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Parallel Augmentation of Hippocampal Long-Term Potentiation, Theta Rhythm, and Contextual Fear Conditioning in Water-Deprived Rats

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The influence of water deprivation on hippocampal long-term potentiation (LTP), theta rhythm, and contextual fear conditioning in rats was examined. In Experiment 1, hippocampal EEG activity and perforant path LTP were assessed in pentobarbital-anesthetized rats. Water deprivation did not affect baseline cell excitability or low-frequency synaptic transmission in the dentate gyrus, but it increased the magnitude of perforant path LTP and elevated the proportion of theta rhythm in the EEG. In Experiment 2, rats were classically conditioned to fear a novel context through the use of aversive footshocks. Water deprivation facilitated the rate of contextual fear conditioning but did not alter the asymptote of learning. Experiment 3 demonstrated that the facilitation of contextual fear conditioning was not due to a change in unconditional shock sensitivity. These results suggest that water deprivation exerts an influence on contextual fear conditioning by modulating hippocampal LTP and theta rhythm and that these processes serve to encode contextual information during learning.

Since the publication of Hebb's seminal work, *The Organization of Behavior* (1949), considerable interest in the role of synaptic plasticity in learning and memory has developed in the neuroscience community. In mammals, the primary experimental model of synaptic plasticity is long-term potentiation (LTP), an enduring enhancement of synaptic transmission induced at excitatory synapses in the brain following brief trains of high-frequency stimulation (HFS; Bliss & Gardner-Medwin, 1973; Bliss & Lømo, 1973). Like memory, LTP is rapidly consolidated, stable over long periods of time (up to weeks in vivo), specific to activated synapses (affording a massive storage capacity), and, under appropriate conditions, associative (Bliss & Collingridge, 1993; Brown, Chapman, Kairiss, & Keenan, 1988). Collectively, these properties have fostered the view that LTP participates in the neural mechanisms of learning and memory (Lynch, Kessler, Arai, & Larson, 1990; Teyler & Discenna, 1984).

In rats, LTP has been demonstrated in several brain regions, but it is most robust and has been studied most extensively in the hippocampus. LTP induction in hippocampal area CA1 and the dentate gyrus typically requires activation of postsynaptic *N*-methyl-D-aspartate (NMDA) receptors, a subclass of receptors for the neurotransmitter glutamate (Collingridge, Kehl, & McLennan, 1983; Maren, Baudry, & Thompson, 1991, 1992; Morris, Anderson, Lynch, & Baudry, 1986). This is generally accomplished by coupling presynaptic neurotransmitter release with strong postsynaptic depolarization during HFS of excitatory afferent fibers (Mayer, Westbrook, & Guthrie, 1984; Nowak, Bregestovski, Asher, Herbet, & Prochiantz, 1984). Calcium influx through activated NMDA receptors triggers a series of intracellular enzymatic cascades and a consequent synaptic modification that maintains the long-term enhancement of synaptic transmission over time (see Baudry, 1991, for a review). The nature of this modification is still a matter of debate, though a number of reports suggest that postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors (a non-NMDA subclass of glutamate receptors) are involved (Davies, Lester, Reymann, & Collingridge, 1989; Manabe, Renner, & Nicoll, 1992; Maren et al., 1992; Maren, Tocco, Standley, Baudry, & Thompson, 1993; Muller, Joly, & Lynch, 1988; Tocco, Maren, Shors, Baudry, & Thompson, 1992).

Given the prominent role of the hippocampus in some forms of learning (Eichenbaum, Otto, & Cohen, 1992; Hirsch, 1974; O'Keefe & Nadel, 1978; Sutherland & Rudy, 1989), considerable effort has been directed at elucidating the role of hippocampal LTP in these processes. Several investigators have demonstrated that pharmacological blockade of NMDA receptors, which prevents LTP induction, impairs learning in a variety of hippocampus-dependent tasks (Kim, DeCola, Landeira-Fernandez, & Fanselow, 1991; Morris et al., 1986; G. S. Robinson, Crooks, Shinkman, & Gallagher, 1989; Shapiro & Caramanos, 1990; Staubli, Thibault, DiLorenzo, & Lynch, 1989). Because these manipulations generally do not affect the performance of previously learned responses, they probably depend on the NMDA receptor-dependent increases in hippocampal AMPA receptor binding that accompany both LTP (Maren, Tocco, et al., 1993; Tocco et al., 1992) and learning (Tocco et al., 1991). Other studies have revealed electrophysiological correlates of LTP in the hippocampus during associative learning (Skelton, Scarth, Wilkie, Miller, & Phillips, 1987;

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Weisz, Clark, & Thompson, 1984) and exploration (E. J. Green, McNaughton, & Barnes, 1990; Sharp, McNaughton, & Barnes, 1989), although the latter may be due to corresponding changes in brain temperature during exploration (Moser, Mathiesen, & Andersen, 1993). LTP-inducing stimulation also serves as an effective conditional stimulus (CS) for classical conditioning (Doyère & Laroche, 1992) and enhances learning rate when administered prior to conditioning (Berger, 1984). Although these collective results do not prove that hippocampal LTP mediates associative learning and memory—NMDA receptor antagonists, for example, may influence learning through some mechanism other than LTP blockade—they are consistent with such a proposal.

If hippocampal LTP is a synaptic memory mechanism, it should be sensitive to conditions that influence learning and memory. For instance, most forms of associative learning are strongly influenced by nonassociative factors such as attention, emotional arousal, and motivation, which collectively define an animal's *endogenous state* (Berlyne, 1960; Bolles, 1975; Mitchell, Kirschbaum, & Perry, 1975; Mitchell, Koleszar, & Scopatz, 1984; Yerkes & Dodson, 1908). Insofar as hippocampal LTP is a neural substrate for learning and memory, one would expect it to be similarly sensitive to endogenous state. In support of this, numerous studies have demonstrated that both hippocampal EEG activity in the theta range (4–12 Hz; Berry & Swain, 1989; Grastyan, Karmos, Vereczkey, & Kellenyi, 1966; J. D. Green & Arduini, 1954; Vanderwolf, 1969) and synaptic transmission (Bramham & Srebro, 1989; Winson & Abzug, 1977, 1978) are modulated by endogenous state. Hippocampal LTP induction is dependent on the phase of theta rhythm (Pavlides, Greenstein, Grudman, & Winson, 1988), optimal with stimulation patterns that mimic theta rhythm (Larson, Wong, & Lynch, 1986), and similarly modulated by endogenous state (Bramham & Srebro, 1989; Diamond, Bennett, Stevens, Wilson, & Rose, 1990; Foy, Stanton, Levine, & Thompson, 1987; Leonard, McNaughton, & Barnes, 1987; Shors, Foy, Levine, & Thompson, 1990). Moreover, individual differences in hippocampal LTP correlate with both serum corticosterone levels (Bennett, Diamond, Fleshner, & Rose, 1991) and behavioral responses to novel environmental stimuli (Maren, Patel, Thompson, & Mitchell, 1993). Both of these measures are sensitive to endogenous arousal levels (Maren, Patel, et al.; Misslin & Cigrang, 1986).

