ELSEVIER

Brain Research 888 (2001) 356–365

BRAIN RESEARCH INTERACTIVE

www.elsevier.com/locate/bres

Interactive report

Estrogen modulates sexually dimorphic contextual fear conditioning and hippocampal long-term potentiation (LTP) in rats[1]

Rishi R. Gupta[a], Srijan Sen[b], Lisa L. Diepenhorst[a], Charles N. Rudick[a,2], Stephen Maren[a,b,*]

[a]*Department of Psychology, University of Michigan, 525 E. University Ave., Ann Arbor, MI 48109-1109, USA*
[b]*Neuroscience Program, University of Michigan, 525 E. University Ave., Ann Arbor, MI 48109-1109, USA*

Accepted 30 October 2000

Abstract

The present study examined the role of ovarian steroids in contextual fear conditioning and hippocampal synaptic plasticity in female rats. In experiment 1, adult female rats were ovariectomized and submitted to contextual fear conditioning, a procedure in which rats received unsignaled footshock in a novel observation chamber; freezing behavior served as the measure of conditional fear. Ovariectomized female rats froze at levels comparable to male rats, both of which froze significantly more than sham-operated female rats. In experiment 2, estrogen replacement in ovariectomized female rats reduced fear conditioning to a level comparable to that of sham-operated females in experiment 1. In experiment 3, the influence of estrogen on the induction of long-term potentiation (LTP) at perforant path-dentate granule cell synapses in ovariectomized female rats was examined. Estrogen decreased both population spike LTP and EPSP-spike potentiation at perforant path synapses. Taken together, these experiments indicate that ovarian steroids regulate both sexually dimorphic behavior and hippocampal plasticity in a fear-conditioning paradigm.

Theme: Neural basis of behavior

Topic: Learning and memory: systems and functions

Keywords: Estrogen; Fear conditioning; Hippocampus; Long-term potentiation; Rat

1. Introduction

Fear is a psychological construct used to describe the various behavioral and physiological changes that take place when an organism is faced with a threatening situation [16,23,35]. As with many behavioral systems [10,12,51], fear-related behavior exhibits a prominent sexual dimorphism in both rodents [7] and humans [31]. Recently, we reported a reliable sex difference in contextual fear conditioning [43], a form of Pavlovian conditioning in which an unconditional stimulus (US, a footshock) elicits freezing behavior (immobility except for breathing) in the context of US delivery. In this study, male rats exhibited significantly higher levels of contextual freezing than female rats. Interestingly, we also discovered a sex difference in perforant path-dentate granule cell long-term potentiation (LTP) [37]. LTP is an enduring form of synaptic plasticity that has been posited to mediate various forms of learning [41,45], including contextual fear conditioning [25,39]. The positive correlation between hippocampal synaptic plasticity and contextual fear conditioning is consistent with the proposed role of the hippocampus in contextual fear conditioning [5,40].

An interesting question concerns the hormonal factors that regulate sexually dimorphic fear conditioning. Although the influence of gonadal steroids on several behavioral systems has been characterized [61], the involvement of these hormones in fear conditioning is poorly

[1]Published on the World Wide Web on 1 December 2000.

*Corresponding author. Tel.: +1-734-936-6532; fax: +1-734-763-7480.

E-mail address: maren@umich.edu (S. Maren).

[2]Present address: Northwestern University, Department of Neurobiology and Physiology, 2153 N Campus Dr., Evanston, IL 60208-0877, USA.

0006-8993/01/$ – see front matter
PII: S0006-8993(00)03116-4

understood. Recently, Anagnostaras et al. found that castration in adult male rats did not affect the sex difference in either fear conditioning or hippocampal LTP [6]. In contrast, a role for ovarian steroids in fear conditioning has been suggested by a study indicating that contextual fear conditioning in female rats varies across the estrous cycle [44]. Specifically, female rats in proestrus (when plasma levels of estrogen are high) exhibit reliably lower levels of conditional freezing compared to females in estrus (when plasma levels of estrogen are low). Consistent with these results, it has been reported that cycling female rats perform more poorly than ovariectomized female rats in an aversively motivated avoidance learning task [19,24]. Moreover, a preliminary report indicates that exogenous estradiol administration in ovariectomized female rats reduces contextual fear conditioning [4]. These data suggest that ovarian steroids and estrogen, in particular, may be important in regulating fear conditioning in adult female rats.

The possible role for ovarian steroids in contextual fear conditioning is interesting in the light of other work implicating estrogen in the regulation of hippocampal neuronal morphology [63–66] and synaptic plasticity [14,17,26] in female rats. In the present experiments we examined the relationship between ovarian steroids and contextual fear conditioning. We conducted three experiments to examine the hypothesis that estrogen exerts an inhibitory influence on both contextual fear conditioning and perforant path-granule cell LTP in adult female rats. Our results reveal an important role for estrogen in modulating contextual fear conditioning and hippocampal synaptic plasticity in adult female rats.

2. Materials and methods

2.1. Experimental strategy

Three experiments were conducted. In experiment 1, the effect of ovariectomy on contextual fear conditioning in adult female rats was examined to test the hypothesis that ovarian steroids in female rats play a role in sexually dimorphic fear conditioning. We compared three groups of adult rats: (1) ovariectomized females, (2) sham-operated females, and (3) sham-operated males. The latter two groups were included to replicate our previous report of sex differences in contextual fear conditioning [43]. If ovarian steroids influence sex differences in the formation of hippocampal-dependent memories then ovariectomized (OVX) rats should show similar contextual fear conditioning as the sham-operated male rats. The sham-operated females should show lower levels of conditional fear than that in the other groups.

In experiment 2, we examined the effects of estrogen replacement on fear conditioning in OVX rats. We hypothesized that estrogen replacement would counteract the effects of ovariectomy on fear conditioning. Specifically, we predicted that estrogen administration prior to fear conditioning would attenuate the acquisition of contextual fear.

In experiment 3, we used electrophysiological techniques to characterize the influence of estrogen administration on the induction of perforant path-dentate gyrus long-term potentiation (LTP) in ovariectomized female rats. Perforant path LTP has been reported to be sexually dimorphic [37,43] and it was therefore of interest to examine the modulation of this form of synaptic plasticity by ovarian steroids.

