen that occurs after the menopause increases the susceptibility of women to Alzheimer's disease (Paganini-Hill and Henderson, 1996), whereas estrogen replacement in postmenopausal women improves verbal memory (Asthana *et al.*, 2001; Resnick and Maki, 2001). Healthy postmenopausal women with estrogen replacement scored significantly higher on tests of immediate and delayed paragraph recall compared with healthy postmenopausal women not taking estrogen replacement (Kampen and Sherwin, 1994). Other evaluations of estrogen-replacement therapy in Alzheimer's disease patients indicate that estrogen does not alleviate cognitive impairment associated with the disease (Mulnard *et al.*, 2000) but it does seem to have a beneficial effect as a preventive treatment (Tang *et al.*, 1996), which is most apparent in younger, postmenopausal women (Henderson *et al.*, 2005).

Estrogen activation and the brain

The nervous system is a major target of hormones and contains receptors specific for many types of steroid hormones, including estrogen, aldosterone, androgen and corticosterone. Cell-fractionation studies that examine 17β-estradiol binding in cytosolic and nuclear fractions show that estradiol–receptor complexes are translocated from the cytosol to the nuclear fraction of cells. Once in the nucleus, the estradiol–receptor complex exerts its effect via alterations in gene transcription, followed by changes in protein synthesis. This constitutes the classic genomic mechanism of action for several steroid receptors that interacts with and alters DNA transcription and gene expression. This process is characterized by a long latency and duration of action on the scale of hours to days. Many of the effects of estrogen and other hormones in the brain are explained readily by this genomic mechanism of action (McEwen and Alves, 1999).

In contrast to the genomic mechanism described above, several steroid hormones have been found to produce rapid, short-term effects, which affect mostly electrophysiological properties of neurons, with latency and duration on the scale of milliseconds to minutes. Because activation of transcriptional and translational mechanisms by intracellular steroid-hormone receptors requires a longer latency, physiological responses occurring with extremely short latencies have been presumed to involve nongenomic, specific membrane actions.

Estrogen and hippocampal electrophysiology

For more than 30 years, electrophysiological reports have shown estrogen to promote changes in synaptic excitability and plasticity within the nervous system. In a pioneering study, decreased thresholds for hippocampal seizures were found in animals primed with estrogen and during proestrus, the time of the estrus cycle when estrogen levels are highest (Terasawa and Timiras, 1968). In humans, changes in electrical activity of nervous system tissue correlate with hormonal factors that appear to play a role in catamenial epilepsy, a form of epilepsy in which the likelihood of seizures varies during the menstrual cycle. Many women with catamenial epilepsy experience a sharp increase in seizure frequency immediately before menstruation, when estrogen concentrations relative to those of progesterone are also at their highest levels (Backstrom, 1976). Changes in hippocampal responsiveness that correlate with estrogen activity have been reported in a study that found that induction of LTP is maximal in female rats during the afternoon of proestrus, when endogenous estrogen concentrations are highest (Warren *et al.*, 1995). Furthermore, ovariectomized rats treated with estrogen have a facilitation for the induction of hippocampal LTP (Cordoba Montoya and Carrer, 1997).

Estrogen and hippocampal excitability

The development of *in vitro* models to study the mechanisms of neuronal plasticity allow researchers to investigate how estrogen regulates synaptic excitability in the nervous system, and, in particular, the hippocampus. However, the binding of tritiated estradiol in the hippocampus does not approach that seen in the hypothalamus and related diencephalic structures (McEwen *et al.*, 1975; McEwen and Alves, 1999). Nonetheless, studies by Teyler and colleagues using *in vitro* hippocampal slice preparations have shown that gonadal steroids dramatically affect neuronal excitability in specific pathways of the rodent hippocampus (Teyler *et al.*, 1980; Vardaris and Teyler, 1980). In initial experiments, extracellular monosynaptic population field responses recorded from area CA1 of hippocampal slices from male and female rats were monitored before and after the application of 17β-estradiol (100 pM) to the slice incubation medium (artificial cerebrospinal fluid; aCSF). In male rats, 17β-estradiol produced a rapid (<10 minute) enhancement of population field responses evoked by stimulation of the afferents to CA1 pyramidal cells (Fig. 1). This was the first published report to demonstrate that picomolar concentrations of the gonadal steroid 17β-estradiol directly enhanced glutamatergic synaptic transmission in hippocampus (Teyler *et al.*, 1980).

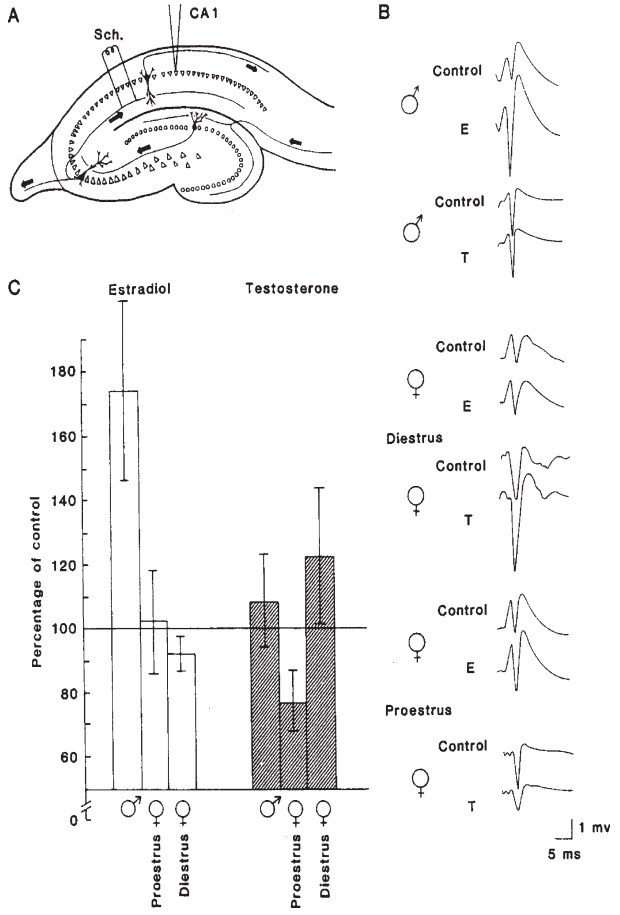

Fig. 1. Hippocampal slice preparation. (A) Diagram of a transverse hippocampal slice. Stimulating electrodes are located in the afferent pathway, which contains the Schaffer (Sch) collaterals. Recording micropipettes are situated in the pyramidal cell body layer in CA1. Cells of this subfield receive monosynaptic input from the CA3 pyramids via the Sch collateral system. (B) Representative field potentials from slice preparations in various experimental conditions. Extracellular population-spike responses to a given stimulus intensity are shown from the control period (before steroid administration) and after the administration of either 10^{-10} M 17β-estradiol (E) or 10^{-10} M testosterone (T). Potentials from slices from males and proestrus and diestrus females are shown for comparison. All potentials are single sweeps recorded at the same voltage and time scales. (C) Summary of the major experimental outcomes. Values on the ordinate are mean percentages of spike amplitudes after steroid administration. Data (mean ± S.E.M.) for each condition are from 6–10 animals, each contributing one slice. Reprinted, with permission, from Teyler *et al.* (1980).