Although the evidence discussed indicates that hippocampal LTP is sensitive to endogenous state, it remains to be determined if there is a corresponding relationship between the influence of endogenous state on LTP and associative learning. To address this issue, we examined the influence of a shift in motivational state induced by mild water deprivation on perforant path LTP, hippocampal theta rhythm, and contextual fear conditioning in rats. Contextual fear conditioning is an ideal behavioral paradigm for this purpose because it depends on the integrity of the hippocampus (Kim & Fanselow, 1992; Phillips & LeDoux, 1992), requires NMDA receptor activation (Kim et al., 1991; Kim, Fanselow, DeCola, & Landeira-Fernandez, 1992), and is sensitive to endogenous state (Fanselow, DeCola & Young, 1993; Phillips & LeDoux, 1992). Because the increase in motivation produced by either food or water deprivation generally enhances the rate (and frequently the asymptote) of learning in a variety of tasks (Barry, 1958; Berry & Swain, 1989; Campbell & Kraeling, 1953; Jensen, 1960; Lewis & Cotton, 1960; MacDuff, 1946), we hypothesized that water deprivation would enhance both hippocampal LTP and theta rhythm and facilitate the acquisition of contextual fear.

Experiment 1

To determine whether water deprivation affects the induction of hippocampal LTP, water-deprived and nondeprived rats were anesthetized and implanted with an extracellular recording electrode in the hilus of the dentate gyrus and a stimulating electrode in the perforant path, the major source of excitatory afferents to the hippocampus (Amaral & Witter, 1989). Single-pulse stimulation of the perforant path evoked robust and reproducible monosynaptic field potentials in the dentate gyrus. In addition to perforant path-evoked responses, hippocampal EEG activity was monitored through the recording electrode in the dentate gyrus. All of the electrophysiological testing was performed under anesthesia to permit the stable recording of both hippocampal EEG activity and perforant path-evoked field potentials independent of ongoing behavior. This was an important consideration because, under some conditions, general activity levels and exploratory behavior in water-deprived and nondeprived animals are considerably different (Bolles, 1975). These behaviors interact with both perforant path-evoked field potentials and hippocampal EEG activity (E. J. Green et al., 1990; Hargreaves, Cain, & Vanderwolf, 1990; Sharp et al., 1989), which may be the result of increased brain temperature in exploring animals (Moser et al., 1993). Following baseline recordings, LTP was induced in the dentate gyrus by applying brief trains of HFS to the perforant path. Perforant path HFS was patterned after the hippocampal theta rhythm, which is optimal for LTP induction (Larson et al., 1986).

Method

Subjects and surgery. Twelve adult male Long-Evans rats (250–300 g; Simonsen Labs, Gilford, CA) maintained in the University of Southern California vivarium served as subjects. The rats were pair housed in hanging plastic tubs on a 12:12-hr light–dark cycle (lights on at 7 a.m.) with free access to food and water. All electrophysiological testing was performed during the light phase of the cycle.

Prior to electrophysiological testing, one group of subjects (deprived, $n = 6$) was placed on a restricted fluid schedule consisting of 1-hr access to water per day for 3 days. A 3-day schedule was used to eliminate any initial nonspecific stress effects associated with the novelty of restricted access to water. The other group of subjects (nondeprived, $n = 6$) remained on ad-lib water. On the day of electrophysiological testing (24 hr following the last 1-hr fluid session), rats were anesthetized with an ip injection of sodium pentobarbital (Nembutal, 65 mg/kg) and mounted in a Kopf stereotaxic frame; the head position was adjusted to place bregma and lambda in the same horizontal plane. 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 elec-

trode 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 mm–3.0 mm ventral to brain surface), and the bipolar stimulating electrode was implanted 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 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 (0.1 ml) of pentobarbital as needed.

Acute electrophysiology. Extracellular hilar field potentials evoked by single-pulse perforant path stimulation (100-μs pulses) were amplified (gain = 100), bandpass filtered (.001–10 kHz), displayed on an oscilloscope, digitized, and written to disk (BrainWave Systems, Colorado Springs, CO). As shown in Figure 1, perforant path-evoked field potentials in the dentate gyrus consisted of a characteristic gradual positive-going excitatory postsynaptic potential (EPSP) with a sharp negative-going population spike (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 population spike 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. Prior to LTP induction, a 2-min time sample (six 20-s epochs) of hippocampal EEG activity was collected. The EEG activity was amplified (gain = 1000), filtered (0.2–20.0 Hz), displayed on an oscilloscope, digitized, and written to disk (BrainWave Systems). Input–output (I/O) functions consisting of three averaged perforant path-evoked field potentials at each of 10 different stimulation intensities were also generated prior to perforant path HFS. The I/O stimulation intensities for each animal were adjusted to elicit a range of field potentials. In general, the lowest stimulation intensity produced a pure subthreshold EPSP (no PS), whereas the highest intensity generated an asymptotic EPSP and asymptotic PS. These I/O functions were used to examine baseline (pre-HFS) EPSPs and PSs, and the relationship between the two, across a range of stimulation intensities. Following collection of the EEG and I/O data, perforant path-evoked field potentials (stimulation current intensity adjusted to elicit a 3-mV population spike) were recorded during a 10-min period before and a 40-min period following 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, that is, they were separated by 200 ms (5 Hz), and each of the 10 pairs of bursts was separated by 10 s.

Data analysis. EEG activity was analyzed to determine the spectral power across a range of frequencies (1–20 Hz). Fast Fourier transforms (FFTs; 1-Hz bin-width) were generated for each 20-s epoch of EEG data, and an average FFT for each rat was generated from the 2-min time sample. Spectral power at each frequency bin was normalized to activity in the 12–20 Hz frequency band by computing a within-subject ratio (Berry & Swain, 1989; Berry & Thompson, 1978); the resultant ratio was termed relative power. This procedure was implemented to reduce the between-subject variance in EEG amplitude. Three low-frequency bands (0.1–3.9, 4.0–7.9, and 8.0–11.9 Hz) were chosen for a priori analysis (Balleine & Curthoys, 1991). The first band served as a low-frequency control, and the latter two bands, representing atropine-sensitive (Type 2, 4.0–7.9 Hz) and atropine-resistant (Type 1, 8.0–11.9 Hz) theta rhythm (Kramis, Vanderwolf, & Bland, 1975), were chosen for analysis because they have been variously implicated in arousal and learning (Balleine & Curthoys, 1991; Berry & Swain, 1989; Berry & Thompson, 1978; J. D. Green & Arduini, 1954; Landfield, 1976; Mizumori, Perez, Alvarado, Barnes, & McNaughton, 1990; Vanderwolf & Leung, 1983; Winson, 1978). Moreover, there is evidence suggesting that hippocampal theta rhythm interacts with LTP induction in vivo (Larson et al., 1986; Pavlides et al., 1988). Group differences in spectral power at these frequency bands were assessed with independent two-tailed t tests ($df = 10$).