2.2. Subjects and surgery

Long–Evans rats were obtained from a commercial supplier (Harlan Sprague–Dawley, Indianapolis, IN). They were individually housed in metal cages located in the vivarium and maintained on a 14:10 h light/dark cycle. Rats had unrestricted access to food and water and were handled daily for 4 days prior to surgery to acclimate the rats to the experimenter. Twenty-five female (150–174 g) and 12 male (175–199 g) were used in experiment 1, 29 female (150–174 g) rats in experiment 2, and 33 female rats (150–174 g) in experiment 3.

In experiments 1–3, rats were anesthetized with methoxyfluorane and were bilaterally ovariectomized; sham-operated males in experiment 1 underwent the same procedure, except that no tissue was removed. Three days after ovariectomy, vaginal lavages were performed on the operated females for 10 days to screen for improperly ovariectomized rats. The presence of cornified epithelial cells in the vaginal smears was evaluated, and a conservative criterion was used for inclusion of animals in the experiments. Sham-operated females and sham-operated males received mock swabs during the 10-day screening period to equate for handling. Sham-operated (randomly cycling) females were used as a control in this experiment to replicate the sex difference in fear conditioning observed by Maren et al., who also used randomly cycling females and intact males [43].

2.3. Hormone administration

In experiments 2 and 3, rats were administered two successive hormone treatments 48 h and 4 h before conditioning (experiment 2) or electrophysiological testing (experiment 3). This injection pattern was chosen to recapitulate the hormonal pattern of normally cycling rats. In experiment 2, the rats were divided into two treatment groups: (1) rats that received peanut oil for both injections (‘oil’ group) and (2) rats that received estradiol benzoate for both injections (‘estrogen’ group). The estradiol administration consisted of a 10-μg dose of estradiol benzoate (Sigma Chemical, St. Louis, MO) dissolved in 0.1

ml of peanut oil and injected subcutaneously in the nape of the neck.

2.4. Electrophysiological procedures

Four hours after their final estrogen treatment, rats in experiment 3 were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and supplemented with pentobarbital throughout the surgical procedure (0.05–0.15 ml) as needed. After inducing surgical anesthesia, each rat was mounted on a stereotaxic frame (David Kopf Instruments, Tujunga, CA). The scalp was exposed, the head was leveled (bregma and lambda were oriented in the same horizontal plane), and small holes (1-mm diameter) were drilled in the skull for placement of recording and stimulation electrodes and a stainless-steel ground screw. The recording electrodes consisted of an Epoxylite-coated stainless steel insect pin (size 00) with a 50-μm uninsulated tip. The bipolar stimulating electrode consisted of two Epoxylite-coated insect pins with 500-μm tips separated by 1 mm. The recording electrode was implanted in the dentate gyrus (3.3 mm posterior to bregma and 2.4 mm lateral to bregma) and the stimulating electrode was implanted in the ipsilateral perforant path (8.1 mm posterior to bregma, 4.4 mm lateral to bregma). The position of the recording electrode was optimized by using the laminar profile of the perforant path-evoked field potentials as a guide. Body temperature was maintained at 37°C with a heating pad.

Extracellular field potentials evoked by biphasic perforant path stimulation (100-μs/cycle) were amplified (gain=100), bandpass filtered (1 Hz–9 kHz), displayed on an oscilloscope, digitized and written to a disk by a computer (DataWave Technologies, Longmont, CO). The perforant path-evoked field potentials in the dentate gyrus consisted of a characteristic gradual positive-going field excitatory postsynaptic potential (EPSP) with a sharp negative population spike (PS) superimposed on the rising phase of the EPSP (see Fig. 3A). The population EPSP reflects synaptic currents at perforant path-dentate granule cell synapses in *stratum moleculare*, whereas the PS reflects the synchronous action potential discharge of granule cell bodies in *stratum granulosum*.

After a stable hilar field potential was maintained for 10 min, perforant path-evoked field potentials (stimulation current, 140–625 μA, adjusted to elicit a 1-mV population spike) were sampled every 20 s during a 10-min period before and a 60-min period after perforant path high-frequency stimulation (HFS). HFS consisted of 10 pairs of 400-Hz bursts (burst duration=25 ms, 10 pulses per burst, 200 ms interburst interval) delivered at the same current used for baseline recording. Each pair of bursts was separated by 10 s. Averages of the perforant path-evoked field potentials were computed for each 10-min block of the 70-min recording period. The percentage of change in the EPSP slope and the PS amplitude was measured from the pre-HFS baseline and the last 10-min block of the post-HFS recording period. All of the data were analyzed using analysis of variance (ANOVA), and post-hoc comparisons in the form of Fisher least significant difference (LSD) tests were used for comparisons of means following a significant omnibus F-test. All of the data are presented as means±the standard errors of the means (S.E.M.s).

2.5. Behavioral apparatus and procedure

In experiments 1 and 2, eight identical observation chambers (28×21×22 cm; Lafayette Instrument Co., North Lafayette, IN) were used for both conditioning and contextual fear testing. The chambers consisted of aluminum (side walls) and Plexiglas (rear wall, ceiling, and hinged front door). The chambers were situated in chests located in a brightly lit and isolated room. The floor of each chamber consisted of 18 stainless steel rods (4 mm diameter) spaced 1.5 cm apart (center to center). The rods were wired to a shock generator and scrambler (Lafayette Instrument Co.) for the delivery of the footshock USs. Each chamber rested on a load-cell platform and was connected to a computer that monitored each animal's motor activity. The chambers were cleaned with 5% ammonium hydroxide solution, and stainless steel pans containing a thin film of the same solution were placed underneath the grid floors before the rats were placed inside. Ventilation fans in each chest supplied background noise (70 dB, A scale).

On the conditioning day, the rats were transported to the laboratory and placed in the conditioning chambers in squads of eight rats; the chamber positions were counterbalanced for each squad and group. Three minutes after placement in the chambers, the rats received three footshocks (2 s, 1.0 mA) with 60-s intershock intervals. Sixty seconds following the final shock, the rats were returned to their home cages.

Freezing was assessed on the conditioning day during the 60-s periods following each of the three footshocks (i.e. immediate postshock freezing), and normalized by subtracting pre-shock freezing [6]. The extinction test was repeated for 4 consecutive days. In all cases, freezing was assessed using a computerized system as described by Maren [38]. The freezing data were transformed to a percentage of total observations, a probability estimate that allows for analysis with parametric statistics. These probability estimates of freezing were analyzed using ANOVA. If a significant omnibus F-ratio was found, then post-hoc comparisons in the form of Fisher LSD tests were performed. All data are presented as means±S.E.M.s.

