Estradiol Modulates Long-Term Synaptic Depression in Female Rat Hippocampus

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Zamani, M. Reza, Nancy L. Desmond, and William B Levy. Estradiol modulates long-term synaptic depression in female rat hippocampus. J Neurophysiol 84: 1800–1808, 2000. Fluctuating estradiol levels in the adult, female rat modify the anatomical and functional organization of the hippocampal CA1 region. When systemic levels of estradiol are low, e.g., on estrus or in ovariectomized (OVX) rats, long-term synaptic potentiation is difficult to induce in vivo. However, little is known about the role of this ovarian hormone in long-term synaptic depression. Using multiple conditioning paradigms, we assess the magnitude of long-term depression (LTD) at CA3-CA1 synapses in vitro from adult, ovariectomized rats as a function of systemic estradiol replacement. In hippocampal slices from control OVX rats with low levels of estradiol, a low-frequency (2 Hz), asynchronous conditioning stimulation protocol does not produce LTD at 1 h postconditioning. However, this same protocol induces robust LTD in slices from estradiol-treated OVX rats. When the conditioning frequency is increased to 4 Hz, slices from both groups of rats show robust LTD in vitro. At an even higher conditioning frequency (10 Hz), the 2-Hz-based observations are reversed; no consistent changes in synaptic transmission are observed in slices from estradiol-treated OVX rats, but those from control rats (OVX + oil) show robust LTD. Thus estradiol reduces the frequency threshold for LTD induction at the CA3-CA1 synapses. Further, regardless of the conditioning frequency employed, where robust LTD is seen, its induction depends on normally functioning N-methyl-D-aspartate (NMDA) receptors during conditioning. The shift in conditioning frequency needed to elicit LTD is consistent with a decrease in NMDA receptor activation with decreasing estradiol levels.

INTRODUCTION

The ovarian hormones, estradiol and progesterone, influence hippocampal physiology and anatomy—and thus behavior—in adult, female rats (see Desmond and Levy 1997; Smith 1994; Woolley 1999 for reviews). Cellular models of memory, such as long-term potentiation (LTP) and homosynaptic long-term depression (LTD), can be expressed at CA3-CA1 synapses in male rats and in female rats when estradiol levels are high [e.g., on proestrus and in estradiol-treated, ovariectomized (OVX + E2) animals]. However, using the same conditioning parameters, LTP and LTD are rarely induced in animals with low levels of estradiol (LTP: Good et al. 1999; Montoya and Carrer 1997; Warren et al. 1995; LTD: Desmond et al. 2000; but see Good et al. 1999). Such observations have been interpreted to suggest that synapses in CA1 are less modifiable when estradiol levels are low (but see Desmond et al. 2000). However, there are other possibilities.

We previously (Desmond et al. 2000) suggested that the frequency dependence of plasticity may shift as a function of estradiol level rather than there being an outright change in synaptic modifiability per se. Studies of LTP and LTD at the CA3-CA1 synapses of developing, adult, and aging male rats hint at just such a possibility. For example, the same conditioning protocol may have different outcomes at various developmental stages (Bliss and Collingridge 1993; Dudek and Bear 1992; Errington et al. 1995; Norris et al. 1998). Such differences may arise from altered activation of N-methyl-D-aspartate (NMDA) receptors or other processes that control intracellular Ca2+.

By systematically varying the frequency of conditioning stimulation here, we establish that robust LTD can be reliably induced in CA1 in vitro when estradiol levels are low or, equivalently, that estradiol lowers the frequency threshold for the induction of LTD. However, regardless of estradiol level, NMDA receptor activation is critical for LTD induction. Portions of this work have been reported in abstract form (Zamani et al. 1998, 1999).

METHODS

Preparation of hippocampal slices

Adult, Sprague-Dawley male and ovariectomized female rats (200–350 g, Charles River) were housed in groups on a 14:10 light:dark cycle. They were handled daily to reduce the level of stress during injection. Six to 10 days after surgery, female rats were injected subcutaneously with either 17β-estradiol benzoate (E2, 10 μg in 0.1 ml sesame oil, referred to as OVX + E2) or vehicle alone (referred to as OVX + oil). The injection regime was repeated 24 h later. Forty-eight hours after the second injection, rats were anesthetized with pentobarbital sodium (~50 mg/kg body wt) in the vivarium before being euthanized.

