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Role of the Cerebellum in an Avoidance Conditioning Task in the Rat

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DAHHAOUI, M., J. CASTON, N. AUVRAY AND A. REBER. *Role of the cerebellum in an avoidance conditioning task in the rat.* PHYSIOL BEHAV 47(6) 1175–1180, 1990.—Adult DA/HAN strain male rats were submitted to an avoidance conditioning procedure. They were divided into two experimental groups, the animals being either conditioned (COC group) or not (NOC group) before cerebellectomy, and two control groups, the animals being either intact (C group) or sham-operated (SO group). Although the NOC rats could be conditioned successfully and the cerebellum is not absolutely necessary for the avoidance conditioning achievement, their scores were significantly lower than the preoperative scores of COC rats. Moreover, the scores of NOC rats (postoperative scores) were significantly lower than the postoperative scores of COC animals, suggesting that the preoperative conditioning makes the postoperative conditioning easier. At last, comparing postoperative scores of COC rats with those obtained by C and SO rats when given the retrieval test and comparing preoperative and postoperative scores in COC animals show that retention of the initial (preoperative) conditioning is partly abolished by the cerebellectomy. Histological controls demonstrated that the entire cerebellum except for the flocculus and the nodulus was removed. These results strongly suggest that the cerebellum is involved in the memory processes that sustain the avoidance conditioning.

Conditioning Cerebellum Rat Memory

FROM the past years, many investigations were devoted to the role of the cerebellum in motor learning and several models were built to try to explain how it can interfere in a learning process (1, 7, 10, 12, 21). Especially, it has been demonstrated that the cerebellum is involved in the adaptation of the vestibulo-ocular reflex to modification of the visual environment (5, 12, 15, 22) and in learning of alternating arm movements in the monkey (11, 24, 25). It is also involved in classical conditioning and, particularly, in classical conditioned nictating membrane response in the rabbit and the cat (2, 6, 14, 16–19, 26). Moreover, in the cat it is possible to elicit the production of a food-procuring conditioned reflex to electrical stimulation of deep cerebellar nuclei (8). These studies include behavioral observations, anatomical lesions of the whole cerebellum or of a part of it (cortex or deep nuclei) and electrophysiological recordings of Purkinje and nuclear cells. To our knowledge, little is known about the eventual role of the cerebellum in the acquisition and retention of a more complex motor behavior such as a conditioned response including the whole body (3, 4, 13, 20, 23). Therefore, the aim of the present study was to test, from a behavioral point of view, whether the cerebellum is involved in an avoidance conditioning task. To this purpose, intact and cerebellectomized rats were submitted to an experimental schedule allowing the animals to avoid an electric shock when hearing a sound; from comparison of the animal's score the role of the cerebellum in the acquisition and the retention of the conditioned behavior could be ascertained.

However, it is well known that cerebellectomy induces ataxia and an eventual deficit of the score observed in cerebellectomized animals could be due either to ataxic disorders or/and to a true learning deficit. To test the relative weight of ataxia and learning deficit in the score reached by cerebellectomized animals, one compared the postoperative scores of rats which were operated while naive and conditioned after cerebellectomy with postoperative scores of rats conditioned before cerebellectomy. Ataxic disorders being similar in both groups, if the postoperative scores of rats conditioned before cerebellectomy are not statistically different from the postoperative scores of naive-operated rats, one can conclude that the former animals have forgotten their preoperative training and that cerebellectomy erases memory. If the postoperative scores of rats conditioned before cerebellectomy are better than the postoperative scores of naive-operated animals, this means that a memory trace of the preoperative conditioning is still present, at least in part.

METHOD

Experimental Device

Experimental sessions were conducted in a shuttle box measuring $42 \times 27 \times 20$ cm and equipped with a grid floor. The two equal compartments of the box were separated by an incomplete partition wall, allowing the animal to pass from one compartment to the other. The floor of each compartment could be electrified separately. A sound was delivered from a buzzer fixed to a wall of the chamber. Sound and electric shocks were generated automatically from an electronic device according to a fixed schedule.

