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15. After this paper was accepted for publication, R. Kiester (Department of Biology, Harvard) kindly brought to our attention independent data tending to confirm the theory. Adult female red deer who fail to breed the preceding year (and are therefore presumably in better than normal condition during the present year) appear to produce a much higher sex ratio than do adult females who bred the preceding year [F. F. Darling, *A Herd of Red Deer* (Oxford Univ. Press, London, 1937), pp. 46–48].
16. Grey seal: J. C. Coulson and G. Hickling, *Nature* **190**, 281 (1961); Weddell seal: I. Stirling, *J. Mammal.* **52**, 842 (1971).
17. It is a common observation of animal breeders that the later one mates a female mammal in her estrous cycle the greater the chance of producing males [(*13a*); also W. H. James, *Lancet* **1971-I**, 112 (1971)]. Late matings minimize the time between copulation and fertilization and would therefore minimize differential mortality by sex of the sperm cell, presumed to operate against male-producing sperm.
18. Deer: Robinette *et al.* (*6*); cows: A. B. Chapman, L. E. Cassida, A. Cote, *Proc. Amer. Soc. Anim. Prod.* **30**, 303 (1938); humans: S. Shapiro, E. W. Jones, P. M. Densen, *Milbank Mem. Fund Quart.* **40**, 7 (1962).
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20. A. C. Stevenson and M. Bobrow, *J. Med. Genet.* **4**, 190 (1967).
21. If members of one sex perform more altruistic acts toward kin of the opposite sex than the other way around, then one can show that parents will be selected to invest, on the average, more than 50 percent of their resources in producing offspring of the more altruistic sex. This factor may be important in explaining the apparent human overproduction of sons (R. L. Trivers, in preparation).
22. G. Elder, *Amer. Sociol. Rev.* **34**, 519 (1969); H. Carter and P. C. Glick, *Marriage and Divorce: A Social and Economic Study* (Harvard Univ. Press, Cambridge, Mass., 1970).
23. R.L.T. and D.E.W. independently conceived the basic theory. The collection of data and writing of the paper were performed by R.L.T. alone. We thank I. DeVore, B. J. LeBouef, and T. Schoener for detailed comments. We thank H. Hare for help finding references. R.L.T. thanks I. DeVore for advice and unfailing support throughout. The work was completed under an NIH postdoctoral fellowship to R.L.T. and partly supported by NIMH grant 13156 to I. DeVore.

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Memory and Hibernation in Citellus lateralis

Abstract. *Squirrels learned to escape from a water bath by making a visual discrimination. Twenty-four hours after reaching criterion for learned behavior, the animals were exposed to the first of two 11-day cold exposures. The animals that hibernated had better retention of the learned behavior.*

Hibernation provides an opportunity to study the effects of low body temperature on memory under natural physiological conditions. We found that hibernation improved retention of learned behavior, a result that could have been predicted because (i) low temperature reduces turnover of protein and other macromolecules likely to be involved in memory, and (ii) external stimuli, which may alter established memory, are reduced in number during hibernation.

Many treatments applied soon after learning impair memory, apparently most effectively by interfering with consolidation of learned material (*1*). New input into an established memory trace may result in disruption of neural processes associated with the original learning. Consolidation theorists suggest that this physical disruption of the memory trace may account for observed amnesic effects (*2*). Electroconvulsive shock and drugs administered to rats have been reported to cause amnesia, possibly by blocking storage and retrieval of new information (*3*).

Facilitation of memory by hibernation raises questions regarding the extent to which memory is dependent upon quiesence in storage area. Most experiments concerned with the effects of lowered body temperature on behavior have involved artificial hypothermia administered to nonhibernators (*4*). Unfortunately there has been little work on the learning ability of hibernators, although a few species have been trained to respond in operant situations (*5*). Others have not investigated the effects of hibernation on retention of learned behavior.

In this study of memory retention in the hibernator *Citellus lateralis* (the golden mantled ground squirrel), we (i) determined the feasibility of training ground squirrels to make visual discriminations in an escape paradigm, (ii) observed the effect of reversal training on performance, and (iii) measured the effect of hibernation on the retention of a learned task. Twenty squirrels were captured in the area of Costilla, New Mexico, and housed in individual cages. The laboratory temperature was 24° ± 3°C. Diet consisted of five pieces of Wayne lab chow (20 ± 2 g) daily and was supplemented weekly with vegetables and peanuts. Animals had free access to water.