After rats were decapitated, their brains were removed and placed in ice-cold, artificial cerebrospinal fluid (ACSF). The brain was dissected and transferred to a Vibratome containing gassed, ice-cold ACSF. Coronal hippocampal slices (400-μm thick) with surrounding cortical tissue were prepared and transferred to a preperfusion chamber where they were continuously gassed for at least 30 min at room temperature for recovery. Slices (mid septotemporal) were then transferred to an interface recording chamber (Medical Systems, BSC-BU) and incubated at 35°C under humidified CO2:O2 (9:5) gas. The

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CA3-CA1 intersection was cut to prevent any propagation of recurrent activity from CA3. The slices were perfused (2 ml/min) with ACSF (35°C), which consisted of, in mM, 127 NaCl, 2 KCl, 2 CaCl₂, 1.5 MgSO₄, 26 NaHCO₃, 1.1 KH₂PO₄, and 10 d-glucose.

In the case of the female rats, the uterus was inspected to confirm the surgical and injection histories of each animal; if the diameter of the uterus was not appropriate for the hormone treatment, data from that animal were excluded. Serum estradiol levels were assayed using the 3rd Generation Estradiol RIA kit (Diagnostic Systems Laboratories) with a sensitivity of 0.60 pg/ml.

Electrophysiological techniques

Electrical stimulation (100 μs, constant current square pulses using WPI 305 stimulus isolation units) of afferents to CA1 pyramidal cells was delivered through twisted, bipolar electrodes (25- or 50-μm-diam tungsten) placed in stratum radiatum 200–400 μm from the recording pipette. The stimulus intensities were initially adjusted to give a test population excitation postsynaptic potential (pEPSP) that was 60–75% of the population spike threshold. This current ranged from 50 to 100 μA.

Extracellular recordings were made through high-impedance preamplifiers (WPI 707 and Axon Instruments Axoclamp-2A). Recording electrodes were made from borosilicate micropipettes (about 5 MΩ) that were filled with ACSF and placed in CA1 s. radiatum. Synaptic responses were monitored at 0.017 Hz for each stimulus pathway. Potentials were band-pass filtered (1 Hz to 5 kHz; WPI LPF-30), displayed on-line by oscilloscopes (Hameg HM 205–3), digitized at 20 kHz and stored in a microcomputer (IBM-PC compatible) for off-line analysis. Data acquisition and analysis (slopes and amplitudes) used in-house software.

Intracellular recording was achieved by impaling CA1 pyramidal neurons using sharp (75–120 MΩ) electrodes filled with potassium methylsulfate (2.5 M). Responses were amplified by an Axoclamp-2A amplifier in bridge mode. In general, a stable input resistance of not less than 25 MΩ, a membrane resting potential of −60 mV or more negative, and an action potential of at least 75 mV were required before the start of an experiment. Responses normally stabilized about 10–20 min after impalement. The input resistance, membrane resting potential, and size of the action potential were frequently assessed throughout the experiment. For on-line oscillographic observation, EPSPs and action potentials were filtered at 5 and 30 kHz, respectively. To measure the input resistance, 0.2- to 0.5-nA hyperpolarizing currents lasting 100 ms were delivered to the impaled neuron every minute, or before each stimulation, and the input resistance was estimated by observing the responses on an oscilloscope in DC-coupled mode.

Conditioning paradigms

Over the last decade, it has become clear that many variables can modulate the induction of homosynaptic LTD at hippocampal synapses. Perhaps not surprisingly, age of the animal strongly influences the experimental conditions required to induce LTD, with LTD being more easily induced in immature hippocampus (Dudek and Bear 1992, 1993; Fujii et al. 1991; Mulkey and Malenka 1992). In some laboratories (e.g., Doyle et al. 1997; Errington et al. 1995; Staubli and Ji 1996; Wagner and Alger 1995; Levy, unpublished in vitro observations), single-pulse, low-frequency conditioning does not reliably induce LTD in adult rats. Other variables are also likely to contribute to paradigmatic differences among laboratories, including strain differences (Manahan-Vaughan and Braunewell 1999), sex differences (Maren et al. 1994), divalent cation concentrations in the ACSF, stress (Kim et al. 1996), and ambient levels of neuromodulators [e.g., acetylcholine (Huerta and Lisman 1996; Kirkwood et al. 1999) and norepinephrine (Kirkwood et al. 1999)].

In the present set of experiments, the age and sex of the animals are determined by our interest in estradiol-dependent changes in synaptic modification in mature animals. The only other major variables are the constituents of the bathing medium and bath temperature. These were chosen to be as simple and as consistent with the literature as possible. Finally, because we found that the 2 Hz paired-pulse, low-frequency conditioning paradigm of Otani and Connor (1995) reliably induced LTD in our laboratory, we initially adopted this conditioning paradigm in the present studies.

