The evidence for a significant cerebellar contribution to cognition has been growing rapidly over recent years. Although the cerebellum has traditionally been labeled a fine-tuning mechanism of motor planning and execution, it has also been shown to be intimately involved in nonmotor processes. Its role in associative learning, including classical conditioning of discrete behavioral responses (McCormick, Clark, Lavond, & Thompson, 1982; McCormick & Thompson, 1984a, 1984b; Swain & Thompson, 1993), has been well established. Cerebellar involvement in “higher order” processes has become more probable with the expansion of the contributing research literature. Studies involving human subjects have suggested an important role of the cerebellum in cognitive processes such as mental imagery (Mell et al., 1996; Parsons et al., 1995; Ryding, Decety, Sjohom, Sternberg, & Ingvar, 1993), linguistic processing (Leiner, Leiner, & Dow, 1993; Silberi, Leggio, & Molinari, 1994; Van Dongen, Catsman-Berrevoets, & Van Mourik, 1994), and executive functions (Botez-Marquard, Elie, & Attig, 1989; Bracke-Tolkmitt et al., 1989; Grafman et al., 1992), to name but a few.

Spatial abilities have been a particular focus of recent cerebellar research. Neuropsychological studies have revealed that patients with cerebellar lesions or atrophy display behavioral deficits in spatial learning (Botez et al., 1989; Botez-Marquard & Botez, 1993; Botez-Marquard & Routhier, 1995; Fehrenbach, Wallesch, & Claus, 1984; Kish et al., 1988). In the rat, cerebellar lesions have been shown to disrupt performance in visuospatial learning on the Morris water maze (MWM; Ghandi, Kelly, Wiley, & Walsh, 2000; Petrosini, Leggio, & Molinari, 1998; Petrosini, Molinari, & Dell’Anna, 1996). Of particular note, Joyal et al. (1996) demonstrated that lateral, but not midline, cerebellar lesions significantly impaired the rats’ ability to locate the invisible platform without producing a concurrent deficit in visible platform learning. The results suggest an important role of the lateral deep nuclei in visuospatial learning that is independent of visuomotor skills. A follow-up study (Joyal, Strazielle, & Lalonde, 2001) showed that focal lesions of the cerebellar dentate nuclei, alone, could produce the same general deficit in visuospatial processing.

The severity of the visuospatial deficits produced by cerebellar lesions lends itself to a number of empirical questions, some of which have already been experimentally challenged. (a) What are the crucial cerebellar regions involved in the resulting cognitive impairment? Joyal et al. (2001) suggested that the dentate nuclei, which receive input from gamma-aminobutyric acid (GABA)-ergic Purkinje cells of the lateral cerebellar cortex, are of primary importance in mediating visuospatial processing. (b) What, if any, efferent pathways that serve as physiological substrates of visuo-spatial learning are disrupted by the cerebellar lesions? Several brain regions are known to be of pivotal importance in visuospatial learning and have some degree of connectivity with the cerebellum. The posterior parietal cortex, for example, receives cerebellar input via the ventrolateral and intralaminar thalamic nuclei (Schmahmann & Pandya, 1990, 1997). A more provocative possibility lies in the cerebellar–prefrontal cortical pathway, the significance of which is still being explored (Middleton & Strick, 1994, 1997, 2001). (c) What degree of plasticity remains following damage to the lateral cerebellum? The answer to this question speaks largely to rehabilitative potential following cerebellar trauma.

The purpose of the present study was to investigate the effects of focal lateral cerebellar lesions on visuospatial learning and to assess the degree to which the resulting impairment could be reversed with pretraining on a spatial working memory task. Visuospatial learning is a complex process involving multiple brain regions and correspondingly diverse cognitive components, including allocentric mapping, spatial memory, and working memory. Lesions of distinct brain regions, including the hippocampus (Kolb, Macintosh, Whishaw, & Sutherland, 1984; Morris, Garrud, Rawlins, & O’Keefe, 1982) and parietal cortex (Crowne, Notovny, Maier, & Vitols, 1992; Dimattia & Kesner, 1988; Kolb, MceWane, McDonald, & Sutherland, 1994; Kolb, Sutherland, & Whishaw, 1983), produce deficits in spatial learning. Evidence for

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Following bilateral lesions targeting lateral deep cerebellar nuclei, rats were subjected to a bridge test as a measure of visuomotor coordination and were trained on the Morris water maze (MWM) as a measure of visuospatial processing. Lesioned rats were significantly impaired in visuospatial processing, but not visuomotor coordination, relative to sham rats. In a 2nd experiment, rats were pretrained on a delayed spatial alternation task (T maze) before MWM training. Pretraining reversed the visuospatial deficit caused by the lesions as compared with nonpretrained rats. Results suggest that lateral deep cerebellar nuclei contribute to visuospatial processing with a negligible contribution to visuomotor skills and that visuospatial deficits resulting from lateral nuclei damage can be reversed with pretraining on a spatial working memory task.
cerebellar contributions to visuospatial learning suggests a network of neural involvement that extends to the cerebellum. In the present study, rats were trained on a spatial working memory task following cerebellar lesion and prior to visuospatial assessment. Delayed spatial alternation is a spatial working memory task that is highly sensitive to brain lesions that also affect MWM performance. Just as lesions of any one structure within the network could impair visuospatial processing, postlesion pretraining could potentially strengthen the contributions of remaining brain regions or perhaps revitalize surviving pathways projecting to and from the lesioned area.