Estrogen and nongenomic mechanism of action

Although the genomic mechanism of action of estrogen is the traditional framework for interpreting the mechanisms of the effects of estrogen on cell function, an increasing number of reports document the effects of acute applications of estrogenic steroids that are too rapid (occurring in <10 minutes) to be accounted for exclusively by a genomic pathway. In particular, the existence of rapid estrogenic steroid-induced changes in neuronal excitability indicates that other, nongenomic mechanisms that involve direct interactions with sites on the plasma membrane either alter or regulate several ion channels and neurotransmitter transporters (Pfaff and McEwen, 1983; Wong *et al.*, 1996). Although the mechanism of action of gonadal steroids in the hippocampus is not understood entirely, there is reason to believe that it is receptor-mediated. There is no facilitation of the field responses when the inactive estrogen 17α-estradiol is added to the hippocampal slice medium (Foy and Teyler, 1983), and the further addition of 17β-estradiol no longer results in an increased response, as observed in the presence of 17β-estradiol alone (Foy and Teyler, 1983; Wong and Moss, 1991; Wong and Moss, 1992). Similar results were found when the estrogen receptor antagonist tamoxifen is applied to hippocampal slices before addition of 17β-estradiol (Foy, 1983). The ability of 17α-estradiol and tamoxifen to block the effects of 17β-estradiol on hippocampal excitability provides strong evidence that the rapid physiological modulation of gonadal hormones is most likely to be caused by a plasma-membrane, receptor-mediated phenomenon.

Estrogen and AMPA receptor activation

In vitro intracellular recordings of CA1 neurons from adult ovariectomized female rats have shown that the administration of 17β-estradiol increases synaptic excitability, in part, by enhancing the magnitude of α-amino-3-hydroxy-5-methyl-4-isoxazoleproprianate (AMPA) receptor-mediated responses (Wong and Moss, 1992). The rapid onset of this increased excitability and its blockade by 6-cyano-7-nitroquinaxaline, an AMPA receptor antagonist) but not by D-2-amino-5-phosphonovalerate (D-APV), a competitive N-methyl-D-aspartate (NMDA) receptor antagonist, supports a postsynaptic membrane site of action that is most likely to be mediated by non-NMDA glutamate receptors. Later studies using whole cell recordings found that acute 17β-estradiol application potentiated kainate-induced currents in a subpopulation of CA1 cells (Gu and Moss, 1996), but a direct interaction between 17β-estradiol and the receptor channel was not indicated (Wong and Moss, 1994).

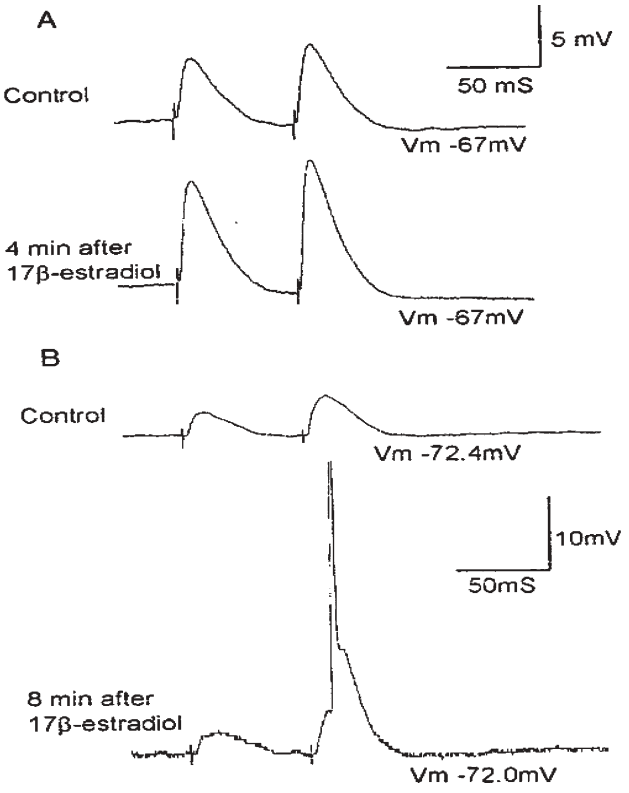

Fig. 2. Fast potentiation by 17β-estradiol perfusion of pharmacologically isolated, non-NMDA-receptor-mediated EPSPs. The AMPA receptor was isolated pharmacologically by applying the NMDA-receptor antagonist D-APV (50 μM) and the GABA-receptor antagonist bicuculline (5 μM) to the bath. (A) 1 nM 17β-estradiol potentiates non-NMDA-mediated EPSPs in aCSF containing D-APV and 1.0 mM Mg^{2+}. (B) Some cells showed action potentials during continuous perfusion with 17β-estradiol but not in control conditions (before 17β-estradiol perfusion). Reprinted, with permission, from Foy *et al.* (1999).

