Effects of Cerebellar Nuclear Inactivation on the Learning of a Complex Forelimb Movement in Cats

JIAN-JUN WANG, YURY SHIMANSKY, VLASTISLAV BRACHA, AND JAMES R. BLOEDEL
Division of Neurobiology, Barrow Neurological Institute, Phoenix, Arizona 85013

Wang, Jian-Jun, Yury Shimansky, Vlastislav Bracha, and James R. Bloedel. Effects of cerebellar nuclear inactivation on the learning of a complex forelimb movement in cats. J. Neurophysiol. 79: 2447–2459, 1998. The purpose of this study was to determine the effects of inactivating concurrently the cerebellar interposed and dentate nuclei on the capacity of cats to acquire and retain a complex, goal-directed forelimb movement. To assess the effects on acquisition, cats were required to learn to move a vertical manipulandum bar through a two-segment template with a shape approximating an inverted “L” after the injection of muscimol (saline for the control group) in the interposed and dentate cerebellar nuclei. During training periods, they were exposed progressively to more difficult templates, which were created by decreasing the angle between the two segments of the template. After determining the most difficult template the injected animals could learn within the specified time and performance constraints, the retraining phase of the experiment was initiated in which the cats were required to execute the same sequence of templates in the absence of any injection. This stage of the experiment assessed retention and determined the extent of any relearning required to execute the task at criterion levels. Next, the animals were overtrained without any injection on the most difficult template they could perform. Finally, to determine the effects of nuclear inactivation on retention after extensive retraining, their capacity to perform the same template was determined after muscimol injection in the interposed and dentate nuclei. The findings show that during the inactivation of the dentate and interposed nuclei the animals could learn to execute the more difficult templates. However, when required to execute the most difficult template learned under muscimol on the day after injections were discontinued, the cats had to “relearn” (reacquire) the movement. Finally, when the cerebellar nuclei were inactivated after the animals learned the task in the absence of any injections during the retraining phase, retention was not blocked. The data indicate that the intermediate and lateral cerebellum are not required either for learning this type of complex voluntary movement or for retaining the capacity to perform the task once it is learned. Nevertheless, when the cerebellum becomes available for executing a task learned in the absence of this structure, reacquisition of the behavior usually is necessary. It is hypothesized that the relearning observed after acquisition during muscimol inactivation reflects the tendency of the system to incorporate the cerebellum into the interactions responsible for the learning and performance of a motor sequence that is optimal for executing the task.

INTRODUCTION

In his pioneering theoretical study, Marr (1969) proposed that the cerebellum serves as a “learning machine” critical for acquiring novel motor skills. Since that time several experimental findings have implicated the cerebellum in the processes required for the learning and modification of different movements, including conditioned eye blink reflexes (Thompson 1986), visuomotor behavior (Gauthier et al. 1979), the vestibulo-ocular reflex (Ito 1982; Li et al. 1995; Lisberger et al. 1984; Robinson 1976), and the adaptation to image displacing prisms (Martin et al. 1996; Thach et al. 1992; Weiner et al. 1983). Changes in the activity of cerebellar cortical neurons during the adaptive modification of a simple wrist joint movement also were found to be consistent with this hypothesis (Gilbert and Thach 1977). Based on these and other related data as well as on inferences derived from the anatomy and physiology underlying the neuronal interactions of the cerebellar cortex and nuclei, the hypothesis of Marr was applied to the learning of complex volitional limb movements. Specifically, it was proposed that the cerebellum is responsible for combining simple movement elements into more complex synergies and storing them in a memory using the cerebellar circuitry (Thach 1996; Thach et al. 1992).

Although data obtained from cerebellar patients during the past decade and a half suggests a role of the cerebellum in learning complex voluntary motor tasks including multijoint voluntary movements, this issue has not been studied rigorously using well-controlled animal models, particularly with objectives focused on determining precisely what aspects of the learning process are influenced by this structure. Certainly many possibilities exist in addition to storage. For example, during learning the cerebellum could contribute to acquisition-related, consolidation-related, and recall-related processes without serving as a storage site for the memory trace. In fact, recent experiments in our laboratory have implicated this structure in the acquisition process (Milak et al. 1995). These studies demonstrated that the event-related modulation of several simultaneously recorded cerebellar nuclear neurons progressively increased as a novel, complex volitional task was practiced and subsequently reached a peak as the behavioral sequence first was performed successfully (Bloedel et al. 1993a,b; Milak et al. 1995). Thus the maximal amplitude of the event-related modulation of nuclear neurons was highly correlated with a critical component of the acquisition process. A different series of experiments assessed the cerebellum’s role in the retention of the same behavior (Milak et al. 1997). These data demonstrated that the selective inactivation of any of the three major nuclear regions (fastigial, interposed, or dentate) with muscimol failed to disrupt the retention of the learned behavior, a finding that is clearly inconsistent with the cerebellar storage hypothesis.