This project was divided into two separate experiments. The first experiment examined the effect of focal lesions of the lateral deep cerebellar nuclei on visuospatial learning and visuomotor coordination. The conclusion of the experiment coincided with the publication of the Joyal et al. (2001) project and is, in part, a replication of their important study. The second experiment was designed to expand on the results of the first by exploring the degree to which pretraining on a spatial working memory task could reverse the visuospatial deficits resulting from the aforementioned lesions. Rats were trained to criterion on a delayed spatial alternation task prior to visuospatial assessment. Thus, any improvement in the functions of the cerebellar dentate nuclei is likely to improve the performance on the visuomotor tasks; that is, the bridge test and the visible platform condition of the MWM. If, however, the contribution is predominantly cognitive, impairments should be reflected in the rats’ performance on the T-maze and invisible platform condition of the MWM, but not in the visuomotor domain. Invisible platform learning is particularly dependent on nonmotor processes such as memory, allocentric mapping, and navigational strategy. The delayed spatial alternation task was selected as the pretraining condition because of its relatively negligible motor component. The demands of the T-maze task are largely dependent on spatial working memory and do not require the extensive motor contribution of maze swimming. Thus, any rehabilitative effect of the pretraining could be attributed to the learning component more so than exercise.

Method

Experiment 1

Subjects. Subjects were 13 male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing between 250 and 300 g. The rats were housed individually in a colony room with an ambient temperature of 20 °C and a 12-hr light–dark cycle (lights on at 0700). Standard laboratory chow and water were available ad libitum.

Surgery. The rats were anesthetized with an intraperitoneal injection of 30 mg/kg sodium pentobarbital and placed in a stereotoxic apparatus (Stoelting, Wood Dale, IL). The head was shaved and an incision made, exposing both bregma and lambda. Holes were drilled above the cerebellar lateral nuclei, and coordinates were measured from the bregma (Paxinos & Watson, 1998; AP = −11.5, −12.5; L = ±3.6; V = −7.0). Coated electrodes, with 300–500-μm exposed tips, were lowered to the appropriate depth for 1 min prior to lesion in order to accommodate tissue compression. An electrolytic current was applied (2 mA for 1 min) at each coordinate. Following lesions, the electrodes were removed and the skull cavities were filled with bone wax. The skin was sutured, and the rats were allowed to recover on a heating pad until they awoke. They were then allowed to heal for 3 weeks prior to behavioral testing. Sham-operated rats received the same surgical procedure without electrode insertion. Thus, sham rats received no lesions of any kind. The final distribution of rats was as follows: lesioned, n = 6; sham, n = 7.

Bridge test. The bridge test was slightly modified from the procedure of Joyal et al. (1996). The rats were placed in the center of a square wooden beam (length = 86 cm, width = 1.3 cm, thickness = 1.3 cm) marked with 2.5-cm segments. The beam was elevated to a height of 50 cm above a soft cushion. A piece of cardboard (21 × 26 cm) was placed at each end of the beam to prevent escape. Three separate parameters were measured as an assessment of gross psychomotor skills (60-s cutoff point): (a) number of segments traversed (distance), (b) time spent with all four paws on the beam (equilibrium), and (c) time spent on the beam before falling (fall latency). Two observers took measurements over a 5-day period with two trials per day.

MWM. The MWM protocol was adapted from Joyal et al. (1996). The rats were placed in a circular pool (diameter, 184 cm) with white inside walls (height, 36 cm). The pool was filled with water (depth, 19 cm; temperature, approximately 24 °C) covered by Styrofoam pieces (approximately 3 × 2 cm) hiding an escape platform placed 3 cm below the surface of the water (invisible platform condition) or with the platform elevated 1.5 cm above the water surface without the Styrofoam pieces (visible platform condition). Numerous external visual cues were present surrounding the pool, which was divided into four quadrants (NE, SE, SW, and NW). Two experimenters conducted each trial, remaining at the same location next to the pool each time.

The rats were placed in the water with their snouts close to and facing the midpoint section of each wall labeled N, E, S, and W. The rats were allowed to swim freely until finding the platform, where they were allowed to remain for 5 s. If the rat failed to find the platform within a 60-s time frame, the experimenter placed the rat on the platform for the 5-s interval. The rats received six trials per day. The starting position for each trial was rotated, beginning with the N position and proceeding clockwise (i.e., N, E, S, W, N, E). The intertrial interval was approximately 2 min, during which time the rat was placed in its home cage next to a space heater. For the first 3 days of testing, the submerged platform was placed in the NW quadrant of the pool, then in the SE quadrant for the following 2 days. After Day 5, the rat was allowed to rest for 7 days at which time it was retested with the platform in the same location as on Day 5. One day later, the rat was retested in the visible platform condition (Styrofoam removed, platform elevated slightly above water level in SW quadrant).

