Effects of Inactivating Individual Cerebellar Nuclei on the Performance and Retention of an Operantly Conditioned Forelimb Movement

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Effects of inactivating individual cerebellar nuclei on the performance and retention of an operantly conditioned forelimb movement. J. Neurophysiol. 78: 939–959, 1997. These experiments were designed to examine the effects of inactivating separately each of the major cerebellar nuclear regions in cats on the execution and retention of a previously learned, operantly conditioned volitional forelimb movement. The experiments test the postulates that the cerebellar nuclei, and particularly the interposed nuclei, contribute substantially to the spatial and temporal features of the interjoint coordination required to execute the task and that the engram necessary for the retention of this task is not located in any one of the cerebellar nuclei. All cats were trained to perform a task in which they were required to reach for and grasp a vertical bar at the sound of a tone and move the bar to a reward zone through a template consisting of two straight grooves in the shape of an inverted “L.” After the task was learned, the effects of inactivating separately each nuclear region (the fastigial, interposed, and dentate nuclei) using muscimol microinjections were determined. Data were analyzed by quantifying several features of the movement’s kinematics and by determining changes in the organization of the reaching component of the movement using an application of dimensionality analysis, an analysis that examines the correlation among the changes in joint angles and limb segment positions during the task. The retention of the previously learned task also was assessed after each injection. Injections of each nuclear region affected temporal and spatial features of the learned movement. However, the largest effects resulted from inactivating the interposed nuclei. These effects included an increased length of the reach trajectory, an accentuated deviation of the wrist trajectory from a straight line, cyclic movement of the distal extremity as the target was approached, a difficulty in grasping the bar, altered temporal features of the movement, and a highly characteristic change in the dimensionality measurements. The changes in dimensionality reflected a decreased correlation (linear independence) of the joint angular velocities coupled with an increased correlation among the linear velocities of markers located on the joints themselves. Related but less consistent changes in dimensionality resulted from fastigial injections. The motor sequence required to negotiate the template could be executed after the nuclear microinjections, indicating that retention of the motor sequence was not affected by the inactivation of any of the cerebellar nuclei. However, in two of the five animals, some decreases in performance were observed after dentate injection that were not characteristic of changes related to an effect on retention. These data suggest that the cerebellum plays an important role in regulating the consistent, stereotypic organization of complex goal-directed movements, including the temporal correlation among joint angle velocities. The data also indicate that the retention of the task is not dependent on any of the individual cerebellar nuclear regions. Consequently, these structures are unlikely to be critical storage sites for the engram established during the learning of this task.

INTRODUCTION

The cerebellum is known to play a significant role in generating the coordinated performance of complex movements. Interestingly, the relationship between the structural compartmentalization of the cerebellum into sagittal zones and the regulation of posture and movement is not well understood. Initially, three sagittal zones were described (Jansen and Brodal 1940) in which separate regions of the cerebellar cortex were associated with the three primary cerebellar output nuclei: the fastigial nucleus, interposed nuclei, and dentate nucleus. To obtain insights into the functional basis for these zones, Chambers and Sprague (1955a,b) performed selective electrolytic lesions in each nuclear region and determined the classes of motor behaviors most affected when the output from one of the zones is eliminated or substantially reduced. One of the many merits of these studies was the fact that the deficits were described over a wide range of motor behaviors including a variety of reflexes, volitional movements, and postural support. A comparable series of studies employing permanent lesions of each specific cerebellar nucleus was not performed in primates. Nevertheless, insights regarding the distinction between neocerebellar, paleocerebellar, and vestibulocerebellar functions were obtained in primates based on less selective ablations of the cerebellar nuclei and ablations of the cerebellar efferent projections coursing in the brachium conjunctivum (Bottrell and Fulton 1938a,b; Carrea and Mettler 1947; Carrea and Mettler 1955; see Bloedel 1992; Gilman et al. 1981 for review). Although these studies in both cats and primates provided key insights into cerebellar nuclear ablation syndromes and certain aspects of cerebellar function, the variability in the studied behaviors made it difficult to compare quantitatively the effects of lesions in different cerebellar nuclei. Furthermore, because the lesions were chronic, it was impossible to differentiate between the effects of the lesions themselves and the consequences of the compensatory changes occurring as the animal recovered (see Bloedel and Bracha 1995 for discussion).

Using a different approach, Brooks and colleagues compared the function of the interposed and dentate nuclei by inactivating these structures temporarily using a reversible cooling probe (Brooks et al. 1973; Uno et al. 1973). A distinct difference in the abnormalities resulting from cooling in the vicinity of each of these two structures during the
performance of a single joint movement was demonstrated. Using a tracking task in which a monkey was required to move a manipulandum between two alternate targets, hold it within a given target zone for a minimum period of time, and then move it to the alternate one, cooling the dentate nucleus produced multiple changes in the features of the movement (Brooks et al. 1973). During cooling, markedly dysmetric movements were performed with velocity profiles ranging from those with a single peak to those with several peaks. Peak velocity of smooth movements during both flexion and extension was greater during cooling than during control periods. Changes also occurred in the time during the movement at which deceleration was initiated as the targets were approached. The task’s performance also became more dependent on the availability of additional external cues signaling when the manipulandum was within the target zone, suggesting that when the dentate nucleus is dysfunctional, eye/hand coordination alone is not sufficient for performing and controlling this movement. These data are consistent with those of Vercher and Gauthier (1988) in which permanent lesions of the dentate nucleus decreased the coupling of coordinated eye/hand movements. In contrast to the effects of cooling the dentate nucleus, cooling the interposed nuclei during an alternating tracking task resulted in hypometric movements with lower peak velocities (Uno et al. 1973).

Interestingly, the changes in forelimb movements produced by cooling transiently the dentate and interposed nuclei are different from those observed by Goldberger and Growdon (1973) after permanent lesions of these structures. In this study, monkeys were required to reach for and track the position of a piece of food on the end of a stick and, in another task, to grasp and release a rod held in different positions. In contrast to the studies employing temporary lesions reviewed above, these authors reported that interposed nuclear lesions resulted in the performance of very marked hypermetric movements involving the proximal musculature. In contrast, dentate lesions produced abnormalities primarily restricted to the distal extremities. Alterations in movement velocity were not described. Although the differences between these findings and those of Brooks and colleagues may be the consequence of employing chronic lesions, the fact that the lesions in the Goldberger and Growdon study sometimes included more than one nucleus also may have contributed.

More recently Thach and colleagues (as reviewed in Thach et al. 1992; see also Goodkin et al. 1993) performed a critical series of experiments in which the effects of selective inactivation of the three major cerebellar nuclear groups was observed in two very different classes of movements—simple, predominantly single-joint movements and more complex behaviors involving a combination of body support and limb movements requiring proximal to distal coordination of the extremity. These investigators demonstrated that even though these temporary lesions produced only minimal effects on the simple behaviors, their effects on the more complex behaviors were profound. Fastigial lesions produced modifications in posture and gait; interposed lesions resulted in the appearance of a severe 3- to 5-Hz tremor during reaching movements, and dentate inactivation produced abnormal angulation of shoulder and elbow joints during reaching as well as abnormalities in employing the digits. These experiments were the first to use temporary, selective inactivation of the output of each of the three major cerebellar sagittal zones to support the argument that the contribution of each zone to the organization and performance of complex movements may be uniquely different. These studies also emphasized the importance of implementing complex tasks in assessing cerebellar nuclear function.

The purpose of the experiments reported here was twofold: to provide one of the first quantitative comparisons of the effects produced by inactivating selectively fastigial, interposed, and dentate nuclei on a single complex, operantly conditioned forelimb behavior in cats and to examine the effects of selective nuclear inactivation on the retention of a motor sequence required to execute a complex volitional movement. The task required the execution of a reaching movement to a vertical bar with the grasp of the manipulandum and a subsequent movement through a two-segment template to a target zone. This motor sequence was selected because it is fairly complex and yet is quantifiable both temporally and spatially.

