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Research report

Sex differences in hippocampal long-term potentiation (LTP) and Pavlovian fear conditioning in rats: positive correlation between LTP and contextual learning

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Abstract

Three experiments investigated sex differences in hippocampal long-term potentiation (LTP) and Pavlovian fear conditioning in rats. Experiment 1 revealed a robust sex difference in the magnitude of LTP induced at perforant path synapses in the dentate gyrus of pentobarbital-anesthetized rats. This sex difference in LTP was evident in rats of 35 and 60 days of age and was not the result of pre-LTP sex differences in perforant path synaptic transmission; 20-day-old rats did not show LTP. An analysis of field potentials evoked during LTP induction revealed a sex difference in the magnitude of *N*-methyl-D-aspartate (NMDA) receptor activation that was highly correlated with the magnitude of LTP. Experiment 2 showed that males condition more fear, measured as freezing, to the contextual conditional stimuli (CSs) of a conditioning chamber compared to their female counterparts. This sex difference in conditional freezing was apparent with both low and high unconditional stimulus (US, footshock) intensities. Experiment 3 revealed that the enhanced fear conditioning in males was specific to contextual CSs, and consisted of a more rapid rate of conditioning. Together, these experiments reveal a positive correlation between the magnitude of hippocampal LTP and a form of learning that depends on the hippocampus. Furthermore, they suggest a neural basis for sex differences in hippocampus-dependent learning tasks.

Keywords: Long-term potentiation; Hippocampus; Pavlovian fear conditioning; Sex difference; Contextual learning; (Rat)

1. Introduction

Long-term potentiation (LTP) in the hippocampus is an enduring form of synaptic plasticity that has been implicated in mammalian learning and memory [5,6]. Strong support for a role of LTP in learning comes from studies of the behavioral effects of *N*-methyl-D-aspartate (NMDA) receptor antagonists, drugs which prevent LTP induction both in vitro [8] and in vivo [24,25,30]. In particular, a number of studies indicate that the effects of NMDA receptor antagonists parallel the effects of hippocampal lesions and impair processes that depend on the hippocampus, such as spatial navigation [30,33,36], olfactory discrimination [37], habituation to a novel environment [11,18], and contextual learning [14,22]. Importantly, the effects of NMDA receptor antagonists are specific to the *acquisition* of information, because they do not influence either the consolidation or performance of learned responses [22].

Further support for a role of hippocampal LTP in learning comes from our recent study examining the influence of water deprivation on hippocampal neurophysiology and the acquisition of Pavlovian fear conditioning to contextual conditional stimuli (CSs), a form of learning that requires the hippocampus [22,32,44]. Specifically, we found that motivation induced by mild water deprivation augments perforant path LTP induction, elevates hippocampal theta rhythm, and greatly facilitates the rate of fear conditioning to contextual stimuli [27]. The enhanced fear conditioning in water-deprived rats was specific to contextual CSs, because conditioning to discrete tone CSs, which is not dependent on the hippocampus, was not affected by water deprivation [28]. Collectively, these experiments suggest that hippocampal LTP underlies the encoding of contextual representations during fear conditioning,

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and that the strength of LTP is a determinant of the rate at which they enter into associations with aversive stimuli [27,28].

For over 75 years it has been known that there are prominent sex differences in hippocampus-dependent behaviors including spatial navigation, open field activity, and active avoidance learning in rats [1,3,9,10,17, 20,34,38,42]. It has been suggested that these sex differences are the result of different anatomical organization of hippocampal circuits in males and females [19,21]. In view of the proposed role of hippocampal LTP in contextual learning, another possibility is that sexually dimorphic behavior in hippocampus-dependent tasks results from sex differences in hippocampal LTP and contextual representation. The existence of sex differences in LTP would both provide strong correlational support for its role in hippocampus-dependent learning and reveal a physiological basis for sex differences in this type of learning. To address this issue, we compared hippocampal LTP at perforant path-dentate granule cell synapses in both adult and developing male and female rats. In addition, we examined Pavlovian fear conditioning to contextual and discrete cues in adult male and female rats to understand the role sex plays in the acquisition of, respectively, hippocampus-dependent and hippocampus-independent forms of fear conditioning. If hippocampal LTP underlies sex differences in hippocampus-dependent learning, we would expect to find a positively-correlated sex difference in both LTP and context conditioning.

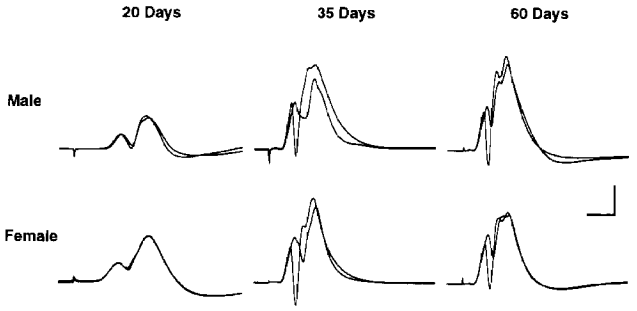

Fig. 1. Averaged pre- (−10–0 min) and post-high-frequency stimulation (HFS, 20–30 min) perforant path-evoked field potentials recorded in the dentate gyrus of representative pre-weanling (20 days old), pre-pubescent (35 days old), and adult (60 days old) male and female rats under pentobarbital anesthesia. Each waveform is an average of 30 perforant path-evoked potentials; pre- and post-HFS responses are superimposed. 35- and 60-day-old male subjects showed greater long-term potentiation (LTP) of excitatory postsynaptic potential (EPSP) slope (measured on the rising phase of the positive-going field potential) than their female counterparts. However, the potentiation of population spike (PS) amplitude (the amplitude of the sharp negative-going potential superimposed on the field EPSP) did not differ between males and females at any age. Note that 20-day-old rats did not exhibit LTP. Calibration: 5 ms, 2 mV.