Animals

The animals used in this study were 3–4-month-old DA/HAN

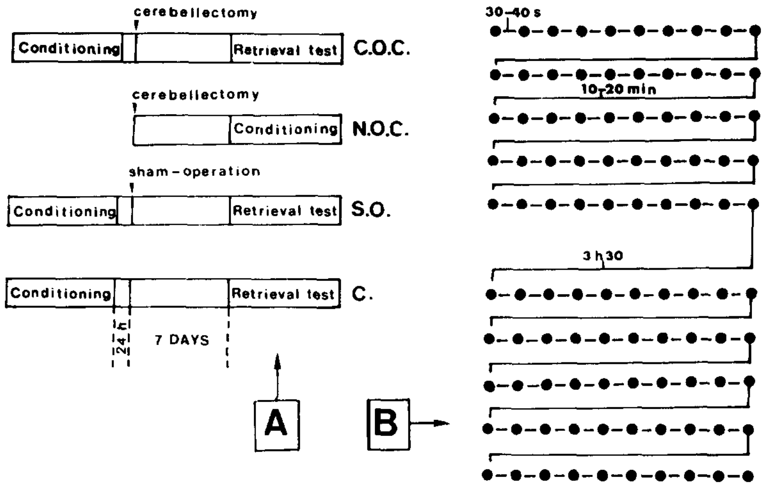

FIG. 1. (A) Schematic representation of the experimental conditions. COC: conditioned-operated-conditioned rats; NOC: naive-operated-conditioned rats; SO: sham-operated rats; C: control rats. (B) Diagram of the conditioning schedule. Each dot represents a trial, two consecutive trials being spaced by a 30–40-sec interval; each line represents a series of 10 trials, two consecutive series being spaced by a 10–20-min interval; each group of 5 lines represents a session of 50 trials, two consecutive sessions being spaced by a 3-hr 30-min interval.

strain male rats (pigmented rats) weighing about 220 g and housed in standard conditions (12-hr light, 12-hr dark, 22°C, food and water ad lib). Twenty animals divided into 4 groups of 5 rats (2 experimental groups and 2 controls) were studied (Fig. 1A).

The two experimental groups were the following:

COC group (conditioned-operated-conditioned): the animals were conditioned and cerebellectomized 24 hr after the conditioning was stabilized; then, after a 7-day delay, they were tested and eventually conditioned again.

NOC group (naive-operated-conditioned): the animals were cerebellectomized while naive and were conditioned after a 7-day delay. This group differs from the COC one by the lack of conditioning before cerebellectomy.

The two control groups were as follows:

C group (control): the animals were conditioned; then, 8 days after the conditioning was stabilized, they were tested and eventually conditioned again.

SO group (sham-operated): the animals were conditioned and sham-operated 24 hr after the conditioning was stabilized; then, 7 days later, they were tested and eventually conditioned again.

Cerebellectomy and Sham Operation

The animals were anesthetized by IP injection of Nembutal (pentobarbital sodium) 35 $mg \cdot kg^{-1}$ and secured in a stereotaxic apparatus. The skin of the skull was incised, the occipital bone drilled and removed. After cutting the dura, the cerebellum was removed by suction and the cavity filled with gelfoam. Then the skin was sutured.

Sham-operated rats were submitted to the same protocol except for removing the cerebellum which was left intact.

Experimental Procedure

The avoidance conditioning was conducted as follows. The rat was placed in one compartment of the shuttle box and allowed to explore the whole box for 15 min. Then it was submitted each day to two sessions of 50 trials (one in the morning, the other in the afternoon) spaced by a 3-hr 30-min interval as a mean. The 50 trials of each session were split in 5 series of 10 trials spaced by a 10–20-min interval. Each trial, spaced from the next one by 30–40 sec, consisted in a sound delivered from a buzzer for 3 sec, followed one second later by an electric shock (about 30–40 V) which lasted up to 30 sec (Fig. 1B). Shock intensity had to be high enough to make the animal greatly disturbed but not too high so that the animal did not experience pain as revealed by its behavior.