The experiments first examined the effects of selectively inactivating one cerebellar nuclear region with muscimol on the temporal and spatial features of this behavioral sequence. Next, retention was evaluated by assessing the capacity of cats to execute the previously learned motor sequence required to negotiate the template after the microinjection of muscimol into each individual cerebellar nuclear region. Based primarily on studies of the substrates underlying the acquisition and retention of the classically conditioned eyeblink reflex and adaptation of the vestibuloocular reflex, the cerebellar cortex and/or nuclei have been proposed as sites of the plastic changes required for storing the memory traces underlying these behaviors (see Du Lac et al. 1995; Raymond et al. 1996; Thompson and Krupa 1994 for recent reviews of these data). Interestingly, the cerebellar storage hypothesis derived from these data has been extrapolated to volitional limb movements (Deuschl et al. 1996; Fiez et al. 1992; Glickstein 1992; Leiner et al. 1993; Raymond et al. 1996; Thach et al. 1992). Although this view already has been expressed in general terms in textbooks addressing cerebellar function (Kandel et al. 1996), there are very few experimental data directly supporting this concept. The several studies examining the learning of complex behaviors by cerebellar patients (Bracke-Tolkmitt et al. 1989; Canavan et al. 1994; Daum and Ackermann 1995; Deuschl et al. 1996; Seitz et al. 1994 for review and examples) are not applicable, because these experiments almost exclusively address the issue of acquisition rather than retention. The experiments reported here provide observations directly related to the issue of retention using the template task introduced above.

Preliminary results were reported previously in abstract form (Milak et al. 1992).

METHODS

Pretraining

Experiments were performed using food-motivated cats that were trained to reach for a vertical manipulandum bar and move
Surgical procedures and retraining

After careful insertion of the injecting cannula to the predetermined depth of the spine over the scapula. The three-dimensional positions of these IREDs were recorded during the movement at a frequency of 100 Hz.

Microinjection procedures

Once the retraining was complete, the optimal site for injecting muscimol into each nucleus was determined along the path of each guide tube. This was done by assessing the effect of muscimol microinjections at successive 0.5-mm steps along each of the four tracks. All injections were made at a rate of 0.1 µl/min using a solution consisting of 800 ng of muscimol in 1 µl of buffered, pH-controlled saline. The microinjection system consisted of a 33-gauge, stainless steel, hypodermic tube connected to a Hamilton syringe by a calibrated transparent plastic tube used to control the amount of muscimol solution injected. The optimal injection site was determined based on two criteria. First, the effect had to be clinically apparent based on an assessment of posture, reflex changes, and reaching behavior, including changes in kinematics of the reaching behavior apparent in the on-line readout of the Optotrak data. Second, the onset time for the effect had to be almost immediate, reaching near peak effect in only a few minutes. Because the effects of injecting the interposed nuclear region were quite dramatic, the location of the injection site for this region was determined first and used as a guide for approximating the depths of the other injection sites, a procedure that reduced the total number of injections required in each animal.

Once the optimal injection site was found for each of the three nuclear regions, the effects of injecting 1 µl of the muscimol solution and, on a different day, 1 µl of the saline vehicle were compared. Injections of each nucleus were performed on successive days. The saline injections never were administered in a given nucleus on the day immediately after a muscimol injection in the same nucleus. To rule out any permanent effects after the injections, all animals were evaluated clinically for the persistence of any cerebellar deficits after the muscimol and saline injections. This complication never occurred.

Experimental protocol

After careful insertion of the injecting cannula to the predetermined depth (i.e., the depth at which the maximal effects were observed), a minimum of 50 preinjection trials were run during which the movement times, the EMG recordings, and the cinematics of the reaching behavior apparent in the on-line readout of the Optotrak data. Second, the onset time for the effect had to be almost immediate, reaching near peak effect in only a few minutes. Because the effects of injecting the interposed nuclear region were quite dramatic, the location of the injection site for this region was determined first and used as a guide for approximating the depths of the other injection sites, a procedure that reduced the total number of injections required in each animal.

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Surgical procedures procedure and retraining

The implantation procedure was conducted while the animals were anesthetized fully with isoflurane under aseptic surgical conditions. Stainless steel 26-gauge guide tubes were inserted stereotaxically to a point 2 mm above each of the following cerebellar nuclei: fastigial, posterior interposed, anterior interposed, and dentate nuclei ipsilateral to the trained forelimb. After being inserted into a small craniectomy site over the ipsilateral cerebellum, the guide tubes were secured in place using dental cement. Next, bipolar electrodes consisting of multistranded, Teflon-coated, stainless steel wires were implanted into several forelimb muscles to obtain data to be reported in a subsequent publication. The wires were led under the skin to a multipin connector, which also was cemented into the glue cap on top of the skull. The guide tubes were covered by a removable cap system that could be attached to a chamber base also implanted into the cement cap.

One week after recovery from surgery, animals were retrained on the task. Retraining continued until the animal had completed all of the movements in three trials listed above achieved presurgical values. In addition to monitoring movement times, the kinematics of the performing limb’s movement was analyzed for each trial. For this purpose, Velcro pads were pasted over several key skeletal landmarks on the ipsilateral performing forelimb to attach the infrared emitting diodes (IREDs) for use with an Optotak (Northern Digital) system, as described below. IREDs were located over the paw dorsum, just lateral to the fifth metacarpal bone, the wrist, the elbow, the shoulder, and...
Data analysis

Experiments were performed using eight animals. Completely analyzed data sets were obtained from five cats for all nuclei except the fastigial nucleus for which four complete data sets were acquired. For each trial, several temporal features of the movement defined above (response time, reach duration, movement duration through the first and second segments of the template) and overall movement duration (the time from paw liftoff to arrival in the target zone of the template) were calculated and tabulated. The kinematic characteristics of the forelimb’s movement were determined from the recording of the IRED positions. In addition to examining qualitatively the changes in the trajectory of the limb from stick figures derived from the changes in IRED locations in three dimensions over the time course of each movement, the peak velocity and peak acceleration of the reach and trace components of the movement (reach, first segment of the template, second segment of the template) were calculated. Acceleration was calculated as the first derivative of the velocity. For the interposed injections, peak velocity and acceleration during the reach were calculated for the initial component ending just before the onset of the terminal ataxia as well as for the entire reach. Additional measurements included: grasp height, the height of the paw when grasping the manipulandum measured relative to the paw’s position before reach onset; trajectory length for the reach and trace components of the movement, the overall length of the path taken by the wrist during each of these components of the task (for the reach this measurement included all ataxic movements before bar contact); reach elevation, the maximum height of the trajectory measured from the point of paw lift-off; and trajectory deviation, the maximum deviation of the paw from a straight line connecting the points of paw lift-off and bar contact.

The data analysis also required the measurement of several joint angles in the reaching limb. Four planes were used to define the wrist angles: plane PTW was determined by the three points joining the IREDS on the paw dorsum, toe and wrist; plane Q passes through the wrist and elbow IREDS and is perpendicular to the PTW plane; plane P goes through the same diodes and is perpendicular to the Q plane; plane WES is determined by the diodes on the wrist, elbow, and shoulder. Wrist supination is defined as the angle between planes WES and P. It increases as the wrist supinates; wrist extension is measured as the angle between plane P and plane PTW. Wrist radial deviation is the angle between the plane Q and the wrist-paw segment. It increases with wrist radial deviation, with “zero” corresponding to a position at which there is considerable radial deviation of the wrist due to the locations of the wrist and paw IREDS. Elbow flexion is the angle between the elbow-shoulder and the elbow-wrist segments. It is equal to 180° when the elbow is fully extended and decreases with flexion.

The definition of shoulder joint angles requires the specification of another plane, this one determined by the IRED locations on the elbow, shoulder, and scapular region (plane ESB). Shoulder lateral rotation is the angle between plane ESB and plane WES. It increases when the shoulder rotates inward. Shoulder abduction is measured between the vertical axis and the plane WES. It is zero when the forelimb is down, and increases when it is moving away from the body. Shoulder flexion is defined as the angle between the shoulder-body and the shoulder-elbow segments. It is zero when the shoulder is flexed fully and increases when the forelimb moves forward.

These data were analyzed statistically in a manner that permitted a comparison of the effects of muscimol and saline microinjections in each nucleus across all of the animals in the study. For all dependent variables, the data from each experiment were normalized by expressing the value of each pre- and postinjection measurement as a percentage of the average of the preinjection values.

This normalization made it possible to retain the variance in all pre- and postinjection measurements while making it possible to compare the data based on the percent change in the measurement resulting from the manipulation. To statistically evaluate the findings, changes in each variable were assessed using an analysis of variance (ANOVA) based on a three-factor design. Factor 1 (nuclei) had three levels (fastigial, interposed, and dentate), factor 2 (treatment) had two levels (muscimol and saline), and factor 3 (observation period) had two levels (pre- and postinjection). Post-hoc analysis comparing the pre- and postinjection changes as well as postinjection changes produced by the two treatments was based on the Neumann-Keuls test. Although all P values are reported as calculated, differences were considered to be statistically significant if P < 0.01.