To examine further the cerebellum’s role in the acquisition and retention of volitional forelimb movements, the present experiments were performed to determine the effects of inac-
tivating unilaterally both the dentate and interposed nuclei on the learning of a variant of the template task employed by Milak et al. (1995, 1997). On the basis of our previous data, it was hypothesized that, although the cerebellum may contribute to the normal acquisition of the template task, it is not essential for this process nor is it required for the task’s retention. The findings support this hypothesis by showing that cats with the dentate and interposed nuclei inactivated can learn the sequence of movements required to perform the template task and that the learning is condition dependent: after learning the behavior during nuclear inactivation, the animals had to reacquire the task when it was performed on a subsequent day with the cerebellum completely functional. The study also demonstrates that the cerebellum is not required for retention, even when the task is learned in the functionally intact animal. An abstract of this study has been presented previously (Shimansky et al. 1994).

METHODS

Paradigm and protocol

Adult food-motivated cats initially were trained to stand on four platforms (9-cm diam) and pull a vertical bar toward a food-reward zone through a horizontal posterior-anterior groove in which the lower end of the bar was inserted. The groove, which was identical to the final segment of the template shown in Fig. 1, was positioned at the animal’s midline. The bar (10-cm long, 12-mm diam) was covered with a surface soft enough for a cat to grasp it. Cats were required to start the reach for the bar after a tone ‘‘go’’ signal and had 3 s to complete the task by moving the bar to the reward position (Fig. 1), located 180–200 mm anterior to a line through the center of the forelimb platforms, where a reinforcement consisting of baby food was extruded onto a planchet located at the top of the manipulandum bar. During the intertrial interval, which varied pseudorandomly between 4 and 6 s, the cats were required to stand quietly while being restrained only by a net-like harness extending around the thorax and attached to two horizontal beams on both sides of the animal. The grooved template was located 60 mm above the level of the foot platform.

The animals were divided into two groups, one (n = 4) in which animals were injected with muscimol during learning and the other, the control group (n = 3), which received saline injections during the same part of the experiment (Fig. 2). Initially, three animals were selected for each group. However, the interesting heterogeneity of the data from the muscimol-injected animals required the inclusion of a fourth cat in this group. Because of the difficulty of this task for cats, particularly when the cerebellar nuclei were inactivated, larger groups were not feasible for this study.

The protocol was identical for all animals in each group. After mastering the single groove task, four stainless steel guide tubes (0.4 mm OD) for the injection cannulae were implanted in the right half of the cerebellum ipsilateral to the reaching limb in each animal under sterile surgical procedures. Each tube was tilted posteriorly at a 28° angle in the sagittal plane, and their lower ends were positioned 3 mm above the center of the dentate, anteriorly.
interposed, posterior interposed, and fastigial nuclei using standard stereotaxic coordinates for these structures. In the experiments reported here, the fastigial nucleus was not injected. Wire electrodes were implanted for electromyographic (EMG) recording in the following muscles of the right (ipsilateral) forelimb: extensor digitorum communis, palmaris longus (PL), brachialis (Bra), lateral triceps (Tri), and spino-deltoides and in the PL, Bra, and Tri of the left forelimb. The EMG recordings will be reported in a subsequent manuscript examining the specific muscle synergies employed in executing the task under different experimental conditions. After surgery, 6–7 days were allowed for recovery. Then the training was resumed on the single groove task until the animals reached the presurgical level of performance.

Next, the most effective site for a muscimol injection (1 μl of 800 ng/μl per nucleus) was determined along the track extending below each guide tube by assessing the action of muscimol at one site each day. The initial depth of the injection was 2 mm above the expected center of the corresponding nucleus, and it was increased by one step of 0.5 mm per day until the maximum effect of the injection was obtained. At each site, muscimol was injected slowly during a 15-min period, during which the animal’s head was fixed. Twenty minutes after the injection was completed, the head was released, and the injection cannulae were left in place for the remainder of the experiment. Injection effects were evaluated both quantitatively and qualitatively by comparing different preinjection and postinjection characteristics of the animals’ performance on the single groove task as well as by testing placing, hopping, and magnet postural reactions immediately after the experimental session. Because of the marked clinical deficits associated with the inactivation of the anterior interposed nucleus (for additional details, see Bloedel and Bracha 1995; Kolb et al. 1997; Milak et al. 1997), the immediate onset of limb ataxia and abnormalities in the cutaneous reflexes were used to help establish the optimal site for injecting this structure. In addition, the stereotaxic location of this site also was used for determining the relative locations of the injection sites in the other nuclei.