The rats were considered as being conditioned when they reached at least 80% of successful trials (going from one compartment to the other when given the sound) before the fifth series of a given session; the conditioning was considered as stabilized when the animals reached the above-mentioned criterion for 5 consecutive sessions.

Retrieval Test and Reinforcement

Eight days after the initial conditioning was stabilized, COC, C and SO animals experienced a retrieval test and, eventually, a reinforcement in order to know the number of trials needed to reach the same conditioning criterion as the one previously defined and, therefore, to test the retention of the initial conditioning.

Treatment of the Results

For each group, the number of trials (x) needed for each rat to reach the conditioning criterion was averaged (mean $= \Sigma x/5$), the mean value being given $\pm$SEM ($\sigma/\sqrt{n}$). Comparison between groups was made according to the Mann-Whitney test at $p \leq 0.05$ or $p \leq 0.01$.

Histological Controls

After completion of the experiments, the animals were overdosed with Nembutal; then the brain was quickly removed and placed in 10% formalin for at least 10 days. Some preparations were embedded in paraffin; serial sections (10 μm) stained with methyl blue were examined to ascertain the extension of the

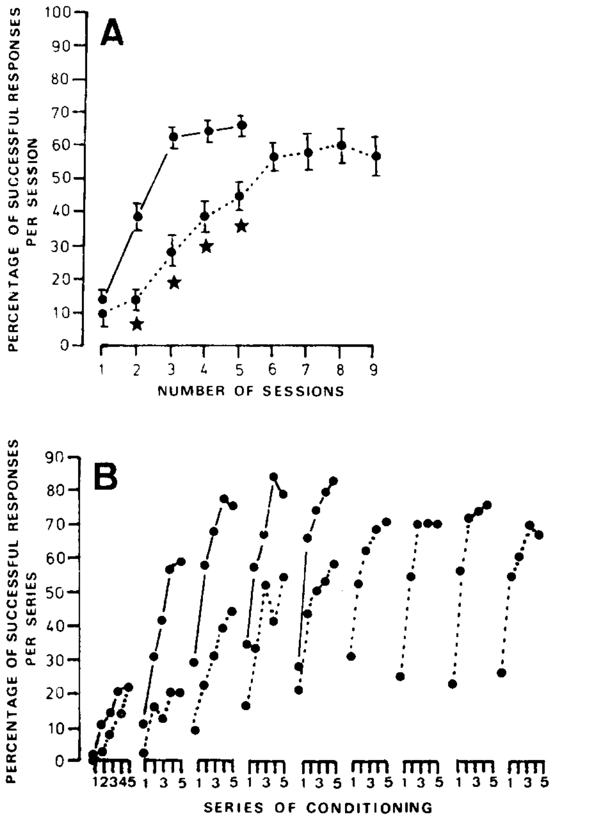

FIG. 2. (A) Percentage ($\pm$SEM) of successful responses per session in intact (full line) and in NOC (dotted line) rats. Stars: significant difference at $p \leq 0.05$. (B) Percentage of successful responses per series in an intact (full line) and a NOC (dotted line) rat.

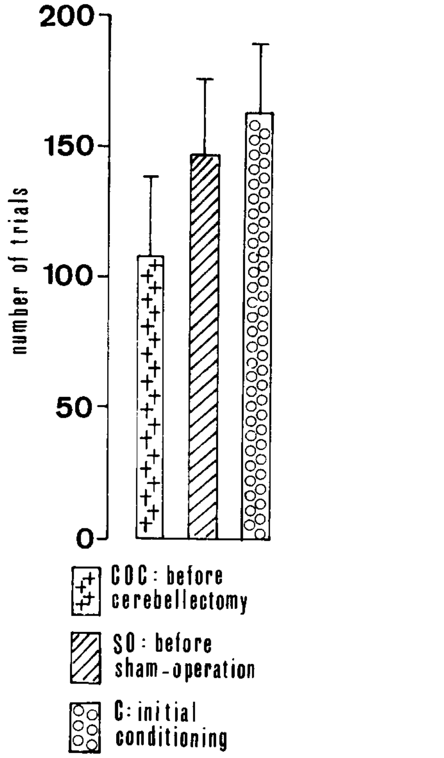

FIG. 3. Initial conditioning: mean number of trials ($\pm$SEM) needed for the achievement of the initial conditioned response in COC, SO and C rats.

cerebellar lesion. The others were examined under the operative microscope.

