

Water Deprivation Enhances Fear Conditioning to Contextual, but Not Discrete, Conditional Stimuli in Rats

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Water-deprived and nondeprived rats were fear conditioned with a discrete tone conditional stimulus (CS) and an aversive footshock unconditional stimulus (US). Twenty-four and 48 hr following conditioning, conditional fear to the tone CS and the context cues of the conditioning chamber, respectively, were assessed by measuring freezing behavior. Water deprivation had no effect on baseline responding to either tone or contextual stimuli. Following either 1 or 3 tone–shock pairings, however, water deprivation selectively enhanced conditional freezing to the contextual cues of the training chamber; conditional freezing to the tone was unaffected by water deprivation. These results are consistent with the view that water deprivation affects fear conditioning via an influence on the hippocampus.

Behavioral performance in many learning situations is affected by motivational state (Bolles, 1975). In food (or water)-rewarded instrumental tasks, for example, hungry (or thirsty) rats acquire operant responses faster and exhibit a greater number of responses than do sated rats (Barry, 1958; Campbell & Kraeling, 1953; Jensen, 1960; Lewis & Cotton, 1960; MacDuff, 1946). Drive-shift studies, in which behavioral training and testing occur under different motivation levels (see Spence, 1956), have shown that high levels of motivation during training markedly facilitate performance of learned responses under normal motivation levels (Capaldi, 1972; Capaldi & Hovancik, 1973; Eisenberger, Myers, & Kaplan, 1973; Hovancik, 1978). Hovancik (1978) has suggested that this phenomenon is due to a stronger association formation in deprived animals, although the neural mechanisms by which motivational state interacts with associative learning are unknown.

We recently examined the influence of water deprivation on Pavlovian conditioning of contextual fear using aversive footshocks and a drive-shift design (Maren, DeCola, Swain, Fanselow, & Thompson, in press). We found that water deprivation during conditional stimulus (CS, context)–unconditional stimulus (US, footshock) pairings enhanced conditional freezing to the contextual cues of the training chamber tested 24 hr following conditioning under normal levels of motivation. This enhancement of conditional fear occurred with one, but not with three, CS–US pairings (Maren et al., in press, Experiment 2) and was not due to a water deprivation–related change in unconditional footshock sensitivity (Maren

et al., in press, Experiment 3). The similar asymptotic learning in the three-shock groups and US sensitivity in water-deprived and nondeprived rats suggested that the enhanced learning in deprived rats resulted from greater salience of the contextual CS in these rats, although an effect of water deprivation on the formation of the CS–US association could not be ruled out. These findings extend those from instrumental paradigms and demonstrate that motivational state influences Pavlovian as well as instrumental conditioning.

In addition to its effects on contextual fear conditioning, water deprivation affected hippocampal neurophysiology in anesthetized rats (Maren et al., in press). Specifically, water deprivation augmented hippocampal EEG activity in the theta range (4.0–7.9 Hz) and increased the magnitude of perforant path–dentate granule cell long-term potentiation (LTP) (Maren et al., in press, Experiment 1). Because the hippocampus is thought to play a role in contextual learning (Blanchard, Blanchard, & Fial, 1970; Hirsch, 1974; Kim & Fanselow, 1992; O’Keefe & Nadel, 1978; Phillips & LeDoux, 1992), we proposed that water deprivation accelerated contextual fear conditioning by modulating hippocampal theta rhythm and LTP in a manner that endowed water-deprived rats with superior processing and encoding of contextual CSs. However, we could not provide a compelling case for a *selective* enhancement of contextual CS processing in water-deprived rats, because we did not determine whether or not water deprivation augmented fear conditioning to other classes of novel CSs. To further address this issue in the present experiment, we assessed fear conditioning to both novel contextual and discrete stimuli in water-deprived and nondeprived rats. If water deprivation results in a selective enhancement of fear conditioning to contextual conditional stimuli, it would suggest that the effects of water deprivation are mediated through a hippocampus-dependent process. Alternatively, an effect of water deprivation on both tone–shock and context–shock conditioning would indicate a more general effect of water deprivation on learning.