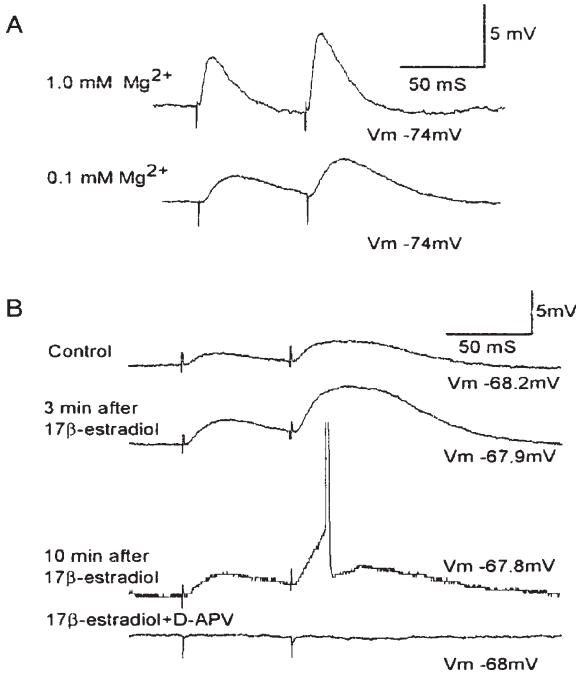

Fig. 3. EPSPs in CA1 pyramidal cells. The amplitude of NMDA-receptor-mediated EPSPs in CA1 pyramidal cells increases shortly after addition of 1 nM 17β-estradiol to the perfusion medium, which contains 5 μM bicuculline. (A) *Top*: EPSPs evoked when slices are perfused with medium that includes 1.0 mM Mg^{2+} but not the non-NMDA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX). *Bottom*: NMDA-receptor-mediated EPSPs evoked 10 minutes after medium was switched to include 0.1 mM Mg^{2+} and 10 μM DNQX. (B) 1 nM 17β-estradiol potentiates the isolated EPSPs within 3 minutes. Potentiation, which occurred in 9 out of 12 cells, was observed in EPSPs evoked by paired-pulse stimulation and peaked within 10 minutes. In 5 out of 9 cells, the potentiated EPSPs reached threshold and generated action potentials during 17β-estradiol perfusion. The potentiated EPSPs were blocked by the NMDA receptor antagonist D-APV. Reprinted, with permission, from Foy *et al.* (1999).

An example of this effect from our work (Fig. 2) shows intracellular recordings of excitatory post-synaptic potentials (EPSPs) from a CA1 pyramidal neuron in hippocampal slices from an adult male rat in response to brief depolarizing pulses separated by 67 mseconds in a medium containing 50 μM D-APV and 1.0 mM Mg^{2+} (to block NMDA receptors). There is a rapid, dramatic increase in EPSP amplitude within 4 minutes of 17β-estradiol infusion, and some cells exhibit depolarization-induced action potentials within 8 minutes of infusion (Foy *et al.*, 1999).

Estrogen and NMDA receptor activation

A large body of evidence demonstrates that regulation of synapse formation by 17β-estradiol is dependent on NMDA receptor activation. Morphological studies of neurons in culture prepared from embryonic-day-18 rat embryos show that estrogenic steroids exert a growth-promoting, neurotrophic effect on hippocampal and cortical neurons by a mechanism that requires activation of NMDA receptors (Brinton *et al.*, 1997a; Brinton *et al.*, 1997b). Studies *in vivo* using adult ovariectomized female rats also reveal an increased number of dendritic spines in hippocampal CA1 pyramidal cells after 17β-estradiol treatment, which is prevented by blockade of NMDA receptors but not by AMPA receptor and muscarinic receptor antagonists (Woolley and McEwen, 1994). Other reports using adult ovariectomized female rats provide evidence that chronic treatment with 17β-estradiol increased the number of NMDA receptor-binding sites and NMDA receptor-mediated responses (Gazzaley *et al.*, 1996; Woolley *et al.*, 1997). These studies indicate that estrogen and NMDA receptors are heavily involved in synapse formation.

Estrogen and NMDA receptor-mediated EPSPs

The possibility that 17β-estradiol might directly regulate NMDA receptor-mediated synaptic transmission might not have been detected previously (e.g., Wong and Moss, 1992) because tests of this hypothesis were not conducted under optimal conditions. Because of the voltage-dependent blockade of the NMDA receptor channel by Mg^{2+} and the slow kinetics of the channel opening relative to that of the AMPA receptor, NMDA receptor stimulation mediates only a minor component of the excitatory postsynaptic potential (EPSP) evoked by low-frequency stimulation of glutamatergic afferents. This NMDA receptor component is enhanced by either low Mg^{2+} concentrations or using high-frequency stimulation patterns to induce the depolarization accompanying the summation of overlapping EPSPs (Xie *et al.*, 1992). In experiments using low Mg^{2+} concentrations and in the presence of the AMPA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX), acute application of 17β-estradiol in hippocampal slices from adult male rats results in a rapid increase in the amplitude of NMDA receptor-mediated EPSPs evoked by