3. Results

3.1. Experiment 1: ovariectomy eliminates the sexual dimorphism in contextual fear conditioning

One rat died during surgery and five rats were excluded

due to incomplete ovariectomy, leaving the following group memberships: sham-males ($n=12$), sham-females ($n=11$), and ovariectomized females ($n=8$). Freezing on the conditioning day was averaged across the three post-shock periods and is shown in Fig. 1A. All groups exhibited immediate postshock freezing and the level of postshock freezing was not different between the groups. This observation was confirmed in an ANOVA by a non-significant main effect of group ($F(2,28)<1$, $P=0.55$). Thus, ovariectomy did not affect either the performance of the freezing response or the short-term memory for contextual fear conditioning.

Freezing to the context of the conditioning chamber during the 8-min extinction tests conducted on 4 consecutive test days is shown in Fig. 1B. For clarity, the 8-min means for each of the three groups are shown. All of the groups exhibited robust conditional freezing on the first day of extinction testing. However, the sham-operated female group extinguished their fear more rapidly than the sham-operated male and ovariectomized females. This effect is apparent on days 3 and 4. A two-way ANOVA with factors of test day and group revealed a significant main effect of day ($F(3,84)=37.3$, $P<0.0001$) and a significant group×day interaction ($F(6,84)=3.0$, $P<0.05$). Ovariectomized female rats extinguished at the same rate as males, and both of these groups extinguished more slowly than sham-operated females.

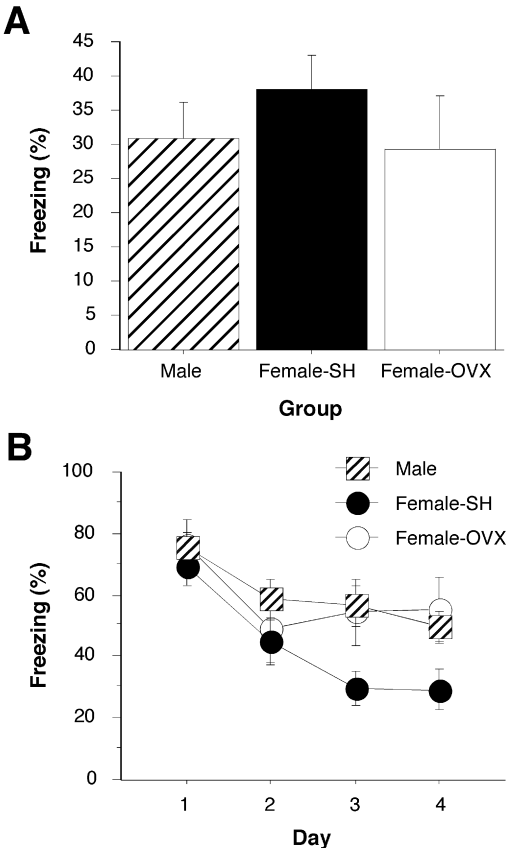

Fig. 1. (A) Mean (±S.E.M.) percentage of freezing on the conditioning day in female rats that received sham surgery (filled), female rats that were ovariectomized (open), and male rats (hatched). The values were normalized to pre-shock freezing and averages across the three 1-min post-shock periods. (B) Mean (+S.E.M.) percentage of freezing collapsed across the 8-min of the context extinction test over the 4 consecutive days of testing for the groups described in (A).

These data suggest that sham-operated female rats exhibit a selective deficit in long-term memory for contextual fear conditioning, insofar as their short-term memory for contextual fear was intact on the conditioning day. Alternatively, it is possible that intact females exhibit more rapid extinction of an equally strong long-term memory. As discussed below, we favor the former interpretation in the light of our previous results [6,43]. We did not examine the relationship between estrous cyclicity in the intact females and contextual fear conditioning [44]. However, as we have previously reported [43], intact female rats exhibit contextual freezing deficits (relative to males) when fear conditioning and testing occur randomly with respect to the estrous cycle.

The present data replicate and extend those of Maren et al., who showed that male rats exhibit higher levels of freezing than randomly cycling female rats after contextual fear conditioning [43]. In Maren et al., however, the sex difference in conditional freezing was detected during the first 8-min extinction test [6,43], whereas it was not apparent until the third extinction test in the present study. It is likely that a ceiling effect contributed to this pattern of results, insofar as conditional freezing during the first two extinction tests was near asymptote. In addition to replicating previous studies [6,43], these data demonstrate that circulating ovarian steroids regulate fear conditioning in adult female rats. Indeed, ovarian steroids appear to exert an inhibitory influence on contextual fear conditioning, insofar as ovariectomy increased conditional freezing during extinction testing. These data are consistent with those from Markus and Zecevic, who showed that contextual fear conditioning in females varied across the estrous cycle [44]. In this study, fear conditioning was maximal during estrus, when estrogen levels are relatively low. Therefore, it seems likely that ovariectomy facilitated contextual fear conditioning in our experiments because it eliminated the inhibitory effect of estrogen on this form of learning. This hypothesis was tested in experiment 2.

3.2. Experiment 2: estrogen treatment attenuates contextual fear conditioning in ovariectomized rats

Two rats died during surgery and 15 rats were excluded due to incomplete ovariectomies leaving the following group memberships: oil ($n=6$) and estrogen ($n=6$). Freezing on the conditioning day is shown in Fig. 2A. Both groups of rats exhibited robust immediate postshock freezing, and the level of freezing did not differ between the groups. This observation was confirmed in an ANOVA by a non-significant main effect of group ($F(1,10)<1$, $P=0.96$). Thus, estrogen treatment did not affect either the