RESULTS

Evolution of the Initial Scores of C, SO and COC and of the Scores of NOC Rats

The evolution of the initial scores of C, SO and COC rats and of the scores of NOC rats is pointed out in Fig. 2. From Fig. 2A, which shows the evolution of the scores during the following sessions of 50 trials, it can be seen that, in intact animals, the percentage of successful trials sharply increases up to the third session to reach a maximal value of 60–65%. In NOC rats, the score increase from session to session is much slower and a maximal value of 55–60% of successful trials is reached only by the sixth session. For any given session, except for the first one, the differences between the scores of intact and cerebellectomized animals are significant at $p \leq 0.05$. Although each animal, whatever the group, is able to reach the given conditioning criterion (80% of successful trials before the fifth series of a session), it is obvious from Fig. 2A that the maximal score obtained during a whole session of 50 trials is always smaller than that. The reason is that during the first series of a given session the score is always much lower than during the last series of the preceding one, as can be seen from Fig. 2B. For intact animals, the score at the beginning of a session is about 25–35%, while for NOC animals it is only 15–30%.

Comparison of Scores Between Groups

Comparison of the initial scores in COC, SO and C rats. During the initial conditioning the rats of these three groups were identical: their cerebellum was intact, they were submitted to the same experimental protocol and they were all naive before the first conditioning session. Therefore, one can expect their scores to be similar. In fact, the number of trials needed by the animals to reach at least 80% of successful responses was 106 ± 29.6 (COC rats), 146 ± 29.5 (SO rats) and 163 ± 26.6 (C rats), values which are not significantly different (Fig. 3).

Influence of cerebellectomy on the avoidance conditioning achievement. Such an influence can be pointed out by comparing the initial scores obtained by COC rats (preoperative scores) and those obtained by NOC rats (postoperative scores in animals which were never submitted to an initial conditioning). Figure 4A illustrates that the number of trials needed to reach 80% of successful responses is about 3.4 times greater in NOC rats (362 ± 55.0) than in COC rats before cerebellectomy (106 ± 29.0); the difference is statistically significant at $p \leq 0.01$.

Therefore, while it is clear that NOC animals can be conditioned successfully and that the cerebellum is not absolutely necessary for the avoidance conditioning achievement, the above-mentioned comparison suggests that it is easier when the cerebellum is intact.

Influence of the preoperative conditioning on the postoperative score. The influence of the preoperative (initial) conditioning on the postoperative score can be ascertained by comparing the postoperative scores of NOC and COC rats: the first animals were naive before the cerebellectomy while the second ones were not. From Fig. 4B, it can be seen that the number of trials needed to