Finally, a method based on principal component analysis was employed to assess the effects of inactivating individual cerebellar nuclei on the kinematics characterizing the forelimb movement during this task. This technique calculates a continuous estimate of the degree of independence among changes in related kinematic parameters during the reaching phase of the behavior (e.g., angular velocities of different joints or the velocity of the different segments of the limb).

Mathematically, the extent of linear dependence among waveforms can be estimated as the dimensionality of the waveform set viewed as a set of vectors (Glaser and Ruchkin 1976). For example, if among n input waveforms, m are mutually orthogonal, and the others are linearly dependent on them, then the actual dimensionality of the input set is equal to m. This number can be calculated as the number of nonzero eigenvalues of the corresponding cross-correlational matrix. Because for waveforms obtained experimentally the relationships of orthogonality and linear dependence usually are only approximate, the significant dimensionality of a waveform set is calculated based on principal component analysis. It usually is determined as the number of significant principal components corresponding to the largest eigenvalues whose sum is equal to 90–95% of the total sum of all eigenvalues (Glaser and Ruchkin 1976). Assessing dimensionality in this way with no additional features to the analysis has at least two disadvantages. First, only integer values are provided, making it very difficult to detect and quantify small, graded changes in dimensionality. Second, the results of the analysis can be subjective because of the difficulty in defining a meaningful threshold for significance. These problems can lead to incorrect estimates of dimensionality across a group of waveforms (see APPENDIX).

To make the estimate of dimensionality (D) continuous and more precise, we modified its definition, defining it as

\[ D = \sum_{i=1}^{n} \min (\lambda_i, 1) \]

where \( \lambda_1, \lambda_2, \ldots, \lambda_n \) are the eigenvalues of the cross-correlational matrix. This estimate can be called the continuous significant dimensionality (CSD) of a given set of waveforms. It is shown in the APPENDIX that this calculation results in a graded, continuous set of values for CSD ranging from 1 to the maximum number of possible principal components that could characterize a specific set of waveforms. Stated differently, CSD is equal to a real number representing the extent of mutual linear independence of the waveforms in the whole set even if there is no orthogonality among them. Using this convention, CSD = 1 exists for a condition in which all the waveforms of the set are the same, and CSD equals the total number of waveforms when they are all orthogonal to each other.

In this paper, the CSD estimate is described for two sets of continuous waveforms obtained from the kinematic analysis of the forelimb movement. The first set consists of the profiles of angular velocities at the shoulder, elbow, and wrist joints. The second set
includes the profiles of linear velocities of the locations marked by the infrared diodes attached to specific locations on the animal’s forelimb. Both waveform sets correspond to the reach component of the forelimb trajectory. The CSD estimate for these sets was designated the angular velocity dimensionality (AVD) and the linear velocity dimensionality (LVD), respectively. These estimates were calculated for each trial separately.

Histological analysis

At the conclusion of each experiment, animals were given a lethal dose of barbiturate and perfused transcardially with saline followed by a 10% formalin solution. Microinjection sites were labeled premortem by injecting a marker dye at the location in each tract at which the maximum effect of the muscimol was obtained (the site at which the control saline injections also were made). At different stages of the experiment, different markers were employed. In the first studies, colloidal gold was injected. In subsequent animals, biocytin was injected. After the injection of the marker, the cerebellum was removed and placed in a 10% formalin, 30% sucrose solution for 3 days. Using a freezing microtome, 40-μm sections then were made through the extent of the cerebellum. Tissue was stained using luxol blue and neutral red. Biocytin injection sites were labeled using the method employed by Helm et al. (1993), and the injected colloidal gold was visualized using the silver intensification method of Menetry (1985). Microinjection sites were reconstructed based on the location of the dye injections and the tracks made by the injecting cannulae.

R E S U L T S

For the purposes of conveying an understanding of the effects produced by microinjections of muscimol in the fastigial, interposed and dentate nuclei, specific alterations of the movement kinematics will be illustrated from representative data both for the reach as well as for the movement through the template. The group data characterizing the findings from all of the animals in the study will compare statistically the findings resulting from saline and muscimol injections in the same cats.

Effects of nuclear inactivation on movement kinematics

SPATIAL FEATURES OF THE REACH PHASE. The effects of microinjecting 800 ng of muscimol in 1 μl of saline into each of the three different nuclear regions on the paw’s movement from the time it leaves the platform to the time it contacts the vertical bar on the manipulandum is shown from the side view in Fig. 1 and from the top view in Fig. 2. All plots in these and subsequent figures illustrating kinematic features of the movement were selected in an effort to show typical changes in the performance of the template task produced by the nuclear inactivations. The plots in A–C and D–G in Figs. 1 and 2 were obtained before and after the microinjections, respectively. Before injection, reach trajectories often were reasonably straight with some deviations from a straight line occurring as the manipulandum was approached. The control reach of one animal is shown in Figs. 1A and 2A, and that of another cat is shown on two different days in Figs. 1, B and C, and Figs. 2, B and C.

The microinjection of muscimol into the fastigial nucleus had only minimal effects on the spatial features of the reach. As seen in Figs. 1 and 2, A and D, the trajectory of the paw became more curved, an effect best seen in the top view (Fig. 2D). In contrast, microinjections in the interposed region produced a striking effect on the trajectory of the reach. First of all, there was always a substantial ataxia as the animal’s paw approached the bar (Figs. 1 and 2E). This ataxia was characterized by a succession of circular movements that ceased only when the bar was grasped. In addition, there was a notable increase in the variability of the paw’s position just before initiating the reach. Notice the spread in the starting positions of the reach both from the side view (Fig. 1F, dashed oval) and the top view (Fig. 2F). Observations between trials (between paw touch down and the initiation of the next reach) revealed that this variability is likely due to the inability of the cat to replace the paw effectively on the surface of the stand after moving the manipulandum through the template. In addition, there was an increase in the variability of the paw’s reach trajectory, best seen in Fig. 2F, which illustrates the trajectory without the terminal ataxia included. Inactivation of the dentate region had comparatively little effect on the spatial features of the reach. Figures 1 and 2, C and G, illustrate the similarity between the pre- and postinjection trajectories. As described in METHODS, measurements characterizing
reaching movement (Fig. 3, A and D). Similarly, there was very little change in the relationship of more proximal joints, as seen in the plots of the shoulder and elbow joint angles (Fig. 3, B and E). Furthermore, the relationship between two rotational angles, wrist supination-pronation and shoulder rotation, did not show a clear change (Fig. 3, C and F). The effects of microinjections in the dentate nucleus on the same angle/angle relationships (Fig. 3, P–U) also were quite unremarkable, producing only modest changes in the relationship of the shoulder and elbow joint angles (Fig. 3, Q and T).

In contrast, microinjections of muscimol in the interposed nuclear region produced two specific modifications in these angle/angle relationships. First, comparison of all pre- and postinjection plots reveals that the inactivation of this region increased substantially the variability of these relationships. (Compare Fig. 3, G with J and M, H with K and N, and I with L and O.) Notice that this variability was particularly dramatic during the terminal ataxia (Fig. 3, J–L). Second, the relationship between the two rotational angles, wrist supination-pronation and shoulder rotation, became very erratic (compare Fig. 3, I with L and O). This was the most dramatic change in a specific angle/angle relationship seen in these experiments.

To summarize, the most dramatic effects on the reach in this experiment were produced by inactivating the interposed nuclear region. The overall trajectory length was increased substantially, likely reflecting the dramatic dysmetria all cats displayed in the terminal stage of the reach as the target was approached. However, this measurement also increased for the component of the reach prior to the onset of the terminal dysmetria. In addition, the inactivation of this nuclear region produced a substantial increase in the deviation of the paw’s path from a straight line and increased the elevation of the trajectory. Finally, there were specific changes in the angle/angle relationships observed after muscimol inactivation.

As shown in Fig. 5, the effects of fastigial and dentate inactivation on spatial features of the reach were appreciably smaller than those produced by interposed inactivation. Nevertheless, some changes were statistically significant. These included a decrease in trajectory length and grasp height after muscimol injections in the dentate nucleus and an increase in grasp height after fastigial injections. Also in contrast to the effects of interposed injections, quantitatively much smaller (but significant) changes in the deviation of the reach trajectory were produced by inactivating these two nuclei.

