Sexually dimorphic perforant path long-term potentiation (LTP) in urethane-anesthetized rats

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Abstract

Sex differences in perforant path long-term potentiation (LTP) have been reported in pentobarbital-anesthetized rats. Because this effect may be due to the prominent sex difference in barbiturate sensitivity, the present report examined perforant path-dentate granule cell synaptic transmission and long-term potentiation (LTP) in adult male and female rats anesthetized with urethane. Spectral analysis of hippocampal electroencephalographic (EEG) activity indicated that urethane induced similar levels of anesthesia in male and female rats, and there were no sex differences in the latency or amplitude of extracellular field potentials evoked in the dentate gyrus following perforant path stimulation. In contrast, there was a robust sex difference in the magnitude of LTP induced in the dentate gyrus following high-frequency stimulation (HFS) of the perforant path. This sex difference in LTP was paralleled by a sex difference in the magnitude of N-methyl-D-aspartate (NMDA) receptor activation generated by perforant path HFS. These results demonstrate a sex difference in hippocampal LTP that cannot be explained by a sex difference in level of anesthesia.

Keywords: Hippocampus; Dentate gyrus; Glutamate; Synaptic transmission; Plasticity; Sex difference; Rat

In rats, several forms of learning and memory that require the hippocampus are sexually dimorphic. For example, there are prominent sex differences in maze learning [1,2] and contextual fear conditioning [3]. The different performance of males and females in these learning tasks is generally interpreted in terms of underlying sex differences in hippocampal anatomy and physiology. In this regard, I have recently proposed [3] that sex differences in hippocampus-dependent learning tasks may be the result of sexually dimorphic hippocampal long-term potentiation (LTP), a form of enduring synaptic plasticity that serves as an experimental model of associative learning and memory [4]. Using pentobarbital-anesthetized rats, I discovered a robust sex difference in the induction of perforant path-dentate granule cell LTP [3]. This sex difference consisted of a greater magnitude of LTP in male compared to female rats and was paralleled by a similar sex difference in N-methyl-D-aspartate (NMDA) receptor activation during LTP induction. Behavioral experiments revealed a sex difference in the ac-

quisition of Pavlovian fear conditioning to contextual cues [3], a form of learning that may require hippocampal LTP [5,6].

One explanation for the sex difference in LTP is the different sensitivity of male and female rats to barbiturate anesthetics, including sodium pentobarbital. Specifically, the lethal dose of sodium pentobarbital is lower in female rats compared to male rats, and female rats exhibit a greater depth and duration of anesthesia following a typical anesthetic dose of sodium pentobarbital [7]. Thus, the greater sensitivity of female rats to sodium pentobarbital could account for their relatively low level of perforant path-dentate granule cell LTP (if it is assumed that LTP magnitude varies as a function of depth of anesthesia). However, three points argue against this possibility. Firstly, baseline perforant path evoked field potentials in the dentate gyrus were not different in male and female rats. Secondly, there was no difference in the levels of stimulation required to evoke dentate field potentials in male and female rats. Thirdly, the sex difference in LTP was specific to the population excitatory postsynaptic potential (EPSP) evoked in the dentate gyrus; population spike (PS) LTP was similar in male and female rats. Col-

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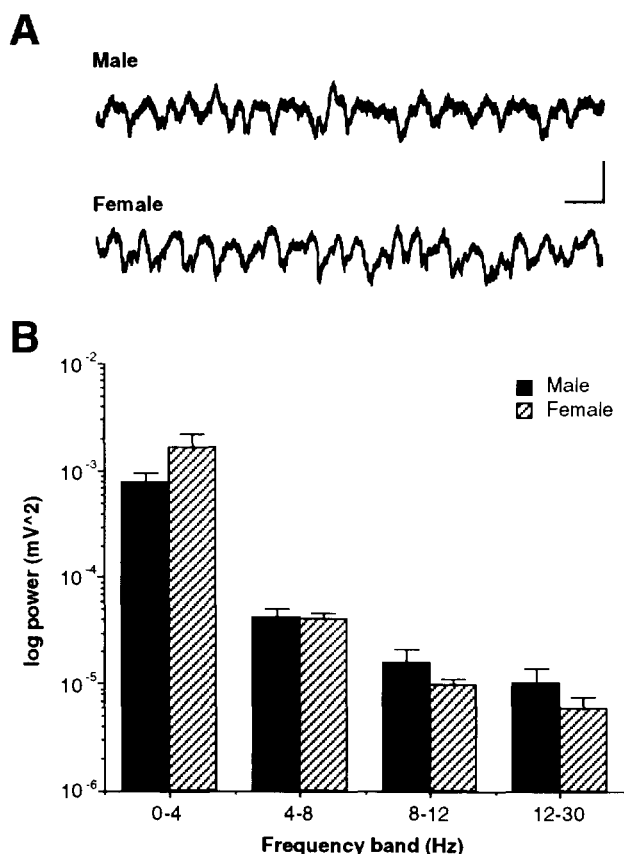E-mail: maren@psych.ucla.edu.