Cerebellar histology and lesion verification. Following completion of the experiment, the rats were given a lethal dose of pentobarbital and were perfused transcardially with 4% paraformaldehyde. Their brains were removed and stored in 4% paraformaldehyde for approximately 48 hr and then transferred into 30% sucrose until saturated. Following fixation, the cerebellum of each rat was sliced into 52-μm coronal sections, mounted onto slides, and allowed to dry overnight. Slides were stained with Cresyl violet and Prussian blue.

Lesion placements were verified by using a light microscope to identify approximate anterior–posterior lesion location of each section as compared with template images (Paxinos & Watson, 1998). Each section was projected onto photocopies of template images (sequential coronal sections of cerebellum) by using an image enlarger (Bogen Photo Corp., Englewood, NJ). Each lesion was traced onto the photocopied images, allowing visualization of each hemispheric lesion in its entirety. The extent of damage to the dentate nuclei was quantified by first creating a transparent overlay of a dot matrix. The matrix was placed over the top of each lesion schematic. The number of pixels was counted within the nuclei and within the traced lesions, allowing for quantification of dentate destruction. The percentage of ablation of the dentate nuclei was averaged across rats, providing a fairly accurate estimate of lesion accuracy.
Statistical analyses. Three different dependent variables were analyzed with the bridge test: (a) latency (seconds to fall), (b) equilibrium (seconds with all four paws on beam), and (c) distance (number of segments traversed). All bridge test analyses were performed using a two-factor analysis of variance (ANOVA) in a 2 × 5 mixed design, with surgical treatment (sham vs. lesion) and day (1–5) as the independent variables. Three different dependent variables were analyzed with the hidden platform condition of the MWM: (a) latency (number of seconds to find the platform), (b) search strategy (number of quadrants traversed), and (c) search success (number of times the rats successfully found the platform). Data collected from the invisible platform condition were subjected to a two-factor ANOVA in a 2 × 5 mixed design, with surgical treatment (sham vs. lesion) and day (1–5) as the independent variables. A comparison of performance on Days 5 and 13 was analyzed separately as a measure of information retention. Data from the Days 5 and 13 comparison were subjected to a one-way between-groups ANOVA, with surgical condition as the between-groups variable and training day as the within-group variable. The Scheffe test was utilized for post hoc comparisons. Search success was measured by counting the number of times rats successfully located the hidden platform within the 60-s time frame averaged across the six trials each day. Success data were subjected to a one-way between-groups ANOVA, with surgical condition as the between-groups variable. Data from the visible platform condition were subjected to a one-way between-groups ANOVA because data were collected on only one day for both lesioned and sham rats. Surgical condition was the between-groups variable.

Experiment 2

Subjects. Subjects were 53 male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing between 350 and 400 grams. Rats were necessarily larger than the first experiment because of the unavailability of younger rats. They were housed individually in a colony room with an ambient temperature of 20 °C and a 12-hr light–dark cycle (lights on at 0700). Standard laboratory chow and water were available ad libitum prior to training. Rats were randomly assigned to one of eight groups according to the surgical condition and behavioral training regimen. Experimental groups and corresponding sample sizes are listed in Table 1. Some of the rats were not exposed to the MWM because they were initially included to balance the experimental design for subsequent neurochemical analyses (data not included in this study). They were, however, included in the T-maze analysis.

Surgery. The rats underwent surgery as described in Experiment 1, except that the rats were anesthetized with halothane gas rather than sodium pentobarbital as in Experiment 1. They were anesthetized with an induction dosage of 4% halothane in oxygen (4 L/min), which was adjusted to a steady maintenance flow of 2% halothane (250–300 ml/min) for the duration of the surgery. Prior to awakening, the rats were administered 100% oxygen for several minutes. Behavioral training began 3 weeks postsurgery.

<table>
<thead>
<tr>
<th>Subgroup number and size (n)</th>
<th>Surgical condition</th>
<th>Pretraining condition</th>
<th>Water maze</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (7)</td>
<td>Lesion</td>
<td>T-maze trained</td>
<td>Yes</td>
</tr>
<tr>
<td>2 (7)</td>
<td>Sham</td>
<td>T-maze trained</td>
<td>Yes</td>
</tr>
<tr>
<td>3 (6)</td>
<td>Lesion</td>
<td>T-maze control</td>
<td>Yes</td>
</tr>
<tr>
<td>4 (5)</td>
<td>Sham</td>
<td>T-maze control</td>
<td>Yes</td>
</tr>
<tr>
<td>5 (7)</td>
<td>Lesion</td>
<td>Sedentary control</td>
<td>Yes</td>
</tr>
<tr>
<td>6 (7)</td>
<td>Sham</td>
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</tr>
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<td>7 (7)</td>
<td>Lesion</td>
<td>T-maze trained</td>
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</tr>
<tr>
<td>8 (7)</td>
<td>Sham</td>
<td>T-maze trained</td>
<td>No</td>
</tr>
</tbody>
</table>