2. Materials and methods

2.1. Electrophysiology

2.1.1. Subjects

For Expt. 1, the subjects were 16 male and 16 female Sprague-Dawley rats (Simonsen Labs, Gilford, CA) selected from three age groups: 15 to 20 days old (pre-weanling; $n = 5$ per sex), 32 to 35 days old (pre-pubescent as determined by day of vaginal opening; $n = 5$ per sex), and 55 to 60 days old (adult; $n = 6$ per sex). They were housed on a 12:12 h light:dark cycle in separate-sex groups of 3–4 rats in standard plastic tubs with free access to food and water.

2.1.2. Surgery

For electrophysiological testing, the rats were anesthetized with sodium pentobarbital (65 mg/kg body weight) and implanted using stereotaxic techniques with a stimulating electrode in the perforant path and a recording electrode in the ipsilateral hilus of the dentate gyrus. After retraction of the scalp, burr holes of approximately 2-mm diameter were drilled unilaterally in the skull for the placement of stimulating and recording electrodes. The electrodes consisted of Epoxylite-coated stainless-steel pins (size 00) with the recording and stimulating surfaces formed by removing the insulation at the conical tips; tip lengths were 50 and 500 μm for the recording and stimulating electrodes, respectively. The bipolar stimulating electrode consisted of two adjacent insect pins with a tip separation of 1 mm. The electrode coordinates were both age- and sex-dependent (see Ref. [43] for the coordinates used in the present study), and the ventral locations of both the recording and stimulating electrodes were adjusted to maximize the amplitude of the perforant path-evoked dentate hilar field potentials. Reference and ground electrodes consisting of small stainless-steel screws were affixed to the skull in an area overlying the nasal sinus. Body temperature was kept at 37°C with a heating pad and surgical anesthesia was maintained with booster injections (0.05–0.15 ml) of pentobarbital as needed.

2.1.3. Acute electrophysiology

Extracellular dentate hilar field potentials evoked by single-pulse perforant path stimulation (100-μs pulses) were amplified (gain = 100), bandpass filtered (1 Hz–10 kHz), displayed on an oscilloscope, digitized, and written to disk (DataWave Systems, Colorado Springs, CO). As shown in Fig. 1, perforant path-evoked field potentials in the dentate gyrus consisted of a characteristic gradual positive-going field EPSP with a sharp negative-going PS superimposed on the rising phase of the EPSP. 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.

Electrophysiological testing began after stable hilar field potentials had been recorded for at least 30 min. Once stable, perforant path-evoked field potentials (stimulation current intensity adjusted to elicit a 3-mV PS) were recorded during a 10-min period before and a 30-min period after HFS; field potentials were sampled at 20-s intervals. High-frequency perforant path stimulation consisted of 10 pairs of 400 Hz bursts (burst duration = 25 ms, 10 pulses per burst) delivered at the same current intensity used for baseline recording. Bursts within a pair were delivered at the theta rhythm (5 Hz, interburst interval = 200 ms) and each of the 10 pairs of bursts was separated by 10 s.

2.1.4. Histology

After electrophysiological testing the rats were perfused through the heart with physiological saline followed by 10% formalin. The brains were sectioned on a cryostat, mounted on slides, and stained

with Cresyl violet to verify electrode tracks. All electrode placements were found to be accurate.

2.1.5. Data analysis

Averaged perforant path-evoked field potentials were generated for each 10-min block of the 40-min recording period (30 waveforms per average). Several parameters were extracted from the pre-HFS averaged field potentials yielding the measures described in Table 1. For analysis of LTP, the percentage of change in EPSP slope and PS amplitude was computed from the 10-min pre-HFS baseline to the final 10 min of the test period (20–30 min post-HFS). These values were submitted to a two-way analysis of variance (ANOVA) with variables of age (3 levels) and sex (2 levels). Post-hoc comparisons in the form of Newman-Keuls tests were used for comparisons of means following a significant omnibus F test. All data are presented as means ± the standard errors of the means (S.E.M.s).

2.2. Behavior

2.2.1. Subjects

In Expt. 2, the subjects were 30 adult male (65–75 days old) and 30 adult female (65–75 days old) Long-Evans rats born and maintained in the University of California, Los Angeles colony on a 14:10 h light:dark cycle. Five days prior to the start of the experiment, the rats were individually-housed in standard hanging stainless steel cages and provided with ad lib access to rat chow and tap water. All rats were handled daily during this time and all procedures were performed during the light phase of the cycle. In Expt. 3, the subjects were 16 male (65–75 days old) and 16 female (65–75 days old) Long-Evans rats housed and maintained as in Expt. 2.

2.2.2. Apparatus

Four identical observation chambers (28 × 21 × 10.5 cm; Lafayette Instrument Co., North Lafayette, IN) were used for both conditioning and contextual fear testing in Expts. 2 and 3. The chambers were situated in chests located in a well-lit and isolated room. A videocamera placed in front of the observation chambers allowed each subject's behavior to be observed and recorded by an experimenter in an adjacent 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) for the delivery of footshock unconditional stimuli. The chambers were cleaned with 5% ammonium hydroxide solution before rats were placed inside. Background noise (78 dB, A-scale) was supplied by ventilation fans and shock scramblers.