Three low-frequency conditioning paradigms were used to evaluate LTD at the CA3-CA1 synapses from females. The stimulation frequency was 2, 4, or 10 Hz. Two of these protocols used paired-pulse low-frequency stimulation (ppLFS), the 2- and 4-Hz paradigms. Each bout in the 2- and 4-Hz ppLFS paradigms consisted of 900 trains of asynchronous paired pulses applied through two (unless otherwise indicated) independent stimulators (S1 and S2) positioned on either side of the recording pipette in CA1 s. radiatum. The S1-S2 interpulse interval was 150 ms, and each paired-pulse-train occurred at either 2 or 4 Hz. Prior to conditioning, the independence of S1 and S2 was assessed using an 80-ms paired-pulse test; if paired-pulse facilitation occurred, the stimulators were repositioned. The third protocol, the 10-Hz paradigm, consisted of 1,800 single pulses delivered via one pathway at 10 Hz. To achieve asymptotic LTD, each paradigm consisted of three bouts of conditioning at test intensity that were separated by 10-min intervals of test stimulation. Note that the total number of pulses was identical in all three conditioning paradigms.

High-frequency conditioning stimulation (HFS), consisting of 10 trains of five pulses delivered at 100 Hz (200-ms intertrain interval), was applied after obtaining stable baseline responses. The conditioning stimulation was delivered at the test intensity.

In light of the novel forms of LTD-inducing paradigms reported here (i.e., 10 Hz in OVX + oil rats and 4 Hz in both groups of rats) and the differential functionality of NMDA receptors reported in CA1 of OVX + E₂ and OVX + oil rats (Woolley et al. 1997), we examined the involvement of NMDA receptors in all three paradigms but only using those groups that exhibited robust LTD by any one of the aforementioned paradigms. The aim of these experiments was two-fold: to document that this LTD involves normal synaptic transmission and to assess the extent to which NMDA receptor activation contributes to the induction of LTD in CA1 from female rats with low or high estradiol levels. (±)-2-Amino-5-phosphonopentanoic acid (APV), a specific NMDA receptor antagonist, was generally added to the bathing solution 20 min after a stable baseline was obtained. Twenty minutes later, slices were conditioned using one of the preceding paradigms. APV was washed out 10 min after the end of the conditioning, and test responses were collected for a further 50 min. In some cases, an alternative method was used to ensure the absence of APV in the bathing medium. Here, two adjacent slices were conditioned using either the 2- or the 4-Hz paradigm. One slice was used as a control while the other was incubated in APV from 20 min before to 1 h after conditioning. In some experiments, control slices were tested after those exposed to APV to eliminate any possible order effect.

In all experiments, a stable baseline was obtained prior to conditioning, and test responses were recorded for 1 h after the last set of trains. The responses during the last 10 min postconditioning were compared with the baseline test responses and are presented here as the mean percent change (±SE). Thus, in the RESULTS, mean values greater than 100% indicate potentiation, and values less than 100% indicate depression. For field responses, peak pEPSP amplitude was measured; for intracellular responses, the early slope of the EPSPs was measured. Statistical assessments (Student’s t-test) were carried out on these averaged responses, with P < 0.05 needed to achieve statistical significance.

Drug application

Where indicated, APV (100 μM, Sigma) was bath applied.
Estradiol levels from a subset of animals were 13.4 ± 0.5 pg/ml for males \((n = 4)\), 14.8 ± 1.0 pg/ml for OVX + oil animals \((n = 14)\), and 39.1 ± 9.7 pg/ml for OVX + E2 animals \((n = 10)\) at the time they were euthanized. On the day of the experiment therefore, the estradiol concentrations in serum of OVX rats were comparable to those of corresponding cycling rats (Smith 1994).

**Estradiol enhances LTD in female rats**

Immediately after 2-Hz ppLFS conditioning, there was a transient depression in CA1 from both groups of female rats. pEPSP amplitudes decreased to 65 ± 2% and 74 ± 1% of their preconditioning baseline values in OVX + oil rats and OVX + oil rats, respectively, during the first 10 min after the third bout of stimulation.