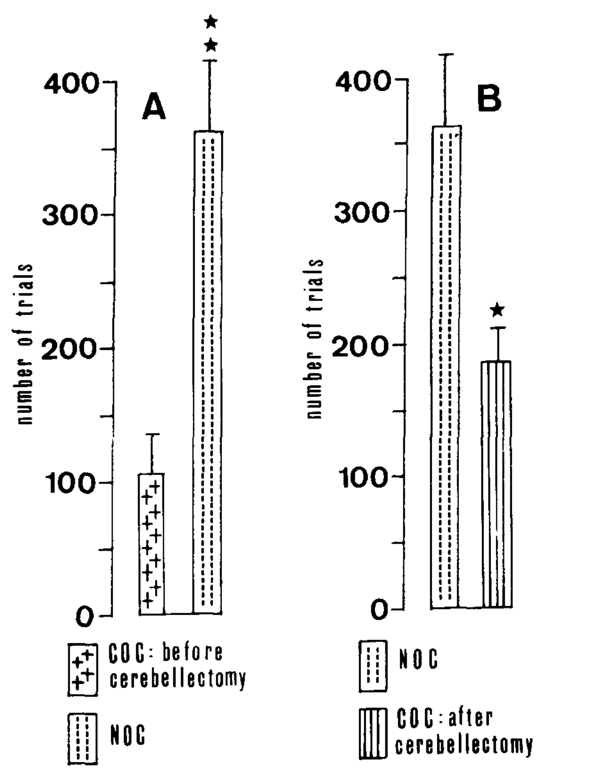

FIG. 4. (A) Influence of cerebellectomy on the avoidance conditioning achievement: comparison of the scores obtained by COC rats before cerebellectomy and by NOC rats. Stars: significant difference at $p \leq 0.01$. (B) Influence of the preoperative conditioning on the postoperative score: comparison of the scores obtained by NOC rats and by COC rats after cerebellectomy. Stars: significant differences at $p \leq 0.05$.

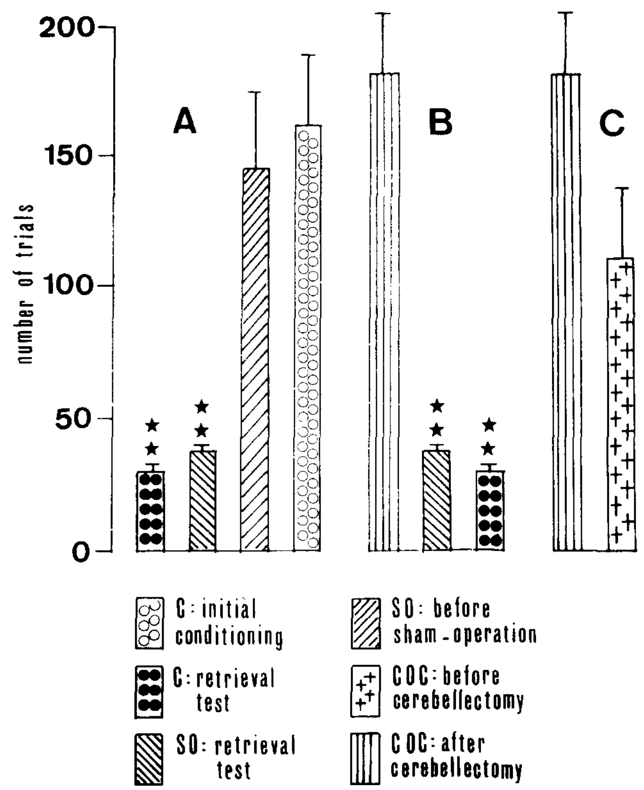

FIG. 5. Role of the cerebellum in retention of the initial conditioning. (A) Comparison of the initial and of the retrieval scores in C and SO rats. Stars: significant difference at $p \leq 0.01$. (B) Comparison of the postoperative scores in COC rats and of the retrieval scores in C and SO rats. Stars: significant difference at $p \leq 0.01$. (C) Comparison of the pre- and postoperative scores in COC rats.

reach the conditioning criterion is about twice greater in NOC rats (362±55.0) than in COC rats (182±24.5); the difference is statistically significant at $p \leq 0.05$. Then it can be stated that the postoperative conditioning is facilitated when the animal has experienced an initial conditioning before cerebellectomy.

Role of the cerebellum in retention of the initial conditioning. Retention in control rats. Retention of the initial conditioning in control rats can be estimated by comparing the number of trials they needed to reach the conditioning criterion when given the retrieval test and when submitted to the initial conditioning, respectively.

The number of trials needed to reach 80% of successful responses is about 5.5 times less in C animals eight days after the initial conditioning (30±2.8) than when submitted to the initial sessions (162±26.6; Fig. 5A). For SO animals, this number of trials is 38±1.8 and 146±29.5, respectively (Fig. 5A). These differences are statistically significant at $p \leq 0.01$. The number of trials needed to reach the conditioning criterion when the retrieval test was given is not significantly different in C and SO rats (30±2.8 and 38±1.8, respectively), showing that the sham operation did not affect the retention of the initial conditioning.