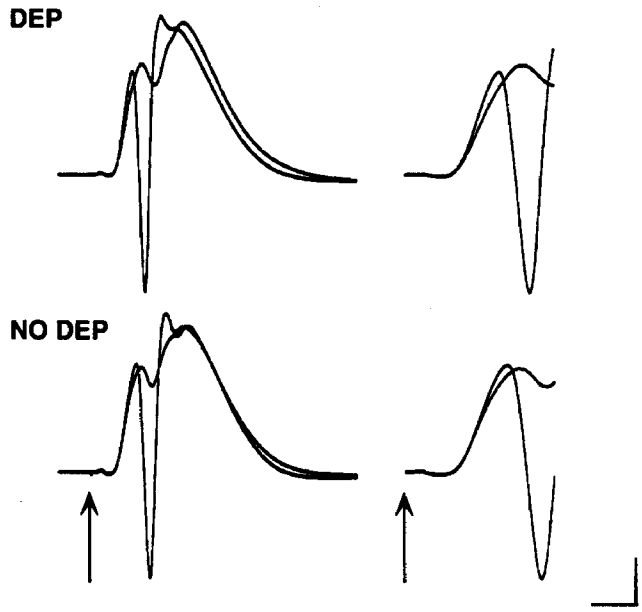

Figure 1. Perforant path-evoked hilar field potentials from representative water-deprived (DEP) and nondeprived (NO DEP) subjects. Pre- and post–high-frequency stimulation field potentials are superimposed; arrows indicate stimulus onset. Each waveform is an average of 30 single-pulse evoked potentials. The deprived subject showed greater potentiation of excitatory postsynaptic potential (EPSP) slope, measured on the rising phase of the field potential, and a greater decrease in the peak latency of the population spike (PS, the sharp negative-going potential superimposed on the field EPSP). Traces on the right have an expanded time base (2×) to illustrate the greater synaptic (EPSP) potentiation in the water-deprived subject. Calibration: 4 ms (2 ms for traces on the right), 3 mV.

Averaged perforant path-evoked field potentials were generated for each 10-min block of the 50-min recording period (30 waveforms per average). Several parameters were extracted from the averaged field potentials yielding the measures described in Table 1. The percentage of change of each of these measures was computed from the 10-min pre-HFS baseline to the final 10 min of the test period (30 min–40 min post-HFS). Within each treatment group, LTP was assessed by comparing baseline and post-HFS measures with two-tailed t tests for repeated measures ($df = 5$). Group differences in LTP and baseline responses were evaluated by comparing the percentage of change measures with independent two-tailed t tests ($df = 10$). The baseline I/O data were treated as described above and analyzed using analysis of variance (ANOVA) with a between-subjects variable of group (2 levels, deprived and nondeprived) and a within-subjects variable of stimulation intensity (10 levels, intensities 1–10). All data are presented as means ± the standard errors of the means (SE_M).

Results and Discussion

The mean pre-HFS perforant path stimulation intensity ($\pm SE_M$) was 90.2 ± 13.4 μA and 88.9 ± 15.5 μA for

Table 1
Mean (± SE_M) Waveform Measures Extracted From Perforant Path-Evoked Field Potentials in Water-Deprived and Nondeprived Subjects

Measure	Nondeprived (n = 6)			Water-deprived (n = 6)		
	Baseline	Post-HFS	%Δ	Baseline	Post-HFS	%Δ
EPSP slope (mV/ms)	6.0 ± 0.6	7.2 ± 0.9[a]	18.9 ± 2.2	5.5 ± 0.4	7.3 ± 0.5[a]	32.0 ± 2.0[b]
PS area (mV · ms)	3.9 ± 0.4	16.9 ± 1.8[a]	348.4 ± 63.4	4.0 ± 0.3	18.5 ± 1.0[a]	372.4 ± 44.7
PS amplitude (mV)	3.0 ± 0.3	16.7 ± 2.2[a]	476.2 ± 87.2	3.1 ± 0.2	20.0 ± 1.5[a]	548.7 ± 55.1
PS peak latency (ms)	5.1 ± 0.1	4.6 ± 0.1[a]	−9.1 ± 1.6	5.3 ± 0.1	4.5 ± 0.1[a]	−13.7 ± 1.0[b]
PS threshold (mV)	9.8 ± 1.0	9.5 ± 1.1	−3.2 ± 3.2	9.8 ± 0.7	9.3 ± 0.7	−5.5 ± 2.5

Note. Baseline and Post-HFS are 10-min blocks immediately prior to perforant path high-frequency stimulation (HFS) and 30–40 min post-HFS, respectively. %Δ is the percent change from the baseline to post-HFS blocks. Excitatory postsynaptic potential (EPSP) slope was measured at a fixed interval (4–6 ms) from the stimulus artifact; population spike (PS) area and amplitude were measured with reference to a line drawn between the EPSP peak amplitude and PS onset; PS peak latency was the time between stimulus artifact and the peak negativity of the PS; PS threshold was defined as the voltage at PS onset. [a]Significantly different from baseline. [b]Significantly different from nondeprived subjects. There were no significant differences between deprived and nondeprived animals on any of the baseline measures.

water-deprived and nondeprived subjects, respectively. As shown in Table 1, water deprivation had no effect on any measure of baseline (pre-HFS) perforant path-evoked field potentials, all $ts < 1.0$. This was confirmed by an analysis of baseline I/O functions (see Figure 2). Both EPSPs and PSs were similar in water-deprived and nondeprived subjects, $Fs(1, 10) < 1$, and increased significantly across the range of stimulation intensities, $Fs(9, 90) > 80, ps < .01$. In addition, the relationship of EPSP slope to PS amplitude, a measure of cell excitability derived from the pre-HFS I/O functions, was also similar in water-deprived and nondeprived subjects, $F(1, 10) < 1$, (see Figure 3). These results indicate that prior to LTP induction low-frequency synaptic transmission and postsynaptic excitability in the dentate gyrus were comparable in water-deprived and nondeprived rats.

As shown in Figure 1, high-frequency perforant path stimulation induced robust LTP in the dentate gyrus of both water-deprived and nondeprived subjects. This was indicated by significant increases from baseline in EPSP slope, PS area, PS amplitude, and a significant decrease in PS peak latency, in both water-deprived subjects, all $ts > 10.0, ps < .01$, and nondeprived subjects, all $ts > 4.0, ps < .01$, 30–40 min following HFS (see Table 1). However, as shown in Figure 4, HFS induced significantly more LTP, measured as the post-HFS percentage of change from baseline, in water-deprived compared with nondeprived animals. Thus, water-deprived subjects exhibited both a significantly greater potentiation of EPSP slope, $t = 4.4, p < .01$, and a significantly greater decrease in PS peak latency, $t = 2.4, p < .05$, relative to nondeprived subjects (see Table 1). This augmentation of EPSP slope and PS peak latency LTP by water deprivation is also apparent in the averaged perforant path-evoked field potentials shown in Figure 1.

As described previously, LTP induction requires NMDA receptor activation, thus any group differences in NMDA receptor activation evoked by perforant path HFS could account for the enhancement of LTP with water deprivation. Field potentials evoked during HFS exhibit a late component (~10-ms onset latency) that is largely NMDA receptor mediated (Maren et al., 1992). An analysis of this NMDA receptor-mediated depolarization revealed no significant differences between water-deprived and nondeprived subjects (data not shown). It is therefore unlikely that the different magnitude of perforant path LTP in water-deprived and nondeprived subjects is due to a difference in the initial, NMDA receptor-dependent phase of LTP induction. Alternatively, water deprivation may be exerting an influence on either the stabilization or expression of LTP. Water deprivation has been reported to increase glutamate receptor binding in the hippocampus (Meeker, Greenwood, McGinnis, & Hayward, 1992). And, as mentioned earlier, hippocampal LTP is expressed, in part, by an increase in postsynaptic glutamate receptor binding (Maren, Tocco, et al., 1993; Tocco et al., 1992). These data suggest that water deprivation may have exerted an influence on hippocampal LTP by modulating the expression mechanisms mediated by postsynaptic glutamate receptors.