After determining the most effective muscimol injection site for each tract, the acquisition part of the protocol was initiated. Each cat was injected concurrently in the anterior and posterior interposed and dentate nuclei either with muscimol or saline, depending on the experimental group (Fig. 2). Each animal then was required to learn a sequence of templates consisting of two segments: an initial medio-lateral segment oriented at an angle $90^\circ + \alpha$ relative to a second antero-posterior segment, which was positioned in the midline (Fig. 1). The learning usually started with $\alpha = 20^\circ$. Each cat was trained on a specific template until it was able to execute it within 3 s in 90% of trials in 20 trial blocks. Once this was achieved, the task difficulty was increased one step, defined as a decrease in the template angle (i.e., smaller angle relative to the antero-posterior grooves). The following template angles were used: $\alpha = 20^\circ$, $10^\circ$, $5^\circ$, $0^\circ$, $-5^\circ$, $-10^\circ$, and $-20^\circ$. During training, if the animal could not or would not execute the next smaller angle (usually failing in a few attempts), the angle was increased one step, and the cat again had to meet criterion at that angle. This procedure helped to ensure that the animals did not become frustrated during the experiment. As training continued, the most difficult template the cat could perform was determined, defined as the smallest angle the injected animal could learn within ~800 trials presented over a period not longer than 7 days. Each day of training concluded when the cat was no longer willing to continue reaching for the manipulandum bar and move it through the template. On each day of training the initial angle used was the same as that employed in the last trials of the previous day.

Next, the retraining phase of the protocol was initiated (Fig. 2). In this phase, the retention of the behavior learned during muscimol inactivation was evaluated, and the extent of the retraining and relearning required to execute the same templates was ascertained in the absence of any injections. Each un.injected animal was required to execute successively the same sequence of templates using the same rule for increasing template difficulty as employed during the initial learning phase. Retention was judged based on the cat’s capacity to complete 18 of 20 trials satisfactorily as the template difficulty was increased for each successive 20-trial block. Thus the minimum number of trials at a given angle was 20.

A final stage of the experiment examined whether nuclear inactivation affected the retention of the task after it had been practiced extensively in the absence of any injection. Once the most difficult template that an uninjected animal could execute was determined, the cat was overtrained on this template during 2–3 successive days. During this stage, each cat was required to perform the same template until it could move the bar quickly and smoothly to the target zone (without decelerating to zero velocity at the template corner) in most of the trials. Once the task became well practiced, its retention during nuclear inactivation was assessed by testing the effects of muscimol microinjections on the cats’ capacity to execute the same template they had learned during overtraining in the absence of any injections.

Data acquisition

Kinematics of the movements in each trial were assessed using an Optotak motion analysis system. Infrared emitting diodes (IREDs) attached with Velcro were used to monitor the position of the following sites: base of the lateral toe, paw dorsum, lateral aspect of the wrist joint, lateral aspect of the elbow joint, shoulder, and a reference point over the scapula. Several sensors were used to record the time of specific events related to the movement: paw lift-off was recorded based on a microswitch mounted in the ipsilateral forelimb platform, initiation of bar movement (“bar start”), arrival of the bar at the template corner, and arrival of the bar in the reward zone were recorded using light-sensitive diodes that were mounted underneath the groove and activated by a light on the bottom of the manipulandum bar.

Data analysis

For the purpose of data analysis, the task was divided into a reach phase, lasting from paw lift-off to contact with the manipulandum, and a trace phase, consisting of the time required to move the bar from its initial position to the reward zone. The temporal parameters measured were: the duration of the reach (from paw lift-off to bar contact), the movement time through the first segment of the template, duration of the trace phase, and the overall movement time. The length of paw trajectory during the initial template segment was calculated as the length of the corresponding path traveled by the paw dorsum IRED from the time the paw contacted the manipulandum to the time it arrived at the template corner. The speed of the paw and manipulandum bar for each component of the movement was calculated as the magnitude of the first derivative of the corresponding IRED’s position. The percentage of successful trials (trials completed within the required 3 s) was calculated within consecutive blocks of 10 trials and tabulated for plotting learning curves. To characterize the forelimb position at the moment of grasping the manipulandum bar, the angle between the elbow-wrist segment and its projection on the sagittal plane, defined as the forearm laterality angle, was calculated.

For each animal, the extent of the learning during each phase of the experiment was determined based on the smallest template angle mastered. The extent of retention at two stages of the experiment was assessed at the beginning of a session by determining whether the animal could execute the most difficult template mastered on the previous day. If the cat could no longer execute this template, successively less difficult templates were tried until suc-
cessful completion was observed. Plots displaying the paw trajectories were used to illustrate the effects of muscimol injections on the reach and the modifications in the trajectories that occurred during learning. The rate of task acquisition for the two experimental groups was not compared statistically because of the variability in learning rates among cats on this type of task and the difficulty in obtaining large numbers of animals willing to attempt the task while the interposed and dentate nuclei were inactivated. Furthermore, the learning rate per se was not a critical issue in our experiments. The Mann-Whitney U test was used to compare the maximum level of difficulty attained by the two groups of animals, and a standard t-test (CSS Statistica) was used to compare performance of control and muscimol-injected animals in the final retention test.