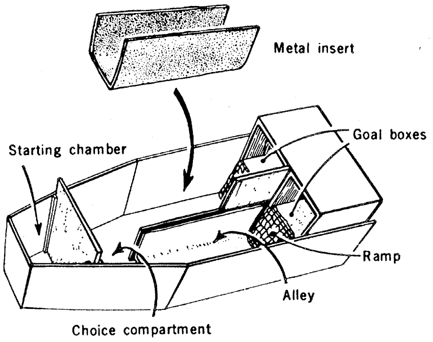

Fig. 1. The training apparatus. Each alley was 45 cm long. The distance from the starting chamber to an escape ramp was 80 cm. The water depth was 27.4 cm. The apparatus had a capacity of 80 liters. The water temperature was 34° ± 1°C.

The water-filled discrimination apparatus consisted of a starting chamber and two parallel alleys (Fig. 1). The entire apparatus with the exception of the goal boxes was painted gray. The two alleys had removable metal inserts, one black and the other white. When these were in position, the walls and floors were covered. By exchanging these inserts the color of the alleys could be changed, which ruled out a position effect. The goal box for the incorrect color choice was obstructed by a piece of glass covering the ramp. Liquid detergent (65 ml) was added to the 80 liters of water to decrease the amount of air trapped in the fur; this treatment prevented passive floating and made it necessary for the animals to actively swim.

The animals' level of drive during training was estimated to be high. The animals never adapted to the water, and escape was always rewarding.

The "correction" method was applied throughout discrimination and training. If a squirrel made an incorrect choice, it was allowed to correct its error. If the squirrel did not attempt to swim back within 5 minutes, the trial was terminated. This method was preferred to the noncorrection method because pilot data demonstrated that *C. lateralis* learn with fewer trials by correction methods.

During the acquisition stage each animal was introduced into the apparatus. Behavior was shaped such that each subject would orient itself with respect to the goal boxes. Eventually all the animals learned to swim down either alley and to climb the wire ramps into the goal box. The colored metal inserts were not employed during these training sessions. If any side preferences appeared, they were shaped out during the training by forcing the animal to swim down the unpreferred alley. Criterion was an uninterrupted approach without a place preference. A trial period started at the time the sliding door to the starting chamber was lifted and ended when the squirrel entered the goal box. The animals were timed with a stopwatch; when the goal was reached in 10 seconds or less, the criterion for acquisition had been met.

For the discrimination training, two groups of animals, five of each sex, were trained to swim down either a black or white alley to gain access to an unshielded escape ramp and goal box. The position of the correct stimulus color was varied randomly. The criterion was responding correctly in 80 to 85 percent of trials; this criterion guarded against overlearning. The discrimination training continued for 7 weeks; at this time all animals were responding correctly in 85 percent of trials.

After all the animals had reached criterion for the discrimination task, they were trained in the reversal task. Now the previously correct choice was incorrect. The procedures outlined for the original discrimination training were followed for this stage of the training. The squirrels were trained the reversal situation to determine if they had really learned what was intended. If they had learned something other than the color preference, reversing the color should have had no effect on their responding. After an 8-week period all the animals were responding correctly in 85 percent of trials.

The overall protocol involved nine consecutive events of indicated duration: (i) acquisition, 2 weeks; (ii) intertraining period, 1 week; (iii) discrimination training, 7 weeks; (iv) reversal training, 8 weeks; (v) first cold exposure, 11 days; (vi) test A, done 24 hours after cold exposure; (vii) second 11-day cold exposure, started 48 hours after test A; (viii) test B, done 24 hours after cold exposure; and (ix) test C, done 21 days after test B.

Twenty-four hours after reaching criterion on the reversal training, all animals were given food, water, and bedding in individual cages and were placed in the hibernaculum. Beginning 30 October 1971, the animals were exposed to 7° to 10°C with a light cycle of 12 hours of light followed by 12 hours of darkness for an 11-day period. All animals spent the same amount of time in the cold. For the first few days the animals had free access to food. Subsequently, food was withdrawn in varying amounts to encourage entrance into hibernation. Some animals hibernated while others did not. Bouts of hibernation were monitored by placing bits of sawdust on the animals and checking to see whether they were displaced. At the end of the 11 days in the cold all animals were housed in the laboratory for 24 hours before testing for retention (test A), and given free access to food and water. Two days after testing the animals were returned to the hibernaculum for another 11-day cold exposure and retested 24 hours later (test B).

Table 1. One-way analysis of variance on retention 48 hours after the first (A) and second (B) cold-exposure periods. Abbreviations: H, hibernation; NH, no hibernation; ♂, male; ♀, female.

