Differential Effects of Deep Cerebellar Nuclei Inactivation on Reaching and Adaptive Control

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INTRODUCTION

Many experimental and clinical studies show that the cerebellum has a mediolateral anatomic and functional organization (for review, see Thach et al. 1992). Composed of medial, intermediate, and lateral divisions, each has different afferent inputs and cortico-nuclear connections (Jansen and Brodal 1940; Voogd and Bigaré 1980). Selective inactivation or lesion of each division can produce distinctive motor impairments, suggesting different motor control functions (for review, see Chambers and Sprague 1955; Milak et al. 1997; Thach et al. 1992). The medial division, consisting of the vermis and fastigial nucleus, plays a predominant role in axial muscle control and balance (Chambers and Sprague 1955). The intermediate cerebellum is composed of the paravermal cortex and interpositus nucleus. Moment-to-moment control of limb movement becomes impaired profoundly with lesion or inactivation of the interpositus nucleus (Chambers and Sprague 1955; Milak et al. 1997; Thach et al. 1992). Although the interpositus nucleus consists of separate anterior and posterior subdivisions, it is unclear if they serve distinct motor control functions (Mason et al. 1998) or if they each represent different body regions (Thach et al. 1992). The lateral cerebellum consists of the lateral cortex and the dentate nucleus. It is the least well understood of the three divisions. In the monkey, different studies report a range of impairments after dentate inactivation, from relatively minimal performance disruption (e.g., Beaubaton and Trouche 1982; Trouche and Beaubaton 1980) to more severe effects (Thach et al. 1992). In the cat, dentate inactivation consistently has a minimal effect (Chambers and Sprague 1955; Milak et al. 1997; Thach et al. 1992).

Both the intermediate and lateral cerebellar divisions exert control over limb movement through differential projections to the red nucleus (Gibson et al. 1987) and, via the ventral thalamus, to cortical motor areas (Anderson and DeVito 1987; Asanuma et al. 1983; Hoover and Strick 1999; Jörntell and Ekerot 1999; Nakano et al. 1980; Rispal-Padel and Latreille 1974; Shinoda et al. 1985). Comparison of the topography of these projections with the effects of focal inactivation of red nucleus and motor cortex could reveal insight into the particular movement control roles of the cerebellar outputs. For example, we have shown in an earlier study (Martin and Ghez 1993) that focal inactivation within the forelimb representation of rostral motor cortex profoundly impairs prehension, and within that subregion, different impairments are produced from inactivation of different sites. Because the cerebellar thalamic relay nuclei provide the major input to rostral motor cortex (Anderson and DeVito 1987), it is plausible that there are similarities between the form of impairments produced by focal inactivations of the cerebellar nuclei and those produced in rostral motor cortex.
In this paper, we examine the contributions of the intermediate and lateral cerebellum (i.e., interpositus and dentate nuclei, respectively) to the control of reaching in cats. Using microinjection of the GABA agonist muscimol, we selectively inactivated the anterior interpositus (AIP), posterior interpositus (PIP), and dentate (DN) nuclei. We determined the changes in reaching produced by inactivation. We chose to study reaching because it is a multijoint movement. Compared with a single-joint movement, a multijoint movement is a more sensitive motor task for examining cerebellar function (Thach et al. 1992). We examined two reaching tasks. In the standard reaching task, cats reached to grasp a small cube of meat from a narrow food well (Martin and Ghez 1993). We studied how nuclear inactivation changed trajectory form and variability. Important insights into the neural control of multijoint movement have been obtained by studying this behavior in the cat (Alstermark et al. 1981, 1993a,b; Gorska and Sybirska 1980; Martin et al. 1995a). In the obstructed reaching task, cats reach over a bar that is placed in the limb’s path. We examined how inactivation impaired the use of somesthetic (and visual) information in trajectory adaptation. Our earlier work has shown that performance in this task depends on control signals from a restricted portion of the forelimb representation of rostral motor cortex (Martin and Ghez 1993).

Our experiments addressed two questions. First, does selective inactivation of AIP and PIP produce different trajectory impairments during reaching? Several studies in the cat have shown that AIP and PIP receive differential cerebellar cortical (Trott and Armstrong 1987; Trott et al. 1998) and olivary (Gellman et al. 1983) inputs. In turn, AIP and PIP project on different parts of magnocellular red nucleus (Gibson et al. 1987) and influence the excitability of different motor cortical regions (Jörntell and Ekerot 1999; Rispal-Padel and Latreille 1974; Shinoda et al. 1985). Whereas in the monkey selective inactivation of PIP and AIP impairs reaching and grasping, respectively (Mason et al. 1998), trajectory control and adaptation have not been examined. Second, does DN inactivation have different effects on trajectory control and adaptation? We reasoned that a facultative (or minimal) role in limb control during performance of some tasks (e.g., standard reaching) could convert to an obligatory (or major) role when animals must rapidly reprogram their movements to reach over an obstacle. We focused on inactivation of the AIP, PIP, and DN because in pilot studies fastigial nucleus (FN) inactivation did not significantly impair reaching performance but, as reported previously (Chambers and Sprague 1955), impaired balance.

We will show that inactivation of AIP and PIP produced different trajectory control and adaptation impairments. These distinctive impairments are similar to those produced by selective inactivation of different zones in the forelimb area of cat motor cortex (Martin and Ghez 1993). This suggests that AIP and PIP are part of separate output channels from the intermediate cerebellum with differential movement control functions. We also will show that performance in both the standard and obstructed reaching tasks was unimpaired by inactivation of the DN. Some of the results were published in abstracts (Cooper and Ghez 1991; Cooper et al. 1993; Martin et al. 1995b), a doctoral thesis (Cooper 1997), and in a general review article on the method of reversible inactivation (Martin and Ghez 1999).