T-maze training. The T-maze procedure was based on a modified protocol of Verma and Moghaddam (1996). The T maze was constructed from Plexiglas. Walls were 30 cm high, and the width of the alleys was 15 cm. The length of the main alley was 50 cm, and the length of the side alleys was 40 cm. Side alleys were opened and closed with movable, transparent guillotine doors. At the end of each side alley, a 2-cm high barrier concealed the food reward from view. A third movable door was mounted in the main alley, providing a start box. The inside of the T maze was painted black in order to reduce reflection. The T maze was cleaned with a 10% ethanol solution between the rats, but not between trials. The food reward was a single Froot Loop cereal piece.

The training procedure for delayed spatial alternation is as follows. The rats were handled for 1 week before exposure to the T maze. On 2 consecutive days, each rat was allowed to explore the maze with all doors raised for 10 min, at which time the food reward was present in both arms. After these 2 days, the rats were partially food deprived (20 g of food per day). After 2 days of food deprivation, the adaptation process was continued by placing the rat in each side alley with the doors closed and with the food reward present at the end of the alley as well as behind the barrier for 5 min. The rat was then placed in the opposite arm, which also contained the food reward, for 5 min. This adaptation was continued for 2 days.

Next, the actual training was initiated. Each rat received 10 trials per day. During the first trial of each day, both arms were baited. During the next 9 trials, the arm opposite the one the rat entered on the previous trial was baited, except when it went to the nonbaited arm on the previous trial. In that case, the food reward was left in place. Thus, the baited side was changed only after the rat had alternated alleys. After the rat entered either arm, the door was closed behind it. After 10 s, rat was removed and returned to a holding cage for 10 s. Training continued until the rat reached a criterion of at least 80% correct choices on 2 consecutive days. The rats that did not reach criterion by 20 days were not included in the remainder of the study. It was anticipated that lesioned rats would take longer to reach criterion than sham rats. Thus, some rats in each group were subjected to subsequent water maze training sooner than others, based on the latency with which they reached criterion on the T maze.

T-maze control. The rats in the T maze control groups were yoked to the T-maze-trained rats. That is, each rat in the control condition was kept alive and subjected to the T-maze apparatus for an equivalent number of days as its yoked counterpart. During the T-maze control condition, the rats were allowed to explore the main alley for 5 min with the guillotine doors closing off each side alley (5 min being substantially greater than the average amount of time T-maze-trained rats spent in the apparatus). No food rewards were presented at any point. Rats in the T-maze control groups were also food deprived during the course of training.

Sedentary control. Rats in the sedentary control groups were left in their home cages and were not exposed to any of the aforementioned behavioral conditions. Sedentary control rats were food deprived as in the other experimental groups. They were yoked to the T-maze-trained and T-maze control rats, such that those rats that received subsequent water maze training began their water maze training on the same day as their yoked counterparts and were killed the same number of days after training was completed as their counterparts.

MWM. Water maze training was conducted according to the protocol of Experiment 1 and began 2 days following the cessation of the pretraining condition (i.e., T-maze training vs. T-maze control vs. sedentary control). Immediately following cessation of the pretraining condition, the rats were provided with food ad libitum.

The two groups of rats not subjected to MWM following pretraining (see Table 1) were left in their home cages while their yoked counterparts underwent water maze training.

Cerebellar histology and lesion verification. Histology and lesion verification were performed according to the protocol of Experiment 1, except that the rats were killed with CO2 gas instead of pentobarbital.
Statistical analyses. The T-maze data were analyzed within a one-way between-groups ANOVA. Surgical condition (sham vs. lesion) was the between-groups variable. The dependent variable for T-maze training was the average number of days to reach the 80% criterion.

As in Experiment 1, three dependent variables were measured from the rats’ performances in the Morris water maze: (a) latency, (b) search strategy, and (c) search success. Data from the hidden platform condition of Experiment 2 were subjected to a three-factor ANOVA within a mixed design \((2 \times 3 \times 5)\). Between-groups comparisons were made for surgical condition and pretraining condition (T-maze trained vs. T-maze control vs. sedentary control), with repeated measures across days (1–5). A separate \(2 \times 3 \times 2\) mixed ANOVA was used to analyze the rats’ performances between Days 5 and 13 in order to assess a possible retention deficit. Between-groups variables were surgical condition and pretraining condition, whereas the within-group variable was day of training (Days 5 and 13). Finally, with regards to behavioral training, search success data were analyzed within a \(2 \times 3\) between-groups ANOVA, with surgical condition and pretraining condition as the two variables. The dependent variable for search success was the number of times the rats successfully found the platform within the 60-s time frame, averaged across trials. Post hoc comparisons were made using the Scheffe test for all of the aforementioned analyses.