The long-term effect of 2-Hz ppLFS conditioning, however, differed between the two groups of female rats. In E2-treated OVX rats, 2-Hz ppLFS induced a statistically significant and long-lasting synaptic depression in both the S1 and S2 pathways in s. radiatum (Fig. 1). On average, by 60-min postconditioning, the responses had decreased to 78.6 ± 2.6% \((S1)\) and 69.6 ± 5.6% \((S2)\) of the preconditioning baseline \((P < 0.05, n = 8)\). In contrast, in CA1 of OVX + oil rats, only a small decrease remained 1 h after conditioning \([95.4 ± 4\% (S1) and 92.1 ± 1.9\% (S2) of baseline, \(P < 0.05, n = 10\), Fig. 1\]. These results confirm our earlier report (Desmond et al. 2000) and suggest, as have previous in vivo studies of LTP (Good et al. 1999; Montoya and Carrer 1997; Warren et al. 1995), that estradiol enhances long-term plasticity in CA1 of female rats. Thus elevated estradiol levels might seem to lead to greater LTD in vitro.

In agreement with Otani and Connor (1995), this 2-Hz ppLFS conditioning protocol led to significant LTD at 60-min postconditioning in CA1 from adult, male rats \((70.3 ± 4.1\% of the S2 baseline response, \(n = 3\), data not shown). High-frequency conditioning 1 h after 2-Hz ppLFS conditioning induced significant synaptic potentiation in both groups of rats as measured 1 h after HFS \((167.5 ± 3.5\% for OVX + E2 and 155 ± 16\% for OVX + oil, see Fig. 7). Higher conditioning frequencies can induce LTD in CA1 from OVX + oil rats

If estradiol enhances synaptic modifiability by increasing NMDA receptor function (Woolley et al. 1997), higher frequency stimulation might compensate for the lower NMDA receptor function in OVX + oil animals. That is, by virtue of greater depolarization at higher frequencies and the voltage requirement for NMDA receptor activation (Mayer et al. 1984; Nowak et al. 1984), higher conditioning frequencies may help increase calcium influx through NMDA receptors in oil-treated OVX animals and thereby enable the induction of robust LTD in CA1.

In agreement with the preceding hypothesis, 4-Hz ppLFS conditioning resulted in a statistically significant synaptic depression in CA1 from both OVX + oil and OVX + E2 rats (Fig. 2). In OVX + oil rats, the responses decreased to 78.8 ± 1.8% and 77.3 ± 1.8% of baseline \((n = 8, P < 0.05)\) in the two conditioned pathways measured 1 h postconditioning. As shown in Fig. 2, this protocol also induced significant LTD in CA1 from OVX + E2 animals \((75.2 ± 7\% and 74.5 ± 6\% of the S1 and S2 baseline responses, respectively, \(n = 4, P < 0.05)\). Thus given the appropriate conditions, oil-treated OVX rats can express LTD that is very similar in magnitude to that in estradiol-treated OVX rats.

To determine whether even larger synaptic changes can be induced in CA1, a higher \((10 \text{ Hz})\) conditioning frequency was employed. In this case, the two female groups differed (see Fig. 2). A significant decrease in synaptic responses was observed 1 h after 10-Hz conditioning in the OVX + oil group \((78.6 ± 2.7\%, n = 7)\) but not in the OVX + E2 group \((92.2 ± 5.6\%, n = 8)\). In addition, no significant changes were seen in the

**RESULTS**

**Serum estradiol measurement**

The effectiveness of the estradiol treatments was confirmed. Estradiol levels from a subset of animals were 13.4 ± 0.5 pg/ml for males \((n = 4)\), 14.8 ± 1.0 pg/ml for OVX + oil animals \((n = 14)\), and 39.1 ± 9.7 pg/ml for OVX + E2 animals \((n = 10)\) at the time they were euthanized. On the day of the experiment therefore, the estradiol concentrations in serum of OVX rats were comparable to those of corresponding cycling rats (Smith 1994).

**FIG. 1.** Estradiol facilitates long-term depression in ovariectomized (OVX) rats. 2-Hz paired-pulse low-frequency stimulation (solid bars) induces significant synaptic depression in CA1 from estradiol-treated OVX (OVX + E2) rats (filled square, \(n = 8\)). The same conditioning protocol, however, induces significantly less depression in CA1 from OVX + oil rats (open circle, \(n = 10\)). The timeline shows the normalized peak population excitatory postsynaptic potential (pEPSP) amplitudes for 1 (S2) of the 2 independent pathways for each group. Similar results were obtained from the other conditioned pathway (see text). Each waveform is the average of 10 pEPSPs taken immediately before conditioning (1 and 2) or 50–60 min after conditioning (3 and 4).