In addition to the conditioning chambers described above, four different observation chambers (28 × 21 × 10.5 cm; Lafayette) were used to assess fear conditioning to a tone CS in Expt. 3. The chambers were situated in chests located in a dimly-lit and isolated room, and were modified to minimize their similarity with the conditioning chambers. The floor of each chamber consisted of 18 staggered 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) for the delivery of footshock unconditional stimuli. The chambers were cleaned with 1% acetic acid hydroxide solution before rats were placed inside. Background noise was not supplied. A videocamera placed in front of the observation chambers allowed each subjects' behavior to be observed and recorded by an experimenter in an adjacent room.

2.2.3. Procedure

In Expt. 2, male and female rats were randomly assigned to one of three groups ($n = 10$ per group): no shock, low shock intensity (0.4 mA), and high shock intensity (0.8 mA). On the conditioning day, the rats were placed in the conditioning chambers in 15 sets of 4 animals; chamber position was counterbalanced across sex and shock condition. After 3 min, the rats in the low and high shock intensity groups received 3 footshocks (1-s duration, 0.4 or 0.8 mA) spaced 20 s apart; rats in the no shock groups did not receive footshock. Thirty seconds following the last shock, the rats were returned to their home cages. 24 h following conditioning, the rats were returned to the chambers for an 8-min test. Freezing, a defensive posture consisting of immobility except that necessitated by breathing, to the contextual cues of the chambers was assessed during the test by using a time sampling procedure in which each rat was scored for immobility every 8 s. Thus, a total of 64 observations was made for each subject during the 8 min test. All scores were transformed to a percentage of total observations.

In Expt. 3, rats were placed in the conditioning chambers on the training day in 8 sets of 4 animals; chamber position was counterbalanced across sex and trial condition. After 3 min, the rats received either 1 or 3 tone (10-s duration, 78 dB, 2 kHz)-shock (1-s duration, 0.4 mA) pairings. For rats receiving three tone-shock trials, the interstimulus interval was 60 s. 30 s following the last shock, the rats were returned to their home cages. A no-shock group was not included in this experiment, because it is well documented that there

Table 1
Mean (±S.E.M.) waveform parameters in male (M) and female (F) pentobarbital-anesthetized rats of 20, 35, and 60 days of age

	Age (days)					
	15–20		32–35		55–60	
	M	F	M	F	M	F
EPSP slope (mV/ms)	0.5 ± 0.1	0.6 ± 0.2	2.2 ± 0.7	2.4 ± 0.4	2.5 ± 0.4	1.9 ± 0.2
EPSP amplitude (mV)	2.3 ± 0.2	3.0 ± 0.5	5.9 ± 1.8	6.6 ± 1.3	6.0 ± 0.9	4.9 ± 0.6
EPSP latency (ms)	5.1 ± 0.5	4.9 ± 0.3	2.2 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.0 ± 0.1
PS amplitude (mV)	1.3 ± 0.4	1.2 ± 0.1	2.5 ± 0.6	2.4 ± 0.7	2.1 ± 0.2	1.8 ± 0.2
PS latency (ms)	8.3 ± 0.6	8.4 ± 0.3	5.5 ± 0.2	5.8 ± 0.2	5.2 ± 0.5	5.3 ± 0.4
PS threshold (mV)	0.9 ± 0.2	1.2 ± 0.3	4.1 ± 1.2	3.9 ± 0.5	4.0 ± 0.9	3.1 ± 0.5
FV amplitude (μV)	12.2 ± 9.5	12.2 ± 3.9	61.0 ± 10.9	92.8 ± 10.6	89.5 ± 11.7	95.6 ± 12.8

Values were extracted from extracellular perforant path-evoked field potentials in the dentate gyrus collected during a 10-min pre-high-frequency stimulation (HFS) period. EPSP slope was measured at a fixed interval (4–6 ms) from the stimulus artifact; EPSP amplitude was measured as the peak EPSP amplitude from baseline; PS amplitude was measured with reference to a line drawn between the EPSP peak amplitude and PS onset; PS latency was measured as the time between stimulus artifact and the peak negativity of the PS; PS threshold was defined as the voltage at PS onset; FV (fiber volley) amplitude was measured as the peak amplitude of the short-latency potential associated with perforant path-evoked fiber depolarization. There were no reliable sex differences in any baseline measure, only age differences; all measures in the 15–20-day-old age group were significantly different from those in both groups of older animals.

is minimal unconditional freezing to tones (e.g., Ref. [23]). 24 h following conditioning, fear conditioning to the tone CS was assessed in the novel chambers. Three minutes following placement in the chambers, a tone identical to that used on the training day was presented for 8 min. Freezing was scored as in Expt. 3. 24 h following the tone test, the rats were returned to the chambers that served as the training context. Freezing was assessed during an 8-min test as described in Expt. 3. All freezing data were converted to a percentage of total observations.

2.2.4. Data analysis

In Expt. 2, freezing data were submitted to a two-way ANOVA with variables of sex (2 levels, male and female) and shock (3 levels, no shock, low shock, and high shock). In Expt. 3, freezing data were submitted to a two-way ANOVA with variables of sex (2 levels, male and female) and trial (2 levels, 1 or 3 trials). All data are presented as means ± S.E.M.s.