Retention in cerebellectomized rats. Retention of the initial conditioning in cerebellectomized rats can be ascertained, first by comparing the postoperative scores of COC animals with those obtained by C and SO rats when given the retrieval test, second by comparing preoperative and postoperative scores in COC animals. From Fig. 5B, it is clear that the number of postoperative trials needed by COC animals to reach 80% of successful responses (182±24.5) is about 6 times and 5 times greater than those needed by C (30±2.8) and SO (38±1.8) animals, respectively, when given the retrieval test; the differences are statistically significant at $p \leq 0.01$. From Fig. 5C, it can be observed that the number of trials needed to reach the conditioning criterion is greater after cerebellectomy (182±24.5) than before (106±29.6); however, the difference is not statistically significant. The results show that cerebellectomy greatly alters the retention of the initial conditioning.

Histological Controls

Brains of COC and NOC rats were subjected to histological controls and to examination under the operative microscope in order to estimate the extent of the lesion. In all cases, the cerebellum was almost completely removed except for the flocculus and the nodulus which were left intact. An example is given in Fig. 7. Moreover, the brain stem exhibited no sign of lesion.

DISCUSSION

Evolution of the Initial Scores

From Fig. 2A it is clear that in intact as well as in cerebellectomized animals the percentage of successful trials per session increases regularly with time and reaches a plateau indicating that the animals are conditioned. However, retention of the task is partly lost from one session to the next one since during the first series of any given session, the scores of intact and cerebellectomized animals fall down to about 25–35% and 15–30%, respec-

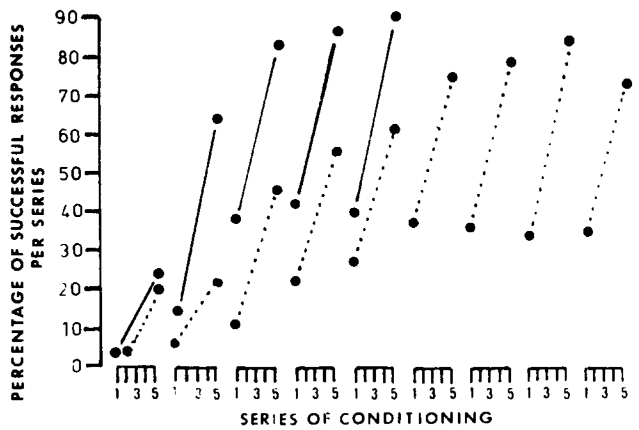

FIG. 6. Regression lines of the curves from Fig. 2B, showing the evolution of the scores in an intact (full line) and a NOC (dotted line) rat. The equations of the regression lines are the following: for the intact rat, from session 1 to session 5: $y = 5.14x - 1.58$; $y = 12.48x + 1.82$; $y = 11.28x + 27.42$; $y = 11.42x + 29.44$; $y = 12.59x + 27.81$; for the NOC rat, from session 1 to session 9: $y = 5.60x - 7.60$; $y = 4.00x + 2.00$; $y = 8.80x + 2.00$; $y = 8.40x + 13.60$; $y = 8.60x + 18.60$; $y = 9.60x + 27.60$; $y = 10.80x + 25.20$; $y = 12.60x + 22.20$; $y = 9.72x + 22.16$.

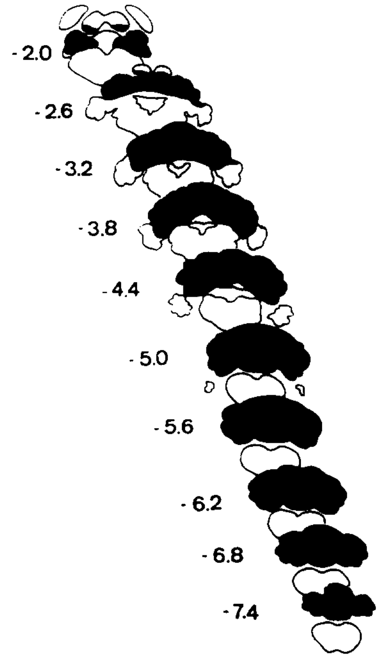

FIG. 7. Tranverse section of the cerebellum and the brain stem showing, in one rat, the parts of the cerebellum (filled in black) which were removed; figures on the left indicate the antero-posterior coordinates with respect to the De Groot system.

tively (Fig. 2B), and the animals need reinforcement to reach 80% of successful responses.

