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BRIEF COMMUNICATION

# Appetitive Motivational States Differ in Their Ability to Augment Aversive Fear Conditioning in Rats (*Rattus norvegicus*)

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The present experiments compared the effects of 2 appetitive motivational states on the acquisition of Pavlovian fear conditioning in rats (*Rattus norvegicus*). In Experiment 1, rats were deprived of either water or food prior to contextual fear conditioning, which consisted of the delivery of a single footshock in a novel observation chamber. Conditional fear to the contextual cues of the conditioning chamber was measured by observing freezing behavior. The results revealed that water, but not food, deprivation enhanced conditional freezing to contextual stimuli paired with footshock. Experiment 2 indicated that the different impact of food or water deprivation on the acquisition of conditional freezing was not due to differential generalization decrements during extinction testing. Together, these experiments suggest that the modulation of fear conditioning by deprivation state is specific to certain motivational systems.

It is well known that motivational states influence behavioral performance in a number of learning tasks (Bolles, 1975). Consistent with this, we recently reported that an appetitive motivational state induced by acute water deprivation enhances the rate of Pavlovian fear conditioning to contextual cues in rats (Maren, DeCola, & Fanselow, 1994; Maren, DeCola, Swain, Fanselow, & Thompson, 1994). In these experiments, rats were acutely water deprived during training, which consisted of unsignaled footshock in a novel context, but were fluid replete during contextual fear testing. Because the conditioning task required no instrumental behavior, the motivational state was irrelevant to task performance. Nonetheless, we found that water deprivation greatly facilitated the rate at which rats acquired conditional freezing. This effect was selective for conditioning to contextual conditional stimuli (CSs); water deprivation did not accelerate acquisition of conditional fear to an auditory CS.

The facilitation of contextual fear conditioning by an irrelevant motivational state suggests that these states play an important role in Pavlovian conditioning. Indeed, classical eyeblink conditioning is also influenced by irrelevant motivational states (Berry & Swain, 1989; Stanton, Freeman, & Skelton, 1992). Given this interaction between motivational state and learning rate, the question arose as to how irrelevant motivational states come to enhance Pavlovian conditioning. With regard to contextual fear conditioning, one possibility is that irrelevant motivational states have a general action on the neurobehavioral system mediating contextual fear conditioning. For example, motivational states may energize exploratory activity directed at locating desired outcomes and consequently enhance the encoding of contextual stimuli. Alternatively, water deprivation may have some specific action on contextual fear conditioning that is not produced by other motivational states. For instance, water deprivation enhances hippocampal long-term potentiation (LTP), a neural process that appears to be involved in contextual fear conditioning (Maren, DeCola, & Fanselow, 1994; Maren, DeCola, Swain, et al., 1994).

To discriminate between these two hypotheses, in the present experiments, we compared the effects of two appetitive motivational states induced by either water or food deprivation on the acquisition of contextual fear conditioning in rats. If motivational states have a nonspecific influence on contextual fear conditioning, then either food or water deprivation should have similar effects on learning rate. However, if water deprivation is unique in its ability to augment the acquisition of contextual fear conditioning, then food deprivation should not affect learning rate. Interesting to note, both food and water deprivation produce similar effects on consumption; that is, both deprivation states are associated with equivalent decreases in food and water consumption (Verplank & Hayes, 1953). In other words, imposed food deprivation reduces voluntary water consumption, and imposed water deprivation reduces voluntary food consumption. Nonetheless, the psychological states induced by food or water deprivation are different. Rats exhibit feeding or drinking, respectively, following food or water deprivation, despite similar decreases in both food and water

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consumption under both states. The similar effects on consumption, but different psychological profiles, of the two deprivation states permit us to examine the specificity of these states in modulating Pavlovian fear conditioning.

## Experiment 1

### Method

*Subjects.* The subjects were 90 adult male Long–Evans rats (*Rattus norvegicus;* 300–500 g) born and reared in the Department of Psychology vivarium at the University of California, Los Angeles. After weaning, the rats were group housed in same-sex cohorts. At the beginning of the experiment, the rats were individually housed in standard stainless-steel hanging cages on a 14:10-hr light–dark cycle (lights on at 7:00 a.m.) and had free access to food and tap water. After individual housing, the rats were handled daily (10–20 s per rat) for 5 days to acclimate them to the experimenter.