Histological procedures

At the end of experiments, the cats were anesthetized with barbiturate and injected with biocytin (100 nL of 5% biocytin in 0.05 M tris (hydroxymethyl)aminomethane buffer, pH 7.6) in all the nuclei at the same stereotaxic locations used previously for muscimol or saline injections. Then the animals were anesthetized deeply with an overdose of barbiturate and were perfused through the left ventricle with 2% paraformaldehyde + 1% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.4). Blocks through the cerebellum were cut at 50 μm in the frontal plane, biocytin was localized by staining with diaminobenzidine, and the tissue was counterstained with luxol blue and neutral red.

RESULTS

Task acquisition

All animals in which the interposed and dentate cerebellar nuclei were inactivated simultaneously (n = 4) could learn the template task at progressively greater levels of difficulty. Two of the four muscimol-injected animals implemented a movement strategy comparable with that of many functionally intact cats observed in other experiments using the template paradigm (Milak et al. 1995, 1997). This strategy, designated the “normal” strategy, is illustrated in Fig. 3, C and D. Similar qualitatively to the movement sequence employed by control animals (A and B), this strategy consisted of grabbing the bar with the paw on the right of the bar and pulling it through the initial template groove (the most difficult, critical part of the task).

All cats across both groups used the normal strategy during initial training on the straight groove. However, during the learning of the more difficult templates (those with 2 segments), two cats in the muscimol injection group employed “alternate” strategies that deviated from this pattern. One of the animals in the alternate group contacted the bar on the left and pushed it from left to right. This strategy was designated the pushing strategy. The trajectory of the IRED on the dorsum of the paw while performing this task is shown in Fig. 3, E and F. Notice that the paw passed to the left of the bar before the pushing was initiated. In this trial, the grip was lost and had to be reestablished at the end of the first segment. The other animal in the alternate group adopted the strategy of batting the bar energetically to propel it through the initial part of the template. This strategy, referred to as the batting strategy, allowed the cat to complete the movement rather quickly once it initiated the bar’s movement. As indicated in Fig. 3, G and H, this motion was cyclic, with the paw passing to the right of the bar and contacting it forcefully in successive cycles (2 shown) in an effort to propel it to the corner of the template. As evidenced by the characteristics of the reach trajectory, this animal was quite ataxic.

To obtain insights into the features of the movement that were modified during this acquisition period and to demonstrate that, in fact, such modifications occurred, specific features of the movement kinematics were evaluated as the task was practiced. One of the most difficult components of the
FIG. 4. Paw and bar speed profiles of a muscimol-injected and a control cat while moving the bar through the template. Both used a normal strategy. A–D: single trials illustrating typical differences in these profiles in one of the 1st trials (trial 27 in A and B and trial 3 in C and D) at $\alpha = 10^\circ$ and $\alpha = 0^\circ$ for the muscimol-injected and the control cat, respectively. Contact of the paw with the bar was frequently broken in the muscimol-injected cat (A), after which the animal had to regrab the bar. In contrast, the control cat maintained contact with the bar as it was moved through the template groove (C). E and H: single trials for the same 2 animals obtained late in the training period (trial 135 in E and F and trial 42 in G and H). Time 0 corresponds to the 1st contact of the paw with the manipulandum bar. Vertical dashed lines show the time the bar contacted the template corner ($a$) and the reward zone ($b$).

task was moving the bar from the origin to the corner through the medio-lateral segment of the template. The progressive improvement in this part of the task is illustrated in the paw and bar speed profiles in Fig. 4. As the muscimol-injected cat executed the task in an early trial during learning (A and B), the bar was moved in a discontinuous manner, with comparatively high peaks of the paw velocity profiles reflecting the paw movements required to regrasp the bar after intermittently losing contact with it. In some animals, the loss of bar contact appeared to be a habitual part of their behavior. Nevertheless, this tendency definitely was accentuated in animals with inactivated nuclei. Although it could not be documented, the prolonged duration of this part of the task also may reflect the substantial friction generated between the bar and the template when there was a large angle between the pulling direction on the bar and medio-lateral template segment (see Fig. 5, C and D). Notice the absence of the multiple peaks in the velocity profiles characterizing the performance of the same movement by a control cat (Fig. 4, C and D). The performance of both animals was improved substantially as the task was practiced. In the example shown in Fig. 4, after substantial practice, the muscimol-injected cat lost contact with the bar only once during the transverse movement through the template (E and F), resulting in a much shorter movement duration. Similarly, practice resulted in a decreased movement time through the template for the control animal (G and H).