Spatial features of the reaching movement before and after muscimol microinjections of each nuclear region were compared statistically across the animals in the study. As shown in Fig. 5, there were statistically significant changes in these measurements, depending on the nuclear region injected. Although the interposed injections produced the most dramatic effect on trajectory length (TrL) due to the terminal ataxia, this measurement also was increased significantly relative to saline controls after fastigial injections and decreased significantly after dentate injections. The height at which the bar was grasped (GH) increased significantly relative to controls after fastigial and interposed injections and decreased after dentate injections. Deviation of the reach trajectory from a straight line (SD) was the largest after interposed injections, although dentate injections also resulted in a deviation that was significantly different from saline controls. However, the difference in the dentate measurements when compared before and after the muscimol injection was not significant statistically. Interposed injections also resulted in a significant increase in trajectory height (Elev). Compared with saline controls, dentate injections produced a significant decrease in this measurement.

The effect of injecting the three nuclear regions on the relationship of joint angles in the forelimb during the reaching movement is shown in Fig. 3. After the injection of the fastigial nucleus, there was only a modest change in the relationship between the wrist and elbow angles during the

**FIG. 2.** Top view of the changes in position of IREDs located on paw, elbow, and shoulder, each indicated in A. L, lateral displacement in horizontal plane. Other abbreviations are the same as in Fig. 1.
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FIG. 3. Effects of microinjections of muscimol in fastigial nucleus (FN, A–F), interposed (IN, G–O), and dentate (DN, P–U) on angle/angle plots for 3 relationships: shoulder extension versus elbow angle, wrist dorso-ventral flexion versus elbow angle, and shoulder rotation versus wrist supination-pronation. →, direction of plot as movement proceeds from beginning of reach to bar contact. Relationships for entire reach are shown in J–L. Those in M–O show plots of the reach up to the point at which oscillatory movements began.

Figure 3 shows the effects of microinjections of muscimol on angle/angle plots for three relationships: shoulder extension versus elbow angle, wrist dorso-ventral flexion versus elbow angle, and shoulder rotation versus wrist supination-pronation. The plots are shown for the entire reach (J–L) and up to the point where oscillatory movements began (M–O).

Measurements characterizing changes in the spatial characteristics of the reach component were limited to trajectory length (Fig. 5). Injection of all three nuclear regions resulted in a significant change in this quantity, relative to both control and preinjection measurements. This likely reflects the difficulty encountered by the cats in maintaining their grasp on the bar after nuclear inactivation. Muscimol microinjections had no consistent effects on the joint angle relationships during the trace phase and therefore are not illustrated.

TEMPORAL FEATURES IN REACH AND TRACE PHASES OF THE MOVEMENT. The most dramatic effects on the temporal characteristics of the reach were produced by the inactivation of the interposed nuclei. After these injections, statistically significant increases (relative to saline controls) were observed in peak wrist velocity and peak wrist acceleration over the entire duration of the reach, including the terminal segment in which the ataxia occurred, peak wrist
duration likely reflects the animal’s failure to maintain grasp of the bar in a number of trials as the manipulandum was moved through the template. Although this deficit was present to some degree after the injection of each nucleus, behaviorally it was most consistent during inactivation of the dentate nucleus. The injection of the interposed nuclei produced a comparatively large increase in peak wrist velocity and acceleration that was significant statistically. Despite the intermittent tendency to lose the grasp on the bar, these changes were large enough to result in a reduction of movement time that was statistically significant relative to control measurements. Injection of the fastigial nucleus also produced a significant increase in peak wrist velocity and peak wrist acceleration.

Effects of muscimol microinjections on dimensionality

The effects of inactivating the output of the three major cerebellar nuclear regions also were examined by determining the changes in dimensionality during the reach (defined in METHODS) for two measurements, linear velocities of points located over the limb’s major landmarks and the angular velocities of the limb’s joints. Before addressing the changes in dimensionality, an example of the changes in joint angles will be described for an experiment in which an injection was made in the interposed region. Figure 6 shows the changes in seven different angles for reaches performed in individual trials before and after the injection of muscimol. The magnitude of these changes is typical of those seen in most experiments.

Dimensionality analysis requires that the angular velocity at the joints and linear velocities of positions on the limb be determined and then, for each set of calculations, normalized. Plots of normalized linear velocities and angular velocities are shown in Figs. 7 and 8, respectively. Notice first of all that the profiles of the linear velocities (Fig. 7) temporally are more heterogeneous before each duration. The increases in reach duration undoubtedly were related to the changes in the trajectory resulting from the ataxia as the paw approached the bar. In contrast, microinjections in the fastigial nucleus produced a significant decrease in peak wrist velocity associated with an increase in reach duration. Dentate injections did not produce statistically significant modifications in either peak velocity or peak acceleration, although a decrease in reach duration was significant both with respect to saline controls and preinjection measurements. In addition, there were modest but significant changes in the deviation and height of the trajectory.

Injections of each of the nuclei also had effects on the temporal features of the trace phase of the task. Microinjections in the dentate nucleus produced a statistically significant increase in movement duration even though the peak acceleration showed a small but significant increase as well. Consistent with the increased trajectory length, the increased velocity and peak wrist acceleration for the component of the reach before the initiation of the ataxia (1st Seg.), and reach duration. The increases in reach duration undoubtedly were related to the changes in the trajectory resulting from the ataxia as the paw approached the bar. In contrast, microinjections in the fastigial nucleus produced a significant decrease in peak wrist velocity associated with an increase in reach duration. Dentate injections did not produce statistically significant modifications in either peak velocity or peak acceleration, although a decrease in reach duration was significant both with respect to saline controls and preinjection measurements. In addition, there were modest but significant changes in the deviation and height of the trajectory.

Injections of each of the nuclei also had effects on the temporal features of the trace phase of the task. Microinjections in the dentate nucleus produced a statistically significant increase in movement duration even though the peak acceleration showed a small but significant increase as well. Consistent with the increased trajectory length, the increased
though fastigial injections produced smaller changes in LVD and AVD than interposed injections, statistically significant (relative to saline controls) decreases in LVD and increases in AVD were observed after fastigial inactivation (Fig. 5). The occasional changes in dimensionality that accompanied microinjections in the dentate nucleus were inconsistent across animals (Fig. 5).

The changes in LVD and AVD usually observed after interposed injections (see Fig. 9) often were reciprocal but not inversely proportional. The reciprocal nature of these LVD and AVD measurements (shown in Fig. 9) is apparent in Fig. 10A, where LVD is plotted versus AVD for each individual trial. Clearly, injection of muscimol into the interposed region produced a decrease in LVD and an increase in AVD. The relationship between the mean values of AVD and LVD before and after interposed microinjections for each animal is shown in Fig. 10B. In some animals (B130 and B132), the changes in LVD and AVD roughly were inversely proportional. However, in the other three animals the change in AVD was much greater than that in LVD.

To provide insight into how the changes in dimensionality relate to certain features of reaching movements, movements made with a multijointed device constructed to simulate a forelimb were analyzed using the method employed in determining the AAD and LVD for the movement of the cats’
Effect of nuclear microinjections on retention and adaptation to the motor deficit

Because of the general interest in the cerebellum’s role in motor learning and the specific discussions regarding the possibility that this structure serves as an important storage site for engrams required for the retention of a variety of learned movements (see Introduction), the effect of injecting each of the three nuclear regions on the retention of the motor sequence required to perform the operantly conditioned task was investigated. The percent of successful trials was determined after the injection of each nucleus in each animal. A successful trial was defined as the completion of the task (movement of the manipulandum to the reward zone) in the allotted 3 s.

FIG. 6. Plots of joint angles observed during two individual trials before and after microinjection of muscimol into IN nucleus. An increase in shoulder rotation, shoulder flexion, and elbow flexion angles corresponds to the following directions: latero-medial rotation, shoulder extension, and elbow extension, respectively. Zero point in time corresponds to reach onset. PL, paw liftoff; BC, bar contact (same abbreviations used in subsequent figures). In Figs. 6–8, 300-ms point on the abscissa occurred just prior to BC.