**FIG. 2.** Estradiol modulates the frequency threshold for induction of long-term depression (LTD) in CA1. Two- and 4-Hz ppLFS each induce significant LTD in OVX + E2 animals while 4-Hz ppLFS and 10-Hz conditioning each induce significant LTD in OVX + oil animals. Note the marked difference in the magnitude of the synaptic depression induced by 2-Hz ppLFS and by 10-Hz conditioning between the OVX + E2 and the OVX + oil groups. * significant change in response amplitude 1 h after conditioning \((P < 0.05)\).
control pathway (102.1 ± 3.6% in CA1 of the OVX + E₂ and 94.0 ± 5.7% in CA1 of the OVX + oil groups). Thus rather than altering the occurrence of LTD, estradiol alters the range of stimulation frequencies capable of inducing LTD.

Concerning the mechanism of this shifted frequency dependence, we found that higher conditioning frequencies enhanced the synaptic responses during conditioning. Compared to a single test response, the pEPSP amplitude on the 20th conditioning pulse in CA1 s. radiatum of OVX + E₂ animals was increased to 139 ± 1.8% using 2-Hz, 149 ± 5.9% using 4-Hz, and 166 ± 7.8% using 10-Hz conditioning, respectively (n = 5 each, 2 and 4 Hz; n = 8, 10 Hz, see Fig. 3). Similar results were obtained from CA1 of OVX + oil rats (Fig. 3) and in intracellular observations. Thus as we postulated above, greater synthetically induced depolarization occurs during conditioning at higher frequencies.

**NMDA receptor activation is required for LTD induction in CA1 of female rats**

To confirm a role for NMDA receptors in LTD, we compared the effect of conditioning at each frequency in the presence of APV with that in its absence. Intracellular recording indicated that, on average, the resting membrane potentials from CA1 pyramidal cells were similar in oil- and E₂-treated animals (range, −66 to −85 mV; median, −76 mV for the OVX + oil group and −79 mV for the OVX + E₂ group). In CA1 from OVX + E₂ rats, APV alone did not significantly change membrane properties or fast synaptic responses.

In the presence of APV, no significant LTD was seen in either the S1 or S2 pathway 1 h after 2-Hz ppLFS in CA1 from estradiol-treated OVX rats (Fig. 4; 94.0 ± 2 and 94.0 ± 5% for the conditioned and unconditioned pathways, respectively; n = 4). After APV washout, 2-Hz ppLFS delivered to the same pathway led to significant homosynaptic LTD of the conditioned pathway (77.7 ± 2.8%, P < 0.05) and a small depression in the control pathway (89.4 ± 2.7%, P < 0.05). Results from simultaneous field recordings (data not shown) agreed closely with our intracellular observations.

The size of the short-term depression correlated with the size of LTD in CA1 from OVX + E₂ rats. During the first 10 min after conditioning in the presence of APV, the pEPSP amplitude only decreased to 84.0 ± 4% of its baseline value. In the absence of APV, however, the same conditioning resulted in a large decrement in pEPSP amplitude (49 ± 7% of baseline) during the first 10 min after conditioning.

The smaller magnitude LTD observed in the APV-washout experiments in OVX + E₂ rats (cf. Figs. 4 and 1) might reflect the continued presence of a small amount of APV in the interface chamber. To assess this notion, the same experiment was performed using two slices from each hippocampus, one conditioned in the presence of APV and the other in its absence. When APV was not present, 2-Hz ppLFS led to significant LTD (71.7 ± 1.3%, n = 3, field recording), similar to the results illustrated in Fig. 1. In parallel slices conditioned in the presence of APV, no significant LTD was observed (95.0 ± 5%, n = 3). In sum, LTD induced by 2-Hz ppLFS in OVX + E₂ rats requires NMDA receptor activation during conditioning.