Results

Experiment 1

Histology. Posthistological analysis of the cerebellar lesion sites showed varying degrees of accuracy. Focal lesions typically destroyed more caudal portions of dentate nuclei and surrounding cerebellar cortex, sometimes leaving more rostral portions of the lateral nuclei intact. Most of the lesions spared the interpositus nuclei entirely. Estimated damage to dentate nuclei across rats was 44%. A diagrammatic representation of lesion extent is depicted in Figure 1.

Bridge test. The bridge test means are depicted graphically in Figure 2 for each of the three dependent variables. The pattern of behaviors indicated a gradual improvement in performance across all 5 days for both lesioned and sham rats. Lesioned rats performed comparably to sham rats across all three behavioral measures. With regards to equilibrium, the within-group comparison of the mean time that the rats had all four paws in contact with the beam indicated a significant improvement across the 5 days of training, \(F(4, 44) = 2.69, p < .05\), with no significant interaction effect between surgical condition and days of training \((p = .59)\). The rats improved in terms of latency to fall, such that the within-group comparison of the mean time that the rats were able to remain on the beam revealed a nonsignificant trend over the course of 5 days, \(F(4, 44) = 2.46, p > .06\), with no significant interaction effect between surgical condition and days of training \((p = .70)\). Within-group comparison of the mean number of beam segments that rats traversed across the 5 days showed no significant improvement, \(F(4, 44) = 1.02, p > .40\), and no interaction effect between surgical condition and days of training \((p = .59)\). The between-groups variable of the mixed ANOVAs for each dependent variable indicated no significant difference in equilibrium, \(F(1, 11) = 0.03, p > .85\), latency, \(F(1, 11) = 2.50, p > .14\), and distance, \(F(1, 11) = 0.01, p > .90\).

MWM. Lesioned rats demonstrated less effective search strategies than sham rats as evidenced by longer latency to find the hidden platform, more quadrants traversed while swimming, and less success in finding the platform within the allotted time. See Figure 3 for graphic representation of latency, search strategy, and search success measures. Although the pattern of means showed a gradual improvement for both lesioned and sham rats across training days, sham rats tended to perform better than lesioned rats as early as Day 1 of training; this pattern held for all dependent variables. The rats tended to show a slight deficit in their performance on Day 4, as evidenced by an increase in mean latency and mean number of quadrants traversed because of the relocation of the hidden platform on that day. The rats’ performance in the visible platform condition was comparable between the lesion and sham surgical conditions.

Data for the invisible platform condition were analyzed within a \(2 \times 5\) mixed ANOVA with surgical condition as the between-groups variable and training days (1–5) as the within-group variable. The rats improved their performance across Days 1–5 with regards to both latency and search strategy, such that they took less time in locating the platform and correspondingly traversed fewer quadrants during the search process. Within-group comparison of the mean time in which the rats were able to locate the hidden platform revealed a significant improvement over training days, \(F(4, 44) = 14.62, p < .01\), with no significant interaction effect between surgical condition and training days \((p = .43)\). Within-group comparison of the number of quadrants that the rats traversed also indicated a significant improvement across days, \(F(4, 44) = 10.15, p < .01\), with no significant interaction effect between surgical condition and training days \((p = .43)\).

A separate \(2 \times 2\) mixed ANOVA, with surgical condition as the between-groups variable and Days 5 and 13 as the within-group variable, was conducted in order to compare the rats’ performance across Day 5 and Day 13 (repeated measures variable) for both latency-to-escape measures and search strategy. Within-group comparisons of the amount of time required to find the hidden platform showed no significant difference between Days 5 and 13 for both lesioned rats, \(F(1, 12) = 0.00, p > .05\), and sham rats, \(F(1, 10) = 1.82, p > .20\). Within-group comparisons of the number of quadrants traversed in searching for the hidden platform also demonstrated no significant difference between Days 5 and 13 for both lesioned rats, \(F(1, 12) = 0.01, p > .93\), and sham rats, \(F(1, 10) = 0.94, p > .35\).

An overall significant difference was found between lesioned and sham rats with regards to latency to escape on the hidden platform condition. The between-groups comparison of the mixed ANOVA indicated that lesioned rats took significantly more time across all 5 days in locating the hidden platform than sham rats, \(F(1, 11) = 7.98, p < .02\). Post hoc analyses indicated significant differences \((p < .05)\) between lesioned and sham rats on all days of training. There was no significant difference between lesioned and sham rats in the visible platform condition. A one-way between-groups ANOVA indicated no difference in the amount of time required for lesioned and sham rats to locate the elevated platform, \(F(1, 11) = 0.04, p > .87\).

In terms of search strategy, lesioned rats crossed more quadrants than sham rats while locating the hidden platform. The between-groups component of the mixed ANOVA used to analyze the number of quadrants traversed during hidden platform learning showed a significant difference between lesioned and sham rats, \(F(1, 11) = 11.03, p < .01\). Post hoc analyses identified significant differences \((p < .05)\) on all days of training.