FIG. 7. Normalized linear velocity profiles acquired before and after movement was made in the pendular condition (B) and microinjection of muscimol in IN nuclei in one experiment. Zero point in time corresponds to reach onset. – – –., (a–e) mark 5 peaks of the 5 profiles shown in preinjection column. Similarly, f–i mark time of occurrence of peaks in linear velocity profiles obtained after injection. Location of IRED pertinent to each velocity profile is indicated next to appropriate condition.
CEREBELLAR NUCLEAR INACTIVATION AND REACHING

from injections in the interposed and fastigial nuclei (Fig. 12C). Typically there was only an initial failure to perform the movement within the allotted 3 s in one or two trials in the first few blocks of 10 after the injection. However, in two animals (cats 127 and 130, Fig. 14) the dentate microinjection produced a somewhat more erratic performance of the task across the first several blocks of trials. In the example shown in Fig. 12D, the microinjection produced a slowly progressive decrease in the animal’s capacity to complete the task during the first five blocks. At the sixth block, the cat again was completing the task on virtually every trial. Interestingly, in all of the trials in which these two cats failed to reach criterion within 3 s, they completed the task within the next few seconds. Based on changes in measurements characterizing the movement, many animals improved their performance relative to that generated immediately after the muscimol microinjections. Selected examples are shown in Fig. 13. Changes in trajectory length during the reach after injection of the interposed nuclei are shown in Fig. 13A. The substantial increase in trajectory length observed after interposed injections often decreased progressively as the

![FIG. 8. Normalized angular velocity plots and accompanying correlational matrices illustrating effects of a muscimol microinjection in IN. Format for this figure is identical to that used in Fig. 7. Abbreviations employed to label rows and columns of matrices represent specific joint angles that were measured. WS, wrist supination; WE, wrist extension; WRD, wrist radial deviation; SLR, shoulder lateral rotation; SA, shoulder abduction; SF, shoulder flexion; EF, elbow flexion.](image)

Figure 12 shows typical effects of the muscimol microinjections in each nuclear region. The most dramatic effects produced by injections of the fastigial nucleus (Fig. 12A) or the interposed nuclei (Fig. 12B) consisted only of a few failures to complete the task within each of the first few blocks of 10 trials. Given the fact that several hundred trials were required to learn the task initially, the few failures observed immediately after the injection likely are due to the resulting dysmetria, which was most marked immediately after the injection (see Fig. 13), rather than the loss of the critical engram. In support of this argument, intermittent lengthening of the trial duration to 5 s usually permitted the cat to complete virtually every trial except in rare instances in which the animal completely failed to contact the bar (unpublished observations).

The effects of microinjections in the dentate nucleus in three of the five animals were identical to those resulting

![FIG. 9. Plots illustrating typical effect of microinjections of muscimol in interposed nuclei on linear velocity dimensionality (LVD, A) and angular velocity dimensionality (AVD, B). Vertical lines, time at which muscimol was injected. As defined in METHODS, values of LVD and AVD are unitless and range from 1.0 to an integer equal to total number of waveforms in case in which all are orthogonal to each other.](image)
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FIG. 11. Changes in AVD (A and B) and LVD (C and D) produced by imposed movements of a mechanical “arm” under two conditions, ‘distally driven’ and ‘pendular’ (see text for definitions). Notice that effects of changing movement from distally driven condition to pendular condition produces reciprocal effects in two dimensionality measurements.

FIG. 10. Scatter diagrams illustrating effects of muscimol microinjections in IN nuclei. Relationship of LVD and AVD on individual trials in one experiment before and after injection is shown in A. Changes in average values of these quantities in five cats are illustrated in B, with dashed lines showing correspondence between pre- and postinjection average measurements for each animal.

Histological findings

The location of the injection sites at which the maximum effects were observed are shown in Fig. 14. The three injection sites for each animal are shown on the same standardized set of cross-sections. The very short onset time for the effects after the injection, the relationship of some of the abnormalities to known deficits after the inactivation of these nuclei, and the uniformity of the deficits (note small error bars on the group data in Fig. 5) indicate that the inactivated nuclear region was very consistent across animals. Although care was taken to place the cannulae just at the dorsal border of the nuclei of interest, in some animals the injection site was near the center of the structure (e.g., fastigial and interposed injection in animal 164). Given the additional observation that, in other regions
of the CNS, 1 μl injections of muscimol resulted in the inactivation of neural elements over a radius of ~1.5 mm (Martin 1991), it is very likely that the majority of the desired nuclear region was inactivated in each experiment. This argument is particularly well supported for injections in the interposed nuclei, where the clinical abnormalities related to the cutaneous-muscular and postural reflexes are well known and easily tested (see Bloedel and Bracha 1995 for description). The fact that these interposed-specific deficits were never observed after fastigial and dentate injections attests to the nuclear specificity of the inactivations produced in these experiments.

Despite these precautions, it is possible that some injections may have failed to inactivate their ventral-most regions. It is also possible that the effects of some injections, although exerting their primary effect in the nucleus in closest proximity to the injection site, also may have inactivated small regions of neighboring nuclei. For this reason, the interpretation of the findings below emphasizes the primary differences in the effects of injecting each of the three nuclear regions while acknowledging that some of the observations may reflect the inclusion of small regions of adjacent nuclei.

**Discussion**

As indicated in the Introduction, this is one of the first experiments to examine the selective effects of inactivating individual cerebellar nuclei on a complex, goal-directed forelimb movement by assessing quantitatively its kinematics. The task required the performance of an accurate reach, a movement that guided the manipulandum through the template from a start position to a target zone, and the return of the paw to the support platform. Furthermore, the group effects of muscimol inactivation were evaluated based on a statistical comparison with the effects of control saline injections in the same locations. Consequently, all findings interpreted as statistically significant were based on a post hoc comparison between the postinjection muscimol and postinjection saline observations. Significant effects between preinjection and postinjection saline measurements very likely reflect progressive behavioral changes related to practice, satiation, and/or fatigue (Fig. 5), emphasizing the importance of this control in experiments of this type. Another important feature of these experiments is that long-term com-
pensatory changes, an inevitable result of chronic lesion studies, did not influence the findings in our experiments.

Implications regarding cerebellar function

The implications regarding the specific features of motor behavior controlled by the cerebellum are difficult to derive from ablation experiments because of the inherent problems in drawing conclusions regarding the function of this structure on the basis of abnormal movements generated by remaining brain regions (see Bloedel and Bracha 1995 for discussion). Despite this qualification, several important inferences can be made.

INvolvement of multiple nuclei in the same volitional movement. Our data clearly show that temporary inactivation of each individual cerebellar nuclei, including the fastigial nucleus, can lead to modifications in specific temporal and spatial features of the same volitional, complex forelimb movement. Clearly, a specific nucleus can play a predominant role in the performance of a specific type of behavior. The effects of fastigial injections, which were greatest (based on percent changes) on the trace phase, likely were not attributable to postural instability because the animal was in contact with a sling that stabilized the cat somewhat during the execution of the task. These changes included difficulties with the movement through the transverse part of the template and increased velocity and acceleration of the movement, manifested primarily during the anterior-posterior segment of the trace movement. The deficits associated with interposed inactivation were the most dramatic, consisting of a marked change in reach trajectory, increased velocity and acceleration during both phases, and a clear alteration in the execution of the grasp. The inactivation of the dentate nucleus affected the trajectory only minimally when compared with the interposed injection effects. However, there were marked increases in the duration of the movement through all parts of the trace phase, most likely due to a failure to maintain contact and control of the manipulandum as it was moved through the groove. Thus although the specific functional role of each nucleus in the performance of this complex movement is not known, it is feasible that the processing in the components of the cerebellum associated with each nucleus offers a unique contribution to the planning and execution of this task as well as many other volitional, goal-directed behaviors (Bloedel 1992).

The concept that all the cerebellar nuclei can participate in controlling the execution of these types of movement sequences is not novel as a fundamental postulate (see Bloedel 1992; Bloedel and Courville 1981 for discussion), nor does it minimize an important principle, namely that there are differences in the spectrum of behaviors affected most dramatically by lesions of the different cerebellar nuclei (Chambers and Sprague 1955a,b; Goldberger and Growdon 1973; Thach et al. 1992). Nevertheless, the data reemphasize an important point, namely that the output of each of the three major cerebellar sagittal zones can contribute to the regulation of the same motor behaviors, including the complex task employed in these studies. This argument also is consistent with the observations of MacKay (1988), who examined the relationship between the activity in each of the three major nuclei and the performance of a visually guided reaching task. Task-related modulation was found in each nuclear region, and there were some striking similarities in the patterns of modulation seen across these structures. Primarily, differences in the onset time of responses in each of the nuclei were reported. These findings also are consistent with those of Thach (1978), who found that cells in both the interposed and dentate nuclei responded during the performance of the same complex movement (see also Thach et al. 1993). As in the study of MacKay, differences in the temporal relationships between their modulation and the movement were reported. In a more recent experiment, Schieber and Thach (1985) compared the modulation of dentate and interposed neurons during a slow tracking movement. In this type of task, the modulation of cells in both nuclei displayed somewhat similar characteristics that often were unrelated to specific kinematic features of the movement. These similarities in discharge patterns do not imply that different nuclei perform the same function. However, they do substantiate the involvement of information processing in multiple nuclei during the same task.