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Method

Subjects

The subjects were 32 adult male Long-Evans-derived rats (300–350 g) reared and maintained in the University of California, Los Angeles, vivarium on a 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 procedures were performed during the light phase of the cycle.

Apparatus

Four identical observation chambers (28 × 21 × 10.5 cm; Lafayette Instrument, North Lafayette, IN) were used for both conditioning and contextual fear testing. The chambers were situated in chests located in a well-lit and isolated room. A video camera placed in front of the observation chambers allowed each rat'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) 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.

An additional set of four observation chambers (28 × 21 × 10.5 cm; Lafayette Instrument) were used for testing conditional fear to the tone. The chambers were situated in chests located in a quiet, dimly lit and isolated room. Ambient light in the room was provided by a single lamp equipped with a red lightbulb. The floor of each chamber consisted of 18 staggered stainless steel rods (4-mm diameter) spaced 1.5 cm apart (center to center). In addition, an opaque Plexiglas tent was inserted into each chamber so that the apex of the tent contacted the roof of the chamber and the open base of the tent fit into the bottom corners of the chamber. The chambers were cleaned with 1% acetic acid solution before rats were placed inside. A video camera placed in front of the observation chambers allowed each rat's behavior to be observed and recorded by an experimenter in an adjacent room.

Procedure

Before conditioning, one group of rats (deprived, $n = 16$) was placed on a restricted-fluid schedule consisting of 1-hr access to water per day for 3 days. The other group of rats (nondeprived, $n = 16$) remained on ad-libitum water. During this period, the rats were handled daily and, 2 days prior to conditioning, acclimated to transport from the vivarium to the laboratory where behavioral testing occurred. On the day of conditioning (24 hr after the last 1-hr fluid session), the rats were placed in the conditioning chambers in six 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 rats received either one or three tone (64 s, 76 dB, 2 kHz)–footshock (1 s, 0.5 mA) pairings ($n = 8$ per group; 94-s intertrial interval for the three-shock groups) 3 min after being placed in the chambers. Thirty seconds following the final shock, the rats were returned to their home cages and allowed free access to water for the remainder of the experiment.

Twenty-four hours after training, fear conditioning to the tone CS was assessed. The rats were placed in observation chambers in a different room that were distinct from those used during conditioning and, after 3 min, were presented with a tone identical to that used during conditioning. Conditional fear to the tone was quantified by scoring freezing behavior with a method previously used in this

laboratory (Fanselow, 1980). Briefly, an observer who was blind to the experimental conditions scored each rat for freezing (behavioral immobility except for movement necessitated by respiration) every 8 s during the postconditioning test for a total of eight observations during the tone. Twenty-four hours following the tone test, fear conditioning to the contextual cues of the original training chambers was assessed by returning the rats to these chambers and scoring freezing during an 8-min test for a total of 64 observations per rat. Freezing behavior was quantified in the same manner for the preshock, tone, and interstimulus intervals on the conditioning day. All freezing scores were transformed to percentage of total observations.

Data Analysis

Two subjects were eliminated from the analysis because they were not handled prior to conditioning; this left 6 rats in the three-shock nondeprived group. The preshock freezing data were subjected to a one-way analysis of variance (ANOVA) with group as a variable (two levels, deprived and nondeprived). Freezing on each 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). An additional analysis of freezing during conditioning in the three-shock rats was performed to assess within-subject acquisition. This analysis consisted of a two-way ANOVA with variables of group (two levels, deprived and nondeprived) and a repeated measure of trial (three levels). The rejection criterion was .05. All data are presented as means and the standard error of the means ($\pm SE_{MS}$).