The NMDA receptor dependence of 4-Hz-induced LTD was tested in CA1 from both estradiol- and oil-treated OVX animals using the parallel slice method. As shown in Fig. 5, LTD induced by 4-Hz ppLFS depended on the activation of NMDA receptors during conditioning in CA1 of both estradiol- and oil-treated OVX animals. When conditioned in the presence of APV, synaptic responses of OVX + oil animals were essentially unaltered 1 h after conditioning (98.1 ± 5 and 98.7 ± 1% for the conditioned and control pathways, respectively; n = 4, Fig. 5A). Conditioning parallel slices of OVX + oil hippocampi in the absence of APV induced significant LTD (81.4 ± 1.7 and 77.4 ± 1.6% for S1 and S2, respectively, P < 0.05, Fig. 5A). As with the OVX + oil group, 4-Hz-induced LTD in E₂-treated rats also required activation of NMDA receptors during conditioning. Thus in the presence of APV, no significant changes in synaptic responses were observed (104.7 ± 2 and 101.4 ± 1% for S1 and S2, respectively, n = 4, Fig. 5B). However, when APV was absent, a significant LTD was seen in parallel slices from the same hippocampi.
(75.2 ± 6.6 and 74.5 ± 6.3% for S1 and S2, respectively, Fig. 5A). Figure 5, A and B, shows representative EPSPs of intracellular recordings in control slices exhibiting 4-Hz-induced LTD. CA1 from both OVX + oil (A) and OVX + E2 (B) rats. One hour after the third conditioning bout (▲), significant LTD was observed in CA1 from both OVX + oil and OVX + E2 rats (A and B, ○). In parallel slices, APV (100 μM) was added to the bathing medium 20 min before conditioning (▼), and test responses were collected for 1 h postconditioning in the presence of APV (A and B, ●). Representative timelines for the S2 pathway from each group are shown in A and B. Similar results were observed in the S1 pathway (see RESULTS). The negative-going waveforms are average pEPSPs of the last 10 responses before (1 and 3) and 1 h after (2 and 4) conditioning, as indicated. Representative intracellular waveforms in A and B are from CA1 conditioned in the absence of APV. C: summary of the results of the field EPSP experiments (n = 9 and 4 for OVX + oil and OVX + E2, respectively).

Thus at all conditioning frequencies where LTD could be induced, and regardless of the circulating estradiol level, LTD in CA1 from female rats involves synaptic transmission and requires NMDA receptors during conditioning.

**DISCUSSION**

Our experimental results indicate that the level of estradiol alters the frequency threshold for the induction of LTD in CA1 from female rats. Thus depending on the recent estradiol history of an animal, different conditioning frequencies may be required to induce the same degree of synaptic depression (or synaptic potentiation) in the hippocampal CA1 region. Moreover, under the basal levels of inhibition found in vitro, the induction of homosynaptic LTD requires NMDA receptor activation during conditioning regardless of the frequency of conditioning stimulation employed and regardless of estradiol-dependent alterations of NMDA receptor function (Woolley et al. 1997). We discuss these results in terms of the modulation of synaptic excitation in CA1 by estradiol, possible underlying mechanisms, the relation of these data to theories of synaptic
modification, and the potential behavioral significance of hormonal modulation of synaptic modifiability.

Estradiol modulates the induction of LTD

Schaffer collateral/commissural-CA1 synapses from female rats are modifiable regardless of the level of circulating estradiol. However, the parametric sensitivities of CA3-CA1 synaptic modification do differ as a function of estradiol level. This difference is of particular interest if we conceptualize synaptic modification as a continuum over which the sign and magnitude vary with the frequency of conditioning stimulation (see Fig. 7). As depicted in Fig. 7, long-term synaptic modification does not occur in response to 1-Hz paired-pulse conditioning stimulation in CA1 in vitro for either oil- or estradiol-treated OVX rats. By appropriately increasing the conditioning frequency while holding pulse number constant, LTD occurs. CA3-CA1 synapses from OVX + oil rats show robust LTD to both 4- and 10-Hz conditioning protocols. Estradiol-treated OVX animals show a different frequency dependence. Compared to oil-treated OVX controls, estradiol pretreatment shifts this frequency dependence to the left; robust LTD is observed with 2- and 4-Hz paired-pulse conditioning (see also Desmond et al. 2000) but not with 10-Hz conditioning. Finally, in agreement with our recent report (Desmond et al. 2000), CA3-CA1 synapses from both oil- and estradiol-treated animals show significant LTP to 100-Hz conditioning stimulation.

Although the optimal conditioning frequency for the induction of LTD varies with estradiol level, NMDA receptor blockade during conditioning completely prevents its induction at unmanipulated, in vitro levels of inhibition in CA1 from both oil- and E<sub>2</sub>-treated rats. This observation is consistent with a large number of LTD studies in CA1 using male rats (e.g., Dudek and Bear 1992; Gean and Lin 1993; Holland and Wagner 1998; Hrabetova and Sacktor 1996; Mulkey and Malenka 1992; Thiels et al. 1996; Xiao et al. 1995; but see Kemp and Bashir 1997; Otani and Connor 1995; Yang et al. 1994). Thus despite the differing parametric sensitivities for synaptic modification in vitro, the induction of LTD requires activation of NMDA receptors regardless of estradiol level.