The time course of the changes in measurements reflecting the movement through the template is shown in Fig. 5 for both a muscimol-injected and a control animal. As the animal with the cerebellar nuclei inactivated practiced this task (A), the movement time for the first segment decreased.
FIG. 5. Improvement of movement characteristics during the learning of the task: a comparison of data from one muscimol-injected cat (A, C, and E; \( \alpha = 10^\circ \)) and one control (saline-injected) cat (B, D, and F; \( \alpha = 0^\circ \)). Both used a normal strategy. A and B: time of bar movement through the initial template segment decreased as a function of trial number for both animals with the rate of decrease being slower for the muscimol-injected animal (A) than for the control (B). C and D: forearm laterality angle plotted against trial number. E and F: percentage of successful trials over successive blocks of 10 trials.

The tendency to lose the grip on the manipulandum bar during nuclear inactivation may have been a factor contributing to the development of different, alternative strategies for moving the manipulandum through the template. Interestingly, the batting strategy actually eliminated the necessity to grasp the bar. In fact, even the two animals employing the normal movement pattern used a technique for pulling the manipulandum that was different from that of the control animals. Rather than gripping the bar with the toe and paw, the muscimol-injected animals tended to “hook” the bar with their paw pad, often using a greater flexion of their wrist. Perhaps in an effort to make the hook more effective, the muscimol-injected animals tended to better align the elbow-wrist segment with the direction of the first segment of the template. These features were quantified by measuring this angle as the task was practiced. This was associated with a decreased movement time through the first segment (A) as well as an increase in the percent of successful trials (E). In contrast, the control animals did not undergo a comparable change in the forearm laterality angle (Fig. 5D) as the performance of the task improved. Apparently, the capacity to grasp the bar permitted these cats to maintain their forelimbs in a consistent relationship with the sagittal plane as the movement time through the first segment shortened and the percent of successful trials increased (Fig. 5, B and F).

The time courses of the learning exhibited by an inactivated animal using the normal strategy and a control cat are shown in Fig. 6, A and B, respectively. All inactivated animals exhibited ataxia immediately after the injection. Perhaps as a consequence of this deficit, the animals occasionally became less persistent immediately after the angle was decreased despite success in the previous series of trials.
This feature of the training likely contributed to the variability in the learning curves between animals in both groups (see Fig. 9). After 6 days, the cat trained during nuclear inactivation was performing the $\alpha = 0^\circ$ angle approximately at a 50% success rate, whereas the control animal could perform to criterion at $\alpha = -5^\circ$ after the same period of time (Fig. 6, A and B, respectively).

The time courses and extent of the acquisition for the cats using the pushing and batting strategies are shown in Fig. 7, A and B, respectively. The animal using the pushing strategy almost completed the learning at the highest level of task difficulty ($\alpha = -20^\circ$) with the cerebellum inactivated (Fig. 7A). By contrast, the cat employing the batting strategy mastered only the $-5^\circ$ angle. Because of the individual vari-

This is apparent in Fig. 6A (*), where there was a sudden decrease in percent successful trials. When this occurred, it was necessary to continue training after reducing task difficulty, as explained in METHODS. Even control cats occasionally became less persistent in attempting to solve the task and required a transient decrease in difficulty to maintain their motivation for mastering the template (Fig. 6B, *).

FIG. 6. Time course of learning and retraining for one cat in the muscimol injection group (A) and another cat in the control (saline injection) group (B). These learning and retraining curves are shown for the animal in each group that had the greatest difficulty using the normal (see text for explanation) movement strategy. *, block of trials in which this animal transiently lost its persistence in attempting to execute the next more difficult template. Ordinates on these learning curves as well as those in Fig. 7 are segmented into the percent of successful trials over 10 consecutive trials for each template angle in ascending order of difficulty. In this as well as the next figure, the 1st block of trials for each day of learning and retraining is indicated with a numbered dashed line. Note the difference in the scale on the ordinate.

FIG. 7. Learning curves for the cats that employed unusual strategies while learning the task under muscimol injection and subsequent retraining without injections. Learning and retraining curves are shown for the cat that was using the pushing strategy (A) and for the animal that utilized the batting movement strategy (B). *, block in which the strategy switched from the normal to the batting strategy. Other designations as in Fig. 3. Note difference in the scale on the ordinate. Additional explanation in text.
ability in the rates of learning, nothing specific can be concluded from the difference in learning rates between these two animals. The likelihood that these alternative strategies provided a substantial advantage to the cat for accomplishing the more difficult templates in the task is suggested by the finding that the animal employing the batting strategy was learning quite slowly until this strategy was initiated (Fig. 7B, *). Even though the animal began to learn faster, it still could not achieve the $-10^\circ$ template. In contrast, the cat that used the pushing strategy could almost master the $-20^\circ$ template. This level of achievement is consistent with the fact that the success of this strategy was not likely to be highly dependent on $\alpha$. Although criterion was achieved in some blocks, this level of performance could not be maintained consistently.