Results

Figure 1A shows the percent freezing behavior displayed to the tone CS by water-deprived and nondeprived rats before (preshock) and after one or three tone–footshock trials. An ANOVA of preshock freezing to the tone (i.e., freezing to the first tone presentation in acquisition) revealed no significant differences between water-deprived and nondeprived rats, $F(1, 26) = 1.7, p = .20$. Statistical analysis of freezing to the tone 24 hr after conditioning indicated that water-deprived and nondeprived rats showed comparable levels of fear conditioning to the tone in both the one-shock and three-shock groups, $F(1, 26) = 0.9, p = .35$, and that the levels of fear conditioning were similar with one and three shocks, $F(1, 26) = 1.4, p = .25$. Thus, water deprivation did not influence fear conditioning to a discrete tone CS.

Freezing behavior to the contextual cues of the training chamber is shown in Figure 1B. An ANOVA revealed no reliable differences in the levels of freezing to the chamber cues in the 3-min period prior to tone–shock conditioning in water-deprived and nondeprived rats, $F(1, 26) = 0.4, p = .52$. However, 48 hr following the tone–shock trials, fear conditioning to the contextual cues of the training chamber during the 8-min test was significantly augmented in water-deprived rats compared with nondeprived rats, $F(1, 26) = 6.6, p < .02$. There was no reliable trial effect or Group × Trial interaction ($F_s < 1$), indicating that the enhancement of conditioning occurred uniformly across groups in the 1-shock and 3-shock groups. Because conditional freezing to the tone and contextual CSs was sampled for different time intervals (64 s and 8 min, respectively), the differential results reported for tone and context freezing may have been due to the different behavioral sampling intervals. However, an analysis of the 64-s

period in the context test that corresponded to that used in the tone test revealed a similar pattern of results to the 8-min test (deprived, one shock, $43.8 \pm 14.2\%$; nondeprived, one shock, $14.1 \pm 9.0\%$; deprived, three shocks, $43.8 \pm 14.8\%$; nondeprived, three shocks, $12.5 \pm 7.9\%$). An ANOVA confirmed that the 64-s context freezing data were statistically reliable, $F(1, 26) = 6.0, p < .03$.

The analysis of the freezing data indicates that manipulating the number of conditioning trials did not influence the amount of freezing during the test. This poses two problems: (a) without reliable acquisition data, conclusions cannot be reached on the nature of the enhancement of context conditioning by water deprivation (i.e., rate vs. asymptote); and (b) a manipulation that affects conditioning rate would have little impact on the apparently asymptotic tone conditioning observed during the test. To address these issues, we examined the acquisition of tone and context freezing on the training day in rats that received three shocks. As shown in Figure 2A, tone conditioning reliably accrued over trials, $F(1, 24) = 20.8, p < .01$, and the amount of conditioning was similar in water-deprived and nondeprived rats ($F_s < 1$). Although context conditioning

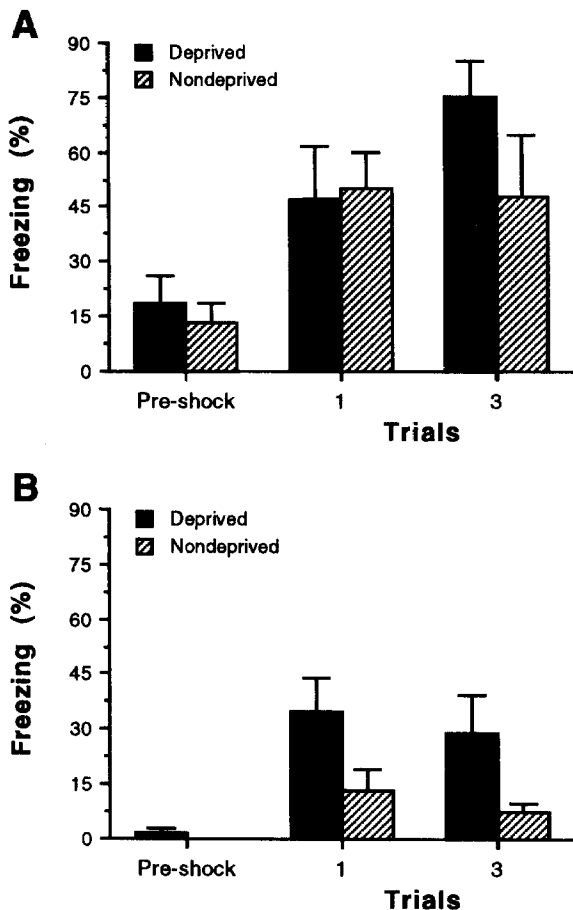


Figure 1. A: Percent freezing ($M \pm SE_M$) to the tone conditional stimulus in water-deprived and nondeprived rats. B: Percent freezing ($M \pm SE_M$) to the context of the conditioning chamber in water-deprived and nondeprived rats.