In terms of search success, sham rats found the hidden platform on Days 1–5 more frequently than lesioned rats. A one-way
between-groups ANOVA of the number of times the rats were able to locate the hidden platform during the allotted 60-s time frame demonstrated a superior performance of sham rats as compared with lesioned rats, \( F(1, 12) = 8.41, p < .02. \)

**Experiment 2**

*Histology.* Lesion accuracy was similar to Experiment 1. Posthistological analysis of lesion placement produced an estimate of 46% damage to the dentate nuclei. A diagrammatic representation of lesion extent is shown in Figure 4.

*T-maze training.* The dependent variable of the T-maze component of behavioral training was the number of days required to reach 80% criterion. The pattern of means indicated a comparable performance of sham and lesioned rats, with both groups averaging approximately 10 days to reach criterion. A one-way between-groups ANOVA with surgical condition as the between-groups variable revealed no significant difference between sham and lesioned rats, \( F(1, 27) = 0.54, p > .46. \)

*MWM.* The rats’ performance on the MWM was measured and analyzed according to the same protocol as in Experiment 1. Both lesioned and sham rats improved in their ability to find the hidden platform over the course of 6 days, as evidenced by a gradual decrease in mean latency to locate the platform and mean number of quadrants traversed in doing so. Sham rats consistently performed better than lesioned rats on all behavioral measures (latency to escape, number of quadrants traversed, and number of times platform found). Measures of the mean latency to escape indicated that pretraining on the T-maze enhanced MWM performance as

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**Figure 1.** Experiment 1: Representative lesion reconstruction of cerebellar damage from electrolytic lesions. Coronal sections are numbered 1–7 from rostral to caudal. Left and right hemispheres (as facing viewer) represent minimal and maximal lesion extent of dentate nuclei, respectively. Reprinted from *The Rat Brain in Stereotaxic Coordinates*, 4th ed., G. Paxinos and C. Watson, Figures 60, 62–66, and 68, Copyright 1998, with permission from Elsevier.
compared with sedentary controls, whereas T-maze control rats performed intermediately between inactive and T-maze pretrained rats. This pretraining effect was magnified within the lesioned group of rats when looking at mean latency to escape, whereas the sham group showed the same general pattern of performance with less difference between the three pretraining groups. See Figure 5 for graphic representation of performance means based on pretraining condition within lesioned and sham rats. In replication of Experiment 1, the sham rats that received no pretraining (sedentary controls) performed better on the MWM across days than did the lesioned rats that received no pretraining. The dependent variable of search strategy showed similar patterns to the dependent variable of mean latency to escape. Sham rats and T-maze-trained rats were able to locate the hidden platform within the 60-s limit on average more times than lesioned rats and non-T-maze-trained rats, respectively. Search success is represented graphically in Figure 6 for all comparison groups. The rats performed comparably on the visible platform condition, regardless of surgical condition or pretraining condition.

Data for the first two dependent variables (latency and search strategy) were analyzed within separate $2 \times 3 \times 2$ mixed ANOVAs, with surgical condition and pretraining condition as the between-groups variables and training days (1–5) as the within-group variable. The between-groups comparison of surgical condition was statistically significant, $F(1, 33) = 4.60, p < .039$, such that the lesioned rats took significantly longer to find the invisible

![Equilibrium](image1)

![Latency](image2)

![Distance](image3)

Figure 2. Mean ($\pm$ SEM) values of equilibrium, latency to fall, and distance traversed on the bridge test for lesioned and sham rats across Days 1–5. Equilibrium was measured as the time (in seconds) rats spent with all four paws on the beam. Latency to fall was measured as the time (in seconds) rats were able to remain on the beam. Distance was measured as the number of segments rats traversed on the beam.

![Latency](image4)

![Search Strategy](image5)

Figure 3. Mean values ($\pm$ SEM) of latency, strategy, and success in locating the Morris water maze hidden platform in Experiment 1 for lesioned and sham rats. Latency was measured as the time (in seconds) to escape onto the platform. Search strategy was measured as the number of quadrants traversed in locating the platform. Success was measured as the average number of times the rats successfully located the platform. All comparisons between surgical conditions were significantly different ($p < .05$).
platform than the sham rats. The between-groups comparison of pretraining condition also showed significance, $F(2, 33) = 5.09, p < .012$. Post hoc analyses of pretraining condition using the Scheffe test showed the following: (a) rats receiving T-maze training took significantly less time to find the invisible platform during water maze training than sedentary rats ($p < .003$); (b) T-maze control rats showed a nonsignificant trend as compared with inactive rats ($p < .067$), such that T-maze control rats took slightly less time to find the hidden platform than sedentary rats; and (c) there was no significant difference between T-maze-trained and T-maze control rats ($p = .30$). The overall pattern of performance on the water maze in terms of mean latency to find the hidden platform across Days 1–5 was the following: T-maze trained $< T$-maze control $<$ sedentary control. The within-group comparison of training day (performance across Days 1–5) showed a significant improvement in water maze learning across surgical groups, $F(4, 132) = 19.37, p < .0001$, with no interaction effects between pretraining condition and surgical condition.

