

"Reminiscence" in the Cold Flour Beetle (*Tenebrio molitor*)

Abstract. Retention of a T-maze task by adults of the species *Tenebrio molitor* was facilitated by exposure to 1.7°C. The facilitation effect was seen after 1, 3, 4, or 5 days of retention, but it was reversed after 2 days. The increment from day 2 to day 5 was termed "reminiscence." That these effects represented alterations in memory and not nonspecific motivational factors was determined by requiring another group of beetles to reverse their learning of the original task. Since reversal performance was inversely related to relearning, it was concluded that "reminiscence" represented alterations in memory. A multistage memory mechanism in the beetle was postulated.

In a series of experiments (1), larvae (meal worms) and adults (beetles) of the species *Tenebrio molitor* were trained on a T-maze position task and then either left at room temperature (23.9°C) or refrigerated (1.7°C) during retention intervals of 1 to 10 days. The retention of the refrigerated animals was superior to that of their warm counterparts. Both warm larvae and warm beetles showed the typical increased forgetting with an increase in retention interval. The retention function of the cold beetles was, however, quite surprising. The performance of the cold beetles was superior to that of warm beetles after 1, 5, and 10 days, but was slightly inferior to that of warm beetles after 2 days. In other words, cold beetles appeared to "remember" the learned response very well after 1 day, rather poorly after 2 days, and very well again after 5 and 10 days. This drop and subsequent recovery in the performance of the cold beetles was termed "reminiscence."

This reminiscence in cold beetles was of great interest because no such anomaly was observed in the warm subjects of either stage of life or in the cold larvae, a fact which suggested that memory storage processes might be different for the two stages of life. However, it was not clear whether the poor performance of the cold beetles after the 2-day retention interval and the subsequent reminiscence represented an effect on memory for the learned response or whether it resulted from some temporary general performance factor, such as amount of motivation.

The study of reversal learning offered a promising approach to this problem. If the originally learned response had really been forgotten after the 2-day interval, then learning to turn in the opposite direction should be relatively easy at that time because of the comparative absence of competing responses from the previous training. However, if the poor performance of the cold animals after 2 days resulted

from some general decrement in performance, reversal learning should also be relatively poor after the same interval. Similar logic would apply to the subsequent reminiscence. If the enhanced performance were related to memory factors, then reversal learning should be impaired; if related to an exacerbated motivational state, then reversal learning might be materially assisted.

With this reasoning, we used a 2 by 5 analysis of variance design in which we varied the type of task (relearning or reversal learning) after original learning and the length of the retention interval (1 to 5 days) between original learning and the second task. Eight adult beetles (age and sex not determined) were used in each of the ten experimental conditions. The subjects lived individually in Styrofoam coffee cups containing a slightly moist mixture of oatmeal and All-Bran. Training took place in a miniature plexiglass T-maze with a floor of fine-grain sandpaper. In this maze, the alleys 0.80 cm wide and 1.27 cm deep, formed a 5.08-cm stem and 7.62-cm arms. Escape into their home cups from the light and heat (35°C on the maze floor) of a 100-watt incandescent bulb, mounted 11.43 cm above the choice point of the maze, provided the reward for making the correct response.

On day 1 of the experiment, the subjects were given a test for turning bias, which consisted of five runs through the maze. In original learning, training was against the subject's measured bias. The training procedures were the same for original learning and subsequent relearning or reversal learning. A trial consisted of one or two runs through the maze. If the subject made the correct turn on the first run, the trial terminated when he entered his home cup which was at the end of the correct alley. If the subject made the incorrect turn, however, he was removed from the end of the incorrect arm and forced to run to the cor-

rect side. With three trials per day, the training criterion for both tasks was six successive trials without error. The animals were refrigerated between the daily training sessions and during retention intervals but were removed from the refrigerator 30 minutes prior to performing in the maze; they were run at room temperature of 23.9°C.