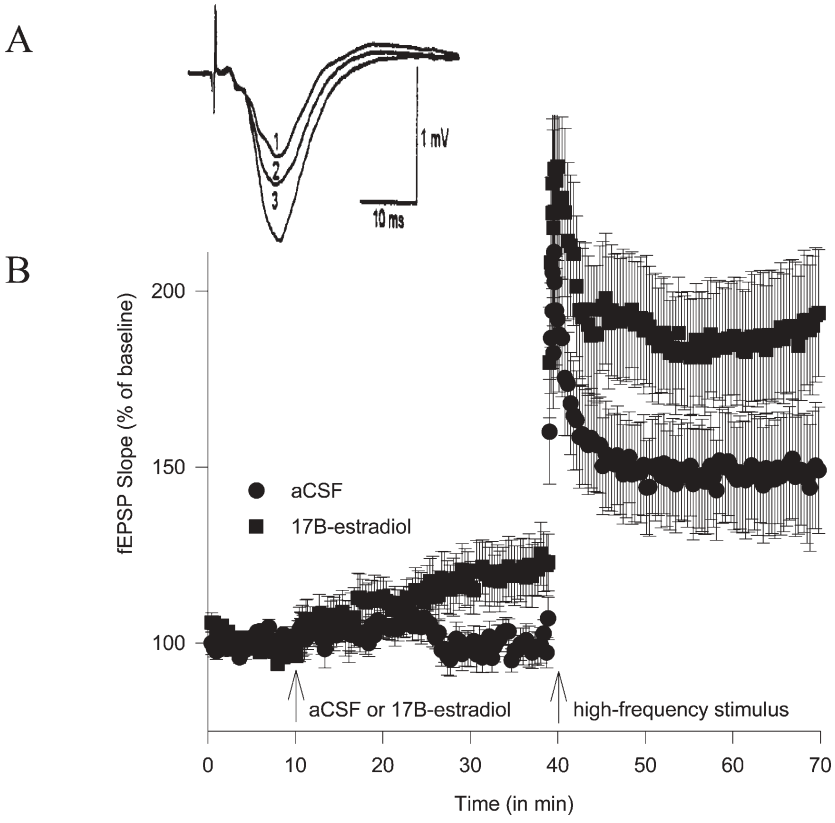

Fig. 4. Field EPSP (f-EPSP) recordings in area CA1. (A) All hippocampal slices were perfused with aCSF for 10 minutes to obtain f-EPSP slope and amplitude percentage baseline data. After 10 minutes of baseline recording, experimental slices were perfused with 100 pM 17β-estradiol. Control slices continued to be perfused with aCSF. 30 minutes later all slices received high-frequency stimulation, designed to induce LTP. (A1) fEPSP recording at the end of the 10-minute baseline period. (A2) fEPSP recording at the end of the 30-minute perfusion with 17β-estradiol. (A3) fEPSP recorded for 30 minutes after high-frequency stimulation in slices perfused with 17β-estradiol. (B) fEPSP slope responses in area CA1. Data points represent averaged fEPSP slope ± S.E. (taken at each 20-second sweep) for experimental (17β-estradiol-treated) and control (aCSF) hippocampal slices. Reprinted, with permission, from Foy *et al.* (1999).

stimulation of Schaffer collaterals (Foy *et al.*, 1999). The effect of 17β-estradiol on pharmacologically isolated NMDA receptor-mediated synaptic responses is such that concentrations of 17β-estradiol >10 nM induce seizure activity in hippocampal neurons, and lower concentrations (1 nM) markedly increase the amplitude of NMDA receptor-mediated EPSPs (Fig. 3).

Estrogen and hippocampal LTP

To investigate the effect of estrogen on synaptic plasticity associated with learning and memory function, estrogen was applied to hippocampal slices from adult male rats before exposure to high-frequency stimulation designed to induce LTP. When LTP was assessed after high-frequency stimulation, fEPSP values increased significantly in 17β-estradiol-treated slices compared with control, aCSF slices (Fig. 4). The mean increases in the slope of fEPSP was 192% (experimental) compared with 154% (control). Thus, hippocampal slices from adult male rats treated with 17β-estradiol exhibited a pronounced, persistent, significant increase in LTP, measured by both population fEPSP slope and fEPSP amplitude recordings (Foy *et al.*, 1999).

To further evaluate the effects of 17β-estradiol on the magnitude of hippocampal LTP, the intensity of afferent stimulation to Schaffer collaterals in slices perfused with 17β-estradiol was decreased to produce baseline values similar to pre-17β-estradiol responses immediately before delivery of the high-frequency stimulation train used to elicit LTP (Bi *et al.*, 2000). Under these conditions, 17β-estradiol still increased the amplitude of LTP from adult male rat hippocampal slices compared with control (aCSF) slices (Fig. 5). These findings, along with other results from this study, indicate that the estrogen-induced enhancement of hippocampal LTP is not caused simply by a change in basal EPSP level, but is more likely to result from biochemical activation of an intracellular cascade, which, presumably, is mediated by activation of a src tyrosine pathway that enhances NMDA receptor function.

Estrogen and hippocampal LTP in females

In another series of studies, estrous-cycle changes in rodents have been correlated with changes in synaptic plasticity. Hippocampal slices from cycling female rats in diestrus (low estrogen concentration) and proestrus (high estrogen concentration) were prepared and recorded in aCSF, followed by LTP induction via high-frequency stimulation. The difference in LTP values between these groups following high-frequency stimulation was dramatic: slices from rats in the proestrus phase exhibited LTP representing ~50% increase over baseline, whereas slices from rats in the diestrus phase had LTP values

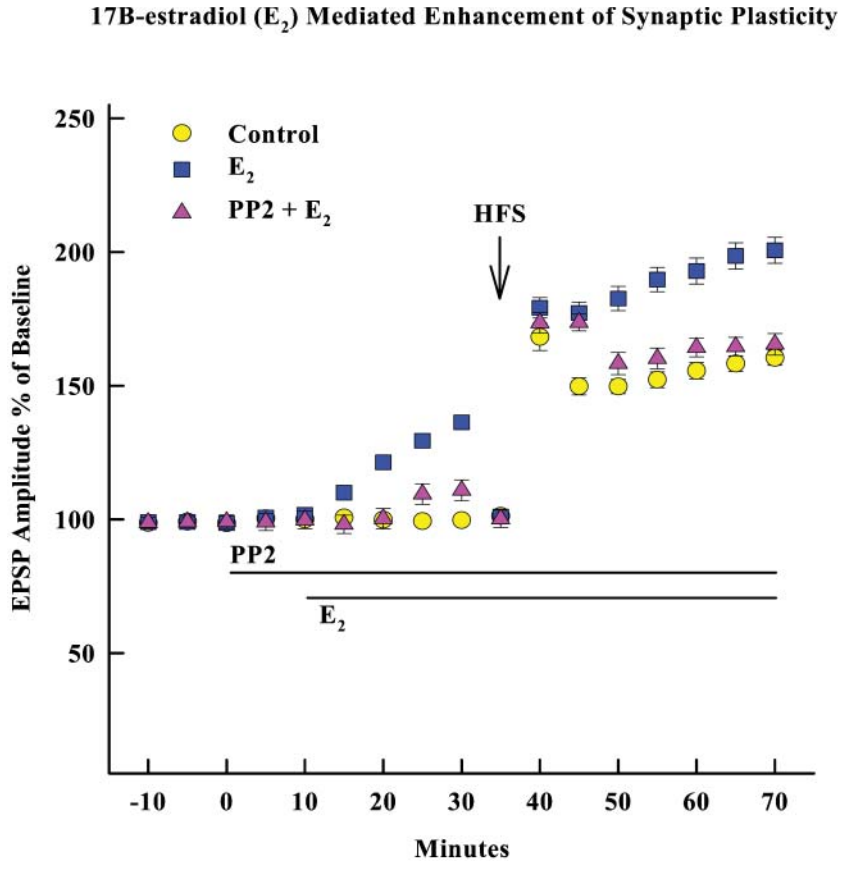

Fig. 5. Effects of src inhibition on 17b-estradiol (E2)-mediated enhancement of EPSP amplitude and degree of LTP in hippocampal slices. A stimulating electrode was located in CA3 and a recording electrode in the stratum radiatum of CA1. Extracellular EPSPs were evoked by stimulation every 30 seconds, and the EPSP amplitude measured. After recording a stable baseline, an inhibitor of src (PP2, 10 μM) and E2 (1 nM) were added at the times indicated. After resetting the stimulation intensity to obtain EPSPs of the same amplitude as before treatment with either PP2 or E2, high-frequency stimulation (HFS) was delivered, and low-frequency stimulation resumed. PP2 blocked the estrogen-mediated enhancement of LTP but did not affect LTP itself. Data (expressed as percentages of predrug values) are mean ± S.E.M. of 6–10 experiments. Reprinted, with permission, from Bi *et al.* (2000).