**Methods**

**Trained behavior**

Five female cats weighing between 2 and 4 kg were used in these experiments. All procedures were approved by the New York State Psychiatric Institute Animal Use and Care Committee. Cats were trained to reach to the back of a narrow food well to grasp a cube of meat (for details; see Martin and Ghez 1993; Martin et al. 1995a). The food well was either a clear plastic box (3 cm high by 4 cm wide by 4 cm deep) or a clear plastic cylinder (3.2 cm diam; 5.0 cm deep) and was instrumented with photocells to detect paw entry. A horizontal cylindrical rod could be placed unexpectedly into the path of the forelimb at a locus that would contact the distal third of the forearm shortly after the beginning of the reach. The rod normally was retracted and, during the first trial in the block of obstructed reaches, was extended in front of the forearm immediately before the reach (either manually or using a pneumatic mechanism). This invariably resulted in the cat’s limb contacting the rod just after the start of the reach. If the animal noticed the obstruction (by contact before reaching or looking toward it), we withdrew the rod and subsequently extended it after a random number of trials (between 5 and 10). The time of contact with this obstacle resulted in an abrupt deceleration of the paw. To minimize trial-to-trial variability in the position of the shoulder relative to the food well, cats were restrained in a cloth vest attached to a hammock. Cats were required to stand on narrow supports to minimize paw placement variability at the start of the reach. The forepaw supports were 5.5 cm in radius and instrumented with a strain gauge for signaling the time when the reaching paw lifted off the support (toe off).

Reaching was elicited during discrete trials initiated when the cat placed all four paws on the supports provided (Martin et al. 1995a). The cat was required to exert sufficient force on the foot-plate strain gauge to hyperextend the metacarpophalangeal joint of the reaching forepaw (as in normal stance) for a period of 0.5–1.5 s, after which it reached to the food well. Normal animals reach into the food well in a single attempt, grasp the bait, and carry the food to the mouth, before replacing the paw on the foot plate to begin the next trial. Trials were run in blocks (typically 20 reaches). In this report, we present data on reaching to a standard target location (14 cm above and 14 cm forward of the foot plate), although in most sessions data were collected for reaches to other locations. Sessions consisted of 100–150 control (i.e., preinjection) trials and 100–300 test or inactivation (i.e., postinjection) trials.

To supplement our quantitative analysis of the effects of cerebellar inactivation on reaching (see following text), we also assessed the following six untrained behaviors: ability to locomote (unrestricted on the lab floor; along a 4 cm wide beam; over obstacles), contact and proprioceptive placing reactions, postural stability (by gently shoving the standing cat to the left or right), facial muscle symmetry, eye movements, and the ability to reposition their limb after imposed lateral limb displacement while standing. For the latter response, we have found that normal cats replace the paw after <2 cm displacement. Movement abnormalities produced by cerebellar nuclear inactivation were documented carefully.

**Data acquisition**

All experiments were videotaped (Panasonic S-VHS, model 960; shutter = 0.01 s). For selected trials during most experiments, we used a telephoto lens to obtain close-up images of grasping movements within the food well. Videotapes of control and dysmetric reaching movements were examined in stop-motion. We used the MacRefl ex motion analysis system (Qualisys) for the quantitative assessment of reaching. This system uses infrared strobes (<800 μs) and video cameras equipped with infrared filters to detect the locations of infrared light reflected from markers attached to the limb. Markers were placed directly on the lateral surface of the distal phalanx of the
fourth digit (paw-tip), on the skin directly over the metacarpalphalangeal (MCP point) joint of the fifth digit, and the styloid process of the ulna (wrist point). To accurately monitor shoulder and elbow joint locations, because the skin slides over the shoulder and elbow joints during forelimb motion, we implanted an orthopedic pin through the bone of the greater tuberosity of the humerus and into the medullary cavity before behavioral training. The pin protruded from the skin surface by 1 cm to provide a stable attachment site for a light-weight metal rod on which markers corresponding to the shoulder and elbow points were mounted (see following text for general surgical procedure) (see also Martin et al. 1995a for details).

Real-time video digitizers computed the x-y coordinates of centers of the markers with a resolution of 8 μm. An overall sampling rate of 100 Hz was achieved by using two 50-Hz cameras, one offset from the other by 10 ms. Custom Matlab (Math Works) software was used to combine data and eliminate the parallax difference between cameras. Sometimes a small amount of residual parallax was visible as horizontal jitter (i.e., x coordinate) of each marker (see Fig. 2A2). The MacReflex cameras were controlled with National Instruments TTL I/O hardware. Force plate and photocell signals were acquired at 100 Hz using 16-bit A/D converters (Macintosh II computer with National Instruments I/O and DMA boards) synchronized with the video cameras.

Analysis

The x-y coordinates of the point markers were processed using custom Matlab software. The upper arm limb segment was defined as joining the shoulder and elbow points; the forearm segment, as joining the elbow and wrist; and the metacarpal segment as joining the wrist and MCP points. The most distal part of the limb was treated as a single phalangeal segment joining the MCP point and the paw tip.

A potential source of error in computing joint angles was movement of the forelimb out of the imaging plane. We detected this motion by measuring changes in the apparent length of limb segments. In our experiments, variations in apparent length were not systematically different between control and inactivation data. The forearm and upper arm segments varied, on average by 4.9 and 1.2%, respectively. Variation in apparent length was greater for distal segments (metacarpal length, by 11.7%; phalangeal length, by 36.5%) due to forearm supination at the end of the lift phase. In their studies of intersegment torques in cat hind limb, Hoy and Zernicke (1986) accepted variations in apparent length of ±10%.