The EEG spectra for water-deprived and nondeprived subjects are shown in Figure 5. The spectra in both groups are fairly typical of EEG activity recorded in anesthetized animals, although the activity in the theta range is lower than that recorded under urethane or ether anesthesia (Kramis et al., 1975). This is probably due to the suppression of theta activity by barbiturate anesthetics, such as pentobarbital (Kramis et al., 1975). Nonetheless, it is apparent in Figure 5A that water deprivation augmented EEG activity in a narrow frequency band (4.0–7.9 Hz) associated with atropine-sensitive theta rhythm, having little or no effect at either lower or higher frequencies. These observations were confirmed by t tests performed on three discrete frequency bands of the EEG spectrum (see Figure 5B). Water deprivation significantly elevated EEG activity from 4.0 Hz to 7.9 Hz, $t = 2.7, p < .05$, but it did not alter EEG activity in either lower (0.1–3.9 Hz) or higher (8.0–11.9 Hz) frequency bands, $ts < 1$. Hence, these data indicate that water deprivation selectively elevated the proportion of low-frequency theta rhythm in the EEG spectrum. The mean total power (±SE_M) of EEG activity recorded from water-deprived (670 ± 80 μV^2) and nondeprived (780 ± 130 μV^2) rats did not significantly differ, $t < 1.0$, indicating that the depth of anesthesia was comparable for subjects in both groups. Thus, the group differences in hippocampal theta

rhythm and LTP described above cannot be attributed to group differences in levels of anesthesia. The augmentation of hippocampal theta rhythm by water deprivation corroborates similar reports of enhanced theta rhythm in water-deprived rabbits (Berry & Swain, 1989) and food-deprived rats (Ford, Bremner, & Richie, 1970). As in the present study, both of these reports found water deprivation-induced elevations of theta activity in a range associated with atropine-sensitive theta rhythm.

The similar augmentation of hippocampal LTP and theta rhythm suggests that the two phenomena are related. Accordingly, an examination of each subject's data revealed a significant Pearson correlation, $r = .88, t(10) = 6.0, p < .01$, between

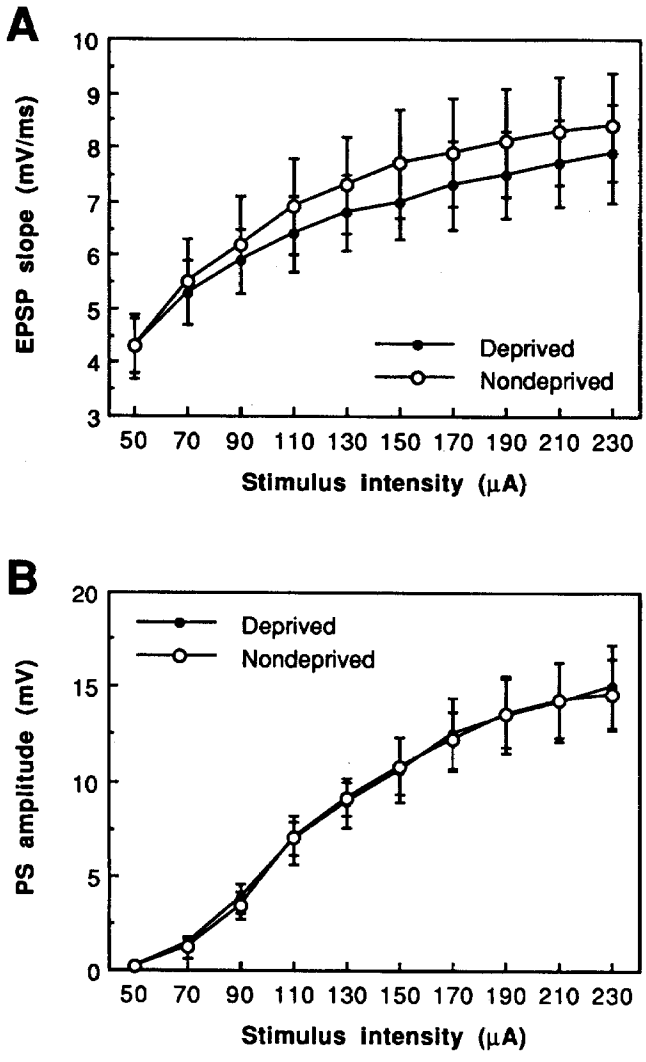

Figure 2. A: Mean ($\pm SE_M$) baseline (pre–high-frequency stimulation) excitatory postsynaptic potential (EPSP) slope and B: population spike (PS) amplitude input–output (I/O) functions for water-deprived (filled circles) and nondeprived subjects (open circles). The I/O functions were generated across a range of 10 stimulation intensities, from intensities that evoked only a pure EPSP to those that evoked an asymptotic EPSP and PS. Each value represents the mean of three observations.

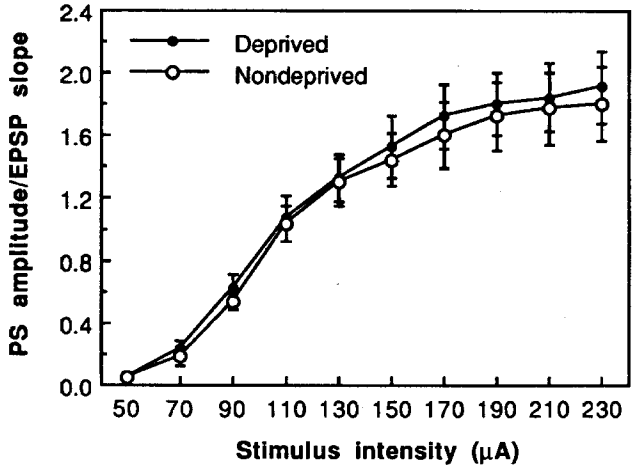

Figure 3. Mean ($\pm SE_M$) ratios of population spike (PS) amplitude to excitatory postsynaptic potential (EPSP) slope input–output data for water-deprived (filled circles) and nondeprived subjects (open circles).

EPSP slope LTP and the proportion of theta activity in the 4.0 Hz to 7.9 Hz frequency band (see Figure 6). These findings, although correlational, allow that there may be some causal relationship between hippocampal theta rhythm and LTP in the dentate gyrus. Consistent with this view, medial septal or mesencephalic reticular formation stimulation, which themselves generate hippocampal theta rhythm, facilitate perforant path LTP induction and expression, respectively (Bloch & Laroche, 1985; G. B. Robinson & Racine, 1982). Hippocampal LTP induction is also sensitive to the phase of the theta rhythm (Pavlides et al., 1988) and is induced preferentially by stimulation patterns that mimic the theta rhythm (Larson et al., 1986). Additionally, hippocampal LTP induction only occurs in behavioral states that are associated with theta rhythm, including waking and REM sleep states (Bramham & Srebro, 1989), and does not occur during slow-wave sleep, which is not associated with theta rhythm (Bramham & Srebro, 1989; Leonard et al., 1987). Thus, hippocampal theta rhythm may at least have a permissive influence on LTP induction, if not an active role in the stabilization or expression of LTP. The correlation between theta rhythm and LTP may also be due to the similar dependence of these processes on NMDA receptor activation (Leung & Desborough, 1988).