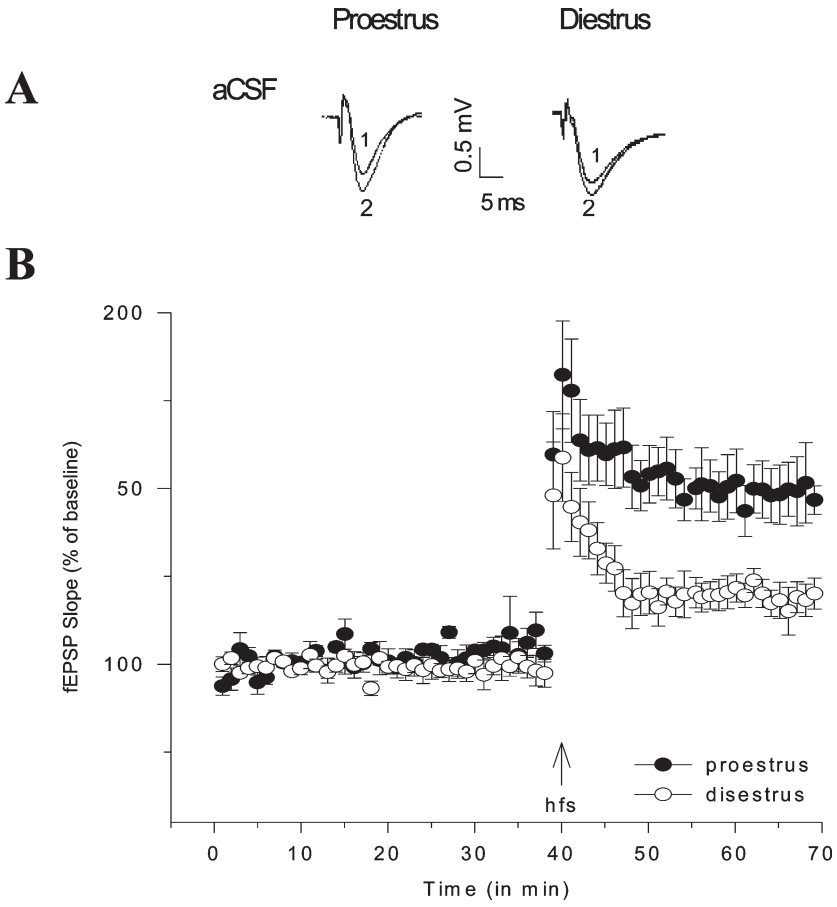

Fig. 6. LTP in field CA1 of hippocampal slices from female rats in proestrus and diestrus. Hippocampal slices from female rats in either proestrus or diestrus were prepared. fEPSP amplitude and slope values were obtained for each slice and averaged across slices to produce one mean before and after the train of high frequency stimulation (hfs). fEPSP amplitudes and slopes were normalized for the 10-minute pre-hfs period for each slice. ANOVA and planned two-tailed *t* tests for pre-hfs and post-hfs periods were used to evaluate the effects of estrous on fEPSP slope and amplitude. (A) Representative waveforms from female rats in proestrus and diestrus for pre-hfs (1) and post-hfs (2) periods. (B) Mean ± S.E.M. of fEPSP slopes recorded in slices from female rats in proestrus (filled circles, $n = 6$) and diestrus (open circles, $n = 5$). Reprinted, with permission, from Bi *et al.* (2001).

representing ~25% increase over baseline (Bi *et al.*, 2001) (Fig. 6). These findings help to support the results from the original work of Teyler *et al.* (1980), who identified changes in baseline synaptic transmission that correlated with the phase of the estrus cycle in female rats at the time of hippocampal-slice preparation.

The electrophysiological study above shows that female rats in proestrus have an increased magnitude of hippocampal LTP compared with female rats in diestrus. Here we report a current study that examines the effect of 17β-estradiol on hippocampal LTP during the same crucial time periods in the rat estrous cycle: proestrus and diestrus. The estrous cycles of adult (3–5 month) Sprague-Dawley rats were monitored for 10 days before physiological experiments, and hippocampal slices were prepared from rats in either proestrus or diestrus. Recording and stimulating electrodes were positioned in the dendrites of area CA1 and Schaffer collaterals, respectively. Baseline stimulation (0.05 Hz, 100 μsec) was adjusted to elicit 50% of the maximum fEPSP amplitude. After 10 minutes of stable baseline stimulation, slices were perfused for 30 minutes with either aCSF or 17β-estradiol (100 pM; experimental group) and LTP induced by a brief period of high-frequency stimulation (5 trains of 20 pulses at 100 Hz). Subsequent synaptic responses were monitored for 30 minutes post-LTP induction. LTP induced in area CA1 was greater in slices from proestrus rats compared with slices from diestrus rats, as seen previously (Bi *et al.*, 2001). However, 17β-estradiol treatment increased LTP in slices from diestrus rats, but decreased LTP in slices from proestrus rats (Fig. 7). These observations indicate that 17β-estradiol alters hippocampal LTP in female rats depending on the state of their estrous cycle (i.e. on the levels of circulating 17β-estradiol). In cycling female rats, when endogenous circulating levels of 17β-estradiol are at their highest levels (proestrus), LTP magnitude is increased and exogenously applied 17β-estradiol decreases LTP magnitude, possibly through the activation of a mechanism that is associated with either an inhibitory or ceiling effect. When endogenous circulating levels of 17β-estradiol are at their lowest levels (diestrus), the situation is reversed from that observed in the proestrus state. Here, LTP magnitude is decreased, and exogenously applied 17β-estradiol increases LTP magnitude.