By examining the videotaped records of the various untrained behaviors and of reaching performance, we selected a subset of experiments for quantitative analysis based on two criteria: the effects were both robust and remained unchanged during the initial one hour postinjection period and the animal remained well motivated and capable of performing ≥150 reaches without long intertrial interruptions during this time period. For each of the experiments selected for quantitative analysis, we compared performance during the period immediately preceding injection with performance during the first hour after injection. We examined stick figures of most unobstructed and obstructed trials (e.g., see Fig. 2) to characterize the form of the defects produced by inactivation. Ensemble averages of time-series data (paw tip and joint speeds; joint angular motions) were computed using a minimum of 15–20 trials for each condition. For the averages, trials were aligned on the time at which wrist speed crossed a threshold of 10% of its peak value on that trial. Onsets and peak values of paw-tip speeds and joint angular velocity were determined for each trial. We constructed a database of movement parameters that contained measured values across sessions and cats (see Table 2). Path, stick figures, and ensemble averages presented in figures are for single representative sessions. Histograms and tabulated findings are for all trials (of a given condition) from sessions quantitatively analyzed. Other analyses are described in Results. Statistical analyses were conducted using the program Statview for the Macintosh computer.

The significance of differences in measured values for control and inactivation conditions (both for individual sessions and across sessions and cats) was tested using unpaired t-tests.

Surgical procedures

After completion of behavioral training, animals were prepared for aseptic surgical implantation of devices for stereotaxic positioning of microelectrodes and the injection cannula and for fixing the head. Before surgery, cats received atropine (0.5 mg/kg im) and a broad-spectrum antibiotic (benzathine penicillin: 300,000 U im). They were sedated with ketamine (20 mg/kg im) and anesthetized with pentobarbital sodium (30 mg/kg iv). Additional doses of pentobarbital sodium (5 mg iv) were administered as needed. During surgery, lactated Ringer solution was administered (80 ml/h iv) and body temperature was maintained at 39° by a heating pad. Animals received Buprenorphine (0.03 mg/kg im) after surgery for analgesia.

Animals were mounted in a stereotaxic head holder (Kopf Instruments), and a craniotomy was made over the cerebellum ipsilateral to the trained limb. We implanted (AP –8.5, ML 4.5, with a 30° posterior angle) a positioner for targeting microelectrodes for electrophysiological identification of the deep nuclei and for directing the cannula for muscimol injections. In four animals we implanted a custom-designed ball-joint positioner that contained a single stainless steel transdural guide tube (711 μm OD), and in one animal, we used a hexagonal grid of seven fixed transdural guide tubes. At the time of cranial implantation, we also implanted a head-fixation clamp that was used during electrophysiological procedures and muscimol injections.

Electrophysiological procedures and reversible inactivation

Sites for nuclear injection were identified by recording the presence of units with large somatic spikes at the appropriate stereotaxic coordinates. At sites in the interpositus nuclei, we recorded unit activity in response to skin contact, movement of limb joints, etc., and evoked muscle contraction and joint movement of the contralateral limbs at threshold currents (<40-μA threshold, 0.5 MΩ etched tungsten electrode, 330-Hz, 45-ms train, 200-μs balanced biphasic pulses). Muscimol (0.25–1.0 μg/μl, in isotonic saline) microinjections were made using a custom-designed cannula (200 μm OD stainless steel hypodermic tubing) (for details, see Martin and Ghez 1993, 1999) connected to a Hamilton microliter syringe with Teflon or polyethylene tubing. For all injections, the microinjection cannula (200 μm OD) was protected by a moveable larger guide tube (457 μm OD). The injection cannula and moveable guide tube were lowered to within 2 mm of the target nucleus. The position of the moveable guide tube was fixed, and the injection cannula was lowered further into the target nucleus.

In most experiments, we used a cannula that was fitted with a microwire (Teflon-insulated 20% platinum-iridium) for recording neurons and for microstimulation at the injection site. Injected volume was checked by measuring movement of the drug-oil meniscus. We mixed Evans blue dye with the aqueous muscimol solution for most injections to facilitate visualizing the drug-oil interface in the injection cannula.

Each inactivation experiment began with 100–150 control (i.e., preinjection) trials. Next, the cat’s head was fixed, and using a hydraulic microdrive, the cannula was lowered to the injection site. After a 4-min wait, the solution was injected during a period of 4–6 min. For AIP and PIP, we typically used 0.25–0.5 μg of muscimol in 1 μl saline; for fastigial and Deiters’ nuclei, we used 0.5 μg of muscimol in 1 μl saline; and for DN, we used 0.75–1.0 μg of muscimol in 1 μl saline. The cannula was left in place after the injection for an additional 4 min to minimize drug spread during withdrawal. The cat’s head then was released from fixation, and behavioral testing resumed. The total delay between the last control trial and the beginning of postinjection testing was 15–20 min; the...
delay between onset of injection and resumption of behavioral testing was no more than 10–12 min. Behavioral effects always lasted much less than 24 h, and injections were never made in the same animal on consecutive days. We observed the behavioral effects of closely spaced injections at the stereotaxic coordinates of the fastigial, anterior and posterior interposed, and dentate nuclei. We made injections adjacent (i.e., within 1–2 mm) to sites where muscimol injections produced behavioral effects (fastigial and interpositus nuclei) to verify the specificity of effects.

**Histological identification of injection sites**

We added a suspension of fluorescent-labeled latex microspheres (rhodamine or fluorescein; LumaFluor, New City, NY) or the dye Evans blue to the muscimol solution as an aid to identifying the center of key injection sites. These agents provided veridical marks of the region of the center of the injection, up to several months after injection. Because diffusion of the microspheres is minimal, the extent of fluorescence does not provide a marker of the extent of drug spread during the inactivation. (See Martin 1991 and Martin and Ghez 1999 for a discussion of drug spread.)