Experiment 2

Contextual fear conditioning, although critically dependent on the amygdala (Blanchard & Blanchard, 1972; Helmstetter, 1992; Hitchcock & Davis, 1986; Iwata, LeDoux, Meeley, Arneric, & Reis, 1986; Maren, Poremba, & Gabriel, 1991), is mediated, in part, by the hippocampus (Blanchard, Blanchard, & Fial, 1970; Kim & Fanselow, 1992; Phillips & LeDoux, 1992). More specifically, hippocampal lesions disrupt the acquisition of conditional fear to contextual stimuli, but not to discrete cues (e.g., tones; Kim & Fanselow, 1992; Phillips & LeDoux, 1992). This observation is consistent with the general role of the hippocampus in processing both contextual (Hirsch, 1974) and spatial information (O'Keefe & Nadel, 1978), which

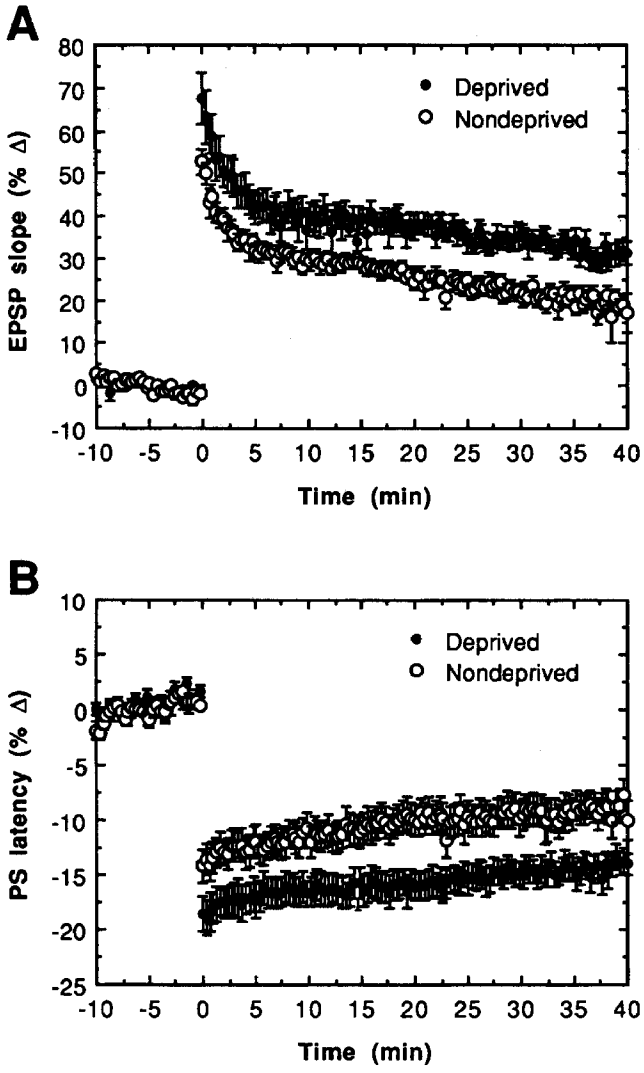

Figure 4. A: Mean ($\pm SE_M$) percentage of change in excitatory postsynaptic potential (EPSP) slope and B: population spike (PS) latency in water-deprived (filled circles) and nondeprived (open circles) subjects over the 50-min recording period. High-frequency stimulation was delivered at $T = 0$.

emphasizes the relational nature of hippocampal information processing (Eichenbaum et al., 1992; Sutherland & Rudy, 1989).

A recent study has implicated hippocampal NMDA receptors, and by inference LTP, in the mediation of contextual fear conditioning (Young, Bohenek, & Fanselow, 1994). If hippocampal LTP is involved in mediating contextual fear conditioning, then manipulations that enhance LTP should facilitate the acquisition of contextual fear. To examine this possibility, water-deprived and nondeprived animals received either one or three footshocks in a distinctive chamber. Varying the number of conditioning trials permitted an assessment of both the rate of conditioning and the magnitude of asymptotic learning. At 24 hr following the context–shock pairings, conditional fear to the context of the chamber was assessed by returning the rats to the chambers and measuring freezing, a species-specific defense reaction characterized by an immobile posture (Fanselow, 1980). Because motivational level can influence both the acquisition and performance of learned responses (Capaldi & Hovancik, 1973), the motivational state of the rats was equated during the freezing test by providing the water-deprived rats free access to water following the conditioning trials.

Method

Subjects. The subjects were 24 adult male Long-Evans rats (250–300 g) reared and maintained in the University of California, Los Angeles (UCLA) vivarium on a 12:12-hr light–dark cycle (lights on at 7 a.m.). The rats were individually housed in conventional hanging stain-

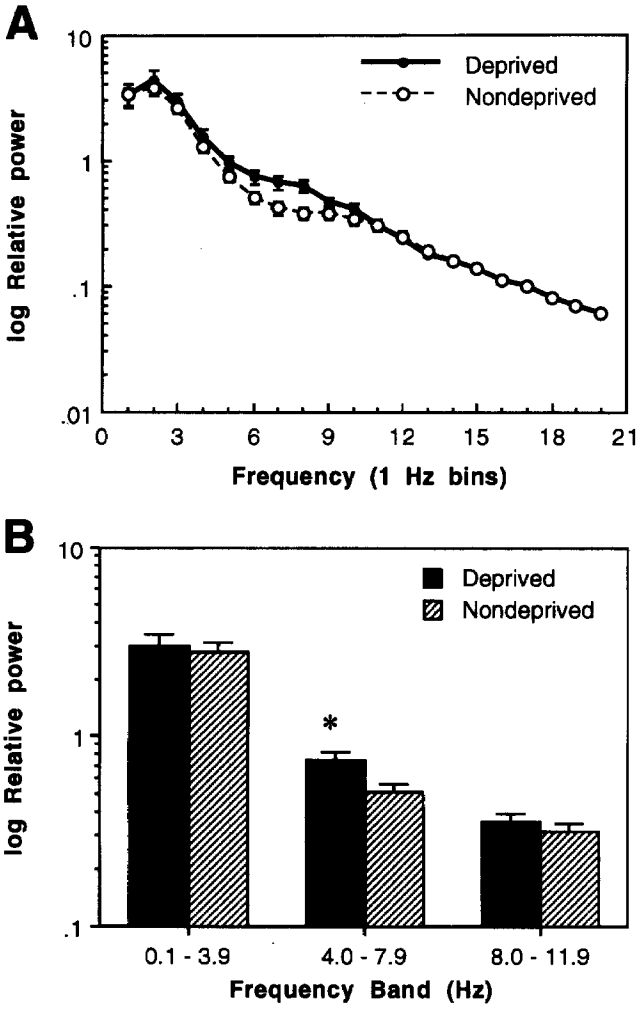

Figure 5. A: Mean ($\pm SE_M$) relative spectral power between 1 Hz and 20 Hz (1 Hz bins) for water-deprived (filled circles) and nondeprived (open circles) subjects. Values are ratios computed relative to the total power in the 12–20 Hz frequency band. B: Mean ($\pm SE_M$) relative spectral power of three frequency bands (0.1–3.9, 4.0–7.9, and 8.0–11.9 Hz) in water-deprived (solid bars) and nondeprived (hatched bars) subjects. The asterisk indicates a significant difference ($p < .05$) between the two groups.

less steel cages with free access to food and water. All behavioral testing was done during the light phase of the cycle.

Apparatus and procedure. Four identical observation chambers (28 × 21 × 10.5 cm; Lafayette Instrument Co., North Lafayette, IN) were used for both conditioning and testing. The chambers were situated in sound- and light-attenuating chests in a well-lit room. A video camera 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 Instrument Co., North Lafayette, IN) for the delivery of footshock unconditional stimuli (USs). 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.