These results indicate that the cyclic changes in estrogen levels occurring during the estrous cycle in female rats are associated with changes in the magnitude of LTP recorded from hippocampal CA1 cells, and corroborate work mentioned earlier indicating that estrogen facilitates LTP induction in ovariectomized female rats (Cordoba Montoya

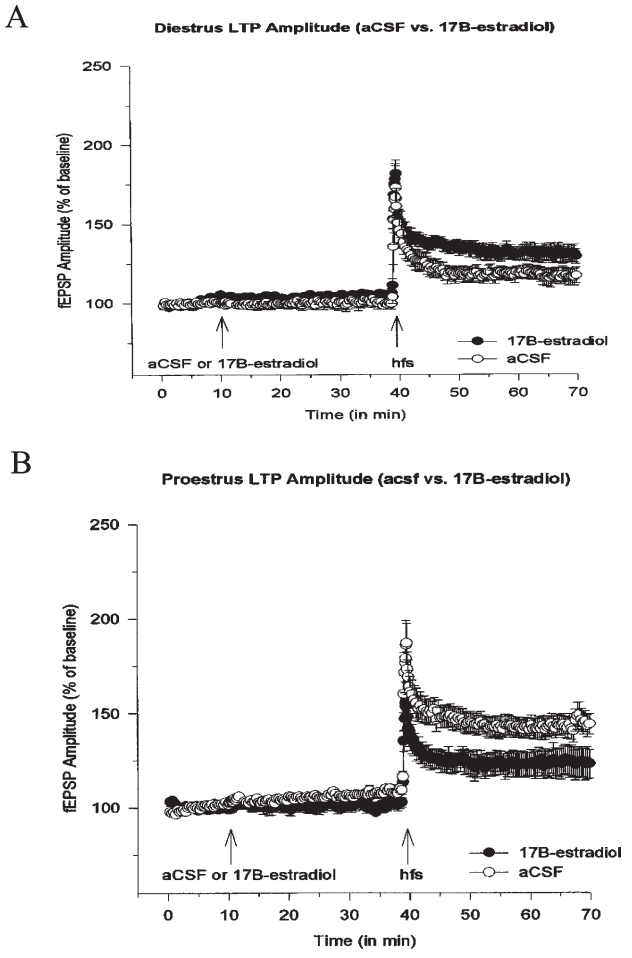

Fig. 7. LTP in field CA1 of hippocampal slices from female rats in proestrus and diestrus during treatment with 17β-estradiol (experimental) and aCSF (control). (A) Mean ± S.E.M. of fEPSP amplitudes recorded following tetanus in slices from female rats in diestrus. 17β-estradiol (filled circles) enhances LTP relative to control aCSF (open circles). (B) Mean ± S.E.M. of fEPSP amplitudes recorded following tetanus in slices from female rats in proestrus. 17β-estradiol (filled circles) impaired LTP relative to control aCSF (open circles).

and Carrer, 1997), and that LTP increases in the afternoon of proestrus of female rats (Warren *et al.*, 1995).

Estrogen, LTP, LTD and aging

It is reported that when memory function declines in aging, the processes of synaptic plasticity in the hippocampus are altered. Specifically, LTP is impaired and the opposite process of long-term depression (LTD) is enhanced (Landfield and Lynch, 1977; Barnes, 1979; Landfield *et al.*, 1986; Barnes *et al.*, 1992; Barnes, 1994; Geinisman *et al.*, 1995; Norris *et al.*, 1996; Foster and Norris, 1997; Norris *et al.*, 1998; Foster, 1999). We replicated this effect of aging on LTD and discovered a profound action of estrogen on this process in aged male rats (Vouimba *et al.*, 2000). LTD was induced in the CA1 region of hippocampal slices using standard conditions (potentiation of Schaffer collaterals at 1 Hz for 15 minutes) in adult (3–5 month) and aged (18–24 month) Sprague-Dawley male (?) rats. In agreement with earlier studies, we found that the standard protocol for inducing LTD caused little or no LTD in slices from adult animals, but in marked LTD in slices from aged animals (Fig. 8A) (Foster and Norris, 1997; Foster, 1999). Infusion of slices with 17β-estradiol caused a slight increase in baseline synaptic transmission, as in previous studies. It had little effect on LTD in slices from adult animals, but markedly attenuated LTD in slices from aged animals (Fig. 8B). Thus, prevention by estrogen of the age-related enhancement of LTD might account, in part, for the protective effects reported in some studies of estrogen on memory functions in aged organisms (see below).

Estrogen and two forms of LTP

As noted earlier, Teyler and associates discovered a form of LTP in CA1 pyramidal neurons that is independent of NMDA receptors and involves voltage-dependent calcium channels (Grover and Teyler, 1990). This form of LTP is induced most strongly by a very high frequency tetanus of Schaffer collaterals (e.g. 200 Hz for 1 second) and is blocked by nifedipine but not by the NMDA receptor antagonist D-APV. The NMDA receptor-dependent form of LTP in CA1, which also involves calcium influx via NMDA receptor channels, is induced best by lower frequencies of tetanic stimulation (e.g. 25 Hz) and is blocked by D-APV but not by nifedipine. The standard tetanus for LTP induction (100 Hz for 1 second) induces both forms of LTP (Cavus and Teyler, 1996; Morgan *et al.*, 2001).

In current work in progress, we are exploring the effects of acute application of 17β-estradiol on both forms of LTP in CA1 hippocampal slices from male rats. Using a 25 Hz tetanus of Schaffer collaterals, 17β-estradiol and nifedipine were infused and extracellular field EPSPs recorded. 17β-estradiol causes the expected increase in synaptic transmission and pronounced enhancement of LTP, but nifedipine has no effect on either process, implying that, under this condition, LTP is mostly dependent on NMDA receptor activation and estrogen facilitates LTP by increasing NMDA receptor-dependent function (Zeng *et al.*, 2004).

Using a 100 Hz tetanus of Schaffer collaterals in the CA1 region of hippocampal slices from adult male rats, we have recorded both extracellular field EPSPs and intracellular EPSPs from pyramidal neurons and infused 17β-estradiol and nifedipine. 17β-estradiol alone causes the expected increase in synaptic transmission and pronounced enhancement of LTP, but both effects of 17β-estradiol are reduced in magnitude by nifedipine. Therefore, under this condition, it seems that 17β-estradiol acts by modulating both L-type voltage-gated calcium channels and NMDA receptors. Intracellularly recorded EPSPs in response to paired subthreshold stimuli with a short interstimulus interval (50 msecond) in the presence of 17β-estradiol indicates an increase in EPSP amplitude to both stimuli without changes in the paired-pulse ratio, which strongly supports a postsynaptic origin of the effects of 17β-estradiol (Akopian *et al.*, 2003).