Groups compared	d.f.	Critical value	*P*
Test A			
H (♂,♀), NH (♂,♀)	1,1	4.60	< .05
H (♂), NH (♂)	1,7	5.59	< .05
H (♀), NH (♀)	1,7	5.59	< .05
H (♂), H (♀)	1,8	5.32	> .05
NH (♂), NH (♀)	1,6	5.99	> .05
Test B			
H (♂,♀), NH (♂,♀)	1,2	4.49	< .05
H (♂), NH (♂)	1,7	5.59	< .05
H (♀), NH (♀)	1,5	4.68	< .05
H (♂), H (♀)	1,9	5.12	> .05
NH (♂), NH (♀)	1,1	4.67	> .05

In the retention tests A, B, and C, each animal was given four trials in the discrimination apparatus. The number of responses to a particular color was recorded for each animal. We wished to determine which color choice prevailed and whether treatment had affected memory for the learned task. To rule out a training effect during the four trials, no responses were punished. Once again the positions of the metal alley inserts were varied randomly.

A one-way analysis of variance was applied to measure the significant ($P < .05$) differences between the means for the experimental groups. Each group of animals served as its own control.

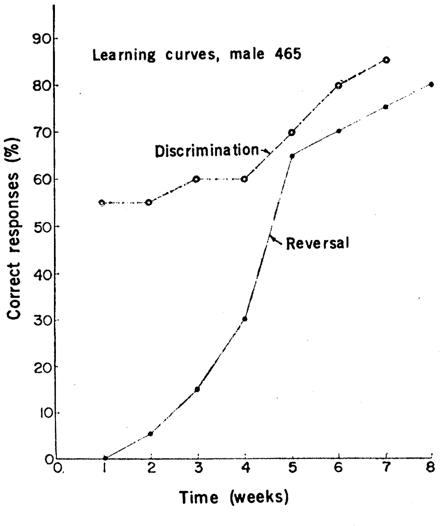

Fig. 2. A learning curve for discrimination and reversal.

During the discrimination training the animals slowly learned to respond selectively to the stimulus colors. The low percentages of response during the first weeks of reversal were an indication that the animals had indeed learned the original discrimination task. Figure 2 shows typical learning curves.

Analysis of the data for discrimination and reversal training periods yielded no differences between the sexes ($F = 4.41$, d.f. = 1,18, $P > .05$) and the color discrimination groups ($F = 4.41$, d.f. = 1,18, $P > .05$). Before the cold-exposure periods there were no differences between those animals that later hibernated and those that did not hibernate ($F = 4.49$, d.f. = 1,16, $P > .05$).

During the first cold-exposure period the mean hibernation period was 12 days for males and 8 days for females. Ten animals had hibernated. One male and one female died during the first 11 days of cold exposure. Animals that hibernated had better retention than did those that did not (test A in Table 1).

During the second cold-exposure period the mean hibernation period was 8 days for males and females; 11 animals had hibernated. Most of the animals that hibernated during this period had also hibernated during the first period. Three females died during the second cold exposure. Once again those animals that hibernated had better retention than those that did not (test B in Table 1).

In test C (21 days after second cold exposure), the animals that hibernated again had better memory retention ($F = 4.75$, d.f. = 1,12, $P < .05$). All animals had a significant loss of memory during the 21 days at room temperature. The test C scores of animals that had hibernated were similar to the test B scores of animals that had not hibernated ($F = 4.60$, d.f. = 1,14, $P > .05$).

The time of onset of hibernation seemed to affect the percentage of recall (6). Animals that started hibernating within 24 hours after being placed in the cold had 100 percent retention of the reversal training task. The inability of nonhibernating animals to remember the acquired information is subject to many interpretations.

Any observed effect of hibernation on retention of learned behavior must be considered as an effect on memory. All animals that hibernated remembered the reversal task. The newest memory to be incorporated was the most susceptible to disruption if the animals did not hibernate. The behavior of animals that did not hibernate may be related to the effects of aversive conditions on memory, reported by Weiskrantz (*7*). Clinical reports of retrograde amnesia indicate that the memory for the most recent experiences is the most susceptible to disruption. Apparently the cold environment acted as a stressful situation to awake animals; this could explain the retrograde effects.

The interference theory of forgetting suggests that material is lost to memory only when it is displaced by some other material. The mere passage of time between initial learning and recall does not cause forgetting. All animals were in the cold for the same periods. Those animals that hibernated blocked out the effects of interfering experiences, whereas those animals that remained alert during the cold-exposure periods were subjected to stimuli that may have interfered with encoding of the original learning task.

A variety of brain electrical activity patterns has been recorded on animals in hibernation (*8*). Chatfield and Lyman (*9*) showed three types of activity in animals aroused from hibernation: (i) bursts of activity at a frequency of 17 $\sec^{-1}$, (ii) bursts of spikes, and (iii) continuous waves of activity. Others reported low-voltage high-frequency desynchronized cortical activity without arousal.