*Apparatus.* Four identical observation chambers (28 × 21 × 22 cm; Lafayette Instrument Co., North Lafayette, IN) were used for both conditioning and contextual fear testing. The chambers were constructed from aluminum (sidewalls) and Plexiglas (rear wall, ceiling, and hinged front door). The chambers were situated in chests located in a brightly lit and isolated room. A videocamera placed in front of the observation chambers allowed each subject's behavior to be observed and recorded by an experimenter in an adjacent room. The floor of each chamber consisted of 18 stainless-steel rods (4-mm diameter) spaced 1.5 cm apart (center to center). The rods were wired to a shock generator and scrambler (Lafayette Instrument Co., North Lafayette, IN) for the delivery of footshock unconditional stimuli (USs). The chambers were cleaned with a 5% ammonium hydroxide solution, and stainless-steel pans containing a thin film of the same solution were placed underneath the grid floors before rats were placed inside. Background noise (70 dB, A-scale) was supplied by ventilation fans in each chest and adjacent shock scramblers.

*Procedure.* Prior to conditioning, one group of subjects (water deprived, $n = 35$) was placed on a restricted-fluid schedule consisting of 1-hr access to water per day for 3 days. Another group of subjects (food deprived, $n = 20$) was placed on a restricted-food schedule consisting of 1-hr access to food per day for 3 days. A third group of subjects (ad lib, $n = 35$) remained on ad lib food and water throughout the experiment. 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. A subset of the rats ($n = 12$ in the deprivation groups and $n = 6$ in the ad-lib group) were weighed daily to monitor changes in body weight as a result of deprivation. On the day of conditioning (24 hr following the last 1-hr fluid or food session), the rats were placed in the conditioning chambers in sets of 4 rats (2 deprived and 2 nondeprived rats per set); the chamber position was counterbalanced for each set and group. Deprived and nondeprived subjects received a single unsignaled footshock (1 s, 0.5-mA) 3 min after being placed in the chambers. Thirty seconds following the shock, the rats were returned to their home cages and allowed free access to food and water for the remainder of the experiment. Thus, the rats in the deprivation groups were water and food replete during extinction testing.

Twenty-four hours following training, fear conditioning to the context CS was assessed. The rats were placed in the conditioning chambers, and conditional 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 context extinction test for a total of 64 observations. All freezing scores were transformed to percentage of total observations.

*Data analysis.* Body-weight data were submitted to a one-way analysis of variance (ANOVA) with a variable of group (three levels; water deprived, food deprived, and ad lib). All data are presented as means plus or minus the standard errors of the means (*SEM*s). Freezing on the extinction test was submitted to a two-way ANOVA with variables of group (three levels: water deprived, food deprived, and ad lib) and test minute (eight levels).

### Results and Discussion

The deprivation schedule reduced the body weight of rats in both the food- and water-deprived groups. The body weight of rats in both the food- and water-deprived groups measured on the conditioning day was reduced to approximately 80–85% of that in the ad-lib group ($M \pm SEM$: ad-lib, 424 ± 15 g; water deprived, 357 ± 6 g; food deprived, 338 ± 6 g). This impression was confirmed by a significant main effect of group in the ANOVA, $F(2, 27) = 23.4$, $p < .0001$. Post hoc comparisons ($p < .05$, Fisher's least significant difference) confirmed that the body weights of both food- and water-deprived rats were lower than those in ad-lib rats, but did not differ from each other. Insofar as hours of deprivation and body-weight loss are considered measures of motivation (Bolles, 1975), both food and water deprivation apparently generated similar levels of motivation.