Figure 9 compares learning rates of all animals in both groups employing the normal strategy. Notice that both control and muscimol-injected animals have a considerable variation in learning rate when assessed on the basis of trials, particularly after the easiest templates are mastered. Furthermore, muscimol-injected animals executed more difficult templates during the retraining period when no injection was made (Fig. 8).

In summary, the data in Figs. 3–9 show that the cats definitely learned to perform the template task when trained while the dentate and interposed nuclei were inactivated. There was no statistical difference in the maximal level of difficulty achieved by the two groups of cats (Mann-Whitney $U$ test). Interestingly, two of the four muscimol-injected animals adopted an alternate strategy as they practiced the task. In both cases, the cats that employed such a strategy could master more difficult templates than the cats that continued to use the normal strategy.

Retention and retraining phase

The next component of the experiment, the retraining phase, evaluated the extent to which cats trained during muscimol inactivation of the nuclei retained what was learned when assessed on the same task in the absence of any injection, namely with the cerebellum functionally intact. Both groups of animals were retrained with the same sequence of templates used during the initial (learning) stage of the experiment. Surprisingly, there was a substantial difference between the retention displayed by the animals that learned the task with the cerebellum inactivated and that demonstrated by the control animals. Cats trained under saline injections attained the maximum level of difficulty as rapidly as the paradigm permitted, i.e., they immediately performed at criterion (90% of successful trials in the first 20 trials at a given $\alpha$ for each progressive step in task difficulty; Figs. 6B and 9B). By contrast, except for the cat that employed the batting strategy, the animals trained during nuclear inactivation could not perform all previously mastered templates at criterion in their first attempts to do so. Rather, it was necessary for them to practice at least the more difficult, previously acquired templates before being capable of executing them at criterion level (Figs. 6A, 7A, and 9B). During the retraining phase, all animals employed the strategy they adopted during the learning phase.

The characteristics of relearning (learning rate, highest level of task difficulty acquired) in each of these three muscimol-injected cats (the 2 using the normal strategy and the 1 using the pushing strategy) were somewhat different from during their original acquisition. There was a tendency to reacquire faster and attain a higher level of difficulty during reacquisition (Figs. 6A and 9B). The plot in Fig. 9B also indicates that, as observed during the initial period of learning, there was a substantial variability in the retraining curves among the animals employing the normal strategy. Only the cat that used the batting strategy performed at criterion level at each successive increase in template difficulty, i.e., this cat did not require relearning to execute the task successfully (Fig. 7B).

Final retention test

The final part of the experiment assessed the effects of nuclear inactivation on the retention of the task after overtraining in the absence of any injections. This was done by determining the consequence of inactivating the dentate and interposed nuclei on the capacity to perform the most difficult template learned during overtraining. After muscimol inactivation (including 20-min wait period), all cats could execute the template within a very few trials (Fig. 10, A and
B). However, immediately after muscimol injection, a brief period occurred during which there was a decrease in the percent successful trials. This phenomenon, which also was observed even after injection of only a single cerebellar nucleus (Milak et al. 1997), may reflect a brief period required for the cat to compensate somewhat for the rapidly acquired cerebellar deficit. This inference also is supported by the findings in Fig. 10, showing that, when the trial length was increased to 5 s (C and D), presumably allowing more time for the ataxic movements to be executed, criterion performance was reached by both groups after fewer trials than when the trial duration was 3 s. Although the difference in the performance of the two groups after muscimol was not statistically significant (P > 0.05, standard t-test between the blocks for trials 1–30 and between the blocks for trials 31–60), the animals that previously had not experienced a muscimol injection (Fig. 10B) on average took more trials to return to criterion performance than the muscimol group.
complex goal-directed volitional movement: 1) cats can learn this task while the interposed and dentate nuclei are inactivated; 2) motor strategies used to execute a task learned during nuclear inactivation are more variable, both within one animal’s performance and across different animals, than those employed when the task is learned with the nuclei functionally intact; 3) most animals that learn the task during nuclear inactivation cannot perform it at criterion levels when tested with the cerebellum intact; and 4) animals that learn the task without any impairment of cerebellar function still can execute the behavior after the cerebellar nuclei are inactivated.

One of the main findings from this experiment is that the functional integrity of the intermediate and lateral cerebellum is not critical for the learning of an operantly conditioned goal-directed forelimb movement. It is highly unlikely that the animals could learn the task because of the incomplete inactivation of the targeted cerebellar nuclei. First, the results of the histological analysis showed that the injections were well localized in all studied animals. Second, the motor deficit observed after muscimol injections was quite substantial and consistent with that described in the literature after lesions in these structures (Gilman et al. 1976). In addition to the well-known ataxia, there was a clear deficit in postural reflexes and reactions, including the forelimb precision placement response (Bloedel and Bracha 1995). Third, the onset of these effects was rapid, suggesting the injections were made in very close proximity to the target nuclei (Kolb et al. 1996). Fourth, a substantial enough portion of the nuclei was injected to produce a condition in which a behavior learned during muscimol inactivation could not be executed in the absence of nuclear inactivation and consequently had to be relearned during the retraining phase.