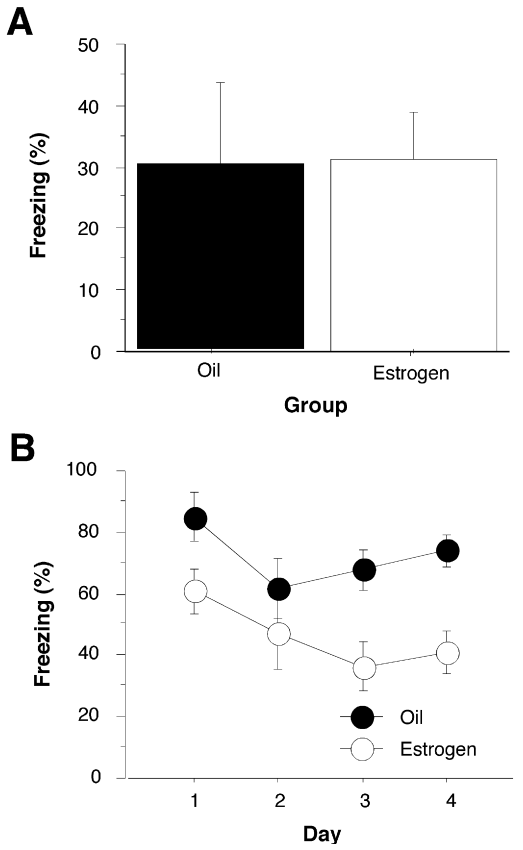

Fig. 2. Mean (±S.E.M.) percentage of freezing on the conditioning day in ovariectomized female rats that received oil (filled) or estrogen (open). The values were normalized to pre-shock freezing and averages across the three 1-min post-shock periods. (B) Mean (+S.E.M.) percentage of freezing collapsed across the 8-min of the context extinction test over the 4 consecutive days of testing for the groups described in (A).

performance of the freezing response or the short-term memory for contextual fear conditioning.

Extinction testing commenced 5 days after training. Freezing to the context of the conditioning chamber during the 8-min extinction tests conducted across the test days is shown in Fig. 2B. As in experiment 1, the 8-min means for each of the three groups are shown. Ovariectomized rats treated with estrogen prior to fear conditioning exhibited less fear conditioning than oil-treated controls. This observation was confirmed by a significant main effect of group ($F(1,10)=10.2$, $P<0.005$) and a significant effect of day ($F(3,30)=3.8$, $P<0.05$). Unlike experiment 1, the difference in freezing behavior was evident during the first extinction test, and there was no difference in extinction rate across the testing days ($F(3,30)<1$, $P=0.51$). These data suggest that pre-training treatment with estrogen attenuates the acquisition of a long-term memory for contextual fear conditioning, a finding that is consistent with a recent report [4]. An alternative possibility is that the reduced freezing in the estrogen-treated rats reflects a state-dependent generalization decrement. That is, rats in this group were tested in a hormonal state that was different from that experienced during training. However, this interpretation seems unlikely insofar as there is little evidence that ovarian hormones yield state-dependent effects in contextual fear conditioning [21].

The results from this experiment suggest that the low levels of freezing in sham-operated female rats in experiment 1 is due to the presence of circulating estrogen at the time of conditioning. That is, estrogen replacement in OVX females reestablishes, at least in part, the pattern of conditioning observed in intact females. Collectively, these data suggest that estrogen may be responsible for the sexual dimorphism in contextual fear conditioning. This is consistent with an earlier report [44]. The stronger conditioning in intact female rats in experiment 1 compared to estrogen-treated OVX rats in experiment 2 may be a function of the estrous cycle stage of the intact females in experiment 1. That is, the intact females in experiment 1 may have been conditioned at a stage of their estrous cycle at which estrogen is relatively low. Alternatively, the large dose of estradiol we used in experiment 2 may have produced higher levels of circulating estrogen than those in intact rats in experiment 1. Nonetheless, the groups are generally comparable, with the exception of the greater levels of freezing in intact females during the initial extinction tests.

3.3. Experiment 3: estrogen treatment reduces hippocampal long-term potentiation in ovariectomized rats

In experiment 3, perforant path synaptic transmission and plasticity was examined in anesthetized rats. Seven rats were not tested due to incomplete ovariectomies, data were not collected from four rats due to technical difficulties, and two rats were excluded based on electrophysiological criteria. This left groups consisting of 10 estrogen-treated females and 10 oil-treated females. Estrogen treatment did not affect baseline synaptic transmission at perforant path-dentate granule cell synapses (see Table 1;

Table 1
Baseline measures of perforant path-evoked hilar field potentials[a]

Measure	Group	
	Oil-treated ($n=10$)	Estrogen-treated ($n=10$)
EPSP slope (mV/ms)	2.00±0.27	2.06±0.34
EPSP latency (ms)	1.80±0.02	1.83±0.03
PS amplitude (mV)	0.98±0.13	1.05±0.11
PS latency (ms)	5.30±0.07	5.35±0.23
FV amplitude (μV)	127±12.8	140±23.0
Stimulation current (μA)	422±55.0	314±54.9

[a] EPSP, excitatory postsynaptic potential; PS, population spike; FV, fiber volley. See Fig. 3A for representative field potentials. All values represent means±S.E.M.

Fig. 3A). Specifically, there was no effect of estrogen treatment on baseline EPSP slope, EPSP latency, PS amplitude, PS latency, or the presynaptic fiber volley (Fs<1, Ps>0.4). Moreover, the average stimulus current required to evoke hilar field potentials did not significantly differ between the groups (Table 1). Normal baseline synaptic transmission after estrogen administration has also been reported in area CA1 in vivo [14].

In contrast to baseline synaptic transmission, estrogen treatment influenced the induction of LTP in the dentate gyrus following high-frequency stimulation of the perforant path. Average waveforms from representative rats in the oil and estrogen groups are shown in Fig. 3A. The percentage of change of both the EPSP slope and PS amplitude of hilar field potentials relative to the 10-min baseline (pre-tetanus) recording is shown in Fig. 3B and C, respectively. The magnitude of EPSP LTP was similar in the estrogen- and the oil-treated groups, and an ANOVA revealed no significant difference between these groups ($F(1,18)=2.3$, $P=0.15$). In contrast, estrogen treatment significantly reduced the magnitude of PS LTP. This observation was confirmed by a significant main effect of group in the ANOVA ($F(1,18)=5.4$, $P<0.05$). EPSP/spike (E/S) LTP, which is the potentiation of the PS beyond which would be expected by potentiation of the synaptic EPSP [2], was also reduced in estrogen-treated females (data not shown, $F(1,18)=6.6$, $P<0.05$). Thus, estrogen treatment did not affect synaptic LTP in the dentate gyrus, but did influence the potentiation of cell excitability manifest by the population spike. This pattern of results is different from that obtained in hippocampal area CA1, where estrogen treatment has been reported to facilitate LTP induction in vivo [14] and in vitro [26], although the latter study was conducted using male brain tissue. In agreement with our earlier results [6,43], these data reveal a positive correlation between hippocampal LTP and contextual fear conditioning. Low levels of conditional freezing and reduced perforant path LTP were observed