Fig. 1. Hippocampal EEG activity in urethane-anesthetized male and female rats. (A) Twenty-second epochs of hippocampal EEG recorded from the dentate gyrus of representative male and female rats. Note the low voltage of the waveforms, the dominance of low-frequency activity (0–4 Hz), and the similarity of the traces in the two rats. Calibration: 2 ms, 200 μV. (B) Mean (±SEM) spectral power of EEG activity from male and female rats. For each subject, spectral power was calculated from six 20 s epochs of EEG activity and the resulting values were averaged and collapsed into four frequency bands (0–4, 4–8, 8–12, 12–30 Hz). There was no sex difference in the spectral power of hippocampal EEG.

lectively, these results suggest that the sex difference in EPSP LTP that was observed in pentobarbital-anesthetized rats was not due to a sex difference in barbiturate anesthesia.

Nevertheless, to eliminate the possible confounding influence of barbiturate-sensitivity on LTP induction, I examined perforant path-dentate granule cell synaptic transmission and LTP in adult male and female rats anesthetized with urethane (ethyl carbamate), an anesthetic that has not been reported to differentially affect males and females. As will be shown, the use of urethane permitted an assessment of perforant path LTP under similar levels of anesthesia in male and female rats.

The subjects were eight male and eight female Sprague–Dawley rats (Simonsen Labs, Gilford, CA), 55–60 days old. They were housed on a 12:12 h light:dark cycle (lights on at 0700 h) in separate sex groups of 3–4 rats in standard plastic tubs with free access to food and

water. For electrophysiological testing, the rats were anesthetized with urethane (ethyl carbamate, 1.6 g/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 recording electrode was implanted in the hilus of the dentate gyrus (3.3 mm posterior to bregma, 2.4 mm lateral to midline, and 2.8–3.0 mm ventral to brain surface) and the bipolar stimulating electrode in the medial perforant pathway (8.1 mm posterior to bregma, 4.4 mm lateral to midline, and 2.0–4.0 mm ventral to brain surface). 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.

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, Longmont, CO). Electrophysiological testing began after stable hilar field potentials had been recorded for at least 30 min. Prior to LTP induction, a 2-min time sample (six 20-s epochs) of hippocampal electroencephalographic (EEG) activity was collected. The EEG activity was amplified (gain = 1000), filtered (0.2–20 Hz), displayed on an oscilloscope, digitized, and written to disk (DataWave Systems, Longmont, CO). After the collection of slow waves, 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, ten 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 ten pairs of bursts was separated by 10 s.