The inactivation of the cerebellar nuclei did not eliminate the animals’ capacity to modify the movement strategy as the task was practiced. As shown in Fig. 5, there were specific changes in movement kinematics as the muscimol-injected animals acquired the behavior. Because they could not secure a grasp of the manipulandum bar with the distal extremity in a normal manner, they hooked the bar with their paw near the wrist (see also Gibson et al. 1994) together with an associated adjustment of the elbow-wrist segment position (see Fig. 5C and the related part in RESULTS). Comparable changes in the same kinematic measurements did not occur in the control animals (Fig. 5, B, D, and F). With the cerebellum inactivated, the movement strategy employed was less coordinated and less successful, requiring the modification of movement kinematics for improving performance. In fact, the two muscimol-injected animals that used the normal strategy during learning failed to attain as high a level of task difficulty as the control animals. The other two muscimol-injected cats actually modified their movement strategy substantially, presumably to overcome the problems related to the motor abnormalities resulting from the extensive cerebellar dysfunction produced by nuclear inactivation. In support of this notion, both the batting strategy and the pushing strategy could be selected by the animal to adapt for...
the motor deficit produced by cerebellar nuclear inactivation, possibly because neither require the complex, precise reach-to-grasp movement employed under normal conditions.

Muscimol-injected cats that used the normal strategy when learning the task achieved a lower degree of task difficulty than the control animals (Figs. 8A and 6–9). Furthermore, during retraining, cats that acquired the task initially during muscimol injections could learn a more difficult template after practicing in the absence of any injection (Fig. 3D and 6–9). However, nuclear inactivation after the retraining period did not suppress the animals’ capacity to perform the template at the same level of difficulty achieved in the absence of muscimol microinjections. Together these facts could be interpreted in support of a learning impairment affecting acquisition of the most difficult templates during cerebellar nuclear inactivation. However, cats that implemented the alternative strategies (batting and pushing) under this condition could learn the more difficult templates (Fig. 8). Consequently, the difference between the difficulty of the task acquired during the initial learning period by the control animals and the muscimol-injected cats that used the normal strategy is more likely due to a motor impairment (performance deficit) of the nuclear-inactivated cats than an impairment in the learning process per se. We propose that, in the naive animal, the prehension deficit together with the ataxia impeded the performance of the more difficult angles and consequently the animal could not learn to execute them within the time limits imposed by the experimental paradigm.

In summary, these findings clearly show that the strategies used to compensate for the loss of cerebellar function could be modified through practice, consisted of a pattern of motor coordination that does not require the normal reach to grasp, and could be executed adequately enough to accomplish the objectives of this difficult motor task.

Phenomenon of relearning

One of the most interesting observations in this study was that three of the four animals that learned the task with the nuclei inactivated had to relearn the task when required to perform it without any injection. Although the mechanisms underlying this relearning phenomenon are not yet known, it provides an important stimulus for considering new concepts related to the cerebellum’s role in the acquisition of goal-directed volitional arm movements. One possibility is that the availability of the cerebellum virtually disables the implementation and/or recall of the previously learned strategy. This seems unlikely because there is no evidence that such a mechanism exists, and it is inconsistent with the fact that one of the animals employing an alternate strategy did not have to relearn. Alternately, we favor a view based on our hypothesis that the cerebellum normally plays a key role in strategy specification during the acquisition of goal-directed volitional arm movements (Bloedel et al. 1996, 1997). According to this view, the relearning observed in these experiments may have occurred to implement a new, cerebellar-dependent algorithm capable of improving the motor pattern with which the task is performed. This proposal is consistent with the behavioral observation that relearning occurred in those cats having an original strategy that was dependent on employing a substitute for the normal grasp and was more variable and appeared less efficient than the strategy employed by a functionally intact cat. Of the muscimol-injected cats, only the one that employed the batting strategy did not have to relearn after muscimol was discontinued. Unlike the other strategies, this one did not require a grasp or a high level of movement accuracy, thus likely reducing its dependence on the cerebellum. Furthermore, the batting strategy consisted of a fairly simple, natural behavior capable of producing a comparatively efficient execution of the task, possibly eliminating an important condition for initiating the relearning.