Task dependency of cerebellar nuclear action. In our view, the eventual understanding of cerebellar nuclear function must explain the fact that the specific features of the movement affected by nuclear ablation appear to be task dependent, namely they are dependent on the behavior being evaluated. For example, the dramatic increase in peak velocities observed by Brooks et al. (1973) after dentate nuclear cooling were not reflected in the movements quantified in our experiment. In fact, we observed a modest but significant decrease (Fig. 5). Furthermore, interposed nuclear cooling produced only minimal effects together with hypometria (Uno et al. 1973), whereas in our experiments, inactivation of this nuclear region produced the largest and most consistent changes in the kinetics of the conditioned limb movements and a marked hypermetria. The behaviors studied by Brooks and colleagues consisted either of controlled movements between targets or oscillating flexion and extension movements between stops. In contrast, the movement studied in our experiment consisted of a rapid reach, a grasp, and the movement of a manipulandum through a two-segment template. Using yet another paradigm, Miall et al. (1985) reported that interposed inactivation produces increases in peak velocity during the performance of a continuous tracking task, an observation that also contrasts with those of Uno et al. (1973). The task-dependent features of cerebellar deficits also are apparent even when the data from the reach and trace phases of this experiment are compared. For example, the percent changes in kinematic measurements were much greater for the trace component than for the reach after dentate inactivation. In fact, the specific kinematic changes in the trace and reach phases were different after the inactivation of each nuclear region.

This task dependency of the changes in specific movement parameters produced by inactivating a single cerebellar nucleus raises an important issue regarding whether the functions of the cerebellum include the regulation of specific movement parameters during the execution of motor behav-
iors. Several investigators have reported that the activity of cerebellar neurons can be correlated highly with temporal and positional features of active and passive movements (Kolb et al. 1987; MacKay and Murphy 1979; Marple-Horvat and Stein 1987; Rubia and Kolb 1978; Schwartz et al. 1987; Thach 1970b, 1978; Van Kan et al. 1993, as examples). In fact, this type of encoding may be very complex. Ebner and colleagues (Ebner and Fu 1997) reported recently that the activity of some Purkinje cells can covary with different kinematic measurements during the time course of a volitional forelimb movement. The task-dependent nature of the changes in movement kinematics observed after cerebellar nuclear lesions and the complex encoding of movement parameters in the activity of cerebellar neurons suggest that the regulation of a specific parameter or related parameters across a variety of movements is not a primary function of the cerebellum. Rather, the task-dependent relationships described above may reflect an intermediate level of processing related to a sensorimotor transformation required for optimizing the performance of a specific movement (see also Ebner and Fu 1997).

**TRAJECORY CONTROL AND THE REACH TO GRASP.** The data also demonstrate that, in addition to affecting the temporal features of the movement, inactivating specific cerebellar nuclear regions also modified spatial characteristics of the trajectories during the reach (Figs. 1–4). The changes were most dramatic and most consistent after the inactivation of the interposed nuclei. Characteristically, these changes resulted in a more curved trajectory and a deviation of the hand path somewhat lateral to the vertical A-P plane. Comparable observations were made by Gilman et al. (1976) after large cerebellar ablations in monkeys (see also Massaquoi and Hallett 1996 for comparable data from human subjects). Despite these changes in trajectory, the movement usually could be performed well enough for its objective to be achieved.

The spatial features of the reach were least affected by the inactivation of the dentate nucleus. This observation relates well to the finding that the activity of neurons in the motor cortex, a structure considered to be intimately involved in the performance of reaching movements (Georgopoulos 1991), is modified very little when a movement is executed during the cooling of the dentate nucleus (Meyer-Lohmann et al. 1975, 1977) or following a permanent lesion of this structure (Spidalieri et al. 1983). Primarily, this procedure produced a shift in the onset of the modulation in the motor cortex, a finding relatable to the well-known observation that the reaction time of movements is prolonged after dentate lesions in monkeys (Beaubaton and Trouche 1982; Spidalieri et al. 1983; Trouche and Beaubaton 1980) as well as in patients with substantial cerebellar pathology (Bonnefoi-Kyriacou et al. 1995; Diener et al. 1989; Nakamura and Taniguchi 1980).

In addition to changes in the path of the wrist, interposed inactivation produced a terminal dysmetria, which included an oscillation of the distal extremity as the bar was approached. This finding often was associated with a failure to properly execute an effective grasp with the paw (Gibson et al. 1994; see also Robinson 1995). An action tremor also was observed by Thach et al. (1992) after interposed inactivation in monkeys. Comparable dysmetria did not occur when the interposed region was cooled during the performance of a tracking task in which a manipulandum was moved between two targets (Uno et al. 1973), indicating again the task-dependent nature of these abnormalities. Possibly the ballistic nature of the reaching movement coupled with the accuracy required to grasp the bar in our experiment enhanced the instability, resulting in the characteristic terminal dysmetria. Interestingly, cerebellar abnormalities in patients were shown recently to produce both an impairment of endpoint accuracy in a reaching task (Bonnefoi-Kyriacou et al. 1995) as well as an exaggerated dysmetria at the end of the reach (Becker et al. 1991).

From a more general perspective, the findings suggest strongly that the cerebellum, particularly the intermediate cerebellum, is critical for the performance of consistent, accurate reaching movements and their coupling with a well-timed grasp of the target. Although the fundamental basis for this deficit may be very complex, there are two general possibilities that should be considered. First, the deficit may relate to a failure to properly organize the relationship between the reach and the grasp phases of the movement so that the handle can be moved effectively. The terminal dysmetria may reflect repeated, poorly coordinated attempts to grasp the bar. There is little known regarding the extent to which the cerebellum regulates the critical temporal relationship between the reach and grasp (Jeannerod 1989). However, initial reports suggest that this relationship may be impaired in cerebellar patients (Haggard et al. 1996).

Second, the failure of the animal to grasp the bar effectively at the termination of the reach may be related to the inadequate processing of sensory cues, including cutaneous cues from the plantar surface of the paw. For example, this processing deficit could reflect inadequate sensorimotor transformations required to translate sensory information into a motor reference frame. Unquestionably, deficits in the coordination of the reach to grasp could reflect inadequate processing of proprioceptive information at either the spinal or supraspinal level (Flament et al. 1984; Gilman et al. 1981; Hore and Flament 1986; Hore and Vilis 1984; Scheiber and Thach 1985). Recent studies in our laboratory (Kolb et al. 1994, 1997) demonstrated that both conditioned as well as unconditioned forelimb withdrawal reflexes are substantially impaired after the inactivation of the interposed nuclei in cats. The deficit in processing these types of sensory cues is reflected in several features of the limb’s behavior. For example, Kolb et al. (1997) recently showed that the precision placement response was affected dramatically after the inactivation of this nuclear region (see also Bloedel and Bracha 1995). After this procedure, cats no longer were able to place the paw back on a small platform in an accurate, consistent manner after completing a phasic forelimb movement. In the present experiments, this deficit is reflected in the comparatively wide distribution of locations from which the reach is initiated due to the fact that the paw is not repositioned in the same location repeatedly after each successive reach (Figs. 1 and 2). Consistent with the reports of Kolb et al. (1996) and Bloedel and Bracha (1995), after...
the completion of the template task, the cat usually required multiple attempts to replace the limb on the pad. In our view, these findings are related to the deficits in contact placing (Amassian and Ross 1972; Amassian and Rudell 1978; Amassian et al. 1972, 1972) and the tendency to drag the dorsum of the foot or paw during locomotion (Chambers and Sprague 1955b; Thach et al. 1992) also observed as a consequence of interposed dysfunction. As argued above, the fact that these effects of interposed injections were not observed after dentate and fastigial injections supports the argument that the observed effects were due primarily to the inactivation of the target structure rather than the spread of muscimol to other nuclei.

This discussion also emphasizes an often-neglected point—the striking similarities between the deficits in reflexes and volitional movements after temporary or permanent lesions of the cerebellar nuclei, particularly the interposed nuclei. As pointed out by Bloedel and Bracha (1995), the inactivation of the interposed nuclei produces a characteristic disorganization of movement applicable to both cutaneomuscular reflexes and volitional movements. We propose that this is due to the loss of a common control strategy normally exerted by the intermediate cerebellum on circuitry serving as partial substrates for the execution of both classes of movements.

Changes in Dimensionality and Implications Regarding Cerebellar Function. We believe that the changes in dimensionality observed in these experiments offer a unifying view regarding the phenomenological basis for the changes in the performance of goal-directed reaching movements produced by the inactivation of individual cerebellar nuclei, particularly the interposed nuclei. Interestingly, the most consistent changes in dimensionality as well as the most pronounced effects on the movement kinematics resulted from the injection of muscimol into this nuclear region. This analysis revealed that the organization of the movement during the reach changed in a very specific way following this procedure: there was a decreased correlation among the angular velocities (increased AVD) and an increased correlation among the linear velocities (reduced LVD) characterizing the limb’s movement.