At the conclusion of experiments, we made electrolytic marking lesions (10 μA × 15 s, 0.5 MΩ etched tungsten microelectrode) at selected locations also to confirm histologically the locations of muscimol injections. Cats were administered a lethal dose of pentobarbital sodium (Nembutal) and perfused through the left ventricle with saline followed by 4% paraformaldehyde solution. The brain was blocked, postfixed, and cryoprotected. Frozen sections (40 μm) were cut, either in the same 30° posterior plane of penetrations or parasagittally. Even-numbered sections were mounted unstained for fluorescence microscopy and odd-numbered sections were processed using either Klüver-Barrera or Nissl-staining methods. Injection sites were reconstructed and nuclear boundaries drawn in accord with cytoarchitectonic criteria (Berman and Jones 1982; Bernston et al. 1978).

**RESULTS**

Results reported in this study are based on 56 muscimol microinjections in five cats in the fastigial, anterior interposed, posterior interposed, and dentate nuclei (Table 1). We quantitatively analyzed data from 20 injections (i.e., trial-by-trial kinematic analyses; see METHODS for selection criteria). We also made control muscimol injections at sites in the cerebellar white matter between nuclei (e.g., see Fig. 1A; sites 2 and 4) dorsal to nuclei, in Dieter’s nucleus (ventral to AIP), and intranuclear saline injections. The effects of selective nuclear inactivation were verified to be consistent by making multiple injections in the various nuclei in each cat, and in two cats, we explored the entire region of the deep nuclei with closely spaced injections.

The effects of muscimol injection varied according to the nucleus in which the injection was made. Microinjections in AIP and PIP (Table 1) in all animals reduced the accuracy of limb movements during both spontaneous motor behaviors and in the trained reaching task. Although FN inactivation interfered with postural stability, it did not impair reaching. DN inactivation did not impair reaching; however, we show in the following text that several performance measures changed (see Table 2).

We verified that the effects were due to the actions of muscimol and not the vehicle. Both performance of untrained behaviors and task performance were unchanged after saline injections into AIP (n = 1 injection), PIP (n = 1), and FN (n = 1). Muscimol injection into the white matter between nuclei produced effects associated with the two adjacent nuclei, although the magnitude of the effects were small and delayed compared with intranuclear injections. For example, injection between AIP and FN (tract 2, Fig. 1) produced a FN defect of ipsilateral falling, and ipsilateral loss of contact placing and AIP reaching defects (see following text). Injection between DN and AIP (tract 4, Fig. 1) produced ipsilateral loss of contact placing and AIP reaching defects. White matter muscimol injections >1 mm dorsal to the nuclei had no effect. We first present a qualitative description of the effects of selective inactivation of the deep nuclei. Then we will describe the changes in paw path kinematics in the two reaching tasks.

**General description of effects of nuclear inactivation**

Muscimol injection in FN (Fig. 1A, end of track 1), produced a severe impairment in balance and in head and trunk control. This prevented the animal from walking unsupported. Contact placing was normal during fastigial inactivation (for comparable results, see Chambers and Sprague 1955). When the animal’s body was supported in the hammock, however, reaching and grasping were unimpaired. We did not observe eye position or eye movement impairments during FN inactivation. Muscimol injection into Dieter’s nucleus produced axial torsion and head rotation (ipsilateral ear-up) and nystagmus.

Muscimol injection in AIP (Fig 1, A, end of track 3, B, and C) impaired ipsilateral limb control and abolished contact and proicoceptive placing but did not affect posture and balance, as reported for interposed lesions (Chambers and Sprague 1955). During locomotion, the ipsilateral fore- and hind paws were dragged and misplaced, causing the animal to fall when it attempted to walk on a narrow beam. During reaching, the paw consistently undershot the food well and paw trajectories were highly variable. However, grasping and bringing food to the

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**TABLE 1. Analysis of data from microinjections**

<table>
<thead>
<tr>
<th>Nuclear Injection Sites</th>
<th>No. of Cats</th>
<th>Video observations</th>
<th>Analyzed quantitatively</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior interpositus (AIP)</td>
<td>5</td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td>C1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Posterior interpositus (PIP)</td>
<td>4</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>C2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dentate (DN)</td>
<td>4</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>C2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fastigial (FN)</td>
<td>2</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>C2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

All experiments/injections listed in Table 1 were verified histologically to lie within the nuclei indicated. Control white matter, intranuclear saline, and Dieter’s nucleus injections are not indicated. Experimental sessions for cats C1 and C2 were not subjected to quantitative analysis.
mouth were not impaired (as assessed in close-up images on videotape).

Muscimol injection into PIP (Fig. 1, B and C) also left posture and balance unaffected but impaired forelimb control during locomotion and reaching, though in different ways than with AIP inactivation. With injections in PIP, there was a characteristic “goose-stepping” (Chambers and Sprague 1955) gait in which the ipsilateral paws were lifted abnormally high during swing. Similarly, during reaching the paw appeared to be aimed above the food well, a finding that contrasted with the systematic undershoot with AIP inactivation. Once the animal’s paw entered the food well (see following text), grasping and food withdrawal were normal. Injections in DN (e.g., Figure 1A, site 5), using up to twice the amount of muscimol for the other nuclei, did not affect posture and balance. Reaches were accurate and grasping was normal. The speed of the reach was reduced slightly (see following text and Table 2). Inactivation of AIP, PIP, or DN did not produce noticeable eye-position or -movement impairments.