Cerebellum and Conditioning

The results of this study clearly show that the cerebellum is not absolutely necessary for the avoidance conditioning achievement. In fact, within a given conditioning session, the evolution of scores is similar in intact and cerebellectomized animals as shown by the regression lines (Fig. 6) drawn from results of Fig. 2B, whose slopes are not significantly different, except for the second conditioning session. This means that within a given conditioning session, cerebellectomized rats learn as fast as intact ones and that the cerebellum is probably not involved in the acquisition of the conditioning. This is in agreement with previous results. In the fish, Bianki (3) demonstrated that electro-defensive conditioned reflexes to light and acoustic stimuli can still be elicited after complete removal of the cerebellum while such reflexes are inconstant. In the dog, the conditioned-avoidance reflex consisting of flexing a limb in response to a sound is formed at the same rate in intact and in cerebellectomized animals (9) and the chief components of complex motor situational food-conditioned reflexes are preserved after removal of the neocerebellum (20). In the rat, discrete cerebellar lesions (lesions of the medial cortex and underlying cerebellar tissue of lobules VI and VII) do not affect performances of visual-learning tasks under conditions of high motivation, while they hinder learning (4). In the rat, too, the effects of destruction of the deep cerebellar nuclei depend on the site of the lesion: bilateral lesions of the fastigial and medial interposed nuclei impaired the acquisition of two-way active avoidance tasks and bilateral lesions of dentate and lateral interposed nuclei facilitated acquisition of the avoidance task, while in both cases the lesions produced transient deficits in posture and movements (23).

While not absolutely necessary for the avoidance conditioning achievement, the cerebellum is involved in the nervous mechanisms that sustain conditioning. From Fig. 4B, it is obvious that after cerebellectomy the number of trials needed to reach the conditioning criterion is twice less in COC animals than in NOC ones. The ataxic disorders being similar in both groups, it can be stated that COC animals have remembered their preoperative experience. However, the retention is much better in intact animals than in cerebellectomized ones, since the number of trials needed to reach the conditioning criterion is 5.5 times less in C animals when given the retrieval test than during the initial conditioning (Fig. 5A). Therefore, one can conclude that, although cerebellectomy does not completely erase memory of the preoperative experience, it greatly alters its consolidation (that is retention of the task from one session to the next one). This conclusion disagrees with results from Lashley and McCarthy (13), who demonstrated the existence of a perfect retention of a maze learning after cerebellar removal. Such a discrepancy can be due to differences in the experimental protocols.

The fact that in our study COC animals after cerebellectomy need statistically as many trials as before the operation (Fig. 5C) seems to be due to both motor ataxia, which develops after cerebellar removal, and partial erase of the memory of the preoperative conditioning.

REFERENCES

1. Albus, S. S. A theory of cerebellar function. Math. Biosci. 10:25–69; 1971.
2. Berthier, M. E.; Moore, J. W. Cerebellar Purkinje cell activity related to the classically conditioned nictating membrane response. Exp. Brain Res. 63:341–351; 1986.
3. Bianki, V. L. Conditioned reflexes to light and sound in fishes deprived of the cerebellum. Zh. Vyssh. Nerv. Deiat. 12:962–968; 1962.
4. Buchtel, H. A. Visual learning deficits following cerebellar damage in rats. J. Comp. Physiol. Psychol. 72:296–305; 1970.