The involvement of NMDA receptors suggests two conclusions. First, conditioning stimulation does not pathologically

FIG. 6. Ten-hertz-mediated LTD in CA1 from oil-treated OVX rats requires NMDA receptor activation during conditioning. A: normalized EPSP slopes of the conditioned pathway across time. APV (100 μM) was added to the bathing medium (solid bar) once stable intracellular EPSPs were obtained from CA1 pyramidal cells. Ten-hertz conditioning (a) was then delivered to 1 pathway in s. radiatum. APV alone did not affect the synaptic response, and conditioning in the presence of APV did not lead to LTD. After APV washout, a second set of conditioning bouts (a) delivered to the same pathway led to significant LTD (n = 4, P < 0.05). The superimposed intracellular waveforms are averages of 10 EPSPs recorded before and after the indicated set of conditioning bouts. B: summary of the percent change in EPSP slope (mean ± SE) after conditioning in the presence of APV and after washout.

FIG. 7. Estradiol lowers the threshold for LTD and LTP induction. Long-lasting changes in synaptic transmission are induced using various conditioning protocols (1, 2, 4, 10, and 100 Hz) in 2 groups of adult, OVX rats. Estradiol-treated rats show a lower frequency threshold for both LTD and LTP. LTD does not occur in response to 1-Hz paired-pulse conditioning in either group of adult, OVX rats. Robust LTD is first seen in OVX + E<sub>2</sub> rats with 2-Hz paired-pulse conditioning, but 4-Hz paired-pulse conditioning is required to induce robust LTD in OVX + oil rats. At 10 Hz, on the other hand, there is no significant change in synaptic transmission; intracellular events involved in the induction of LTD and LTP may be equally initiated, resulting in no net change in synaptic transmission. However, LTD occurs in OVX + oil rats following 10-Hz conditioning. HFS (100 Hz, see METHODS) resulted in significant synaptic potentiation in both groups of rats 1 h after conditioning. Curvilinear lines are hypothetical and resemble the Bienenstock et al. (1982) model.
inactivate presynaptic mechanisms involved in synaptic modification. Second, increased intracellular calcium is involved in the production of this LTD induced by low-frequency stimulation.

It is generally acknowledged that postsynaptic calcium plays a pivotal role in modification of the CA3-CA1 synapses (e.g., Lynch et al. 1983; Malenka et al. 1988; Mulkey and Malenka 1992; Yang et al. 1999), including a dual role where it can lead to LTD, LTP, or no change (Bear et al. 1987; Lisman 1989). Moderate but prolonged (Yang et al. 1999) Ca\textsuperscript{2+} entry produces LTD (a); large Ca\textsuperscript{2+} entry produces LTP (b); and no change results either when too little Ca\textsuperscript{2+} enters to produce LTD or when Ca\textsuperscript{2+} entry straddles (a) and (b) producing LTD and LTD reactions that offset each other. Of course, controlling synaptic modification via postsynaptic intracellular Ca\textsuperscript{2+} is not the exclusive domain of the NMDA receptor (Cavus and Teyler 1996; Christie et al. 1997; Coussens et al. 1997; Kemp and Bashir 1997, 1999; Nicoll et al. 1998; Oliet et al. 1997; Yang et al. 1994).

**Relationship to theories of synaptic modification**

The frequency curve for LTP/LTD (Fig. 7) resembles the curve used to summarize Bienenstock et al.’s (1982) theory of synaptic modification and thus invites consideration of its relevance. Indeed, the most interesting aspect of Bienenstock et al. (1994). Moderate but prolonged (Yang et al. 1999) Ca\textsuperscript{2+} entry produces LTD (a); large Ca\textsuperscript{2+} entry produces LTP (b); and no change results either when too little Ca\textsuperscript{2+} enters to produce LTD or when Ca\textsuperscript{2+} entry straddles (a) and (b) producing LTD and LTD reactions that offset each other. Of course, controlling synaptic modification via postsynaptic intracellular Ca\textsuperscript{2+} is not the exclusive domain of the NMDA receptor (Cavus and Teyler 1996; Christie et al. 1997; Coussens et al. 1997; Kemp and Bashir 1997, 1999; Nicoll et al. 1998; Oliet et al. 1997; Yang et al. 1994).