Although reaching data reported in this study during inactivation were collected within 1 h after cessation of muscimol injection, we continued to assess untrained behaviors and reaching for an additional 4–6 h. In some cases, behavioral abnormalities developed 1–4 h after the injection that were not present during the first hour. These consisted either of typical FN postural instability after interpositus injections, or typical AIP locomotor and paw-placement abnormalities after injections into DN or the white matter. Assuming these late effects to have been due to spread of inactivation, we were able to establish a strong relationship between injection size and the extent of this spread. With 0.5 μg (in 1 μl saline) injections, we never saw spread 1.5 mm from the site of injection, even after 4 h (n = 21 injections). Moreover, spread even as far as 1 mm was very rarely observed earlier than 3 h after the injection (1 of 5 injections). With injections of 1.0 μg, however, spread of 2–4 mm was common in the cerebellum (4 of 7 injections), and no more likely to occur late than early (range 70 min to 4 h).

**Anterior interpositus**

**PATH ANOMALIES.** The inaccuracy resulting from AIP inactivation resulted from both systematic errors and from increased trajectory variability. Systematic errors consisted of path directional biases, increases in path curvature, reduced paw speeds, and loss of distal response components of the reaching synergy. Figure 2A shows the changes in wrist and paw paths in a representative case. The gray traces are control paths before inactivation and demonstrate the typical segmentation of the movement into lift (i.e., where the paw is raised to the height of the target) and thrust (i.e., where the paw is directed forward into the food well, toward the food) phases reported previously (Martin et al. 1995a). During inactivation, wrist and tip paths (black traces) were displaced downward and forward. These biases led the paw to collide with the underside of the food well rather than entering its opening. The cat then retracted its paw along the underside of the well and then thrust it into the opening. These effects were present from the first trial examined after muscimol injection until the end of the experimental session.

AIP inactivation resulted in consistent reductions in peak wrist height and anterior displacement of the wrist paths during individual experiments. These changes were significant across all sessions quantitatively analyzed (for a standard height of 14 cm, reduction in peak wrist height, P < 0.0001; Fig. 3A1; anterior displacement of the wrist path, P < 0.0001; Fig. 3B1). Inactivation also resulted in increases in the variability of these measures (Fig. 3, A and B, right). We also noted a small but consistent downward convexity (i.e., bowing) to both wrist and tip paths, although this effect is modest for the wrist paths illustrated in Fig. 2A. We quantified this effect by computing a measure of path curvature (the difference in path directions from the peak speed and the peak wrist height) for preinjection and postinjection trials. This effect was significant for both wrist and tip (Fig. 3C).
SLOWING OF TRANSPORT AND REDUCED STEREOTYPY. During inactivation, wrist speeds remain bell-shaped, as in control trials, during most of lift phase (Fig. 4A1, thin line for controls, thick for AIP inactivation). There was, however, an immediate slowing of the movement (21% reduction peak wrist speed; Table 2; P < 0.001) and a prolongation in the final deceleration at the end of lift (Fig. 4A1).

With AIP inactivation, movements immediately became more variable. This was evident as an increase in the coefficient of variability of all temporal response measures (wrist speed, transport time from movement onset to target entrance, time to peak wrist speed, and time to peak wrist speed as a percent of the transport time; Table 2). AIP inactivation also increased spatial variability (Fig. 4A, 2 and 3). We plotted the locus at which the peak wrist speed is attained directly on the wrist path (gray circles). Although in controls these points are clustered tightly in space about one-third of the distance to the target (Fig. 4A2), during inactivation these loci are substantially more variable (e.g., Fig. 4A3).

LOSS OF DISTAL RESPONSE COMPONENTS. In our earlier study (Martin et al. 1995a), we showed that during normal reaching the metacarpal-phalangeal (MCP) joint undergoes a complex double flexion movement. Ensemble average of the angular velocity has two speed peaks (Fig. 5A, gray trace and arrows). Figure 5B plots wrist speed directly on MCP joint paths (as an inverted gray scale, with maximal velocity white and zero velocity, black). The two responses correspond to the regions of white pixels (i.e., maximal velocity). The initial peak occurs at toe-off (lower gray arrow, B) and we suggested (Martin et al. 1995a) that it results from elastic restoring forces as the hyperextended joint is unloaded. The later flexor peak (upper gray arrows, B) occurs during the lift as the paw approaches the target and prepares to clear the lower lip of the food-well. Although the initial response at toe-off is unchanged during AIP inactivation (Fig. 5A, black lines; B2, arrow), the second response is abolished. This differential effect on the second response is consistent with being the only one subject to active control by the animal.

OBSTACLE AVOIDANCE: IMPAIRED TRAJECTORY REPROGRAMMING. MCP flexion occurs when the paw is close to the edge of the food well so as to avoid contact. This suggests that the point in space where this response occurs is regulated according to the anticipated location of an obstacle. The loss of this response therefore could reflect a generalized impairment in adapting the paw path to avoid contact with the edge of the food well. Alternatively, as suggested by AIP inactivation in the monkey (Mason et al. 1998), this could reflect AIP’s role in distal control. To distinguish these alternatives, we examined trajectory adaptation to avoid an obstacle (horizontal bar) placed in the limb’s path, which requires reprogramming at both the wrist and shoulder (Cooper 1997; Martin and Ghez 1993).

On unexpectedly contacting the obstacle, the normal animal makes an immediate feedback correction to reach over it by increasing shoulder extension and wrist flexion. On the next and on all subsequent obstructed reaches (Fig. 6A2, black stick figure), shoulder extension and wrist flexion increase phasically during the initial phase of the movement in anticipation of contact (Fig. 6A2) (see also Martin and Ghez 1993). Before adaptation the wrist and tip paths initially are directed toward the target (see Fig. 6A1, gray and black/white and black arrows), but after adaptation, these paths initially are directed away from the target (Fig. 6A2, black arrow) to reach around the obstacle. During AIP inactivation, the animal did not make the appropriate anticipatory adjustments to avoid obstacle contact (Fig. 6B). Instead, it repeatedly reached directly toward the target as if the bar were not present. Compare the gray/black arrow in Fig. 6B1 with the black arrow in Fig. 6B2. The initial wrist and tip paths for all unobstructed and obstructed reaches are the same. The anticipatory increase in shoulder extension that normally accompanies trajectory adaptation to reach over the obstacle failed to occur. After contact with the obstacle, however, animals were able to extend the shoulder and flex the wrist to avoid the bar effectively (shoulder extensor angle during control and inactivation = 198 ± 1.2 and 197 ± 1.5°, NS, mean ± SE; wrist flexor angle during control and inactivation = 120 ± 1.1 and 115 ± 1.4°, P < 0.01—inactivation more flexed than controls). (Although the paw was dragged over the obstacle after contact on the 2nd obstructed trial, in many trials there was as much limb withdrawal after contact as on the 1st obstructed reach in the noninactivated condition.) Thus the animal could increase shoulder extension and wrist flexion after contact, and thus the failure of the animal to produce an anticipatory or adaptive trajectory adjustment to