3. Results

3.1. Experiment 1: ontogeny of perforant path LTP in pentobarbital-anesthetized rats

To assess sex differences in hippocampal synaptic plasticity we examined perforant path-dentate granule cell LTP in pentobarbital-anesthetized rats. Male and female rats from three age groups were tested to examine the possible interaction of age and sex in perforant path LTP induction. Extracellular field potentials evoked by perforant path stimulation were recorded in the hilus of the dentate gyrus before and after perforant path high-frequency stimulation (HFS). LTP was quantified by examining HFS-induced changes in the slope of the field excitatory postsynaptic potential (EPSP) and amplitude of the population granule cell discharge (i.e., the population spike, PS). In addition to measuring LTP in these rats, a number of pre-LTP measures were taken from the perforant path-evoked hilar responses to assess any sex or age differences in baseline synaptic transmission.

3.1.1. Baseline measures

The mean (± S.E.M.) pre-HFS current intensity used for perforant path stimulation was not significantly different in male and female rats (male, 117.1 ± 10.3 μA; female, 149.4 ± 27.2 μA). Table 1 shows the pre-HFS waveform parameters extracted from hilar responses in male and female rats in each age group. There were no significant sex differences in either baseline EPSPs or PSs [Fs(2, 26) < 1.0], but there were developmental changes in these measures. Compared to 20-day-old rats, both 35- and 60-day-old rats exhibited significantly shorter EPSP and PS latencies, greater EPSP, PS and fiber volley amplitudes, and greater PS threshold [Fs(2, 26) > 4.6, Ps < 0.02]. However, there were no significant differences between 35- and 60-day-old rats in the waveform parameters. The changes in perforant path-evoked responses from 20 to 35 days has previously been shown [4,43] and reflect developmental processes including myelination of perforant path axons.

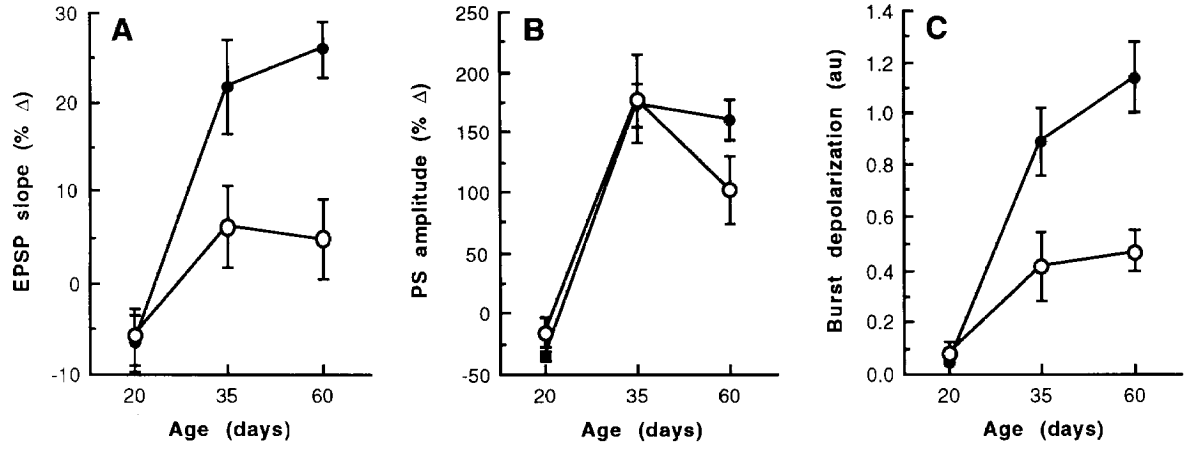

Fig. 2. Ontogeny of perforant path-granule cell excitatory postsynaptic potential (EPSP) slope (A) and population spike (PS) amplitude (B) long-term potentiation (LTP) in pentobarbital-anesthetized male (filled circles) and female (open circles) rats. Values were calculated as the percentage of change from the 10-min pre-high-frequency stimulation (HFS) baseline to the final 10-min post-HFS interval (20–30 min post-HFS) and are represented as means (± S.E.M.s). The labels on the abscissa identify the age of the oldest rats in each group. C: Ontogeny of *N*-methyl-D-aspartate (NMDA) receptor-mediated burst depolarization in pentobarbital-anesthetized male (filled circles) and female (open circles) rats. Data are represented as mean (± S.E.M.) fractional changes in burst area (au, arbitrary units) for rats in each of the three age groups. The labels on the abscissa identify the age of the oldest rats in each group. NMDA receptor-mediated burst depolarization during high-frequency stimulation (HFS) was quantified by subtracting the first from the second burst potential (interburst interval = 200 ms) in each of the 10 burst-pairs in the HFS train. This procedure revealed a late depolarization (onset latency ≈ 10 ms) that was mediated almost entirely by NMDA receptors. The area under the late depolarization was measured, averaged across the 10 burst-pairs, and normalized to the area of the pre-HFS single-pulse evoked field EPSP.

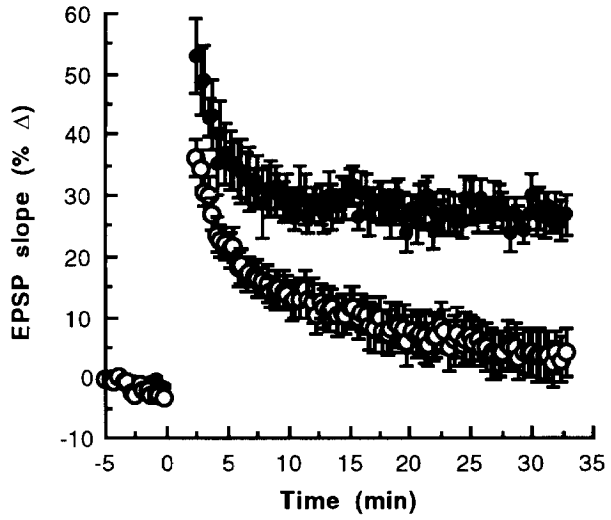

Fig. 3. Mean (±S.E.M.) percent change in excitatory postsynaptic potential (EPSP) slope of perforant path-evoked field EPSPs in adult (60-day-old) pentobarbital-anesthetized male (filled circles) and female (open circles) rats during the 40-min recording period. High-frequency perforant path stimulation was delivered at $t = 0$. In males, HFS induced a robust and non-decremental LTP. In females, HFS induced a rapidly-decaying, short-term potentiation that was significantly smaller than that induced in males.