However, as shown in Figure 1, rats in the three groups differed in their levels of freezing during the 8-min context extinction test that followed contextual fear conditioning. Specifically, water-deprived rats exhibited more freezing than either food-deprived rats or rats on ad-lib food and water. This impression was confirmed in the ANOVA by a significant main effect of group, $F(2, 87) = 5.9$, $p < .005$. Post hoc comparisons ($p < .05$) indicated that water-

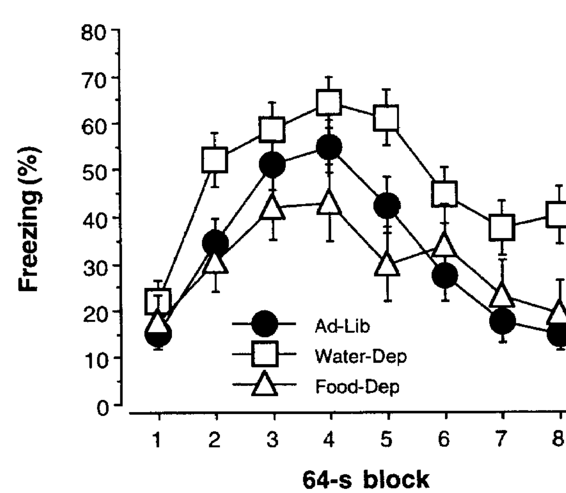


*Figure 1.* Percentage of freezing ($M \pm SEM$) to the context of the conditioning chamber during an 8-min extinction test in ad lib, water-deprived (Water-Dep) and food-deprived (Food-Dep) rats.

deprived rats froze more than either food-deprived or ad-lib rats, which did not differ from one another. Freezing varied over the 8-min test, $F(7, 609) = 27.4, p < .0001$, but did not interact with deprivation state, $F(14, 609) = 1.3, p = .18$. These results indicate that water deprivation, but not food deprivation, augments the acquisition of contextual fear conditioning.

## Experiment 2

It is well documented that food-deprivation cues can control contextual freezing (e.g., Davidson, Flynn, & Jarrard, 1992). In light of this, it might be argued that the food-deprived rats in Experiment 1 suffered a relatively large generalization decrement when shifted to a nondeprived state for extinction testing. If water-deprived subjects experienced a smaller generalization decrement during testing, then one might expect to observe the pattern of results reported in Experiment 1. To address this issue, Experiment 2 examined whether deprivation state (deprived or nondeprived) during extinction testing affects the different levels of freezing obtained in food- or water-deprived rats.

### *Method*

*Subjects.* The subjects were 32 adult male Long–Evans rats (*Rattus norvegicus;* 200–224 g) obtained from a commercial supplier (Harlan Sprague–Dawley, Indianapolis, IN) and housed in the Department of Psychology vivarium at the University of Michigan. After arrival, the rats were individually housed in standard stainless-steel hanging cages on a 14:10-hr light–dark cycle (lights on at 7:00 a.m.) and were provided free access to food and tap water. After housing, the rats were handled daily (10–20-s per rat) for 5 days to acclimate them to the experimenter.

*Apparatus.* Eight identical observation chambers (30 × 24 × 21 cm; MED-Associates Inc., Burlington, VT) were used for both conditioning and contextual fear testing. The chambers were constructed from aluminum (sidewalls) and Plexiglas (rear wall, ceiling, and hinged front door) and were situated in chests located in a brightly lit and isolated room. The floor of each chamber consisted of 19 stainless-steel rods (4-mm diameter) spaced 1.5 cm apart (center to center). The rods were wired to a shock source and solid-state grid scrambler (MED-Associates Inc., Burlington, VT) for the delivery of footshock USs. The chambers were cleaned with a 5% ammonium hydroxide solution, and stainless-steel pans containing a thin film of the same solution were placed underneath the grid floors before the rats were placed inside. Background noise (65 dB, A-scale) was supplied by ventilation fans in each chest.

Each conditioning chamber rested on a load-cell platform that was used to record chamber displacement in response to each rat's motor activity. To ensure inter-chamber reliability, each load-cell amplifier was calibrated to a fixed-chamber displacement. The calibrated output of each chamber's load cell was set to a gain that was optimized for detecting freezing behavior. Load-cell amplifier output from each chamber was digitized and acquired on-line by use of Threshold Activity software (MED-Associates, Inc., Burlington, VT).