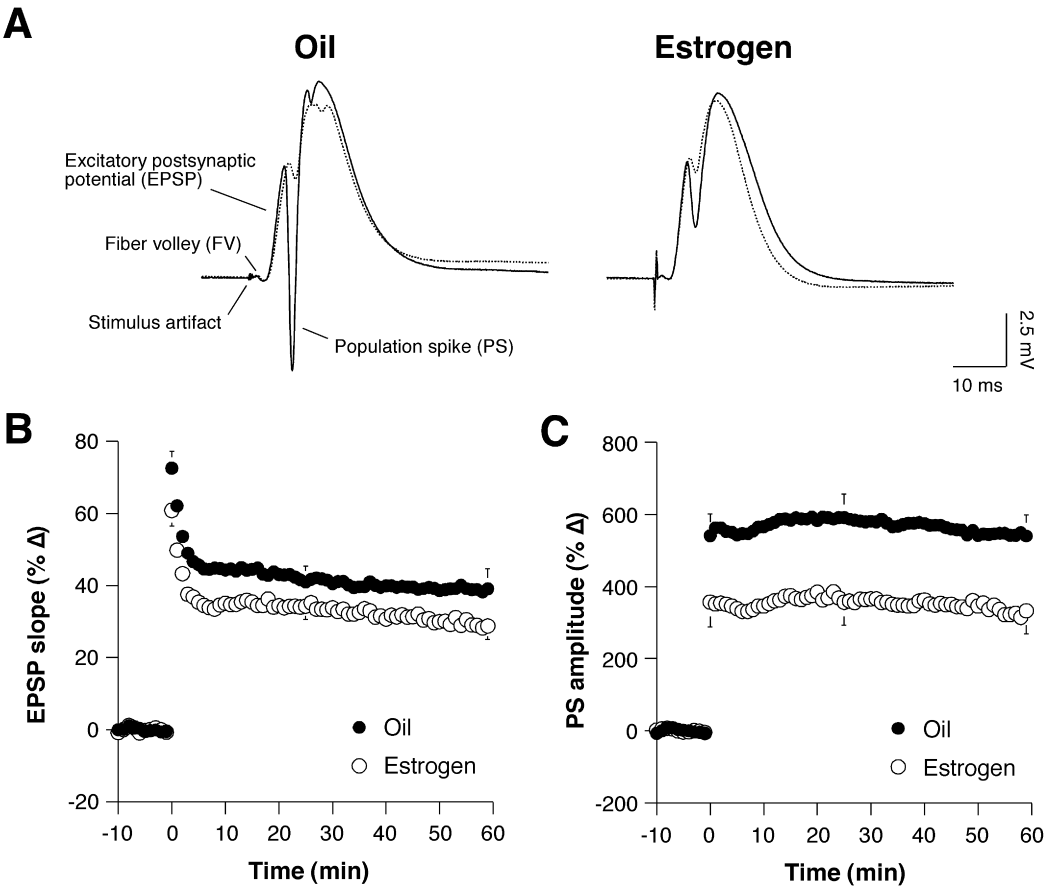

Fig. 3. (A) Representative perforant path-evoked field potentials recorded from the dentate gyrus in anesthetized female rats. Waveforms represent an average of 30 potentials (10 min) collected either before (dotted lines) or 50 min after (solid lines) high-frequency stimulation of the perforant path. Oil or estrogen was administered 4 h prior to the induction of anesthesia. Long-term potentiation of the population spike (PS) was suppressed by estrogen treatment. (B) Mean (±S.E.M.) percentage of change (relative to 10-min baseline) in EPSP slope for the 70-min recording period in ovariectomized female rats that received oil treatment (filled circles) and ovariectomized female rats that received estrogen treatment (open circles). Perforant path HFS was delivered at $t=0$. (C) Mean (±S.E.M.) percentage of change (relative to 10-min baseline) in PS amplitude for the 70-min recording period.

after estrogen treatment. These data are congruent with the hypothesis that perforant path LTP is important for contextual learning and memory.

4. Discussion

The present study examined the role of ovarian steroids in modulating sex differences in fear conditioning and hippocampal LTP. Ovariectomized (OVX) female rats exhibited levels of freezing that were similar to male rats and significantly higher than that in intact female rats. Estrogen treatment in ovariectomized female rats reduced both contextual fear conditioning and hippocampal LTP. Collectively, these data reveal a positive correlation between contextual fear conditioning and hippocampal LTP and suggest that circulating estrogen in female rats modulates both contextual fear conditioning and hippocampal synaptic plasticity.

The role for estrogen in modulating contextual fear conditioning complements a large body of studies indicating that estrogen modulates other forms of hippocampus-dependent learning, such as spatial learning and memory. For example, male rodents typically outperform female rodents in several tasks that assess spatial learning such as the radial arm maze and Morris water maze [60]. A recent study found that ovariectomy in adult female rats eliminated the sex difference in Morris water maze performance, and that estrogen replacement in OVX females reinstated poor performance [27]. Spatial learning and memory are also regulated by the estrous cycle and tend to be poor in females in proestrus, which is associated with high estrogen levels [54] (cf. Ref. [11]). Moreover, spatial learning is seasonally regulated in female deer mice, and tends to be poorer during the breeding season when females are cycling and estrogen levels are high [29]. A similar relationship between plasma estrogen levels and spatial learning has been demonstrated in female meadow voles [28]. In addition to spatial learning, aversively motivated avoidance behavior is also improved by ovariectomy [19,24] and stress appears to impair Pavlovian eyeblink conditioning in female rats through an activational influence of estrogen [62]. In this latter case, however, estrogen in intact females has a beneficial effect on learning. Thus, there appears to be convergent evidence from a number of different behavioral paradigms and species supporting a role for estrogen in the modulation of hippocampus-dependent learning and memory. In many cases, estrogen inhibits hippocampus-dependent learning and memory in female rats [4], although there are recent studies suggesting that estrogen can also facilitate memory under some conditions [46,62].