The demonstration employing the mechanically simulated extremity described in detail in RESULTS indicates that the decreased correlation contributing to the change in AVD can be related to the nonsynchronous change in joint angles associated with the whipping motion. Interestingly, patients with cerebellar pathology show a comparable change in joint angle relationships in which there is a tendency for the angles to change serially in a proximal to distal direction (Becker et al. 1991; Goodkin et al. 1993). The angular velocity profiles in Fig. 8 indicate that the decreased correlation resulting from interposed nuclear inactivation is likely due to a more complex change in the temporal relations of joint angle changes.

This argument also is consistent with the increased variability in the angle/angle plots (Fig. 3), including the appearance of a very erratic relationship in the plots involving rotation angles. The variability in the relationship between proximal angles after muscimol inactivation is greater than occurred under control conditions, greater than has been reported previously for normal human subjects (Soechting 1984), and comparable with those characterizing the reaching movements of cerebellar patients (Goodkin et al. 1993). Although the relationship between proximal joint angles and wrist joint angles displays an inherent variability under normal conditions (Soechting 1984), these relationships became particularly erratic after cerebellar inactivation. These changes coupled with a somewhat unexpected decrease in LVD indicate that the reorganization in a goal-directed reach produced by interposed inactivation is very specific. These modifications, which may reflect an instability in the limb’s control system, could account for the errors in endpoint accuracy discussed above. This argument is consistent with the abnormalities in the kinematic characteristics of limb movements learned by cats during the inactivation of the ipsilateral dentate and interposed nuclei (Shimansky et al. 1994).

Although a complex movement could be acquired during the inactivation of these structures, there were substantial trial-to-trial variations in the motor patterns used to perform the task that were not seen after learning of the task with the cerebellum intact. Based on these studies, we proposed that the cerebellum acts with other central structures first to select and then to optimize the motor pattern employed to execute a specific, novel complex behavior (Bloedel et al. 1996).

The nature of these deficits supports the view that the cerebellum, and particularly the intermediate cerebellum, is critical for optimizing a set of constraints within a control strategy responsible for regulating the relationship between the changes in joint angles and the translation of the limb in space as a reaching movement is performed (see also Thach et al. 1993). The deficits also imply that this cerebellar-dependent control strategy results in a greater endpoint accuracy, enabling the execution of a more effective grasp at the end of the reaching movement. As emphasized above, a corresponding strategy could specify the functional characteristics of the cutaneomuscular reflex system and in fact could act to establish an optimal interrelationship between these reflexes and volitionally determined patterns of movement during the execution of complex behaviors (Bloedel and Bracha 1995).

Implications regarding the cerebellum’s role in the retention of learned motor sequences

The role of the cerebellum in the learning of motor tasks remains a topic for considerable debate. As reviewed extensively elsewhere (Bloedel and Bracha 1995; Ito 1984; Raymond et al. 1996; Thompson and Krupa 1994), studies examining the cerebellum’s role in the modification of certain reflexes, including the vestibulo-ocular, eyeblink, and withdrawal reflexes, have been interpreted as indicating that this structure serves as a necessary and sufficient structure for the storage of the plasticity required for these modifications. Although the results of several experiments suggest that there may be sites in the brain stem involved in the storage of these engrams (Bracha et al. 1991, 1994; Kelly et al. 1990; Lisberger et al. 1994; Welsh and Harvey 1991), this controversy has not
been resolved. Nevertheless, the cerebellar hypothesis has been extended to the learning of operantly conditioned behavior, suggesting that at least a major site of the plasticity required for recalling learned motor behavior is stored in the cerebellum (see INTRODUCTION). Although the primary objective of our study was not focused on this issue, the data are pertinent to this question.

This study assessed the effect of inactivating each cerebellar nucleus individually on the retention of the motor sequence required to execute the required volitional forelimb movement. As shown in Figs. 1–4, the sequence of movements required for this task usually could be performed after nuclear inactivation, although in two cats there was an unusual change in the performance of correct trials immediately after the injection of muscimol, an exception that will be discussed below. The fact that the motor sequence was retained during muscimol inactivation is emphasized by the finding that muscimol injection in any of the nuclei failed to produce an increase in the time required to traverse between two set points before and after the corner of the template. An assessment of this time, expressed relative to the overall duration of the trace phase, revealed that the only significant changes (determined using the ANOVA and posthoc analysis as described in METHODS) were decreases in this measurement after the fastigial and dentate injections, with no significant change observed after interposed injections. The small reduction in successfully performed trials seen during the first 10 trials after most injections very likely reflects the incoordination resulting from the initial effects of nuclear inactivation. This suggestion is supported by the fact that increasing the duration of the trials to 5 s gave the cat enough time to complete the task (unpublished observations). Furthermore, because this task takes several days to learn with or without the inactivation of the cerebellar nuclei (Shimansky et al. 1994), a return to criterion performance after 10 trials is unlikely if the required engram has been rendered dysfunctional by the injection and a new one has to be established. Consequently, it is not likely that the transient decrease in performance illustrated in Fig. 12, A–C, reflects the inactivation of a site critical for the retention of the learned complex motor sequence executed in these experiments.

Not only was retention of the motor sequence unaffected, but the cats often were capable of improving some aspects of their performance with practice during nuclear inactivation, particularly those related to the reach phase (Fig. 13). Because the memory trace was not eliminated after the injection of any nuclear region and because most cats could improve their performance with practice during inactivation, extracerebellar storage sites important for retention likely are established during the learning of this complex forelimb movement. This conclusion is further supported by our recent demonstration that a similar task can be acquired during the simultaneous inactivation of the dentate and interposed nuclei (Shimansky et al. 1994).

In addition to the implications regarding retention of the motor sequence, the findings in two animals suggest that at least a region of the dentate nucleus may be involved in the execution of the learned movement in a manner that is somewhat unique. As described above, in two animals (Fig. 12D), there was a gradual reduction in the percent of conditioned responses over several blocks of trials after the muscimol injection. The time course of this effect suggests it is not due to a disruption of retention (see Bracha et al. 1994 for examples of the rapid, persistent failure to execute a previously learned behavior following muscimol inactivation). Even if the location of the injection was remote enough from the critical site so that the onset of the effect was slow, any inactivation mediated by muscimol on a site critical for retention would be persistent rather than subside after a few blocks of trials (Kolb et al. 1997). Furthermore, in all trials in which the cats failed to perform the task within the allotted 3 s, they were able to complete the task after the time limit had passed.

Alternatively, these findings may indicate that the dentate nucleus contributes to specific features of motor planning and motor preparation that are required for rapid, consistent performance of a previously learned movement. This hypothesis is consistent with recent observations using functional magnetic resonance imaging implicating the dentate nucleus in this class of functions (Kim et al. 1994) and its known projections to the prefrontal cortex (Middleton and Strick 1994), a site involved in working memory (Funahashi and Kubota 1994; Goldman-Rakic 1992, 1995). Because the other three cats that received dentate injections did not display this finding, this paradigm may not have been optimal for illustrating this aspect of the deficit. A performance deficit also could have contributed, because dentate injections clearly affected the capacity to retain the grasp of the bar during the trace phase. Additional experiments directed toward this question will be required to assess this issue systematically.

Summary

In summary, the results of this experiment suggest that each functional component of the cerebellum plays an important role in specifying the central interactions defining the temporal and spatial organization of a volitional, goal-directed limb movement. These interactions may be important for optimizing endpoint accuracy and ensuring stability as the limb decelerates and the target is grasped. Furthermore, the inactivation of individual nuclei does not affect the retention of the motor sequence required to execute this task, and performance could be improved with practice under this same condition. Consequently, it is unlikely that any single nucleus serves as a critical storage site for the plasticity established during the learning of this behavior.

APPENDIX

This appendix demonstrates that the CSD analysis introduced in METHODS gives an intuitively correct measure of the extent of linear independence within a given set of waveforms viewed as vectors. First, we show that the CSD measure is equal to the “classical” integer dimensionality, as defined in linear algebra, in all cases when those measures intuitively should be identical. We can give the general description of such cases based on two intuitively obvi-
ous facts: the dimensionality of a waveform set is maximum and equal to the total number of waveforms when all waveforms are mutually orthogonal, i.e., correlation between any two of them is zero, and the dimensionality of a waveform set does not change if any linear combination of waveforms from the set is added to it as a new waveform. It follows from these two facts that, when a given waveform set contains a subset of mutually orthogonal waveforms (orthogonal basis) and all other waveforms are linearly dependent on them, the dimensionality of the whole waveform set is equal to the number of waveforms in the orthogonal basis. Intuitively this must be true for any definition of the dimensionality of a waveform set. The following theorem asserts that it is true for CSD.