Pengelley and Fisher (*10*) reported that ground squirrels become habituated to stimuli while in hibernation. These experiments indicate that the nervous system of hibernators is capable of functioning at low body temperatures (1°C), at least to the extent that habituated response to a stimulus can be made. It is clear the animal is maintaining an integrated control.

During hibernation the cortex may be nonfunctional. However, cortical silence associated with lowered body temperature does not pose a threat to the survival of the animal because important thermoregulatory centers are subcortical. Lyman and O'Brien (*11*) concluded that peripheral temperature receptors in *C. tridecemlineatus* and *C. lateralis* are not involved in temperature regulation during hibernation, and that accurate sensing of declines in brain temperature allows the hibernator to arouse from lethal cold.

Animals that hibernated had better retention scores. The most reasonable interpretation of this result is that hibernation eliminates or greatly reduces cortical activation and thereby protects memory traces from being eliminated. The significance of memory retention in the natural life history and evolution of the hibernator can now be surmised. Among hibernators, the animals that do not hibernate or hibernate only briefly during a winter may lose behavior patterns that are essential to the survival of the species.

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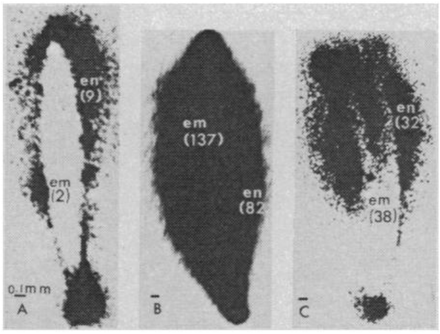
Dichloromethane and Lettuce Seed Germination

Meyer and Mayer (*1*) have proposed the use of dichloromethane (DCM) for introducing substances into dry seeds. If the potential of this method were realized, as pointed out by the authors, it could be useful in overcoming practical problems of seed storage. I therefore investigated the effectiveness of DCM for introducing chemicals into dry seeds. The lack of toxicity of DCM to dry whole seeds, was confirmed; however, under my experimental conditions DCM was not entirely successful in introducing coumarin into the embryo of dry lettuce seeds.

Treating lettuce seeds with DCM (*2*) for 24 hours had little effect on subsequent germination (Table 1). When seeds were treated with 5 or 10 m*M* coumarin in DCM, germination was inhibited, but not as severely as previously reported. Inhibition was reversed by rinsing the seeds for 20 seconds in DCM, an indication that the



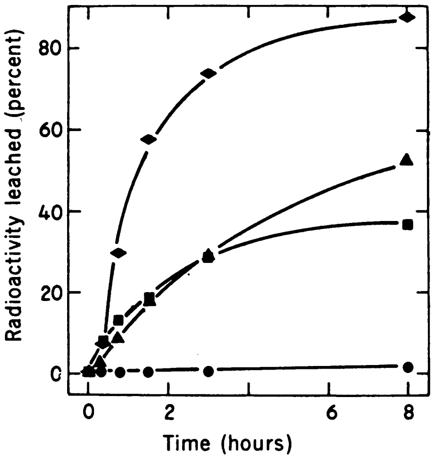


Fig. 1 (left). Autoradiograms of lettuce seeds (Grand Rapids) treated with 5 m*M* [3-^{14}C]coumarin (0.5 μc/ml). (A) Whole seeds treated 24 hours with [^{14}C]coumarin in dichloromethane; (B) whole seeds treated 18 hours with [^{14}C]coumarin in water; and (C) scarified seeds treated with [^{14}C]coumarin in dichloromethane. Treated seeds were cut transversely into three sections with each section mounted on two-faced tape, and the cut surface placed in direct contact with x-ray film for 13 days. Numbers in parentheses represent nanomoles of coumarin associated with the embryo or endosperm complex from ten seeds treated with [^{14}C]coumarin; *em*, embryo; *en*, pericarp-integument-endosperm. Fig. 2 (right). Radioactive material leached from lettuce seeds (Grand Rapids) containing [3-^{14}C]coumarin; whole (●) or cut (■) seeds in dichloromethane and whole (▲) or cut (◆) seeds in water. Seeds imbibed aqueous 5 m*M* [3-^{14}C]coumarin (0.5 μc/ml) for 18 hours, and were then rinsed, blotted, and dried in air. Then ten whole or longitudinally cut seeds were placed in 1 ml of distilled water or dichloromethane. Rate of leaching was determined by removing and replacing each solvent after 0.05, 0.17, 0.67, 1.67, 3, and 8 hours. Radioactivity in the leachate and that remaining in the seeds after 8 hours was determined.