We found that reversal learning was more difficult than relearning and that this difficulty was inversely related to the ease with which relearning took place (Fig. 1). These results appear to support the theory that the reminiscence effect represents an effect on memory (2). As additional support for this view, we looked at the first-trial performance on the second task of all subjects. If the reminiscence curve reflects the operation of memory mechanisms, then for the first-trial performance on the second task the curve should be somewhat similar. This apparently represents the best available index of retention in the present study. Figure 2 shows that retention on days 1, 3, 4, and 5 was above chance, but performance on day 2 was precisely at chance. This result, therefore, supports the view that the reminiscence effect does not involve a nonspecific motivational factor, in that it would be difficult to determine how such a mechanism could guide the correct choice on the first trial of retention.

Statistical analyses were performed to determine the extent to which reversal learning was inversely related to relearning. (i) More errors were made during reversal learning (Fig. 1)

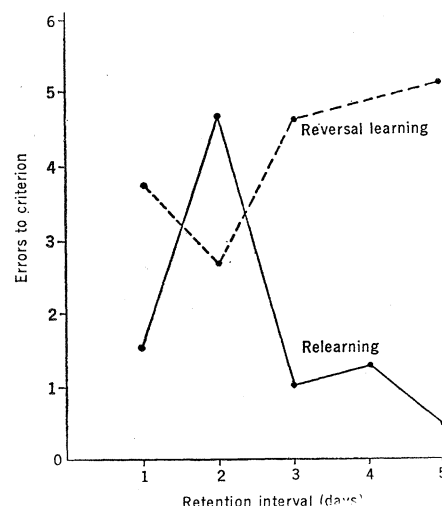


Fig. 1. Mean number of errors prior to attainment of criterion on the second task as a function of retention interval.

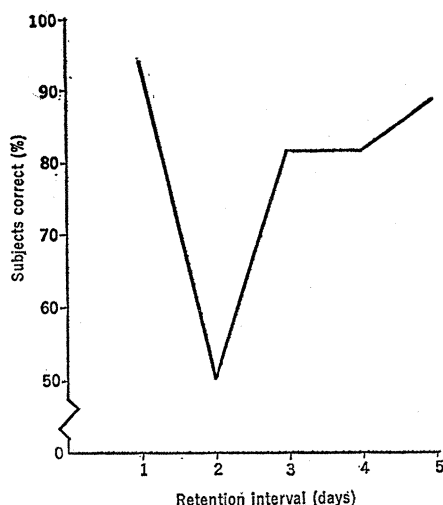


Fig. 2. Percentage of subjects, on the first trial of the second task, making response which was correct in original learning.

than during relearning [ratio of variance (F) = 105.72, degrees of freedom (df) = 1 and 60, $P < .001$] (3). (ii) The linear, quadratic, cubic, and quartic components of the relearning function were significant ($P < .005$, except that $P < .05$ for the quadratic component). The significant linear component indicated a general tendency for fewer errors to be made in relearning with increasing length of the retention interval, while the significance of the other components of the relearning curve reflected the relatively large number of errors made in relearning after the 2-day retention interval. For reversal learning, a significant linear component of the curve ($P < .005$) revealed a general tendency to become more difficult as the retention interval lengthened. Thus, while relearning becomes easier with time, reversal learning becomes more difficult. Moreover, significant cubic and quartic components of the reversal learning curve ($P < .05$) reflected the fact that, as an exception to the general increase in difficulty with time, reversal learning was relatively easy after a cold 2-day retention interval. Therefore it seems reasonable to conclude that the reminiscence effect is a memory-related phenomenon and that it does not appear to be related to nonspecific factors such as amount of motivation.