*Procedure.* The experiment consisted of a 2 × 2 factorial design with deprivation type (water or food) during training and deprivation state during testing (nondeprived or deprived) as the variables. Prior to conditioning, one group of subjects (water deprived, $n = 16$) was placed on a restricted-fluid schedule consisting of 1-hr access to water per day for 3 days. Another group of subjects (food deprived, $n = 16$) was placed on a restricted-food schedule consisting of 1-hr access to food per day for 3 days. On the day of conditioning (24 hr following the last 1-hr fluid or food session), the rats were placed in the conditioning chambers in sets of 8 rats (4 water-deprived rats and 4 food-deprived rats per set); the chamber position was counterbalanced for each set and group. The subjects received a single unsignaled footshock (1-s, 0.5-mA) 3 min after being placed in the chambers. Thirty seconds following the shock, the rats were returned to their home cages. Half of the rats in each group (nondeprived) were placed back on ad-lib food and water, and the other half of the rats (deprived) remained on the deprivation schedule.

Twenty-four hours after training, fear conditioning to the context was assessed by returning the rats to the conditioning chambers and measuring freezing behavior (somatomotor immobility except that necessitated by breathing) during a 4-min extinction test. During the extinction session, each rat's activity was monitored continuously by using the data acquisition system described above. For each chamber, load-cell voltage was digitized at 5 Hz, yielding one observation per rat every 200 ms (300 observations per rat per minute). In all experiments, freezing was quantified by computing the number of observations for each rat that had a load-cell value less than the freezing threshold. The load-cell value for the freezing threshold was determined in a separate group of pilot animals by using an experienced observer's (Stephen Maren's) ratings of freezing behavior. Thus, movements such as grooming, head turning, and sniffing that were not scored by the observer as freezing produced load-cell output that exceeded the freezing threshold. Most important, the freezing threshold is absolute and does not vary from animal to animal. To avoid counting momentary inactivity as freezing, an observation was scored as freezing only if it fell within a contiguous group of at least five observations that were all less than the freezing threshold. Thus, freezing was scored only if the rat was immobile for at least 1 s. For each session, the freezing observations were transformed to a percentage of total observations.

*Data analysis.* Freezing on the extinction test was submitted to a two-way ANOVA with variables of training state (two levels: water deprived and food deprived) and testing state (two levels: nondeprived and deprived). All data are presented as means plus or minus the *SEM*s.

### *Results and Discussion*

As shown in Figure 2, rats trained under water deprivation exhibited more conditional freezing than rats trained under food deprivation whether they were tested sated or deprived. This impression was confirmed in the ANOVA by a significant main effect of training state, $F(1, 28) = 6.6, p < .05$. There was not a reliable Training State × Testing State interaction ($F < 1, p = .98$). Although there was a trend for lower freezing in the deprived groups, the main effect of testing state was not reliable, $F(1, 28) = 2.9, p = .10$. In accordance with Experiment 1, these results indicate that rats trained under water deprivation acquire more contextual fear than those trained under food deprivation. Most important, the state of deprivation during extinction testing did not interact with the differential enhancement of context conditioning by food or water deprivation during training. Thus, the present data indicate that the results of Experiment 1 are not due to different generalization decrements in water- or

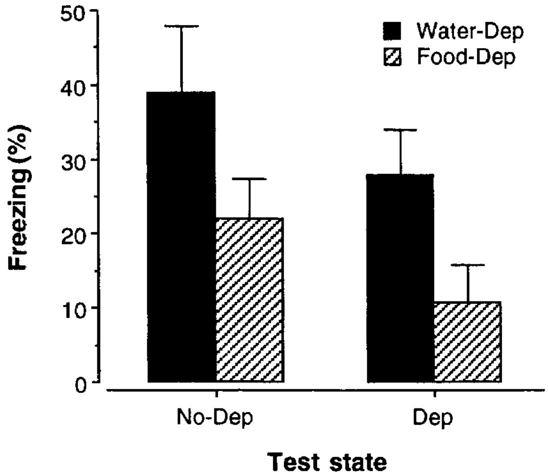


*Figure 2.* Percentage of freezing ($M \pm SEM$) to the context of the conditioning chamber during a 4-min extinction test in rats trained under water deprivation (Water-Dep) or food deprivation (Food-Dep) and tested either deprived (Dep) or nondeprived (No-Dep).

food-deprived rats shifted to a nondeprived state during extinction testing.