**Mechanisms underlying estrogen’s modulation of synaptic plasticity**

The experimental paradigm we used, ovariectomy followed by estradiol replacement, only approximates discrete windows of the estrous cycle. In our hands, serum estradiol levels measured on estrus are similar to those in oil-treated OVX rats. Likewise, estradiol levels on proestrus are similar to those in estradiol-treated OVX rats. Thus the present electrophysiological results using OVX rats predict that the optimal frequency for inducing LTD in CA1 will also vary across the naturally occurring estrous cycle. Because we did not replace progesterone in these experiments, however, we are unable to predict how the rapid increase in progesterone levels on proestrus may modulate LTD at the CA3-CA1 synapses.

The estradiol regimen used here in OVX rats is the same as used by others studying estradiol effects on hippocampus (e.g., Woolley et al. 1997) and involves two injections at 24-h intervals with euthanasia 48 h after the second injection. Because of this time frame, we presume that estradiol acts via a genomic pathway and binds to intracellular estrogen receptors (ER), ER\textalpha and/or ER\textbeta. More specifically, we hypothesize that the effects of estradiol are not due to direct action of estradiol in CA1 pyramidal neurons but instead must involve the interplay of excitatory and inhibitory hippocampal circuitry. Multiple observations support this view. First is the anatomical localization of ER\textalpha and ER\textbeta. The pyramidal cells of the dorsal hippocampus (from where slices for our electrophysiological studies are taken) show low levels of mRNA for ER\textalpha and ER\textbeta (Shughrue et al. 1997). Weiland et al. (1997) observe sparse ER-immunolabeling of hippocampal neurons, and these are likely to be interneurons. Second, tissue culture studies (Murphy et al. 1998) indicate that estradiol downregulates glutamic acid decarboxylase in a manner temporally consistent with increased hippocampal excitability after estradiol treatment. Finally, estradiol-dependent increases in CA1 spine density involve NMDA receptor-dependent mechanisms (Woolley and McEwen 1994). Taken together, it seems unlikely that estradiol acts directly on the CA1 pyramidal neurons we subsequently study.

In our view, any event that would enhance calcium influx, such as enhanced pyramidal cell excitability or enhanced NMDA receptor activation, would tend to shift the thresholds for LTP and LTD based on the hypothesis that calcium influx is critical for such plasticity and that it is controlled in a voltage-dependent manner. Two possible mechanisms are: estradiol decreasing inhibition and/or estradiol increasing NMDA receptor activation (Pozzo-Miller et al. 1999; Woolley et al. 1997) to enhance calcium influx. On the other hand, some studies have suggested a rapid, nongenomic pathway for estrogen action in CA1 (Foy et al. 1999; Gu and Moss 1996, 1998; Teyler et al. 1980; Wong and Moss 1994). Most intriguing is the report (Gu et al. 1999) that estradiol rapidly potentiates kainate-induced currents in CA1 from ER\textalpha-knockout mice. Thus although a nongenomic pathway seems less likely to be involved in the modulation of LTP/LTD by estradiol, we cannot discount the possibility that some of the results reported here are due to nongenomic mechanisms.

**Behavioral significance and conclusion**

Estradiol-dependent modulation of hippocampal synaptic plasticity may be significant for hippocampal-dependent behaviors (see also Desmond and Levy 1997). The hippocampus is considered to be an intermediate-term memory system, and an important class of memories depends on a normally func-
tion hippocampus for their subsequent storage in neocortex (Cohen and Eichenbaum 1993). Furthermore, it is well established that emotional content affects the strength of memories, e.g., the normally functioning amygdala can modulate the strength of hippocampal memories (see McGaugh 2000 for review). Perhaps memories formed during periods of fertility, which would be likely to include mating in the wild, have relatively greater significance. For example, if a female is to practice successful polyandry, then she must remember who her former partners were so that she can seek other partners elsewhere (for thoughts on polyandry, see Eberhard 1996).

Higher estradiol levels, such as occur during periods of fertility, may facilitate the erasure of old memories and the storage of stronger and longer-lasting, new memories. In other words, a lower frequency threshold for LTD, as we have observed when estradiol levels are high, could allow the over-writing of old memories and thereby allow more memory space for information acquired during periods of fertility.

In conclusion, our results indicate that synaptic modifiability is not impaired by low levels of estradiol but that the conditions for synaptic modification in CA1 change as estradiol levels fluctuate. With estradiol pretreatment of OVX animals, the optimal conditioning frequency for LTD shifts downward. The frequency-dependent crossover point from LTD to LTP shifts to the left with estradiol pretreatment. Furthermore, although other processes controlling intracellular calcium should not be ignored, the activity of NMDA receptors during conditioning can be necessary for induction of LTD in female rats.

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