An important question is whether the deficits in fear conditioning observed in intact, cycling female (experiment 1) and estrogen-treated females (experiment 2) are due to associative deficits, or the result of other factors [7,9]. For example, reduced freezing in the cycling female rats in experiment 1 could be the result of an impact of ovarian steroids on the performance of freezing behavior during the extinction tests, rather than on learning and memory per se. There are several reasons why the low levels of freezing in intact females in experiment 1 are not the result of a freezing performance problem. For example, intact females exhibited normal levels of immediate post-shock freezing on the conditioning day, and also exhibited normal and high levels of conditional freezing during their first extinction test. Moreover, intact female rats exhibited normal and high levels of freezing to an auditory CS paired with footshock [43] (cf. Ref. [49]). Thus, it would appear that intact female rats are capable of normal freezing behavior under some conditions.

In experiment 2, it is possible that lower levels of freezing in estrogen-treated rats were due to reduced footshock sensitivity. However, Drury and Gold found that estrogen increases footshock sensitivity in OVX rats [20]. Therefore, it is not likely that a decrease in US processing accounted for the attenuation of fear conditioning in estrogen-treated rats. Alternatively, a state-dependent generalization decrement from training (hormone present) to testing (hormone absent) may have accounted for the poor performance of estrogen-treated rats. As described earlier, this explanation seems unlikely [21]. For example, the deficits in conditional freezing in female rats conditioned during proestrus are not state-dependent [44]. Nonetheless, further experiments are required to determine whether the deficits in contextual fear conditioning associated with estrogen treatment are state-dependent.

Considering all of the evidence, our data suggest that fear conditioning deficits in cycling females and estrogen-treated OVX females are associative in nature. These deficits do not appear to reflect deficits in either encoding or retrieving fear memories, insofar as immediate post-shock freezing (and freezing during the first extinction test in the case of intact females) is normal in these animals. Moreover, estradiol administration in ovariectomized female rats produces selective impairments in contextual fear conditioning; fear conditioning to auditory CSs is not affected by estradiol administration [4]. Hence, it seems more likely that poor performance in intact and estrogen-treated females reflects an impairment in consolidating a long-term memory for contextual fear. We have proposed that impaired induction of perforant path-granule cell LTP may contribute to this deficit in memory consolidation [43], insofar as the hippocampus has been posited to play a role in the consolidation of long-term contextual fear memories [4]. Consistent with this view, we have found in the present study that estrogen treatment reduces the induction of perforant path LTP in vivo.

The attenuation of perforant path LTP induction by estrogen was not expected in the light of studies of examining the influence of estrogen on synaptic transmission and plasticity in hippocampal area CA1. In area CA1,

for example, estrogen increases both NMDA receptor-mediated synaptic responses [26,68] and LTP induction [14,26] and female rats in proestrus exhibit greater CA1 LTP [30,53]. The reasons for the different effects of estrogen on LTP in area CA1 and the dentate gyrus are not known, although LTP in hippocampal area CA1 and the dentate gyrus are differentially influenced by a number of factors [8,15,32,58,59]. Moreover, estrogen does not modulate the morphology of dentate granule cell neurons as it does in area CA1 [67] and estrogen has opposite effects on NMDA receptor binding in area CA1 and the dentate gyrus [56]. Clearly, further work is required to understand the differential regulation of LTP in the dentate gyrus and hippocampal area CA1 by estrogen, insofar as plasticity in both of these structures has been implicated in contextual fear conditioning.

How might estrogen decrease population spike LTP in the dentate gyrus? Perforant path-granule cell LTP has two physiological components: synaptic LTP and E/S LTP. Synaptic LTP is associated with an increase in the field EPSP, whereas E/S LTP is manifest as an increase in PS amplitude beyond that predicted by synaptic LTP alone [1,13,22]. Kairiss et al. suggested that LTP of a perforant path-inhibitory interneuron synapse might be responsible for E/S LTP in the dentate gyrus [33]. Specifically, reduced GABAergic inhibition in the hippocampus has been specifically correlated with E/S LTP [2,13,33]. Estrogen receptors (ERα) are located on interneurons in the dentate gyrus [55] and estrogen can modulate the number of GABA receptors in the hippocampus [36,48,57]. It is therefore possible that the interaction between estrogen and GABA receptors in the dentate gyrus is responsible for reducing E/S LTP after estrogen treatment. Alternatively, estrogen has been reported to decrease NMDA receptor binding in the dentate gyrus [56], and NMDA receptors are critical for perforant path LTP induction [3,42]. An inhibitory influence of estrogen on NMDA receptor binding in the dentate gyrus could account for both the significant decrease in PS LTP and the modest decrease in synaptic LTP that we observed.

In addition to direct interactions with neurotransmitter receptors involved in LTP induction, estrogen might also act to modulate hippocampal LTP through indirect actions on other hormonal systems. For example, hypothalamo-pituitary-adrenocortical activity is modulated by the estrous cycle and both basal and stress-induced adrenocorticotropic hormone and corticosterone release are enhanced during proestrus [34,52]. Importantly, corticosterone has been demonstrated to play a role in both hippocampal LTP induction [18,47] and contextual fear conditioning [50]. Thus, an avenue for future research would be to examine the interaction of estrogen and corticosterone in the modulation of hippocampal plasticity and contextual fear conditioning in female rats. It is also important to note that the sexual dimorphism in contextual fear conditioning and hippocampal LTP might also involve organizational influences of gonadal hormones [61]. Indeed, it is likely that gonadal steroids have an important role in the organization of neural circuits underlying fear [9], and future studies should consider this mode of hormone action on sexual dimorphisms in fear conditioning and hippocampal synaptic plasticity.

Acknowledgements

The research reported here was supported by a National Institutes of Health grant (R29MH57865) to SM. We would like to thank Dr. Jill Becker for helpful advice concerning the design of these experiments and comments on an earlier version of this manuscript.