The data also indicate the possibility that more than one stage of memory may exist in *Tenebrio* adults. Consolidation of memory trace in the adult *Tenebrio* beetle may involve at least two memory storage systems, a theory consistent with increasing evidence of

similar systems in higher animals (4). After the beetle has been in the cold for 2 days, the trace is in the process of being transferred from one system to the other; and, if the animal is warmed or tested (or both) during this critical transfer period, the trace is lost. In fact, the loss of memory on day 2 appears to be complete. (i) The animals on day 2 made a mean of 4.62 errors in relearning, a figure that compares closely with the mean of 4.78 errors made by all the animals in original learning. (ii) The difference in retention between these animals and those with other retention intervals was present on the first trial of the second task (Fig. 2). (iii) The poor retention of the 2-day group was not confined to the first day of relearning, as it might be if some temporary difficulty were involved. The 2-day group made significantly more errors on their 2nd day of relearning than the 3-day group made on their 1st day ($t = 2.75$, $df = 14$, $P < .02$), despite the fact that the 2-day group had had practice for an additional day. Thus the warming of the beetle on day 2 causes apparently total forgetting. In the development of the memory, day 2 may represent a critical period during which the engram is sensitive to temperature manipulation; this period may represent engram transfer to another memory stage.

The nearly perfect retention of the cold beetles on day 5 suggests that the cold may act to protect the memory. The failure to find reminiscence with larvae (1) suggests that whatever mechanism was protected in our study either does not exist in the larvae or has a different temporal course. Finally, we wish to emphasize the simple techniques involved in performing these experiments and the possible use of this preparation in discerning the electrophysiological and biochemical substrates of memory.

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References and Notes

1. T. M. Alloway, thesis, Northwestern University (1966).
2. That our findings were the result of trail pheromones seems unlikely because (i) each animal would be required to distinguish between his own trail and that of 39 other animals, and (ii) it would be necessary to assume that on day 2 there was a reduced pheromone sensitivity which recovered on day 3.

3. Half the subjects in each experimental group were run in each of two independent replications. In the analysis of variance and trend, these replications were considered as a variable orthogonal to the experimental variables. Thus the error term for the F -tests has 60 df , instead of 70 df which would otherwise be the case. There was no statistical significance to replications either as a main effect or as an interaction with the other variables.
4. B. W. Agranoff, *Sci. Amer.* **216**, 115 (June 1967); S. E. Glickman, *Psychol. Bull.* **58**, 218 (1961); J. L. McGaugh, *Science* **153**, 1351 (1966); N. C. Waugh and D. A. Norman, *Psychol. Rev.* **72**, 89 (1965).
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Squirrel Monkey Reproduction: The "Fatted" Male Phenomenon and Seasonal Spermatogenesis

Abstract. Records of reproduction in a Miami colony of squirrel monkeys living in a seminatural state and of monkeys in the field indicate that the male undergoes an annual testis cycle. The spermatogenic phase is associated with the seasonal acquisition of a secondary sexual characteristic which we have termed the "fatted" condition. Body weights, measurements of subcutaneous fat, and testicular histology studied over a 12-month period further establish this cycle. Climatological analyses suggest that this cycle is associated with the precipitation cycles of the respective environments.

Field studies indicate that many non-human primates are seasonally reproductive. Lancaster and Lee (1) discuss cyclic reproduction in Old World primates; however, descriptions of such periodicity in New World primates is almost totally lacking. Carpenter (2) did not report seasonal reproduction in either the howler monkey (*Alouatta palliata*) or the red spider monkey (*Ateles geoffrey*). However, discrete mating and birth seasons have been observed in a colony of squirrel monkeys (*Saimiri sciureus*) living in a seminatural environment at Monkey Jungle, Miami, Florida (3). The concept of reproduction cycles in male simian primates is even more recent. A seasonal spermatogenic cycle has been described in the rhesus monkey (*Macaca mulatta*) on Cayo Santiago (4). We have studied the reproduction cycle in the male squirrel monkey, especially the seasonal acquisition and loss of a secondary sexual characteristic which we have termed the "fatted" state, its relationship to the seasonal spermatogenesis.