**Theorem**

If in a set of \( n \) waveforms \( m \) are mutually orthogonal and the others can be presented as exact linear combinations of them, then

\[
\text{CSD} = \sum_{i=1}^{m} \lambda_i = m,
\]

where \( \lambda_1, \lambda_2, \ldots, \lambda_n \) are the eigenvalues of the matrix \( A \) consisting of the coefficients of cross-correlation between the \( n \) waveforms.

**Proof**

Because any correlational matrix is positive semidefinite, all eigenvalues of \( A \) are nonnegative. Also, under the theorem conditions, exactly \( m \) eigenvalues are nonzero. Consequently, to prove the theorem, it is only necessary to show that all the nonzero eigenvalues of \( A \) are not less than 1.

In the most general case, the correlational matrix of a set of waveforms conforming to the condition stated in the theorem can be presented in the following block form

\[
A = \begin{bmatrix}
I & Y \\
Y^T & B
\end{bmatrix}
\]

where \( I \) is the \( m \)-dimensional identity (the matrix of cross-correlation coefficients for the waveforms of the orthogonal basis); \( Y \) is the \( m \times (n-m) \) matrix of the coefficients of correlation between the \( m \) components of the orthogonal basis and the other \( n-m \) waveforms; \( B = Y^TY \) is the \((n-m) \times (n-m)\) matrix of cross-correlation coefficients for the waveforms not belonging to the basis. Readers who are not familiar with matrix analysis can find all the information necessary to understand the related mathematics of this paper in Horn and Johnson (1986). The eigenvalues of \( A \) can be found from the following characteristic polynomial equation

\[
P_n(\lambda) = \det (\lambda I - A) = 0.
\]

Because the determinant of a matrix does not change after multiplying the matrix by a unitary matrix, \( \det (\lambda I - A) = \det (U\lambda I - A \times U^T) \). By setting

\[
U = \begin{bmatrix}
I \\
(\lambda I - I)^{-1}Y^T
\end{bmatrix}
\]

we have

\[
\det (\lambda I - A) = \det \begin{bmatrix}
I \\
(\lambda I - I)^{-1}Y^T
\end{bmatrix} \det \begin{bmatrix}
\lambda I - 0 \\
-Y^T \\
\lambda I - B
\end{bmatrix} \det \begin{bmatrix}
I \\
(\lambda I - I)^{-1}Y
\end{bmatrix}.
\]

Given that \( B = Y^TY \) it follows that

\[
\det (\lambda I - A) = \det \begin{bmatrix}
\lambda - 1 & 0 \\
0 & \lambda - \frac{1}{\lambda - 1} B
\end{bmatrix}
= \det (\lambda I - I) \det \begin{bmatrix}
\lambda - 1 & 0 \\
0 & \lambda - \frac{1}{\lambda - 1} B
\end{bmatrix}
= (\lambda - 1)^{n-m} \lambda^{-m} \det (\lambda I - I - B).
\]

So all the eigenvalues of \( A \), which are neither 0 nor 1, are among the roots of the polynomial equation

\[
P_{n,m}(\lambda) = \det (\lambda I - (I + B)) = 0.
\]

Notice that this equation describes the set of eigenvalues of the Hermitian matrix \( C = I + B \). According to the Rayleigh-Ritz theorem, the minimum eigenvalue of \( C \) can be estimated as

\[
\lambda_{\min}(C) = \min \{ X^*CX \} = \min \{ (1 + X^*BX) \}
= 1 + \min \{ X^*BX \} = 1 + \lambda_{\max}(B)
\]

where \( X \) is a vector from a complex \((n-m)\)-dimensional vector space and \( X^* \) is the adjoint vector. Because \( B \) is a correlational matrix, \( \lambda_{\max}(B) \geq 0 \), and consequently \( \lambda_{\min}(C) \geq 1 \). Thus all the nonzero eigenvalues of the matrix \( A \) are not less than 1, which proves the theorem, and CSD gives an intuitively correct number under the conditions stated in the theorem.

It should be noted that the integer dimensionality estimate described in Glaser and Ruchkin (1976), despite the fact that it seems to be reasonable and practical, is incorrect in principle and in practice is very likely to give an incorrect number under the above conditions. To illustrate this, let us assume a dimensionality significance limit of \( Q < 1 \). According to the procedure of estimating dimensionality suggested in the above paper, one should find the minimum number of the biggest eigenvalues whose sum, \( S_P = QS \), covers the “principal” part of the total sum \( S \) of the eigenvectors.

\[
S = \sum_{i=1}^{\lambda_n} \lambda_i = n
\]

where the minimum nonzero eigenvalue is equal to \( \lambda \), it will be discarded as “insignificant” when it is less than \( S - S_P = (1 - Q)n \), i.e., when \( n > \lambda/(1 - Q) \). In a realistic case, when \( e.g., \lambda \approx 1 \) and \( Q = 95\% \), the eigenvalue will be discarded, and consequently the integer dimensionality estimate will give an incorrect number (less by 1 than the theoretically correct dimensionality) when the total number of waveforms is greater than only \( n = 1/(1 - 0.95) = 20 \). Thus the larger the number of waveforms in the set, the bigger and more likely the error of this dimensionality estimate.

In a general case, the conditions specified in the above theorem may not be satisfied for a given waveform set. However, they may be almost satisfied. For instance, one can say that two waveforms are almost orthogonal to each other if the coefficient of correlation between them is very close to zero. Or a waveform \( W_n \) may be almost linearly dependent on the waveforms \( W_1, W_2, \ldots, W_n \) if it can be approximated as a linear combination of those waveforms, i.e., if

\[
\delta = \min_{\lambda_1, \lambda_2, \ldots, \lambda_n} \int_{t_1}^{t_2} \left| W_n(t) - \sum_{i=1}^{n} k_i W_i(t) \right|^2 dt \approx 0.
\]

Consequently, if in a waveform set there is a subset of waveforms that are almost orthogonal to each other, and all the other waveforms are almost linearly dependent on them, the measure of mutual linear independence of waveforms in the whole set should be close to the set’s dimensionality when the relationships of orthog-
nality and linear dependence are exact. When normalized waveforms are viewed as vectors, the formula for the Euclidean product of two such vectors is identical to the formula for the coefficient of correlation between the waveforms. The CSD measure can be viewed as one that inherits its properties from both the dimensionality of a vector set as defined in linear algebra and the correlation between two waveforms. In the case of a two waveform set, its dimensionality ranges from 1 (when the cross-correlation coefficient $k$ is equal to 1 or $-1$) to 2 (when $k = 0$). A continuous measure of dimensionality can be obtained in this case by interpolating the dimensionality function of the cross-correlation coefficient between those two endpoints. The simplest, linear interpolation gives the following formula: \( \dim(k) = 2 - |k| \), which seems to be intuitively correct. On the other hand, the eigenvalues of the corresponding \((2 \times 2)\) correlational matrix are \( \lambda_1 = 1 + |k| \) and \( \lambda_2 = 1 - |k| \) (sorted in descending order), which gives \( \text{CSD} = 1 + (1 - |k|) = 2 - |k| \), i.e., exactly the same formula. It will be shown below that the CSD has two important properties that intuitively should be required from such a characteristic.

**CSD measure is a continuous function of the correlation coefficients**

This immediately follows from the fact that CSD is a continuous function of the eigenvalues according to its formula, and the eigenvalues are known to be continuous functions of the matrix elements, i.e., correlation coefficients. Thus CSD, as a continuous function of the cross-correlation coefficients, can be viewed as interpolating between cases when the conditions of the above theorem are satisfied and its value is integer and equal to the rank of the correlational matrix.

**CSD measure is an additive function**

If a linear space containing a given set of vectors can be decomposed into several subspaces so that the original space is the Cartesian product of the subspaces, the dimension of the space is the sum of the dimensions of the subspaces. If a set of waveforms yields such a decomposition, the corresponding correlational matrix can be presented in a block-diagonal form where each block is the correlational matrix for the subset of waveforms corresponding to a linear subspace. Because the set of eigenvalues of the whole matrix is the unification of the sets of the submatrix eigenvalues, one can easily see that the CSD for the whole matrix can be presented as the sum of CSD values calculated separately for each submatrix.

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