Prior to conditioning, one group of subjects (deprived, $n = 12$) was placed on a restricted fluid schedule consisting of 1-hr access to water per day for 3 days. The other group of subjects (nondeprived, $n = 12$) remained on ad-lib water. On the day of conditioning (24 hr following the last 1-hr fluid session), the rats were placed in the conditioning chambers in 6 sets of 4 rats (2 deprived and 2 nondeprived rats per set); the chamber position was counterbalanced for each set and group. Water-deprived and nondeprived subjects received either one or three footshock(s) ($n = 6$ per group; 0.5-mA intensity, 1-s duration; 30-s intershock interval for the latter groups) 3 min after being placed in the chambers. Within 30 s following the final shock, the rats were returned to their home cages and allowed free access to water for the remainder of the experiment. The water-deprived rats were rehydrated so that deprivation-induced increases in activity and exploration would not interfere with the performance of conditional freezing during the retention test.

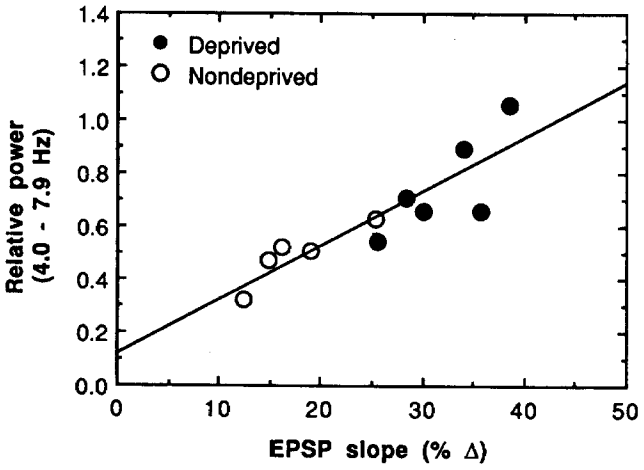

Figure 6. Linear relationship between hippocampal theta rhythm and excitatory postsynaptic potential (EPSP) slope long-term potentiation in water-deprived (filled circles) and nondeprived (open circles) subjects. Relative power values were calculated as described in the legend for Figure 5. EPSP slope values were calculated as the percentage of change from the 10-min pre–high-frequency stimulation (HFS) baseline to the last 10 min of the recording period (30 min–40 min post-HFS). The regression line computed for the subjects revealed a significant Pearson correlation coefficient ($r = .88$). The data from 2 subjects in the nondeprived group overlap; thus it appears that there are data from only 5 nondeprived subjects displayed in the graph, whereas the data from 6 subjects are actually displayed.

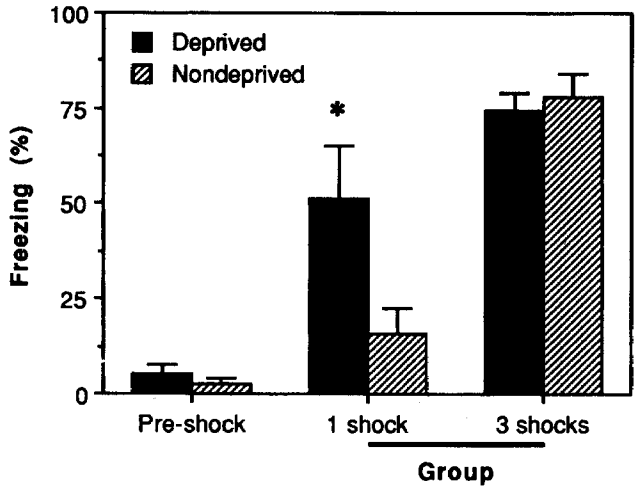

Figure 7. Mean ($\pm SE_M$) percentage of freezing in water-deprived (solid bars) and nondeprived subjects (hatched bars) during the preshock and postconditioning tests. Freezing scores are expressed as the percentage of total observations during each test. The asterisk indicates a significant difference between water-deprived and nondeprived subjects in the one-shock condition.

At 24 hr following training, fear conditioning to the context of the observation chamber was assessed by returning each rat to the chamber for 8 min. Conditioned fear was quantified by scoring freezing behavior with a method previously used in this laboratory (Fanselow, 1980). Briefly, an observer who was unaware of the experimental conditions scored each rat for freezing (behavioral immobility except for movement necessitated by respiration) every 8 s during the 8 min postconditioning test for a total of 60 observations. Freezing behavior was quantified in the same manner for the 3-min preshock period on the conditioning day.

Data analysis. The preshock freezing data were subjected to a one-way ANOVA with group as a variable (two levels, deprived and nondeprived). Freezing on the postconditioning test day was submitted to a two-way ANOVA with variables of group (two levels, deprived and nondeprived) and trial (two levels, one and three shocks). All data are presented as means ± the standard errors of the means (SE_M).

Results and Discussion

As shown in Figure 7, contextual fear conditioning, measured as freezing behavior, varied as a function of both motivational state and the number of context–shock pairings. First, rats that received three shocks during conditioning exhibited substantially more conditional freezing than those that received only one shock, $F(1, 20) = 24.0, p < .01$. Second, although there was not a significant main effect of deprivation state on conditional freezing, $F(1, 20) = 3.3, p = .09$, there was a significant interaction between deprivation state and the number of conditioning trials, $F(1, 20) = 4.9, p < .05$. Post hoc comparisons (Fisher tests, $p < .05$) revealed that water-deprived rats showed significantly more conditional freezing than nondeprived controls following one, but not three, context–shock pairings. Following three context–shock pairings, however, water-deprived and nondeprived animals showed comparable (asymptotic) levels of freezing. In addition, the

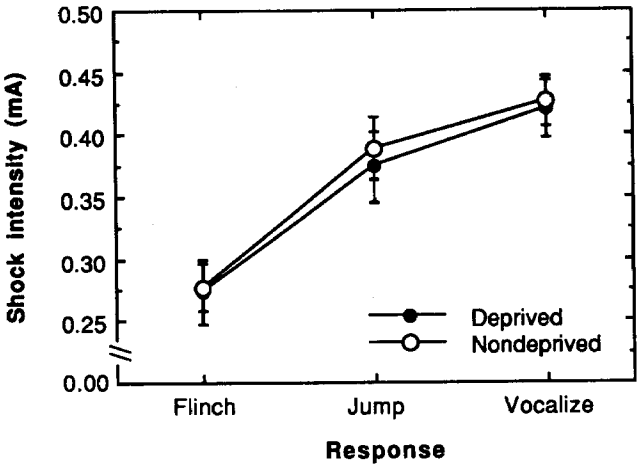

Figure 8. Mean ($\pm SE_M$) footshock intensity (mA) to evoke a flinch, jump, and vocalization in water-deprived (filled circles) and nondeprived subjects (open circles).

degree of freezing exhibited by water-deprived rats in the one-shock condition was not significantly different from the asymptotic levels of freezing shown by the three-shock groups. Thus, the effect of water deprivation was to enhance the rate, not the asymptote, of contextual learning. There were no group differences in baseline (preshock) freezing, $F(1, 22) = 0.6$, $p = .43$, indicating that unconditional fear to the novel conditioning chamber was similar in water-deprived and nondeprived animals.

One might argue that the state change experienced by water-deprived animals in the present experiment, from deprivation on the conditioning day to repletion on the test day, confounds interpretation of the data. However, a state change such as this would be expected to produce a generalization decrement (i.e., performance would be reduced in deprived rats on the test day), and we observed the opposite result.