The relearning phenomenon also emphasizes that the cerebellum could play an important role in the normal acquisition process even though some motor patterns that can be used to execute the task can be acquired in the absence of this structure. The importance of the cerebellum in acquisition also is supported by the recent results of Milak et al. (1995), who investigated the changes in cerebellar nuclear activity during the acquisition and performance of a variant of the template task. They showed that the event-related modulation of ~80% of these cells progressively increased as the task was practiced, reaching a maximum at the time during acquisition when the task was first performed smoothly. Response amplitudes then progressively decreased as the task continued to be practiced. Related observations also have been reported in other studies. For example, a transient increase in the complex spike activity of Purkinje cells was shown to occur as monkeys compensated either for an external load applied during a simple wrist movement (Gilbert and Thach 1977) or for changes in the gain of the manipulandum employed to perform visually guided arm movements (Ojakangas and Ebner 1992). In addition to these electrophysiological data, imaging studies revealed that the highest level of activation within the cerebellum occurs in association with the learning of a new task (Flament et al. 1996; Friston et al. 1992; Jueptner et al. 1997; Seitz et al. 1994). The specific process associated with these transient changes during task acquisition has not yet been clarified. Nevertheless, these data together with the findings from the present experiments point toward a contribution of the cerebellum to acquisition during the period in which its task-related modulation is increased.

The precise process that is reflected by this change in modulation is still unknown. One possibility is that the cerebellum’s output is used by the motor control system to establish an internal representation of the motor plant or specific features of the movement per se as a motor behavior is learned. The argument that such internal representations are integral to motor control is consistent with many findings in the literature (Andersen et al. 1993; Baev et al. 1991; Flanagan and Wing 1997; Gerdes and Happee 1994; Shimansky 1991; Soechting and Flanders 1992; Wolpert et al. 1995). It is possible that the cerebellum contributes to the establishment of optimal internal representations during the learning of voluntary movements because of its importance in the on-line processing of sensory information during movement execution (Bloedel et al. 1996, 1997; Shimansky et al. 1995). If so, an incomplete internal model of the controlled object would be established when a new task is learned during cerebellar nuclear inactivation because the task would
have to be learned through integrative processes that could not employ the information processing normally performed by the intact cerebellum. When the cerebellum becomes available and retraining occurs, an upgraded, appreciably more effective internal model would be formulated through practice, thus accounting for the relearning observed during this phase of the experiment. Stated differently, the relearning phenomenon could reflect the correction of an incomplete internal representation generated while the cerebellar nuclei were inactivated.

One proposal consistent with this view and with the findings of this experiment is that the learning of a motor pattern involves the establishment of an internal model of the controlled object consisting in part of the internal representation of the dynamics of the limbs’ musculo-skeletal system (Baev and Shimansky 1992). After the internal model is formed as the task is learned, this model can serve as the basis for controlling the task’s performance when the cerebellum is dysfunctional as long as the internal model remains valid for the desired movement. This hypothesis is also in good agreement with the transient changes in cerebellar neuronal modulator observed during motor learning (Gilbert and Thach 1977; Milak et al. 1995; Ojakangas and Ebner 1992) because the involvement of the cerebellum would be expected to be much less once the internal model is formed. The idea that the cerebellum’s output is included in the process of establishing the internal representation also is very consistent with the observation that muscimol inactivation of this structure’s output after retraining did not block the performance of the task. Only if the conditions governing the movement or its objectives changed would the internal model become inadequate, and only if the processing of rapid on-line perturbations were required would the internal representation be insufficient. Although this hypothesis is speculative, it is consistent with all of the findings in the experiment.

**Is the cerebellum required for engram storage?**

Two findings in this study strongly challenge the hypothesis that the learning of volitional, goal-directed movements is dependent on the establishment of essential engrams within the cerebellum. First, the template task could be learned during the inactivation of both the interposed and dentate nuclei. Second, and equally important, the task still could be executed when the same inactivation was produced after the behavior was learned with a functionally intact cerebellum. These observations, including the relearning phenomenon, do not exclude the possibility that some plasticity related to learning the optimal strategy for performing the task resides within the cerebellum. For example, it is possible that relearning employs newly available storage sites within the cerebellum that were not accessible during nuclear inactivation. However, in our view, if such storage sites are implemented, they are not essential sites of plasticity because the task learned with the cerebellum intact also can be executed during nuclear inactivation. Consequently, the relearning process very likely involves the cerebellum in a manner comparable with that occurring when the same task is learned in the normal, naive animal. When the cerebellar output becomes available to the rest of the brain, the motor control system, having learned to perform this task without this structure, reestabishes a new control algorithm derived by incorporating the cerebellum into the sensorimotor integration related to movement generation and modification. Through these interactions, the essential plastic changes related to the storage of the internal representation of the movement would be established outside the cerebellum.

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Address for reprint requests: J. R. Bloedel, Division of Neurobiology, Barrow Neurological Institute, 350 W. Thomas Rd., Phoenix, Arizona 85013. Received 24 July 1997; accepted in final form 14 January 1998.

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