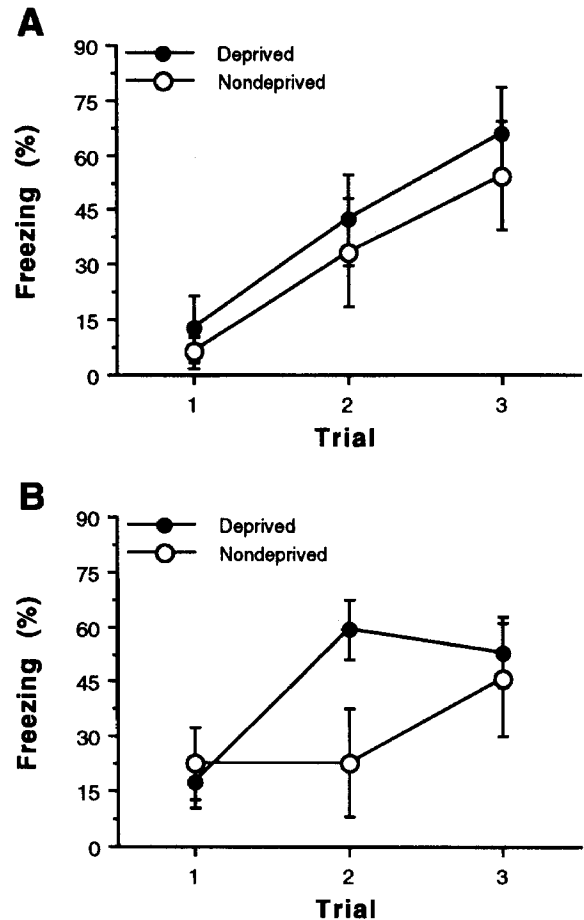


Figure 2. A: Percent freezing ($M \pm SE_M$) to the tone conditional stimulus in water-deprived and nondeprived rats receiving three trials on the conditioning day. B: Percent freezing ($M \pm SE_M$) to the context of the conditioning chamber in water-deprived and nondeprived rats receiving three trials on the conditioning day.

showed a similar significant increase across trials (see Figure 2B), $F(1, 24) = 7.4, p < .01$, there was a significant interaction between water deprivation and conditioning trial, $F(2, 24) = 3.8, p < .04$. Post hoc comparisons indicated that water deprivation significantly accelerated the rate of context conditioning, but it had no effect on the magnitude of asymptotic learning. Thus, water deprivation selectively augmented the rate of conditional freezing to contextual CSs present during discrete tone CS-US pairings.

Discussion

The present results replicate and extend those of our previous report (Maren et al., in press) and reveal that water deprivation enhances fear conditioning to contextual cues present during explicit tone-footshock pairings. This augmentation of contextual fear conditioning by water deprivation was similar following one and three tone-shock trials. Water deprivation did not, however, influence the magnitude of fear conditioning to a discrete tone CS. Thus, water deprivation

selectively enhanced fear conditioning to the contextual cues of the training chambers present during explicit tone-shock conditioning (current results) as well as context-shock conditioning in the absence of tone (Maren et al., in press).

The findings of the present study are in general agreement with Maren et al. (in press). In our previous report, we observed that water deprivation facilitated the rate, but not the asymptote, of contextual fear conditioning. In the present study, there were no reliable differences during the postconditioning tests in conditional freezing to either the tone or context between groups receiving one or three tone-shock trials. For this reason, the postconditioning freezing data do not allow a conclusion to be reached on the nature of the deprivation-related enhancement of context conditioning. An analysis of freezing on the conditioning day in rats receiving three shocks revealed that water deprivation selectively facilitated the rate of context conditioning. This was manifest as greater conditioning in deprived rats following two, but not one or three, conditioning trials. Thus, although these data confirm our earlier results, caution must be taken in the interpretation of these data because of possible unconditional influences of shock and water deprivation on performance of freezing during conditioning.