The enhancement of contextual fear conditioning by a motivational shift induced by water deprivation is consistent with the well-known interaction of endogenous state and learning (Berlyne, 1960; Bolles, 1975; Mitchell et al., 1975; Mitchell et al., 1984; Yerkes & Dodson, 1908). However, it is interesting to note that the present results are unique in showing a facilitation of learning by a motivational state seemingly irrelevant to any dimension of the training situation. For instance, it has been repeatedly demonstrated that food-deprived (or water-deprived) rats will acquire an operant response for a food (or water) reward much faster than nondeprived animals (Barry, 1958; Campbell & Kraeling, 1953; Capaldi & Hovancik, 1973; Jensen, 1960; Lewis & Cotton, 1960; MacDuff, 1946). Whereas these reports show enhanced learning of food-motivated responses in food-deprived animals, our data indicate that the motivational state induced by water deprivation can also affect the learning of classically conditioned responses (i.e., conditioned freezing) unrelated to water availability or reward (see also Berry & Swain, 1989). This suggests that motivational states may nonspecifically enhance information processing in many stimulus modalities and augment associative learning in a variety of situations.

Experiment 3

The magnitude of fear conditioning is influenced by footshock intensity; more painful shocks condition more freezing (Fanselow & Bolles, 1979). Thus, the differential fear conditioning in water-deprived and nondeprived animals in Experiment 2 could have been due to a change in unconditional shock sensitivity produced by water deprivation. If, for example, water deprivation increased sensitivity to the US, water-deprived rats would be expected to exhibit superior conditioning than nondeprived controls. To test this possibility, water-deprived and nondeprived rats were tested for their unconditional sensitivity to footshock under conditions that closely approximated those of conditioning in Experiment 2.

Method

Subjects. The subjects were 20 adult male Long-Evans rats (250–300 g) reared and maintained in the UCLA vivarium on a 12:12-hr light–dark cycle (lights on at 7 a.m.). The rats were individually housed in conventional hanging stainless steel cages with free access to food and water. All behavioral testing was performed during the light phase of the cycle.

Apparatus and procedure. The apparatus was identical to that described in Experiment 1. Prior to shock-sensitivity testing, one group of subjects (deprived, $n = 10$) was placed on a restricted fluid schedule consisting of 1-hr access to water per day for 3 days. The other group of subjects (nondeprived, $n = 10$) remained on ad-lib water. On the day of testing (24 hr following the last 1-hr fluid session), the rats were placed in the observation chambers and were delivered a series of footshocks (1-s duration, intershock interval = 10 s). The footshocks were delivered in an ascending series of intensities from 0 mA to 0.66 mA in .066 mA steps. The rats were scored for their first flinch, jump, and vocalization to the footshocks: a flinch was any observable reaction to the footshock, typically an orienting response directed at the grid floor; a jump was any motoric response to the shock, typically running in place; and a vocalization was any audible vocalization to the shock. Following vocalization, the ascending series was repeated. Three replications of the footshock sensitivity test were performed on each animal within a testing session. Scoring was performed by an observer who was blind to the treatment conditions.

Data analysis. The footshock sensitivity data for each rat were averaged across replications and subjected to a two-way ANOVA with a between-subjects variable of group (two levels, deprived and nondeprived) and a within-subjects variable of response (three levels, flinch, jump, and vocalize). All data are presented as means ± the standard errors of the means (SE_M).

Results and Discussion

As shown in Figure 8, the footshock intensity required to elicit a flinch, jump, and vocalization increased for each successive behavioral response, $F(2, 36) = 150.4$, $p < .01$, but it did not differ between water-deprived and nondeprived animals, $Fs < 1.0$. Hence, the facilitated conditioning in water-deprived animals reported in Experiment 2 cannot be attributed to increased footshock sensitivity in these animals.

Alternatively, it is possible that water deprivation facilitated conditioning either indirectly by augmenting the processing of contextual conditional stimuli (CSs), or directly by modulating the neural systems involved in forming the CS-US association. We favor the former hypothesis because of the more prominent role of the hippocampus in processing contextual information than CS–US associations (Hirsch, 1974).

General Discussion

In Experiment 1, water deprivation enhanced hippocampal theta rhythm and increased the magnitude of perforant path LTP in a correlated manner. Water deprivation did not, however, affect baseline cell excitability or low-frequency synaptic transmission in the dentate gyrus, indicating that the influence of water deprivation on LTP and theta rhythm was not the result of a generalized change in hippocampal function. In Experiment 2, water deprivation facilitated the rate of contextual fear conditioning without affecting unconditional shock sensitivity (Experiment 3). Water-deprived animals reached near asymptotic levels of fear conditioning with only one context–shock pairing, whereas nondeprived controls required three context–shock pairings to reach similar levels of conditioning. Thus, water deprivation produced a parallel augmentation of hippocampal theta rhythm, perforant path LTP, and contextual fear conditioning.

The augmentation of hippocampal theta rhythm by water deprivation corroborates similar reports of enhanced theta rhythm in water-deprived rabbits (Berry & Swain, 1989) and food-deprived rats (Ford, Bremner, & Richie, 1970). Similarly, the enhancement of LTP induction in the dentate gyrus by water deprivation is consistent with other reports of the sensitivity of hippocampal synaptic plasticity to endogenous state (Bramham & Srebro, 1989; Leonard et al., 1987; Diamond et al., 1990; Foy et al., 1987; Shors et al., 1990). However, the present study is the first to report an enhancement of hippocampal synaptic plasticity with a behavioral manipulation that alters arousal levels. That is, to our knowledge, all other reports have reported attenuations of LTP following various manipulations of endogenous state. For instance, Diamond and colleagues (1990) report that exposing rats to a novel environment markedly suppresses the incidence of primed-burst (PB) potentiation (a low threshold form of LTP) in hippocampal area CA1 in vivo. Similarly, severe stress induced by inescapable tail shock impairs LTP induction in area CA1 in vitro (Foy et al., 1987; Shors et al., 1990). It is reasonable to suggest that the differences in the results of these studies and the present study are related to both the nature and levels of arousal induced by the behavioral manipulations used in each study. Insofar as serum corticosterone levels indicate endogenous stress or arousal, both forced exposure to novelty (Diamond et al., 1990) and inescapable tail shock (Foy et al., 1987; Shors et al., 1990) are similar in their ability to evoke robust increases in serum corticosterone levels (Misslin & Cigrang, 1986; Shors et al., 1990). Scheduled water deprivation, on the other hand, is not associated with a significant elevation in serum corticosterone levels (Armario & Jolin, 1986). Thus, the levels of arousal (and corticosterone) associated with acute water deprivation appear to be optimal for hippocampal LTP induction, whereas those associated with novelty- or shock-stress are deleterious for LTP induction.

In addition to augmenting hippocampal theta rhythm and perforant path LTP induction, water deprivation greatly facilitated the rate of contextual fear conditioning. The similar asymptotic learning (Experiment 2) and footshock sensitivity (Experiment 3) in water-deprived and nondeprived animals suggest that water deprivation augmented the acquisition of contextual fear by modulating the processing of contextual CSs. In this view, it is possible that water-deprived animals formed either a more inclusive contextual representation (i.e., a configural contextual representation with a greater number of individual elements) or a stronger contextual representation (i.e., a stronger contextual memory trace) than nondeprived animals. Although the present data do not distinguish between these alternatives, the former possibility may be more congruent with the behavioral data. That is, this alternative suggests that the contextual CS for water-deprived animals may have been substantially different from, and perhaps more salient than, that for nondeprived animals by virtue of its greater elemental complexity (McLaren, Kaye, & Mackintosh, 1989). Such a difference in CS salience could account for the faster rate of learning in water-deprived subjects (e.g., Mackintosh, 1975; Rescorla & Wagner, 1972). In contrast, a stronger CS-US association in water-deprived animals cannot account for the present results, because it would be expected to yield greater asymptotic conditioning in deprived compared with nondeprived animals. Hence, the similar asymptotic learning in water-deprived and nondeprived animals strongly suggests that the faster rate of conditioning in deprived animals was the result of enhanced CS processing.