3.1.2. Excitatory postsynaptic potential LTP

As shown in Fig. 2A, the magnitude of EPSP slope LTP, recorded as an increase in the slope of the field EPSP 30 minutes following perforant path HFS, varied with both the age and sex of the rats. An ANOVA revealed significant main effects of age [$F(2, 26) = 17.8$, $P < 0.01$], sex [$F(1, 26) = 13.3$, $P < 0.01$], and a significant interaction of the two [$F(2, 26) = 4.0, = P < 0.05$]. Post-hoc comparisons ($P < 0.05$) indicated that males exhibited significantly more EPSP slope LTP than females in both the 35-day and 60-day age groups. Rats in the 20-day age group did not show LTP, which is consistent with other reports [4,43]. The time course of LTP induced in adult male and female rats is shown in Fig. 3. From these data, it is apparent that a rapidly-decaying short-term potentiation was induced at perforant path synapses in female rats, whereas an enduring LTP was induced in male rats.

3.1.3. Population spike LTP

As shown in Fig. 2B, age differences in perforant path LTP were also evident in the magnitude of PS potentiation [$F(1, 26) = 43.4$, $P < 0.01$]. Post-hoc comparisons ($P < 0.05$) indicated that rats in both the 35-day and 60-day age groups exhibited significantly more PS amplitude LTP than rats in the 20-day age group. Unlike EPSP slope LTP, however, a sex difference in PS amplitude LTP was not apparent [$F(1, 26) = 0.4$, $P = 0.52$], nor was there an interaction between sex and age [$F(2, 26) = 1.8$, $P = 0.18$]. There was a tendency for greater PS amplitude LTP in male compared to female rats in the 60-day age group, but a planned comparison in the form of a univariate F test revealed that this trend did not reach acceptable levels of statistical reliability [$F(1, 10) = 3.4$, $P = 0.09$].

3.1.4. N-methyl-D-aspartate receptor activation

In the dentate gyrus, LTP induction typically requires activation of NMDA receptors. High-frequency perforant path stimulation evokes substantial NMDA receptor activation in the dentate gyrus, which is manifest as a long-latency (15–50 ms) depolarization in the HFS-evoked field potentials [25]. To address whether sex differences in perforant path LTP were related to events occurring during the initial phases of LTP induction, the magnitude of NMDA-receptor mediated or 'burst' depolarization during HFS was quantified. The NMDA receptor-mediated burst depolarization during HFS was quantified by subtracting the first from the second burst potential (interburst interval = 200 ms) in each of the 10 burst-pairs in the HFS train. The area under the late depolarization was measured, averaged across the 10 burst-pairs, and normalized to the area of the pre-HFS single-pulse evoked field EPSP.

As shown in Fig. 2C, burst depolarization during HFS varied significantly with both age [$F(1, 26) = 27.2$, $P < 0.01$] and sex [$F(1, 26) = 18.6$, $P < 0.01$] in rats anesthetized with pentobarbital. Post-hoc comparisons (Newman-Keuls test, $P < 0.05$) revealed that significant burst depolarization appeared first at 35 days of age, and remained at a similar level through adulthood. In addition, males and females differed in the magnitude of burst depolarization evoked during HFS; males exhibited significantly more burst depolarization than females in both the 35-day and 60-day age groups [sex × age, $F(1, 26) = 6.0, P < 0.01$; Newman-Keuls, Ps < 0.01]. As we have seen, the relatively weak NMDA receptor activation in 35- and 60-day-old females was not sufficient to yield non-decremental LTP (at least of the field EPSP), rather it led to a form of rapidly-decaying short-term potentiation (see Fig. 3). These data indicate a strong correlation between levels of NMDA receptor activation and the magnitude of EPSP LTP in the dentate gyrus.

3.2. Experiment 2: Pavlovian fear conditioning to contextual stimuli in adult rats

The sex difference in hippocampal LTP observed in Expt. 1 has important implications for the performance of hippocampus-dependent behaviors in male and female rats. As mentioned earlier, one form of hippocampus-dependent form of learning is Pavlovian fear conditioning to contextual CSs. Lesions placed in the dorsal hippocampus produce substantial deficits in contextual fear conditioning, yet leave fear conditioning to discrete cues such as tones intact [23,32]. Addi-