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**Table 2. Summary of kinematic changes produced by inactivations**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AIP Preinjection</th>
<th>Postinjection</th>
<th>PIP</th>
<th>Preinjection</th>
<th>Postinjection</th>
<th>DN</th>
<th>Preinjection</th>
<th>Postinjection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wrist speed COV</td>
<td>1.48 ± 0.25</td>
<td>1.18 ± 0.27*</td>
<td>1.13 ± 0.26</td>
<td>1.31 ± 0.29*</td>
<td>1.24 ± 0.21</td>
<td>1.11 ± 0.25*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transport time COV</td>
<td>0.17</td>
<td>0.23</td>
<td>0.23</td>
<td>0.22</td>
<td>0.17</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time-to-peak wrist speed COV</td>
<td>169 ± 39</td>
<td>245 ± 82*</td>
<td>208 ± 47</td>
<td>205 ± 62</td>
<td>179 ± 23</td>
<td>198 ± 38*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COV</td>
<td>0.23</td>
<td>0.33</td>
<td>0.23</td>
<td>0.30</td>
<td>0.13</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTP wrist speed as % transport time COV</td>
<td>79 ± 14</td>
<td>102 ± 32*</td>
<td>80 ± 26</td>
<td>82 ± 21</td>
<td>94 ± 13</td>
<td>99 ± 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COV</td>
<td>0.18</td>
<td>0.31</td>
<td>0.33</td>
<td>0.26</td>
<td>0.14</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Wrist speed corresponds to the measured peak in the wrist speed; transport time is between the onset of wrist motion (10% of peak speed) and entry of the paw into the food well; time-to-peak (TTP) wrist speed corresponds to the interval between wrist speed onset and peak; TTP wrist speed as percent transport time is the interval between wrist onset and peak as a percent of the interval between wrist onset and food-well entry. The number of trials used for these analyses were AIP, 146 preinjection, 200 postinjection; PIP, 152 preinjection, 178 postinjection; DN, 269 preinjection, 272 postinjection. * P < 0.0001; ** P < 0.01; *** P < 0.02.
avoid the obstacle cannot be attributed to an inability to produce the relevant joint motions.

We estimated the degree of adaptation to the obstacle from the differences in the initial directions of the paw tip path before and after introduction of the obstacle. Here, initial direction was taken as the direction of a straight line originating at the paw tip location at the onset of movement and extending to its location in the frame just before impact of the forearm with the bar. There was a significance increase in the mean initial paw tip direction, from $62 \pm 1.2^\circ$ (mean $\pm$ SE) for control unobstructed reaches to $86 \pm 1.9^\circ$ for the trials adapted to reach over the obstacle ($P < 0.0001$). Figure 7 shows the differences between the initial direction of unobstructed and obstructed reaches for controls and during inactivation of the different deep nuclei. Initial directions of unobstructed and obstructed reaches were not significantly different during AIP inactivation ($67.5 \pm 3$ vs. $70.3 \pm 4^\circ$).

**Posterior interpositus**

Reaches also became inaccurate and more variable with PIP inactivation (e.g., posterior site in Fig. 1, B and C). However, the systematic errors were different from AIP inactivation. PIP inactivation produced an initial posterior bias followed by overshooting the target (Fig. 2B) and bumping into the top of the food well. All trials were wildly ataxic as the limb approached the target. Success in placing the paw into the food well was achieved only after several corrective adjustments (Fig. 2B2, thin lines). Histograms in Fig. 3 show trajectory changes across all animals. The increased wrist height (Fig.
3A1) was significant (P < 0.0001) as was the posterior path bias (P < 0.0001; Fig. 3B1). Variability also was increased (Fig. 3, A2 and B2). We noted a consistently larger posterior bias of the tip than wrist path (see Fig. 2B). As seen in Fig. 2B2, during PIP inactivation, wrist path curvature increased only slightly during lift, whereas paw tip paths became highly concave due to increased wrist (and MCP) flexion. As with AIP, these changes were present during the first postinjection trial and lasted until the end of the session. The effects of PIP inactivation on movement speed and transport time were smaller than those produced by AIP inactivation (Fig. 4B1). On average, small increases in wrist speed were produced (Table 2). Variability in wrist speed and in the durations of different movement components also did not change consistently (see Table 2). The most prominent effect was an increase in the spatial dispersion of the locus of the peak wrist speed (Fig. 4B3). Thus AIP and PIP inactivation both produced increased trajectory variability while producing different trajectory biases.

Despite the impairments in trajectory control during unobstructed reaching, the animal’s ability to adapt its limb trajectory to reach over the obstacle was preserved. As shown in Fig. 8A, the animal contacted the obstacle minimally on the first obstructed reach (gray stick figure), a reflection of the large posterior tip path bias produced by PIP inactivation (e.g., Fig. 3B1). On the second obstructed reach (black stick figure) and all subsequent obstructed reaches, the initial direction of paw movement was increased further (Fig. 8A; see arrows) as was the safety margin to clear the bar. The mean angle of inclination of the paw path for unobstructed and obstructed reaches for all experiments was 79.4 ± 2° and 100 ± 3°, respectively. This difference was significant (Fig. 7; P < 0.0001) and similar to that seen during preinjection control reaches.