References

[1] W.C. Abraham, T.V.P. Bliss, G.V. Goddard, Heterosynaptic changes accompany long-term but not short-term potentiation of the perforant path in the anesthetized rat, J. Physiol. 363 (1985) 335–349.
[2] W.C. Abraham, B. Gustafsson, H. Wigstrom, Long-term potentiation involves enhanced synaptic excitation relative to synaptic inhibition in guinea-pig hippocampus, J. Physiol. 394 (1987) 367–380.
[3] W.C. Abraham, S.E. Mason, Effects of the NMDA receptor/channel antagonists CPP and MK801 on hippocampal field potentials and long-term potentiation in anesthetized rats, Brain Res. 462 (1988) 40–46.
[4] M. Altemus, C.D. Conrad, S. Dolan, B.S. McEwen, Estrogen reduces fear conditioning: differential effects on tone vs. context conditioning, Biol. Psychiatr. 43 (1998) 14S.
[5] S.G. Anagnostaras, G.D. Gale, M.S. Fanselow, The hippocampus and contextual fear conditioning: recent controversies and advances, Hippocampus (2000), In press.
[6] S.G. Anagnostaras, S. Maren, J.P. DeCola, N.I. Lane, G.D. Gale, B.A. Schlinger, M.S. Fanselow, Testicular hormones do not regulate sexually dimorphic Pavlovian fear conditioning or perforant path long-term potentiation in adult male rats, Behav. Brain Res. 92 (1998) 1–9.
[7] J. Archer, Rodent sex differences in emotional and related behavior, Behav. Biol. 14 (1975) 459–479.
[8] C.A. Barnes, B. McNaughton L, G.B. Goddard, R.M. Douglas, K. Adamec, Circadian rhythm of synaptic excitability in rat and monkey central nervous system, Science 197 (1977) 91–92.
[9] W.W. Beatty, Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organizational and activational influences, Horm. Behav. 12 (1979) 112–163.
[10] J.B. Becker, Gender differences in dopaminergic function in striatum and nucleus accumbens, Pharmacol. Biochem. Behav. 64 (1999) 803–812.
[11] B. Berry, R. McMahon, M. Gallagher, Spatial learning and memory at defined points of the estrous cycle: effects of performance of a hippocampal-dependent task, Behav. Neurosci. 111 (1997) 267–274.
[12] S.W. Bottjer, A.P. Arnold, Developmental plasticity in neural circuits for a learned behavior, Annu. Rev. Neurosci. 20 (1997) 459–481.
[13] L.E. Chavez-Noriega, T.V.P. Bliss, J.V. Halliwell, The EPSP-spike (E-S) component of long-term potentiation in the rat hippocampal slice is modulated by GABAergic but not cholinergic mechanisms, Neurosci. Lett. 104 (1989) 58–64.
[14] D.A. Cordoba-Montoya, H.F. Carrer, Estrogen facilitates induction