The possibility that 17β-estradiol modulates calcium influx through L-type calcium channels is consistent with the effects of aging on synaptic transmission and plasticity in hippocampus. Thus, aging is associated with enhanced activity of voltage-gated calcium channels in hippocampal CA1 neurons (Campbell *et al.*, 1996), and blocking calcium influx through L-type calcium channels inhibits LTD induction and enhances LTP in aged animals in the CA1 region of hippocampal slices (Norris *et al.*, 1998). Blocking L-type calcium channels in the

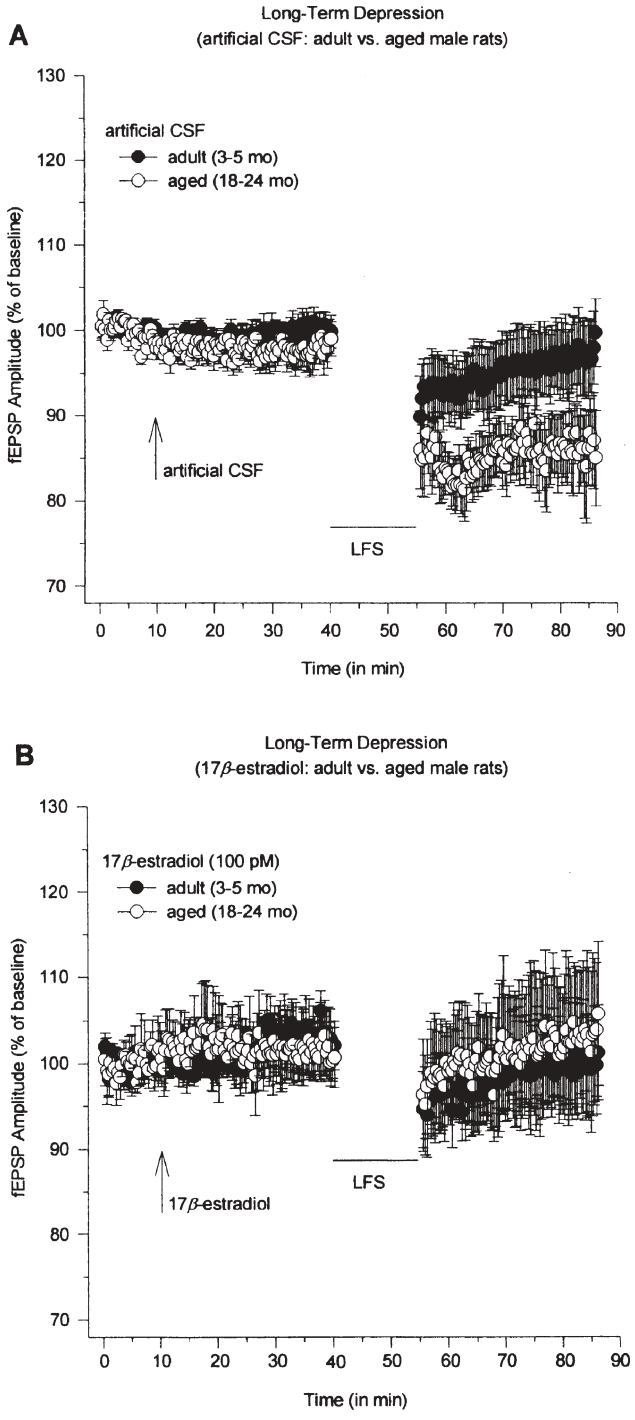

Fig. 8. Long-term depression. (A) Normalized fEPSP amplitudes (percentage of baseline) obtained in slices from adult (filled circles, $n = 11$) and aged (open circles, $n = 8$) rats bathed in normal perfusion media (aCSF). After an initial baseline period, low-frequency stimulation (LFS) induced robust LTD in slices from aged rats, but not adult rats. The horizontal bar indicates when 900 pulses at 1 Hz were delivered. (B) Effect of 17β-estradiol on LTD in slices from aged and adult rats. Before addition of 17β-estradiol, fEPSP amplitudes recorded during the baseline period were similar in both control and experimental groups. LFS delivered to slices from aged rats perfused with 17β-estradiol (open circles, $n = 6$) did not induce robust LTD. Following LFS, slices from adult rats displayed little change in their synaptic response in the presence of 17β-estradiol (filled circles, $n = 6$) compared with their control (aCSF). Reprinted, with permission, from Vouimba *et al.* (2000).

hippocampus is also reported to enhance memory in several paradigms and, particularly, to enhance learning and memory processes in aged animals (Quevedo *et al.*, 1998; Power *et al.*, 2002; Disterhoft *et al.*, 2004).

Estrogen and cellular neuroprotection

In a well-established model of estrogen-induced neuroprotection, primary cultures of dissociated hippocampal neurons are prepared in medium that contains the excitatory amino acid, glutamic acid. Neuronal injury in the cell cultures resulting from glutamate excitotoxicity is assessed by quantifying the release of lactate dehydrogenase into the culture medium. A 5-minute treatment with 100 μM glutamate caused significant cell death compared to control conditions (no glutamate exposure), an effect that decreased significantly following pre-exposure to 17β-estradiol (Nilsen and Brinton, 2002a).

In another *in vitro* study using microfluorimetry and calcium imaging techniques in primary cultures of dissociated hippocampal neurons, 17β-estradiol potentiates the glutamate-induced increase in intracellular calcium by ~70% compared with glutamate exposure alone (Nilsen and Brinton, 2002b). The estrogen enhancement of this glutamate response agrees with other reports showing enhancement of NMDA receptor activation, LTP and memory by estrogens (Foy *et al.*, 1999; Rice *et al.*, 2000). Estrogen-induced neuroprotection against excitotoxic glutamate may involve the mitogen-activated protein (MAP) kinase cascade in primary cultures of cortical neurons (Singer *et al.*, 1999; Nilsen and Brinton, 2003). The impact of estrogen on cellular neuroprotection is specific and dramatic. However, the mechanisms involved in these actions need to be elucidated further to comprehend the complex and indirect ways in which estrogen interacts with cellular signaling pathways.