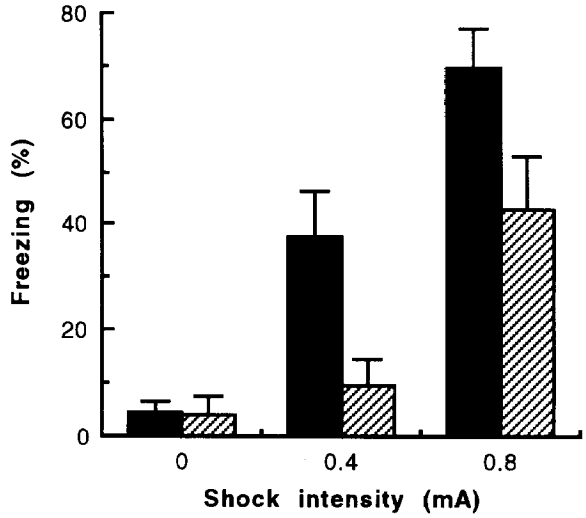

Fig. 4. Mean (±S.E.M.) percent freezing behavior in adult male (solid bars) and female rats (hatched bars) in each of the three shock conditions during an 8-min test. Males showed significantly more freezing than females in both the low and high shock intensity groups.

tional data suggest that contextual fear conditioning may be mediated by hippocampal LTP [22,27]. If LTP is involved in contextual fear conditioning, one would expect sex differences in LTP to be manifest in a similar sex difference in the acquisition of conditional contextual fear. To address this possibility, we examined the acquisition of Pavlovian fear conditioning to contextual cues in male and female rats. In this experiment, male and female rats were conditioned to fear a novel context through the use of aversive footshocks. We used two intensities of footshock (low and high) and a no-shock control. The low footshock intensity (0.4 mA) was chosen to produce minimal levels of conditioning in females and the high intensity (0.8 mA) was chosen to produce strong conditioning in females [45].

Fig. 4 shows the percentage of freezing behavior to the contextual stimuli of the conditioning chambers 24 h following training. Rats that did not receive footshock during training showed little or no freezing to the context during the test. In contrast, rats that received footshock during training exhibited conditional fear to the context during the test, and this fear was greater with 0.8 mA than 0.4 mA shocks [$F(2, 54) = 30.2$, $P < 0.01$]. Although conditioning was evident in both males and females, there was a significant sex difference in its magnitude; males exhibited significantly more conditional freezing than their female counterparts [$F(1, 54) = 11.2$, $P < 0.05$]. The sex difference in freezing did not interact with shock intensity [$F(2, 54) = 2.7$, $P = 0.076$], indicating that males conditioned more fear than females with both low and high shock intensities.

3.3. Experiment 3: Pavlovian fear conditioning to discrete stimuli in adult male and female rats

Experiment 2 indicates that fear conditioning to contextual cues is sexually dimorphic, with males showing greater levels of conditional freezing than females. However, it is well known that female rats are more active than male rats in a variety of test situations including activity wheels, open fields, and emergence apparatuses [1]. Hence, sex differences in fear conditioning may reflect a sex difference in the performance of freezing behavior, rather than in learning the context-fear association. Indeed, Archer [1] has suggested that male and female rats exhibit different species-typical defense reactions to fearful stimuli, with males tending to freeze and females tending to escape in the presence of fear-evoking stimuli.

In Expt. 3, we used a tone-shock conditioning procedure to examine this hypothesis. Instead of receiving unsignaled shock, male and female rats were trained using shocks signaled by a brief tone. Using this procedure, fear conditioning to both the context and the tones can be assessed independently. The absence of a sex difference in tone conditioning would argue against a performance interpretation of Expt. 2. Moreover, a selective sexual dimorphism in contextual fear conditioning would specifically implicate hippocampal LTP in this form of learning. Because differences in the rate, but not asymptote, of contextual fear conditioning have been reported for manipulations that affect hippocampal LTP [27,28], we trained male and female rats in Expt. 3 with either 1 or 3 trials to assess the acquisition of conditional fear.

Freezing during the 8-min tone test was confined to the first 2 min of the tone, so we restricted our analysis to this interval. As shown in Fig. 5A, no sex difference was observed in fear conditioned to the tone CS [$F(1, 28) = 0.1$] and both male and female rats showed greater conditioning with 3 trials compared to 1 trial [$F(1, 28) = 6.1$, $P < 0.05$]. There was no interaction between sex and number of trials, indicating that males and females conditioned to the tone at a similar rate, and reached a similar level of asymptotic conditioning [$F(1, 28) = 0.2$].

Freezing during the 8-min context test is shown in Fig. 5B. It is apparent from these data that males conditioned more contextual fear than females and extinguished this fear more slowly. These impressions were confirmed by an ANOVA, which revealed a significant main effect of sex [$F(1, 28) = 4.5$, $P < 0.05$] and a significant interaction of sex and test minute [$F(7, 196) = 3.9$, $P < 0.01$]. There was not a significant main effect of trial [$F(1, 28) = 0.5$], indicating that levels of context conditioning were similar in rats receiving either 1 or 3 tone-shock trials. However, there was a nearly significant interaction between sex and