**Dentate nucleus**

In contrast to AIP and PIP, DN inactivation (e.g., Fig. 1A, 5) did not impair performance: the animals continued to reach accurately to the target, to grasp the meat, and to withdraw it from the food well effectively. More quantitative assessments,
however, did reveal a small posterior bias in the paw paths (Fig. 2C) and reduction in movement amplitude; only the former was significant ($P < 0.05$) and was more consistent across animals (see Fig. 3B1). Neither wrist path variability nor curvature were increased (Fig. 3). However, there was a slight but significant increase in tip path curvature that was consistent across sessions (Fig. 3C2). DN inactivation produced a small reduction in wrist speed, a corresponding increase in transport time (Table 2; $P < 0.02$) and a small increase in the spatial dispersion of peak wrist speeds (Fig. 4C). It is important to note that none of these kinematic changes impaired performance.

DN inactivation did not impair trajectory adaptation to reach around the obstacle (Fig. 8B). Both the stick figure and paths were indistinguishable before and during inactivation. Moreover, the angle of inclination of the paw paths were significantly different during DN inactivation for unobstructed ($56 \pm 1.5^\circ$) and obstructed ($67 \pm 1.7^\circ$) reaches ($P < 0.0001$; see Fig. 7). It should be noted that for the DN experiments, the obstacle was located slightly farther from initial forelimb position and the animals contacted the bar slightly closer to the paw (see stick figure in Fig. 8B). The effect of this placement was that less limb withdrawal was required to reach over the obstacle.

**DISCUSSION**

There are two principal findings of this study. First, inactivation of AIP and PIP both decreased the accuracy of reaching movements through distinctive biases in distal paths. AIP inactivation consistently produced underreaching and PIP inactivation produced overreaching. Although these results do not necessarily reveal the specific functions of AIP and PIP, the differences in path biases produced by inactivation are likely to reflect differences in the contributions these two nuclei make to trajectory control. The second major finding was that inactivation of AIP, but not PIP, interfered with the animal’s ability to alter adaptively the trajectory to avoid contacting obstacles. During AIP inactivation, animals could not make the necessary kinematic adjustments in anticipation of contact with an obstruction but relied on contact to evoke a corrective response. Inactivation impaired both the animals’ ability to increase shoulder extension and wrist flexion to reach over the obstacle, in the beginning of the reach, as well as to produce MCP flexion to avoid contacting the food well near the end of the reach. This adaptation defect, affecting both proximal and distal response components, is consistent with a generalized impairment in planning movements in extrinsic space.

The effects produced by inactivation were seen on the first
A selective inactivation of AIP and PIP produced different path biases. The impairments Milak and colleagues reported during IP inactivation in the cat (Milak et al. 1997)—increases in maximal reach height and wrist speed, as well as terminal ataxia—are similar to what we report for selective PIP injection. Their injection sites were located between the two nuclei, but the presence of overreaching suggests that they preferentially inactivated PIP. Our finding that selective AIP and PIP inactivations produced distinct kinematic impairments helps to reconcile some of the differences reported by others for generalized interpositus nucleus inactivation. For example, hypometria and slowing (Uno et al. 1973) could reflect preferential AIP cooling, whereas increased speed (Miall et al. 1987) could reflect inactivation of PIP.

We found that inactivation of AIP and PIP both increased the variability of temporal and spatial response measures, although the changes produced by AIP inactivation were more robust. These changes are likely to reflect interjoint coordination defects (Ghez et al. 1996; Martin et al. 1995a). This was especially evident for the increased spatial dispersion of the occurrence of the peak wrist speed on wrist paths. One effect of this increased variability was to make joint excursions and the limb’s position less predictable during movement, thereby minimizing the efficacy of corrective adjustments. Mason and colleagues (1998) also found that inactivation within anterior and posterior sectors of IP (and adjoining parts of dentate) in the monkey both increased endpoint errors, but the effect was more prominent for posterior inactivation sites. Inactivation of anterior sites, by contrast, preferentially degraded distal manipulative skills (Mason et al. 1998). Although our finding of a deficit in controlling the MCP with AIP inactivation appears to be an effect of inactivating a distal somatotopic zone (Thach et al. 1992), our finding that the same inactivation also impaired obstacle avoidance (through proximal and distal limb impairments) indicates a generalized role in adaptive control and in planning in extrinsic space rather than specialization for the distal extremity.

Inactivation of FN and DN did not impair reaching in the tasks we examined. Impairments in reaching during FN inactivation were reported by Milak and colleagues (1997), although of a more modest nature compared with those produced by IP inactivation. In that study, 800 ng (in 1 μL) of muscimol was used to inactivate the nucleus. We find that doses >500 ng results in spread of inactivation to adjacent nuclei within 1 h. Thus the small effect seen by Milak and colleagues (1997) could have resulted from involvement of the adjacent interpositus nuclei, where combined AIP and PIP inactivation could wash out trajectory biases leaving only increased trajectory variability. We found that DN inactivation produced a significant slowing of the movements but did not degrade performance. This modest effect, which could be due to nonspecific disfacilitation of thalamocortical motor projections, was produced using muscimol concentrations that were two to four times greater than those used for AIP and PIP. Our result that dentate inactivation had such modest effects on reaching is consistent with other published studies in cats (Chambers and Sprague 1955; Gorassini et al. 1993; Milak et al. 1997).