Sham rats that were left inactive prior to water maze training took significantly less time ($p < .04$) to find the hidden platform than lesioned rats that were left inactive prior to maze training. In terms of the pretraining condition, lesioned rats showed the following pattern of mean latency to escape: T-maze trained $< T$-maze control $<$ sedentary control. Only the difference between T-maze-trained and sedentary control groups was significant ($p < .001$). The lesioned/T-maze-trained group was as efficient at finding the invisible platform as the sham/T-maze-trained group, such that no significant difference existed between the two groups ($p = .012$).

Figure 4. Experiment 2: Representative lesion reconstruction of cerebellar damage from electrolytic lesions. Coronal sections are numbered 1–7 from rostral to caudal. Left and right hemispheres (as facing viewer) represent maximal and minimal lesion extent of dentate nuclei, respectively. Reprinted from The Rat Brain in Stereotaxic Coordinates, 4th ed., G. Paxinos and C. Watson, Figures 60, 62–66, and 68, Copyright 1998, with permission from Elsevier.
Sham rats showed the same pattern as lesioned rats, with no significant differences between pretraining conditions.

A separate 2 × 3 × 2 mixed ANOVA, with surgical condition and pretraining condition as the between-groups variables and Days 5 and 13 as the within-group variable, was performed in order to determine the presence or absence of a retention deficit. The effect of pretraining was significant, \( F(1, 33) = 3.29, p = .05 \). Post hoc comparisons showed a significant difference between sedentary control and T-maze-trained rats (\( p < .02 \)). No significant difference was found for surgical condition, \( F(1, 33) = 1.10, p > .30 \). No significant difference was found for Training Days 5 and 13, \( F(1, 33) = 0.02, p > .90 \), suggesting the absence of a retention deficit for any of the experimental groups.

In terms of visible platform training (Day 14), no difference was found between any of the experimental groups with regards to latency to escape. Data of the mean latency to escape were subjected to a two-way between-groups ANOVA, with surgical condition and pretraining condition as the two variables. There was no significant main effect of surgical condition, \( F(1, 33) = 0.12, p > .73 \), or pretraining condition, \( F(2, 33) = 1.75, p > .19 \).

Data for the second dependent variable—search strategy—were subjected to a 2 × 3 × 2 mixed ANOVA, with surgical condition and pretraining condition as the two between-groups variables and days (1–5) as the within-group variable. Within-group comparison of Training Days 1–5 was significant, \( F(4, 132) = 17.50, p < .0001 \), indicating substantial improvement of all experimental groups. Between-groups comparisons demonstrated no significant differences between sham and lesioned rats, \( F(1, 33) = 0.34, p > .56 \), or pretraining conditions, \( F(2, 33) = 0.55, p > .58 \), nor were any interaction effects evident for number of quadrants traversed.
Data for rats’ success in locating the hidden platform were measured as the mean number of times rats were able to escape within the 60-s time frame. Data were subjected to a $2 \times 3$ between-groups ANOVA, with surgical condition (lesions vs. sham) and pretraining condition (T-maze trained vs. T-maze control vs. sedentary control) as the two variables. Overall, sham rats found the platform significantly more times than did lesioned rats on Days 1–5, $F(1, 33) = 7.21, p < .02$. Pretraining also produced a significant effect in the rats’ ability to locate the hidden platform, $F(2, 33) = 3.33, p < .05$. Post hoc analyses indicated a significant difference ($p < .05$) between T-maze trained and sedentary rats, demonstrating a superior performance of T-maze-trained rats. However, there was no significant interaction effect between pretraining condition and surgical condition, $F(2, 33) = 0.54, p > .59$.

**Discussion**

The results suggest an important role of the lateral cerebellar deep nuclei in mediating visuospatial learning. Experiment 1 demonstrated that focal lesions of the lateral nuclei impair hidden platform learning on the MWM without producing a corresponding impairment on visible platform and bridge test learning. Lesioned rats took significantly longer, successfully found the platform fewer times, and crossed more quadrants in locating the hidden platform than sham rats, while latency to escape on the visible platform and performance on the bridge test indicated no significant differences between surgical groups. This effect was evident despite only a partial ablation of the dentate nuclei. These findings suggest that the afferent and/or efferent pathways of the lateral nuclei are crucial for processing and learning visuospatial information without necessarily affecting the vestibular and motor components of such task acquisition. Since the inception of this project, research has been published in another laboratory with similar results to those of Experiment 1. Joyal et al. (2001) subjected rats to a number of behavioral tasks following focal electrolytic lesions of the dentate nuclei. These tasks included the MWM as well as other tests of sensorimotor performance, such as the rotarod, wire test, and grid test. In general, rats showed impairment in acquisition of the hidden platform test with little or no effect on the other performance measures. This research provides additional evidence for a key role of the dentate nuclei in visuospatial learning.