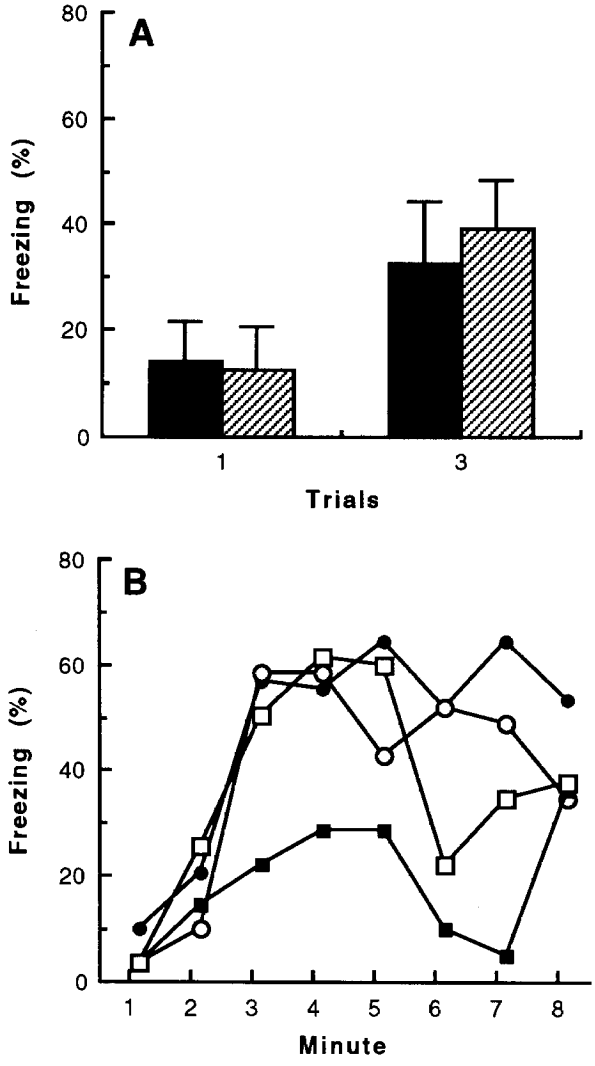

Fig. 5. A: Mean (±S.E.M.) percent freezing behavior to the tone conditional stimulus (CS) in male (solid bars) and female (hatched bars) rats during a 2-min test interval. Rats received either 1 or 3 tone-shock pairings (trials). There were no sex differences in conditional freezing to the tone CS. B: Mean percent freezing behavior to the context conditional stimulus during an 8-min test for the rats shown in A. Male (circles) and female (squares) rats received either 1 (filled markers) or 3 (open markers) tone-shock pairings. Male rats exhibited greater freezing to the contextual CS than female rats, achieving asymptotic levels of conditioning in only 1 trial.

trial [$F(1, 28) = 3.6$, $P = 0.07$], suggesting different rates of acquisition of context conditioning in males and females. Closer examination of Fig. 5B indicates that males reached asymptotic conditioning in 1 trial, whereas females required 3 trials to attain this level of freezing. Collectively, these results indicate that male rats acquire more conditional fear to the context of the conditioning chamber than female rats early in acquisition, but display similar levels of freezing to female rats later in conditioning.

The present data reveal that sex differences in Pavlovian fear conditioning are specific to the acquisition of hippocampus-dependent contextual fear, and consist of a greater rate of conditioning in males. Furthermore, the similar level of freezing in males and females receiving tone-shock pairings indicates that sex differences in context freezing cannot be attributed to a sex difference in the performance of conditional fear. As mentioned earlier, it has been suggested that female rats freeze less than males because females engage in species specific defense reactions different than freezing [1]. Our data confirm this observation that females freeze less than males, but the pattern of data do not support a performance account of this sex difference. Rather, our data are consistent with the interpretation that females show less freezing because they acquire less contextual fear. For example, both female rats and rats with hippocampal lesions rapidly acquire two-way shuttle avoidance. Because reducing fear by lowering shock intensity enhances acquisition of shuttle avoidance (see Ref. [7], pp. 323–366 for a review), the more rapid conditioning in female and hippocampal rats is probably due to their lower levels of contextual fear. Indeed, it has been pointed out that differences in defensive behavior (e.g., freezing or escaping) that are frequently attributed to differences in response selection often turn out, upon closer scrutiny, to be caused by differences in the level of fear [13].

4. Discussion

Experiment 1 demonstrated a sexual dimorphism of synaptic plasticity at perforant path-granule cell synapses in anesthetized rats. The sex difference in LTP was dependent on the age of the rats, specific to field EPSPs, and apparently resulted from greater NMDA receptor activation in male compared to female rats. Experiments 2 and 3 demonstrated a sex difference in the acquisition of contextual fear conditioning, a hippocampus- and NMDA receptor-dependent form of learning. Specifically, males exhibited greater conditional freezing than females to contextual stimuli, a result of a faster conditioning rate in male rats. In contrast, female and male rats conditioned equally well and froze at a high level to a discrete tone CS. We will consider each of these results in greater detail below.

In Expt. 1, age interacted with the sex difference in LTP inasmuch as pre-weanling (20-day-old) rats did not show LTP. The absence of perforant path LTP in 20-day-old rats is consistent with a number of reports [4,43] and is apparently due to insufficient postsynaptic depolarization in dentate granule cells during perforant path HFS. The reason for insufficient depolarization in the dentate gyrus of pre-weanling rats is unclear, but may be related to the incomplete development of the dentate gyrus at this age or the relatively

late functional maturation of NMDA receptors in this region [4]. In the present experiments, these phenomena were evidenced, respectively, by long-latency perforant path-evoked responses and minimal burst depolarization in 20-day-old rats. This profile of perforant path-evoked responses was limited to rats in the 20-day-old group. Perforant path-evoked responses in both 35- and 60-day-old rats were typical of 'mature' responses and, at least in males, exhibited robust LTP. Thus, whatever factor limits postsynaptic depolarization and LTP in 20-day-old rats is absent in rats 35 days of age or older.