Molecular mechanisms of the effects of estrogen in brain

Recent results from several laboratories provide a general framework to understand the mechanisms underlying the multiple effects of 17β-estradiol on synaptic structure and function (Lee and McEwen, 2001). At physiological concentrations, 17β-estradiol interacts with estrogen receptors (ERα and ERβ), to produce both direct and indirect genomic effects. The direct genomic effects are due to the interactions between 17β-estradiol and traditional cytoplasmic receptors followed by the regulation of transcription, through interactions with regulatory estrogen response elements of several genes. In neurons, these genes include anti-apoptotic genes of the bcl-2 family, which are probably responsible for the neuroprotective effects of 17β-estradiol observed in a number of models of neuronal death. In astrocytes, genes include GFAP (downregulated) and laminin (upregulated), which might be involved in the sprouting responses observed following lesions, and in normal astrocyte activation observed in brains from old animals (Kohama *et al.*, 1995). The indirect, non-genomic actions of 17β-estradiol are believed to be mediated through plasma membrane-associated ERs, whose actions are associated with the activation of various protein-kinase cascades (Losel and Wehling, 2003). Subsequent 'non-genomic to genomic signaling' pathways of 17β-estradiol's effects might

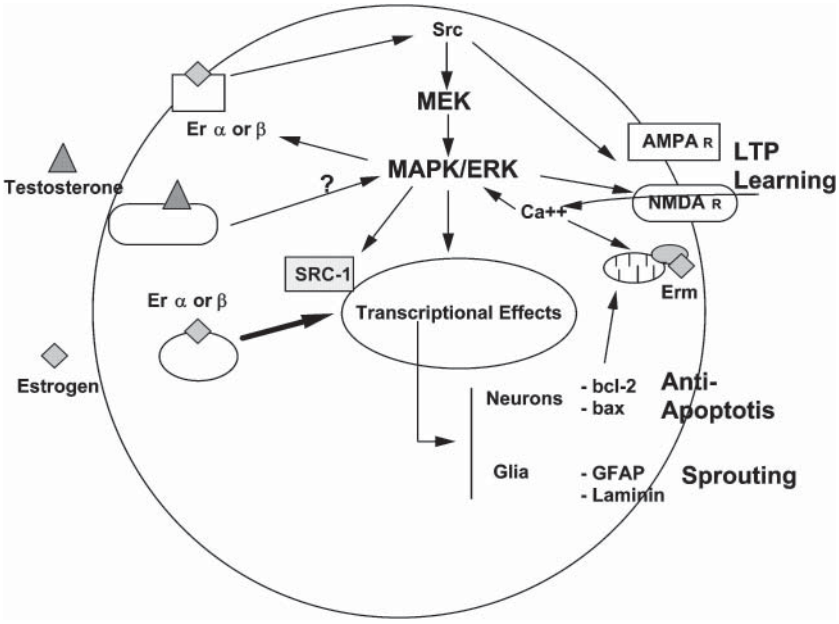

Fig. 9. General hypotheses that link estrogen/testosterone with the MAPK/ERK pathway, NMDA receptors and synaptic plasticity in brain.

be linked to the stimulation of the phosphoinositol-3 (PI_3) kinase/Akt system (Datta *et al.*, 1999; Simoncini *et al.*, 2000) and/or of a G protein, and/or of Src tyrosine kinase and extracellular signal-regulated kinase (ERK)/MAP kinase pathways (reviewed in Bjornstrom and Sjoberg, 2005; Singh *et al.*, 2000).

The MAP kinase pathway occupies a central place in the regulation of synaptic plasticity (Mazzucchelli and Brambilla, 2000; Sweatt, 2001). Pharmacological manipulations directed at blocking this pathway produce consistent impairments in synaptic plasticity, and learning and memory, and this pathway is activated with LTP-inducing tetanus and in different learning paradigms (Brambilla *et al.*, 1997; Berman *et al.*, 1998; Blum *et al.*, 1999; Selcher *et al.*, 1999). We have shown that endogenous estrogen levels in cycling female rats produce a tonic phosphorylation/activation of ERK2/MAP kinase (Bi *et al.*, 2001). In addition, we have shown that this activation of the MAP kinase pathway is linked to the regulation of glutamate ionotropic receptors and might be involved in the 'cognitive enhancing' effects of 17β-estradiol. Indeed, the acute, estrogen-mediated enhancement of LTP is mediated by activation of a src tyrosine kinase pathway (Bi *et al.*, 2000). Thus, acute application of the src inhibitor PP2 in the perfusing medium of hippocampal slices from adult male rats abolishes the estrogen-mediated enhancement of both synaptic transmission and of LTP, but has no effect on LTP itself (Fig. 5). Similarly, this pathway might also be involved in the neuroprotective effects of 17β-estradiol because MAP kinase inhibitors consistently block the neuroprotective effects of 17β-estradiol in several models of neurodegeneration. Moreover, growth factors and other factors providing neuroprotection, such as PDGF, also use the MAP kinase pathway for their neuroprotective effects.

Interestingly, it appears that ERα stimulation is crucial for the neuroprotective effect of estrogen because 17β-estradiol does not protect ERα-knock-out mice against ischemia-induced neuronal damage (Dubal *et al.*, 2001). Furthermore, recent results obtained from the same knock-out mice suggest the possible existence of novel 17β-estradiol receptors that are responsible for the activation of the ERK/MAP kinase pathway (Singh *et al.*, 2000). These results indicate that several steps described in Fig. 9 remain to be elucidated.

Concluding remarks

The studies described here establish several fundamental characteristics of the effects of estrogen on synaptic transmission in the mammalian CNS. Estrogen acts rapidly via presumed membrane mechanisms to enhance both NMDA and AMPA receptor/channel responses elicited by glutamate released from excitatory presynaptic terminals.

17β-estradiol also markedly enhances hippocampal LTP in CA1 neurons of adult, male rats. The enhancement of LTP after acute application of 17β-estradiol is caused by increases in NMDA-receptor and AMPA-receptor functions. Both possibilities are consistent with intracellular data. Changes in estrogen levels in cycling female rats also correlate with changes in synaptic plasticity, measured by changes in LTP magnitude. This finding indicates a mechanism by which natural fluctuations in endogenous hormone levels can impact a cellular model associated with important aspects of learning and/or memory storage in the mammalian CNS.

To the extent that LTP is a mechanism involved in processes of coding and storage of information (i.e. in memory formation), estrogen appears to enhance these processes. Indeed, the estrogen enhancement of LTP indicates a possible mechanism by which estrogen exerts facilitatory effects on memory processes in humans. Clinical evidence indicates that estrogenic steroids enhance cognitive functions in humans, particularly in postmenopausal women (Henderson, 1997; Kawas *et al.*, 1997; Henderson, 2000). However, prospective observational studies do not find a protective effect of estrogen on either cognition and the incidence of dementia (Barrett-Conner and Kritz-Silverstein, 1993; Matthews *et al.*, 1999). Understanding the mechanisms that underlie the age-related changes in the

effects of estrogen will provide a significant advance in understanding of the mechanisms that are involved in the decline in cognitive function with aging.

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