The enhanced salience of contextual CSs in water-deprived subjects may have resulted from both behavioral and neural factors. From a behavioral point of view, it is well known that deprivation states increase exploratory behavior in a variety of situations (Bolles, 1975). The adaptive significance of increased exploratory behavior in deprived rats is obvious; rats in need of food or water must actively seek out repletion in order to survive (Cowan, 1983). Exploration is, therefore, frequently antecedent to other nonexploratory behaviors in food- or water-deprived animals. Indeed, deprived rats will continue to explore an unfamiliar environment even in the presence of food and water (Inglis, 1983). This evidence suggests that water deprivation generates an endogenous state that has been evolutionarily selected to favor the acquisition and processing of contextual information. Although we did not quantify exploratory behavior in the present study, it is possible that water-deprived rats engaged in more exploration when introduced to the training situation. This may explain, at least on a behavioral level, the superior contextual representations and faster contextual conditioning in water-deprived animals.

Many investigatory behaviors, including exploration, are accompanied by theta rhythm in the hippocampal EEG (Macrides, Eichenbaum, & Forbes, 1982; Vanderwolf, 1969; Vanderwolf & Leung, 1983). It is generally believed that theta rhythm reflects a state of active hippocampal information processing involved in information storage (e.g., Eichenbaum et al., 1992). Thus, the emergence of hippocampal theta rhythm during exploration apparently reflects, at least in part, the neural

coding of contextual stimuli in the hippocampus. As was shown in Experiment 1, the proportion of hippocampal EEG activity in the theta range is increased by water deprivation in anesthetized rats, an effect that is also observed in awake, behaving animals (Berry & Swain, 1989; Ford et al., 1970). Collectively, these data suggest that the enhanced theta rhythm in water-deprived rats confers greater hippocampal information processing and, consequently, superior contextual encoding in these rats. Hence, in addition to priming adaptive behavioral responses to deprivation (e.g., exploration), water deprivation produces concomitant changes in the physiology of hippocampal neuronal circuits that apparently afford a greater capacity for hippocampal processing of contextual stimuli.

An important component of this greater capacity for contextual information processing in water-deprived rats is the enhanced hippocampal LTP observed in these subjects. Because hippocampal LTP has been implicated in the mnemonic encoding of contextual (Kim et al., 1991) and spatial information (Morris et al., 1986; G. S. Robinson et al., 1989; Shapiro & Caramanos, 1990), the greater LTP induced in water-deprived subjects is a putative neural mechanism for the presumed superior representation of contextual stimuli in these rats. In this view, contextual representations in the hippocampus are thought to consist of associations between many discrete elemental stimuli in the environment (McLaren et al., 1989). We suggest that during fear conditioning environmental stimuli are acquired during exploration, processed by the hippocampus in discrete theta-locked cycles, and codified into a cohesive contextual representation by associative LTP in coactive hippocampal afferents. Once established, this hippocampal representation of the contextual CS is relayed to neurons in the amygdala, which is the site of convergence and association of CS and US information (Hitchcock & Davis, 1986; Iwata et al., 1986). Water deprivation optimizes both the theta-driven processing of contextual stimuli and the LTP-mediated association of contextual elements, thereby resulting in a more salient CS being conveyed to the critical amygdaloid neurons involved in association formation, and a consequent enhancement of contextual fear conditioning. Collectively, these results provide further evidence for a general role of hippocampal LTP and theta rhythm in the neural mechanisms of learning and memory and suggest a specific role for these processes in the acquisition of contextual representations during classical fear conditioning.

An alternative point of view to the foregoing discussion is that water-deprivation augments the processing of novel stimuli by the hippocampus, rather than specifically augmenting the processing of contextual stimuli. Although it is generally agreed that the hippocampus is not essential for neotic information processing, it appears to play an important role (Mitchell, Maren, & Hwang, 1993). For example, Maren, Patel, et al. (1993) found that emergence neophobia in an exploratory task was significantly correlated with individual differences in hippocampal LTP. Specifically, neophobic animals that were reluctant to enter and explore a novel alley exhibited both a lower threshold for and a greater magnitude of perforant path LTP (Maren, Patel, et al.). On the basis of this, it was hypothesized that neophobic animals were more sensitive to novelty–familiarity dimensions by virtue of their superior neotic information processing and habituation (for a similar argument see Mitchell, 1976). Theoretically, this could confer an advantage in the acquisition of fear conditioning, because novel stimuli generally condition more fear than familiar stimuli. For example, preexposure to discrete to-be-conditioned CSs (e.g., tones) greatly attenuates fear conditioning (Young & Fanselow, 1992). And, although preexposure to the training context initially facilitates fear conditioning (Fanselow, 1990; Young & Fanselow, 1992), extensive context preexposure ultimately impairs conditioning (Kiernan & Westbrook, 1993). The differentiation of context versus neotic processing accounts of the present data would be relatively simple: If water deprivation facilitates neotic information processing generally, then fear conditioning to novel discrete cues should be enhanced to a similar extent as conditioning to novel contextual cues in water-deprived animals. We are currently performing experiments to test this hypothesis.

A still unanswered question concerns the cellular mechanisms of the water deprivation-induced enhancement of hippocampal LTP and theta rhythm. As was suggested earlier, the enhancement of LTP by water deprivation may involve mechanisms mediating the expression of LTP, because the initial events during LTP induction (i.e., NMDA receptor activation) do not appear to be different in water-deprived and nondeprived animals. We have shown that perforant path LTP in anesthetized rats is associated with NMDA receptor-dependent elevations in postsynaptic AMPA receptor binding in the dentate gyrus (Maren, Tocco, et al., 1993; Tocco et al., 1992). These increases in hippocampal AMPA receptor binding provide a plausible mechanism for the expression of enhanced low-frequency synaptic transmission that occurs following perforant path LTP induction (Maren, Tocco, et al.). It has recently been reported that water deprivation also increases glutamate receptor binding in the hippocampus (Meeker et al., 1992). Although it is not yet clear whether these deprivation-related increases in hippocampal glutamate binding resulted from changes in AMPA or NMDA receptors, it is most likely that the changes were limited to AMPA receptors because of the greater sensitivity of these receptors to endogenous state (Tocco, Shors, Standley, Baudry, & Thompson, 1993). Taken together, these findings suggest that water deprivation may have exerted an influence on perforant path LTP by modulating the hippocampal AMPA receptors involved in LTP expression. A similar process may have contributed to the increased theta rhythm in water-deprived animals, which also depends on hippocampal glutamate receptors (Leung & Desborough, 1988). Although speculative, this proposed modulation of LTP and theta rhythm by water deprivation may have been mediated by hormonal factors released during dehydration, such as vasopressin (antidiuretic hormone). Although it is not known whether vasopressin interacts directly with glutamate receptors, it has been shown to have a number of neuroactive effects including altering cell excitability in the hippocampus. Additionally, vasopressin is a putative nootropic (i.e., a cognitive enhancer; de Wied, 1984), which would be consistent with the view that it plays a role in the enhancement of LTP, theta rhythm, and contextual fear conditioning produced by water deprivation.

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