Kitazawa et al. (1993) reported that combined lesion of the interpositus and dentate nuclei produce hypometria and increase the integrated rate of change of acceleration (jerk). Such changes are more likely due to damage to the anterior interpositus than the dentate nucleus. The more consistent (and robust) impairments produced by DN inactivation in monkey (Beaubaton and Trouche 1982; Trouche and Beaubaton 1980) could reflect the more complex tasks these animals perform that often rely on learned arbitrary associations. The sensory information that the DN receives from association cortex (Stein and Glickstein 1992), reflecting, for example, working memory
or behavioral context, may not be important for performance in the tasks used to examine the DN in the cat.

Inactivation of AIP and PIP produced distinctive effects on feed forward control

The form of the defects produced by selective inactivation of AIP and PIP, in relation to the connectivity of the nuclei, may provide insight into the functions normally served by these nuclei. We propose that the trajectory defects reflect impairments in the use of cutaneous and proprioceptive information, by AIP and PIP, respectively, in programming movements.

AIP receives its climbing fiber input from the rostral dorsal accessory olivary nucleus, which transmits predominantly cutaneous information (Gellman et al. 1983). In the obstructed reaching task, animals normally integrate cutaneous information from the site on the limb of obstacle contact with the time of contact during movement, to reprogram effectively the trajectory of the next reach. Deep inputs, by contrast, are less well suited for this function because of the coarser spatial resolution provided by this submodality (i.e., limb segment or joint vs. a specific skin site). A climbing fiber discharge from the dorsal accessory olive could provide a signal for trajectory reprogramming.

The rostral medial accessory olivary nucleus provides the climbing fiber input to PIP and transmits predominantly proprioceptive information (Gellman et al. 1983). The form of the trajectory defect produced by PIP inactivation, with wide spatial deviations in distal paths, is remarkably similar to that seen in patients deprived of limb proprioceptive information (Sainberg et al. 1995). These patients show characteristic interjoint coordination defects that result in increased systematic and variable errors. Such proprioceptive climbing fiber inputs could provide critical timing signals for interjoint coordination.

The form of the defects produced by AIP and PIP inactivation is also consistent with a selective loss of anticipatory rather than feedback control (Vilis and Hore 1980). Normally, the animal chooses between one of two trajectories depending on the presence or absence of the obstruction (at a particular location). This choice occurs rapidly between contact and the second obstructed reach. This rapid switch probably reflects the animals extensive experience in the obstructed reaching task (Martin and Ghez 1993). During AIP inactivation, the...
animals failed to produce the adaptive trajectory in anticipation of obstacle contact. However, they were able to produce an appropriate corrective withdrawal response to obstacle contact. Limb withdrawal after contact resembles the stumbling-corrective reaction (Forssberg 1979), where cutaneous input from the limb (in response to striking an obstruction) triggers increased limb flexion during the swing phase of locomotion.

During PIP inactivation animals could adapt the trajectory to reach over an obstacle but were not able to adaptively reprogram the trajectory to prevent overreaching. Although obstacle avoidance could rely on preserved (cutaneous) feedforward control by the intact AIP, reach height control is apt to depend on feedforward visual (and proprioceptive) guidance. Normal animals readily adjust forelimb flexor and extensor responses to reach accurately to targets of different heights (Ghez et al. 1996; Martin et al. 1995a). Our findings suggest that PIP could play a key role in the visuomotor transformations for accurate reaching.

Comparison of the effects of inactivating the deep nuclei with motor cortex inactivation

Impairments produced by inactivation of different zones within the forelimb representation of motor cortex (Martin and Ghez 1993) are similar to the distinctive effects produced by AIP and PIP blockade. Rostrolateral motor cortex inactivation...
(RL-MCx; Fig. 9, inset, · · ·) produces overreaching, similar to PIP inactivation, and a trajectory adaptation defect, similar to AIP inactivation. Inactivation of adjacent medial sites in rostral motor cortex (just medial to the lateral tip of the cruciate sulcus; RM-MCx, Fig. 9, —) produces under reaching and increases in paw trajectory curvature (bowing), similar to the particular bias and trajectory defects produced by AIP inactivation. The presence of similarities in the form of defects produced by inactivation of the cerebellar nuclei and motor cortex is consistent with the hypothesis that there are distinct functional cerebellar output channels that link cell groups in the AIP and PIP with interconnected downstream (i.e., motor cortex and red nucleus) structures. This is similar to the output channels proposed by Middleton and Strick (1998) for dorsal, lateral, and ventral DN in the monkey.

Electrophysiological studies in the cat suggest that PIP, AIP, and DN influence separate, but overlapping, populations of neurons in the lateral, intermediate, and medial parts of rostral motor cortex (Fig. 9) (Jörntell and Ekerot 1999; Rispal-Padel and Latreille 1974; Shinoda et al. 1985). AIP stimulation produces short-latency excitatory responses that are maximal in an intermediate portion of rostral motor cortex (near the lateral margin of the cruciate sulcus), flanked laterally by the PIP projection zone, and medially by the DN projection zone (see Fig. 9). Jörntell and Ekerot (1999) recently have shown that within the AIP receiving zone in motor cortex, there is a refined topographic order: AIP neurons receiving cutaneous input from the distal limb activate a more lateral portion of the zone than neurons receiving input from the proximal portion of the arm. Focal motor cortical inactivation not only dissociates the effects of AIP from PIP inactivation but also the compound effects of inactivating AIP (i.e., trajectory biases and adaptation defect). This is consistent with the hypothesis that there are separate functional output channels from the intermediate cerebellum for distinct aspects of trajectory formation and control and for trajectory adaptation.

We thank G. Johnson for construction of apparatus and M. Anwar for histology. This research was supported by grants from the National Institute of Neurological Disorders and Stroke (NS-31391) to C. Ghez and the National Science Foundation (1BN-9421582) to J. Martin.

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Received 30 August 1999; accepted in final form 18 November 1999.

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