5. Collewijn, H. The modifiability of the adult vestibulo-ocular reflex. Trends Neurosci. 2:98–102; 1979.
6. Desmond, J. E.; Moore, J. W. A brain stem region essential for classically conditioned but not unconditioned nictating membrane response. Physiol. Behav. 28:1029–1033; 1982.
7. Eccles, J. C. An instruction-selection theory of learning in the cerebellar cortex. Brain Res. 127:327–352; 1977.
8. Fanardjian, V. V.; Papoyan, E. V. Electrophysiological analysis of conditioned reflexes where the conditional stimulus is the stimulation of phylogenetically different parts of the cerebellum. Brain Behav. Evol. 9:458–487; 1974.
9. Gambaryan, L. S. Conditioned avoidance reflexes induced in dogs with cerebellar lesions. Physiol. Bohemoslov. 9:261–266; 1960.
10. Gilbert, P. F. C. A theory of memory that explains the function and structure of the cerebellum. Brain Res. 70:1–18; 1974.
11. Gilbert, P. F. C.; Thach, W. T. Purkinje cell activity during motor learning. Brain Res. 128:309–328; 1977.
12. Ito, M. Synaptic plasticity in the cerebellar cortex that may underlie the vestibulo-ocular adaptation. In: Berthoz, A.; Melvill Jones, G., eds. Adaptive mechanisms in gaze control. Facts and theories. New York: Elsevier Science Publishers; 1985:213–221.
13. Lashley, K. S.; McCarthy, D. A. The survival of the maze habit after cerebellar injuries. J. Comp. Psychol. 6:423–433; 1926.
14. Lavond, D. G.; Hembree, T. L.; Thompson, R. F. Effect of kaïnic acid lesions of the cerebellar interpositus nucleus on eyelid conditioning in the rabbit. Brain Res. 326:179–182; 1985.
15. Lisberger, S. G. Role of the cerebellum during motor learning in the vestibulo-ocular reflex. Different mechanisms in different species? Trends Neurosci. 5:437–441; 1982.
16. McCormick, D. A.; Lavond, D. G.; Clark, G. A.; Kettner, R. E.; Rising, C. A.; Thompson, R. F. The engram found? Role of the cerebellum in classical conditioning of nictating membrane and eyelid response. Bull. Psychon. Soc. 18:103–105; 1981.
17. McCormick, D. A.; Steinmetz, J. E.; Thompson, R. F. Lesions of inferior olivary complex cause extinction of the classically conditioned eyeblink response. Brain Res. 359:120–130; 1985.
18. McCormick, D. A.; Thompson, R. F. Neuronal responses of the rabbit cerebellum during acquisition and performance of a classically conditioned nictating membrane response. J. Neurosci. 4:2811–2822; 1984.
19. McCormick, D. A.; Thompson, R. F. Cerebellum: essential involvement in the classically conditioned eyelid response. Science 223: 296–299; 1984.
20. Malyukova, I. V. Influence of the neocerebellum extirpation on situational conditioned reflexes in dogs. Zh. Vyssh. Nerv. Deiat. 13:1052–1058; 1963.
21. Marr, D. A theory of cerebellar cortex. J. Physiol. (Lond.) 202: 437–470; 1969.
22. Robinson, D. A. Adaptive gain control of vestibuloocular reflex by the cerebellum. J. Neurophysiol. 39:954–969; 1976.
23. Schneiderman Fish, B.; Baisden, R. H.; Woodruff, M. L. Cerebellar nuclear lesions in rats: subsequent avoidance behavior and ascending anatomical connections. Brain Res. 166:27–38; 1979.
24. Thach, W. T. Discharge of Purkinje and cerebellar nuclear neurons during rapidly alternating arm movements in the monkey. J. Neurophysiol. 31:785–797; 1968.
25. Thach, W. T. Discharge of cerebellar neurons related to two maintained postures and two prompt movements in the monkey. II. Purkinje cell output and input. J. Neurophysiol. 33:537–547; 1970.
26. Yeo, C. H.; Hardiman, M. S.; Glickstein, M. Discrete lesions of the cerebellar cortex abolish the classically conditioned nictating membrane response in the rabbit. Behav. Brain Res. 13:261–266; 1984.