Fig. 1A shows representative EEG records from the hippocampus of urethane-anesthetized male and female rats. The pattern of EEG activity in both males and females was typical of an anesthetized preparation, consisting of low-voltage/low-frequency slow waves. The EEG
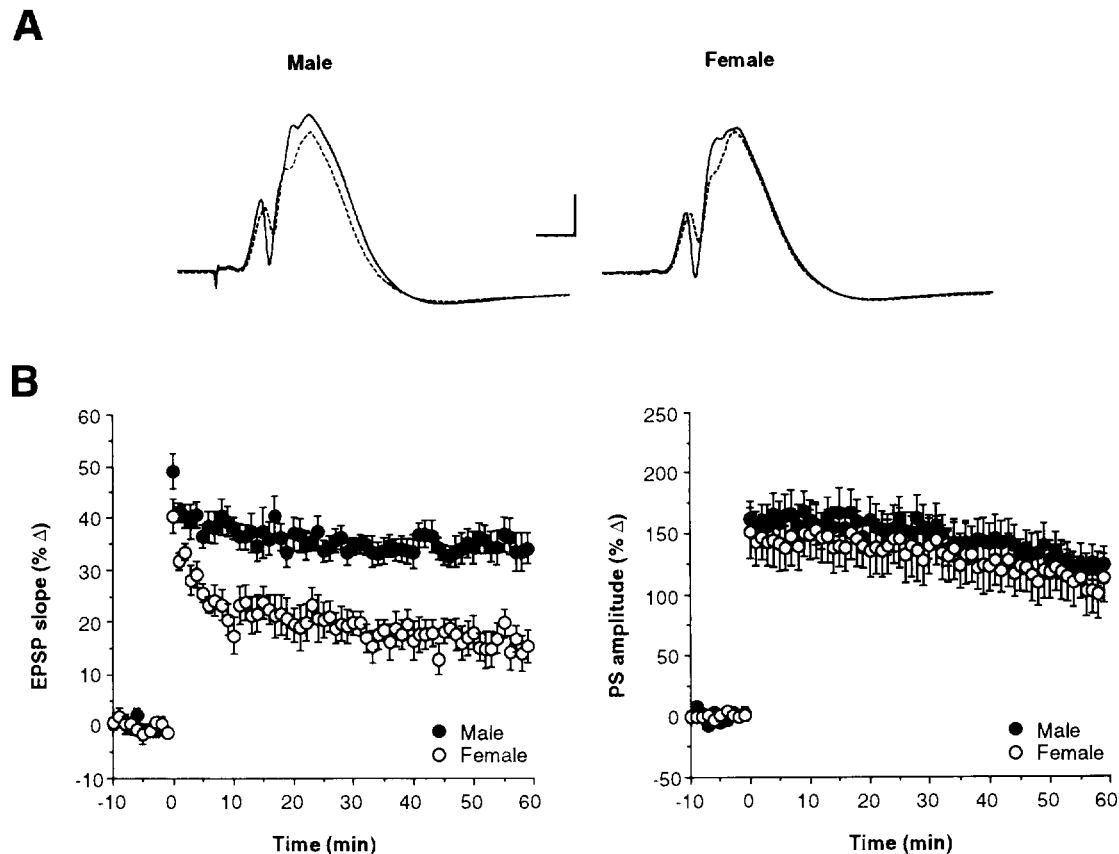
Fig. 2. Perforant path-dentate granule cell LTP in urethane-anesthetized male and female rats. (A) Averaged extracellular field potentials evoked in the dentate gyrus by single-pulse stimulation of the perforant path in representative male and female rats. Waveforms are 10 min averages of field potentials collected 10 min before and 50–60 min following perforant path LTP induction. Pre- (dotted line) and post- (solid line) LTP responses are superimposed. Note the greater level of EPSP slope LTP in the male compared to female rat, despite the similar level of PS amplitude LTP. Calibration: 3 ms, 2 mV. (B) Mean (±SEM) percentage of change (relative to baseline) in EPSP slope and PS amplitude for the 70 min recording period. Perforant path HFS was delivered at $t = 0$. Perforant path HFS induced significantly greater levels of EPSP slope LTP in male compared to female rats; PS amplitude LTP was not significantly different in male and female rats.

activity was analyzed to determine the spectral power across a range of frequencies (1–30 Hz, Fig. 1B). Fast Fourier transforms (FFTs; 1 Hz bin-width) were generated for each 20-s epoch of EEG data, and an average FFT for each animal was generated from the 2-min time sample. Four frequency bands (0.1–3.9, 4.0–7.9, 8.0–11.9, and 12–30 Hz) were chosen for a priori analysis and submitted to an analysis of variance (ANOVA) with factors of sex (two levels, male and female) and frequency band (four levels). The ANOVA revealed a significant main effect of frequency band ($F(3,42) = 15.2$, $P < 0.0001$) indicating that spectral power varied as a function of EEG frequency. Post-hoc comparisons ($P < 0.05$) revealed that spectral power in each frequency band was reliably different from that in all other bands. There were, however, no sex differences in spectral power. This was indicated by a non-significant main effect of sex ($F(1,14) = 1.9$, $P = 0.19$) and a non-significant interaction of sex and frequency band ($F(3,42) = 2.0$, $P = 0.13$). The similar spectral power in male and female rats indicates that the level of anesthesia in male and female rats was comparable.