## General Discussion

The present results reveal that irrelevant appetitive motivational states differ in their ability to modulate Pavlovian fear conditioning. Consistent with our previous demonstrations (Maren, DeCola, & Fanselow, 1994; Maren, DeCola, Swain, et al., 1994), acute water deprivation during training augmented contextual freezing exhibited during an extinction test conducted in replete rats. Although only a single conditioning trial was used in the present study, our previous work indicated that enhanced conditioning in water-deprived rats reflected an enhanced rate of conditioning, as opposed to a greater asymptote of conditional responding. Interesting to note, motivation induced by food deprivation did not affect the acquisition of contextual fear conditioning. Despite the similar weight loss in water- and food-deprived rats, food-deprived rats acquired the same level of contextual freezing as that in ad-lib rats. These results indicate that motivational states do not have a general facilitatory affect on contextual fear conditioning and suggest that water deprivation has a specific effect on the neurobehavioral system mediating fear conditioning.

In our previous work, we found that water deprivation did not influence sensitivity to the shock US (Maren, DeCola, Swain, et al., 1994). This led us to propose that water deprivation modulates fear conditioning by increasing the salience of the contextual CS. We suggested that water deprivation might enhance the salience of the contextual CS by increasing the number of contextual elements that become incorporated into the contextual representation prior to footshock or by increasing the strength of the contextual representation. Either mechanism could account for the effect of water deprivation on the acquisition rate, but not asymptote, of contextual freezing (Maren, DeCola, Swain, et al.). Nonetheless, we favored the former hypothesis, because water deprivation is known to increase or "energize" motor activity in novel environments (Bolles, 1975; Fowler, 1965). Also, although many studies have confounded deprivation-related increases in general activity with exploratory behavior in forced-exploration tasks (e.g., Dashiell, 1925; Montgomery, 1953; Zimbardo & Montgomery, 1957), studies using free-exploration or choice tasks have revealed consistent increases in exploratory behavior in deprived subjects (Fehrer, 1956; Richards & Leslie, 1962; Zimbardo & Miller, 1958). It follows, then, that increased exploratory behavior might permit deprived animals to acquire a more inclusive contextual representation, that is, a contextual representation that contains a greater number of the available stimulus elements. However, because food or water deprivation produces equivalent enhancements in exploratory activity (e.g., Richards & Leslie, 1962), it seems unlikely that increased exploration and its presumed consequences for a more inclusive contextual representation can account for the selective augmentation of conditioning in water-deprived rats. Alternatively, as we suggested earlier, it may be that water deprivation is influencing the strength of contextual representation, rather than the content of that representation.

A number of variables suggest that the influence of water deprivation on contextual fear conditioning is meditated by a specific neural structure, namely, the hippocampus. It is becoming clear that the hippocampal formation is required for contextual fear conditioning (Kim & Fanselow, 1992; Maren, Aharonov, & Fanselow, 1997; Maren & Fanselow, 1997; Phillips & LeDoux, 1992). With regard to the influence of water deprivation on fear conditioning, we have found that water deprivation increases both theta-band electroencephalographic activity in the hippocampus and the capacity for hippocampal LTP (Maren, DeCola, Swain, et al., 1994); LTP is thought to be a physiological substrate of associative learning (e.g., Maren & Baudry, 1995). Furthermore, the specificity of the water-deprivation effect for contextual fear conditioning, as opposed to conditioning to discrete auditory cues (Maren, DeCola, & Fanselow, 1994), suggests a special role for the hippocampus. The finding that water, but not food, deprivation facilitates the acquisition of contextual fear conditioning suggests that only water deprivation produces a physiological state that modulates the hippocampus in a way conducive to the representation of contextual information.

One possibility is that vasopressin, a posterior pituitary hormone that is released during water deprivation, modulates hippocampal LTP. In support of this, it has been reported that vasopressin is released during water deprivation but is not released during food deprivation despite reduced water consumption under both deprivation states (Kiss, Jezova, & Aguilera, 1994). Furthermore, vasopressin has been reported to facilitate LTP induction in hippocampal slices (Rong, Chen, & Du, 1993) and to contribute to the maintenance of LTP in septal slices (van den Hoof, Urban, & de Wied, 1989). Thus, it is possible that a neurohormonal factor such as vasopressin may underlie the enhancement of contextual conditioning by water deprivation. Regardless of the specific mechanism, it is clear that certain types of irrelevant motivation can have selective effects on the acquisition of Pavlovian conditioning.