Maren et al. (in press) suggested that water deprivation-related changes in hippocampal theta rhythm and LTP constituted a neural substrate for enhanced contextual fear conditioning in water-deprived rats by increasing the capacity of the hippocampal system to process and encode contextual CSs. The present results are consistent with this hypothesis and rule out an alternative hypothesis that the augmentation of fear conditioning was due to a more general augmentation of the processing of novel stimuli by the hippocampus. There is some precedent for this latter interpretation as the hippocampus has been suggested to have a role in processing stimulus novelty (Mitchell, Maren, & Hwang, 1993; Vinogradova, 1970), and perforant path-dentate granule cell LTP correlates with exploratory responses to novel environmental stimuli (Maren, Patel, Thompson, & Mitchell, 1993). However, the present data reveal that water deprivation enhances fear conditioning to contextual but not discrete stimuli, despite the relative novelty of both classes of stimuli.

Although contextual fear conditioning depends on the integrity of the hippocampus, both context and tone conditioning appear to critically depend on the amygdala (Applegate, Frysinger, Kapp, & Gallagher, 1982; Blanchard & Blanchard, 1972; Helmstetter, 1992; Hitchcock & Davis, 1986; Iwata, LeDoux, Meeley, Arneric, & Reis, 1986; Kapp, Frysinger, Gallagher, & Haselton, 1979; Maren, Poremba, & Gabriel, 1991; Phillips & LeDoux, 1992). In addition, anatomical and physiological studies indicate that the amygdala is a locus of convergence for contextual, auditory, and somatosensory information (Mello, Tan, & Finch, 1992; Ottersen, 1982; Romanski, Clugnet, Bordini, & LeDoux, 1993). If water deprivation was modulating the strength of the CS-US association presumably formed in the amygdala during fear conditioning, one would expect that both context and tone conditioning would be enhanced to a similar degree. The lack of this effect provides further evidence that water deprivation is not modulating CS-US associative strength and suggests that the amygdaloid

systems involved in association formation during fear conditioning are insensitive to deprivation state.

As suggested earlier, accelerated context conditioning in water-deprived rats could be explained by the existence of superior contextual CS representations in these rats. In this view, water-deprived rats, by virtue of their greater hippocampal theta rhythm and LTP, may be in a position to assemble a more inclusive contextual representation of the individual elements of the conditioning chamber than their nondeprived counterparts (for more theoretical background, see McLaren, Kaye, & Mackintosh, 1989). Such a representation may serve as a more salient contextual CS and enter into associations more rapidly, which could account for the facilitated rate of context learning in water-deprived rats (Mackintosh, 1975; Rescorla & Wagner, 1972). In general, this view may have important implications for other hippocampal-dependent processes that rely on contextual CS-processing, such as latent inhibition. Furthermore, it is not unreasonable to suggest that enhanced contextual learning in food- or water-deprived animals reflects an evolutionarily adaptive strategy for ensuring successful foraging when food or water resources are depleted. Foraging animals that could rapidly identify and remember the location of food caches in their environment, for example, would be more likely to survive both food shortages and predation during foraging. Indeed, the importance of the hippocampus in food-caching behavior in certain species of birds has been indicated by a number of studies (e.g., Healy & Krebs, 1993).

In conclusion, the results of the present study indicate that changes in motivational state induced by mild water deprivation selectively influence the acquisition of fear conditioning to contextual stimuli. In particular, water deprivation appears to accelerate the rate of context conditioning, having little or no effect on the levels of asymptotic learning. These results together with those reported previously provide evidence for a role of the hippocampus in the augmentation of contextual fear conditioning by water deprivation, and, more generally, in the mediation of contextual learning.

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