Perforant path synaptic transmission in male and female rats was assessed by recording extracellular field potentials in the dentate gyrus during a 70-min recording period. As shown in Fig. 2A, perforant path-evoked field potentials in the dentate gyrus consisted of a 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. The EPSP slope and PS amplitude of these potentials were measured and the resultant values were normalized to the average of the 10 min baseline. Fig. 2B shows the percentage of change (relative to baseline) values for EPSP slope and PS amplitude averaged across 1 min intervals. To assess basal perforant path synaptic transmission in male and female rats, several parameters were extracted from the pre-HFS (10 min baseline) average and submitted to one-way ANOVAs with factors of sex (two levels). There were no reliable differences between males and females on any of the pre-HFS waveform measures

Table 1

Mean (±SEM) waveform parameters from perforant path-evoked responses in the dentate gyrus of urethane-anesthetized male and female rats

	Male	Female	F	P
EPSP slope (mV/ms)	1.8 ± 0.4	1.6 ± 0.2	0.3	0.60
EPSP amplitude (mV)	5.2 ± 1.0	4.5 ± 0.4	0.4	0.55
EPSP latency (ms)	2.19 ± 0.02	2.15 ± 0.06	0.5	0.49
PS amplitude (mV)	1.7 ± 0.3	1.8 ± 0.2	0.1	0.81
PS latency (ms)	4.4 ± 0.1	4.5 ± 0.1	0.4	0.53
PS threshold (mV)	2.7 ± 0.7	2.1 ± 0.3	0.5	0.47
FV amplitude (μV)	64.1 ± 15.9	54.9 ± 6.92	0.3	0.61

Values were extracted from extracellular perforant path-evoked field potentials in the dentate gyrus collected during a 10-min pre-HFS period. EPSP slope was measured at a fixed interval (4–6 msec) 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.

(see Table 1), which indicates that there is not a sex difference in perforant path synaptic transmission in urethane-anesthetized rats.

It is apparent from Fig. 2A that perforant path HFS induced a robust LTP of both EPSP slope and PS amplitude in male and female rats. For analysis of perforant path 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 (50–60 min post-HFS). These values were submitted to a one-way ANOVA with a factor of sex (two levels). The ANOVA revealed a significant sex difference in the level of EPSP ($F(1,14) = 22.4$, $P < 0.0003$), but not PS ($F(1,14) = 1.3$, $P = 0.28$), LTP 60 min following HFS. This analysis indicates that perforant path HFS induces a greater magnitude of LTP in the dentate gyrus in male compared to female rats. Interestingly, this sex difference in LTP was specific to the synaptic (EPSP) component of the field potentials; population spike LTP, which may be the result of different neuronal induction mechanisms, was not sexually dimorphic.

In the dentate gyrus, LTP induction typically requires activation of NMDA receptors [4]. 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 [8]. 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 'burst depolarization' during HFS was quantified. This parameter was quantified by subtracting the first from the second burst

potential (interburst interval = 200 ms) in each of the ten burst-pairs in the HFS train. The area under the late depolarization was measured, averaged across the ten burst-pairs, and normalized to the area of the pre-HFS single-pulse evoked field EPSP. Consistent with the levels of EPSP slope LTP, male rats exhibited greater burst depolarization than female rats (mean ± SEM (in arbitrary units): male, 1.8 ± 0.1; female, 1.4 ± 0.1). An ANOVA confirmed this effect ($F(1,14) = 5.3$, $P < 0.04$).

In summary, the present results reveal a robust sex difference in perforant path LTP under an anesthetic that induces comparable levels of anesthesia in male and female rats. As in an earlier report [3], this sex difference in LTP was specific to the EPSP component of the dentate field potentials and was paralleled by a sex difference in NMDA receptor activation; there were no sex differences in basal perforant path synaptic transmission. It is concluded that sex differences in perforant path LTP cannot be explained by levels of anesthesia. Sexually dimorphic LTP in intact rats may underlie sex differences in forms of learning and memory, such as contextual fear conditioning, that require the hippocampus.

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