of long term potentiation in the hippocampus of awake rats, Brain Res. 778 (1997) 430–438.
[15] R.C. Dana, J.L. Martinez Jr., Effect of adrenalectomy on the circadian rhythm of LTP, Brain Res. 308 (1984) 392–395.
[16] M. Davis, Neurobiology of fear responses: the role of the amygdala, J. Neuropsychiatry Clin. Neurosci. 9 (1997) 382–402.
[17] N.L. Desmond, D.X. Zhang, W.B. Levy, Estradiol enhances the induction of homosynaptic long-term depression in the CA1 region of the adult, ovariectomized rat, Neurobiol. Learn. Mem. 73 (2000) 180–187.
[18] D.M. Diamond, M.C. Bennett, M. Fleshner, G.M. Rose, Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation, Hippocampus 2 (1992) 421–430.
[19] G. Diaz-Veliz, V. Soto, N. Dussaubat, S. Mora, Influence of the estrous cycle, ovariectomy and estradiol replacement upon the acquisition of conditioned avoidance responses in rats, Physiol. Behav. 46 (1989) 397–401.
[20] R.A. Drury, R.M. Gold, Differential effects of ovarian hormones on reactivity to electric footshock in the rat, Physiol. Behav. 20 (1978) 187–191.
[21] D.L. Ebner, R. Richardson, D.C. Riccio, Ovarian hormones and retention of learned fear in rats, Behav. Neural Biol. 33 (1981) 45–58.
[22] M.S. Evans, K.E. Viola-McCabe, Midazolam inhibits long-term potentiation through modulation of $GABA_A$ receptors, Neuropharmacology 35 (1996) 347–357.
[23] M.S. Fanselow, Neural organization of the defensive behavior system responsible for fear, Psychon. Bull. Rev. 1 (1994) 429–438.
[24] S.A. Farr, J.F. Flood, J.F. Scherrer, F.E. Kaiser, G.T. Taylor, J.E. Morley, Effect of ovarian steroids on footshock avoidance learning and retention in female mice, Physiol. Behav. 58 (1995) 715–723.
[25] M. Fendt, M.S. Fanselow, The neuroanatomical and neurochemical basis of conditioned fear, Neurosci. Biobehav. Rev. 23 (1999) 743–760.
[26] M.R. Foy, J. Xu, X. Xie, R.D. Brinton, R.F. Thompson, T.W. Berger, 17beta-estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation, J. Neurophysiol. 81 (1999) 925–929.
[27] C.A. Frye, Estrus-associated decrements in water maze task are limited to acquisition, Physiol. Behav. 57 (1995) 5–14.
[28] L.A.M. Galea, M. Kavaliers, K.P. Ossenkopp, E. Hampson, Gonadal hormone levels and spatial learning performance in the Morris water maze in male and female meadow voles, *Microtus pennsylvanicus*, Horm. Behav. 29 (1995) 106–125.
[29] L.A.M. Galea, M. Kavaliers, K.P. Ossenkopp, Sexually dimorphic spatial learning in meadow voles *Microtus pennsylvanicus* and deer mice *Peromyscus maniculatus*, J. Exp. Biol. 199 (1996) 195–200.
[30] M. Good, M. Day, J.L. Muir, Cyclical changes in endogenous levels of oestrogen modulate the induction of LTD and LTP in the hippocampal CA1 region, Eur. J. Neurosci. 11 (1999) 4476–4480.
[31] J.A. Gray, Sex differences in emotional behaviour in mammals including man: endocrine basis, Acta Pscyhol. 35 (1971) 29–46.
[32] K.M. Harris, T.J. Teyler, Age differences in a circadian influence on hippocampal LTP, Brain Res. 261 (1983) 69–73.
[33] E.W. Kairiss, W.C. Abraham, D.K. Bilkey, G.V. Goddard, Field potential evidence for long-term potentiation of feed-forward inhibition in the rat dentate gyrus, Brain Res. 401 (1987) 87–94.
[34] J. Kitay, Effects of various hormones on the pituitary-adrenal axis, in: L.W. Hedlund, J.M. Franz, A.D. Kenny (Eds.), Advances in Experimental Medicine and Biology, Vol. 54, Plenum Press, New York, 1975, pp. 155–167.
[35] J.E. LeDoux, Emotion circuits in the brain, Annu. Rev. Neurosci. 23 (2000) 155–184.
[36] A. Maggi, J. Perez, Estrogen-induced up-regulation of γ-aminobutyric acid receptors in the CNS of rodents, J. Neurochem. 47 (1986) 1793–1797.
[37] S. Maren, Sexually dimorphic perforant path long-term potentiation (LTP) in urethane-anesthetized rats, Neurosci. Lett. 196 (1995) 177–180.
[38] S. Maren, Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala, J. Neurosci. 18 (1998) 3088–3097.
[39] S. Maren, Long-term potentiation in the amygdala: a mechanism for emotional learning and memory, Trends Neurosci. 22 (1999) 561–567.
[40] S. Maren, S.G. Anagnostaras, M.S. Fanselow, The startled seahorse: is the hippocampus necessary for contextual fear conditioning?, Trends Cogn. Sci. 2 (1998) 39–42.
[41] S. Maren, M. Baudry, Properties and mechanisms of long-term synaptic plasticity in the mammalian brain: relationships to learning and memory, Neurobiol. Learn. Mem. 63 (1995) 1–18.
[42] S. Maren, M. Baudry, R.F. Thompson, Effects of the novel NMDA receptor antagonist, CGP 39551, on field potentials and the induction and expression of LTP in the dentate gyrus in vivo, Synapse 11 (1992) 221–228.
[43] S. Maren, B. De Oca, M.S. Fanselow, Sex differences in hippocampal long-term potentiation (LTP) and Pavlovian fear conditioning in rats: positive correlation between LTP and contextual learning, Brain Res. 661 (1994) 25–34.
[44] E.J. Markus, M. Zecevic, Sex differences and estrous cycle changes in hippocampus-dependent fear conditioning, Psychobiology 25 (1997) 246–252.
[45] S.J. Martin, P.D. Grimwood, R.G.M. Morris, Synaptic plasticity and memory, an evaluation of the hypothesis, Annu. Rev. Neurosci. 23 (2000) 649–711.
[46] M.G. Packard, Posttraining estrogen and memory modulation, Horm. Behav. 34 (1998) 126–139.
[47] C. Pavlides, Y. Watanabe, B.S. McEwen, Effects of glucocorticoids on hippocampal long-term potentiation, Hippocampus 3 (1993) 183–192.
[48] J. Perez, I. Zucchi, A. Maggi, Sexual dimorphism in the response of the GABAergic system to estrogen administration, J. Neurochem. 47 (1986) 1798–1803.
[49] C.R. Pryce, J. Lehmann, J. Feldon, Effect of sex on fear conditioning is similar for context and discrete CS in Wistar, Lewis and Fischer rat strains, Pharmacol. Biochem. Behav. 64 (1999) 753–759.
[50] C.R. Pugh, D. Tremblay, M. Fleshner, J.W. Rudy, A selective role for corticosterone in contextual-fear conditioning, Behav. Neurosci. 111 (1997) 503–511.
[51] D.F. Sherry, L.F. Jacobs, S.J. Gaulin, Spatial memory and adaptive specialization of the hippocampus, Trends Neurosci. 15 (1992) 298–303.
[52] V. Viau, M.J. Meaney, Variations in the hypothalamic–pituitary–adrenal response to stress during the estrous cycle in the rat, Endocrinology 129 (1991) 2503–2511.
[53] S.G. Warren, A. Humphreys, J.M. Juraska, W.T. Greenough, LTP varies across the estrous cycle: enhanced synaptic plasticity in proestrus rats, Brain Res. 703 (1995) 26–30.
[54] S.G. Warren, J.M. Juraska, Spatial and nonspatial learning across the rat estrous cycle, Behav. Neurosci. 111 (1997) 259–266.
[55] N.G. Weiland, C. Orikasa, S. Hayashi, B.S. McEwen, Distribution and hormone regulation of estrogen receptor immunoreactive cells in the hippocampus of male and female rats, J. Comp. Neurol. 388 (1997) 603–612.
[56] N.G. Weiland, Estradiol selectively regulates agonist binding sites on the *N*-methyl-D-aspartate receptor complex in the CA1 region of the hippocampus, Endocrinology 131 (1992) 662–668.
[57] N.G. Weiland, Glutamic acid decarboxylase messenger ribonucleic acid is regulated by estradiol and progesterone in the hippocampus, Endocrinology 131 (1992) 2697–2702.
[58] J. Winson, C. Abzug, Gating of neuronal transmission in the hippocampus: efficacy of transmission varies with behavioral state, Science 196 (1977) 1223–1225.

[59] J. Winson, C. Abzug, Neuronal transmission through hippocampal pathways dependent on behavior, J. Neurophysiol. 41 (1978) 716–732.
[60] C.L. Williams, A.M. Barnett, W.H. Meck, Organizational effects of early gonadal secretions on sexual differentiation in spatial memory, Behav. Neurosci. 104 (1990) 84–97.
[61] C.L. Williams, W.H. Meck, The organizational effects of gonadal steroids on sexually dimorphic spatial ability, Psychoneuroendocrinology 16 (1991) 155–176.
[62] G.E. Wood, T.J. Shors, Stress facilitates classical conditioning in males, but impairs classical conditioning in females through activational effects of ovarian hormones, Proc. Natl. Acad. Sci. USA 95 (1998) 4066–4071.
[63] C.S. Woolley, Estrogen-mediated structural and functional synaptic plasticity in the female rat hippocampus, Horm. Behav. 34 (1998) 140–148.
[64] C.S. Woolley, B.S. McEwen, Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat, J. Neurosci. 12 (1992) 2549–2554.
[65] C.S. Woolley, B.S. McEwen, Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat, J. Comp. Neurol. 336 (1993) 293–306.
[66] C.S. Woolley, B.S. McEwen, Estradiol regulates hippocampal dendritic spine density via *N*-methyl-D-aspartate receptor-dependent mechanism, J. Neurosci. 14 (1994) 7680–7687.
[67] C.S. Woolley, E. Gould, M. Frankfurt, B.S. McEwen, Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons, J. Neurosci. 10 (1990) 4035–4039.
[68] C.S. Woolley, N.G. Weiland, B.S. McEwen, P.A. Schwartzkroin, Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density, J. Neurosci. 17 (1997) 1848–1859.