In addition to developmental differences in perforant path LTP, there were also prominent sex differences in LTP in both pre-pubescent and adult rats. Interestingly, these sex differences in LTP were specific to perforant path-evoked EPSPs; LTP of PSs was not sexually dimorphic. Thus, there was not an absolute absence of LTP in the female hippocampus, it merely took a different form from that in the male hippocampus. It is possible that these sex differences in EPSP LTP induction were related to the well known sexual dimorphism in pentobarbital sensitivity [39,41]. However, neither PS LTP nor baseline perforant path-evoked EPSPs were different in males and females. Thus, it does not seem probable that sex differences in levels of pentobarbital anesthesia were responsible for the present results, although this possibility cannot be discounted until our experiments are replicated in awake rats. One factor that may have contributed to the sex difference in perforant path LTP is the sex difference in NMDA receptor activation generated by perforant path HFS in adult rats. As in young rats, the insufficient NMDA receptor activation in adult female rats was probably due to insufficient postsynaptic depolarization evoked by perforant path HFS. In this case, the sex difference in postsynaptic depolarization may have been due to the regulation of amino acid receptor-mediated excitation and inhibition by gonadal steroids [40], which have an important role in both organizing and activating sexually dimorphic neural circuits [2]. For example, adult female rats have much higher levels of the progesterone metabolite, allopregnanolone, than their male counterparts [31]. Because allopregnanolone potentiates γ-aminobutyric acid (GABA) receptor-mediated inhibition and acts synergistically with pentobarbital, it may have contributed to both the well known sex difference in pentobarbital sensitivity [39,41] and the failure to induce EPSP LTP in anesthetized female rats. In other words, high levels of GABAergic inhibition in the female hippocampus may have shunted the critical depolarization required for NMDA receptor activation and LTP induction. Although it is not clear how this mechanism could account for the selective sexual dimorphism in EPSP compared to PS LTP, it nonetheless provides a starting point for further studies into the regulation of LTP in male and female rats.

In accordance with sex differences in LTP, the presents experiments demonstrated a sex difference in the acquisition of Pavlovian fear conditioning to contextual cues, a form of learning that depends on both the hippocampus and NMDA receptors. The sex difference in fear conditioning consisted of a faster rate of conditioning in male rats, because male rats showed superior context conditioning compared to their female counterparts following 1 but not 3 tone-shock pairings. It was not due to a sex difference in freezing, because both males and females exhibited comparable and high levels of freezing to tones that had been paired with footshock. This pattern of results is similar to that reported for other forms of learning that depend on the hippocampus. For instance, sex differences have been reported in a number of spatial learning tasks, including the radial arm maze and Morris water maze [20,34,42]. In these tasks, sex differences also take the form of an increased acquisition rate in males. Some authors have suggested that these sex differences in spatial behavior are due to sex differences in hippocampal morphology [19,21]. While not ruling out this interpretation, the present findings suggest that sex differences in hippocampal physiology, namely hippocampal LTP, also play an important role in generating sexually dimorphic behavior in hippocampus-dependent tasks.

Our current working model of the neural mechanisms of Pavlovian fear conditioning illustrates a mechanism whereby sex differences in hippocampal LTP could impact contextual (spatial) learning. The model has been formulated from a number of empirical observations reported by several laboratories and is elaborated in greater detail elsewhere [12]. In brief, the model posits that when a rat is placed in a novel place it explores, thereby acquiring contextual information which is represented in the hippocampus by LTP. When the rat experiences an aversive event in the place, the context representation in the hippocampus enters into association with the representation of the aversive stimulus in the amygdala. This view is supported by studies showing that lesions of the hippocampus selectively attenuate fear conditioning to contextual cues [23,32], whereas lesions of the amygdala attenuate fear conditioning to contextual [15,32], auditory [32], and visual cues [16]. Because the hippocampus is a component of the contextual CS pathway, our model predicts that any factor that modulates LTP in the hippocampus will affect the salience of contextual CSs and hence the rate of context conditioning (see Refs. [27,28] for elaboration of this prediction). Consistent with this prediction, we have observed in the present study a prominent sexual dimorphism in hippocampal LTP that is paralleled by a similar dimor-

phism in the rate of contextual learning. The nature of the sexual dimorphism in LTP suggests that EPSP LTP is particularly important in establishing the salience of context representations and therefore the rate of learning. In female rats, PS LTP may underlie a less salient representation which does not favor fast learning but can support asymptotic performance following strong shocks or multiple trials. It is interesting to note that the sex differences in fear conditioning appear to be hippocampus-specific, because conditioning to a discrete tone CS was similar in male and female rats. This suggests that the mechanisms of CS-US association in the amygdala, which are also presumably mediated by LTP [29], may not be sexually dimorphic.

Water deprivation augments both perforant path-dentate granule cell LTP and the rate of contextual fear conditioning [27,28]. The present results reveal a similar positive correlation between perforant path LTP and the acquisition of conditional contextual fear: male rats showed substantial perforant path LTP and rapid context conditioning, whereas female rats showed relatively less LTP and slower context conditioning. Despite these apparent similarities, however, the molecular mechanisms underlying the effects of water deprivation and sex differences are probably different. For instance, NMDA receptor activation induced by LTP-inducing HFS is similar in water-deprived and nondeprived rats, whereas the present data reveal different levels of NMDA receptor activation in male and female rats. Additionally, recent evidence indicates that water deprivation increases the number of high-affinity α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors (S. Maren, S. Standley, C. Aquino, M. Baudry and R.F. Thompson, unpublished observations), a subclass of glutamate receptors that plays a role in the expression of LTP [26]. Together, these data suggest that water deprivation interacts with the AMPA receptor-mediated expression of LTP, whereas sex interacts with the NMDA receptor-mediated induction of LTP. Although the molecular mechanisms underlying sex differences in LTP and contextual learning and the effects of water deprivation on these processes are probably different, both data sets are congruent with a role for hippocampal LTP in the acquisition of contextual information during fear conditioning.

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