## References

Berry, S. D., & Swain, R. A. (1989). Water deprivation optimizes hippocampal activity and facilitates nictitating membrane conditioning. *Behavioral Neuroscience, 103,* 71–76.

Bolles, R. C. (1975). *Theory of motivation.* New York: Harper & Row.

Dashiell, J. F. (1925). A quantitative demonstration of animal drive. *Journal of Comparative Psychology, 5,* 205–208.

Davidson, T. L., Flynn, F. W., & Jarrard, L. E. (1992). Potency of food deprivation intensity cues as discriminative stimuli. *Journal of Experimental Psychology: Animal Behavior Processes, 18,* 174–181.

Fanselow, M. S. (1980). Conditional and unconditional components of post-shock freezing. *Pavlovian Journal of Biological Sciences, 15,* 177–182.

Fehrer, E. (1956). The effects of hunger and familiarity of locale on exploration. *Journal of Comparative and Physiological Psychology, 49,* 549–552.

Fowler, H. (1965). *Curiosity and exploratory behavior.* New York: MacMillan.

Kim, J. J., & Fanselow, M. S. (1992, May 1). Modality-specific retrograde amnesia of fear. *Science, 256,* 675–677.

Kiss, A., Jezova, D., & Aguilera, G. (1994). Activity of the hypothalamic pituitary axis and sympathoadrenal system during food and water deprivation in the rat. *Brain Research, 663,* 84–92.

Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behavioral Brain Research, 88,* 261–274.

Maren, S., & Baudry, M. (1995). Properties and mechanisms of long-term synaptic plasticity in the mammalian brain: Relationships to learning and memory. *Neurobiology of Learning and Memory, 63,* 1–18.

Maren, S., DeCola, J. P., & Fanselow, M. S. (1994). Water deprivation enhances fear conditioning to contextual, but not discrete, conditional stimuli in rats. *Behavioral Neuroscience, 108,* 645–649.

Maren, S., DeCola, J. P., Swain, R. A., Fanselow, M. S., & Thompson, R. F. (1994). Parallel augmentation of hippocampal long-term potentiation, theta rhythm, and contextual fear conditioning in water-deprived rats. *Behavioral Neuroscience, 108,* 44–56.

Maren, S., & Fanselow, M. S. (1997). Electrolytic lesion of the fimbria/fornix, dorsal hippocampus, or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats. *Neurobiology of Learning and Memory, 67,* 142–149.

Montgomery, K. C. (1953). The effect of the hunger and thirst drives upon exploratory behavior. *Journal of Comparative and Physiological Psychology, 46,* 129–133.

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience, 106,* 274–285.

Richards, W. J., & Leslie, G. R. (1962). Food and water deprivation influences on exploration. *Journal of Comparative and Physiological Psychology, 55,* 834–837.

Rong, X.-W., Chen, X.-F., & Du, Y.-C. (1993). Potentiation of synaptic transmission by neuropeptide AVP4-8 (ZNC(C)PR) in rat hippocampal slices. *NeuroReport, 4,* 1135–1138.

Stanton, M. E., Freeman, J. H., Jr., & Skelton, R. W. (1992). Eyeblink conditioning in the developing rat. *Behavioral Neuroscience, 106,* 657–665.

van den Hoof, P., Urban, I. J., & de Wied, D. (1989). Vasopressin maintains long-term potentiation in rat lateral septum slices. *Brain Research, 505,* 181–186.

Verplank, W. S., & Hayes, J. R. (1953). Eating and drinking as a function of maintenance schedule. *Journal of Comparative and Physiological Psychology, 46,* 327–333.

Zimbardo, P. G., & Miller, N. E. (1958). Facilitation of exploration by hunger in rats. *Journal of Comparative and Physiological Psychology, 51,* 43–46.

Zimbardo, P. G., & Montgomery, K. C. (1957). The relative strengths of consummatory responses in hunger, thirst, and exploratory drive. *Journal of Comparative and Physiological Psychology, 50,* 504–508.

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