Experiment 2 demonstrated that pretraining on a T maze reverses the lesion deficit as indicated by improved subsequent performance on the water maze task. This effect was evident in measures of latency to escape and success in locating the hidden platform. The attenuation in performance deficit was substantial to the extent that no significant difference existed between sham and lesioned rats that had received pretraining on the T maze. In other words, the spatial impairment caused by destruction of the lateral cerebellar deep nuclei was diminished via learning of the delayed spatial alternation task. The lack of statistical difference between groups on the search strategy variable was likely due to the larger size and correspondingly slower swim speed of the rats as compared with the first experiment, which minimized the number of quadrants the rats could traverse within the 60-s time frame. Further analysis in both experiments indicated no difference in performance between Day 5 and Day 13 of the invisible platform condition. This finding suggests that the spatial learning impairment caused by the focal lesions was not likely due to a retention deficit, but rather to a spatial processing deficit.

Mutant mice have provided a widely used and effectual model of cerebellar contributions to visuospatial processing. Degeneration of cerebellar Purkinje cells, as exemplified by lurcher, staggerer, and Purkinje cell degeneration (pcd) mice, is consistently characterized by MWM deficits with varying degrees of corresponding motor impairment (Goodlett, Hamre, & West, 1992; Hilber, Jouen, Delhaye-Bouchard, Mariani, & Caston, 1998; Lalonde, 1987). Some of the mutants are, in fact, able to learn the MWM task, albeit with relative impairment (Caston et al., 1999; Hilber et al., 1998; Lalonde, 1987), although evidence exists for the implementation of alternative search strategies, suggesting that the cerebellar cortex is perhaps less necessary for escape learning than for acquisition of a cognitive map (Hilber et al., 1998).

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**Figure 6.** Mean ($\pm$ SEM) values of the number of times rats were able to successfully locate the hidden platform during Experiment 2. All comparison groups are depicted. Asterisks indicate significant differences ($p < .05$) between sham and lesioned rats (surgical condition; top panel) and between T-maze-trained and sedentary control rats (pretraining comparisons; middle panel).
The results of the present study offer further support to research using mutant mice, with the seeming exception that the lesion effects of the present study were not quite as pronounced as those resulting from some cerebellar mutations. The hallmark study by Goodlett et al. (1992), for example, found that pcd mice were significantly impaired in distal cue (hidden platform) learning with negligible impairment in proximal cue (visible platform) learning. Their results gain support from the present study that showed a similar pattern of performance deficit. However, unlike the Goodlett study, the rats in the present study were able to acquire effective search behavior over the course of distal cue training. This important difference can potentially be explained by considering the global neuroanatomical implications of each type of cerebellar manipulation. In addition to the near total loss of Purkinje cells, pcd mice evidence diffuse secondary degeneration in thalamic nuclei, cerebellar granule cells, and the inferior olivary complex, as well as neurochemical changes in deep cerebellar nuclei, although these secondary changes have a much slower time course (Ghetti, Alyea, & Muller, 1978; Ghetti, Norton, & Triarhou, 1987; O’Gorman & Sidman, 1985; Roffler-Tarlof, Beart, O’Gorman, & Sidman, 1979). The exception is degeneration of the inferior olivary complex, which shows substantial cell loss by postnatal Day 24 (Ghetti et al., 1987; Shojaeian, Delhyaye-Bouchaud, & Mariani, 1988; Triarhou & Ghetti, 1991), a week younger than Goodlett et al.’s most undeveloped group of pcd mice. This may be of importance in light of recent evidence showing a significant spatial impairment following lesion of the inferior olivary complex (Gasbarri, Pompili, Pacitti, & Cicirata, 2003). Given that the electrolytic lesions in the present study were only moderately successful in ablating the dentate nuclei, with corresponding infringement on cerebellar cortex, the ability of lesioned rats to acquire effective search behavior could be subserved by surviving terminals from the lateral cerebellar cortex. When put within the context of the Goodlett et al. (1992) study, the relatively smaller magnitude of the visuospatial impairment in the present study could reflect an inverse correlation with the number of surviving neurons in the dentate nuclei and overlying cortex. One point of interest, however, is that nonmutant mice with large cerebellar lesions have been reported to learn the MWM task (Hilber et al., 1998), raising the possibility that it is not cerebellar impairment precluding learning of the task, as indicated by Goodlett and colleagues, but rather extracerebellar degeneration secondary to the pcd mutation. Similarly, the cognitive and motor enhancement in lurcher mice, following rearing in an enriched environment, has been attributed to possible plasticity in extracerebellar regions taking over lost cerebellar function (Caston et al., 1999). Outside of its theoretical explanatory power, however, it must be reemphasized that, given its slower time course, secondary degeneration in pcd mice cannot fully account for the complete spatial impairment of Goodlett et al.’s youngest group of mice.

No difference was found in Experiment 2 between lesioned and sham rats in latency to reach criterion on the T maze, raising the possibility that the lateral deep cerebellar nuclei are more essential in processing of visuospatial information than in contributing to working spatial memory. This finding conflicts with other studies showing concurrent MWM and T-maze deficits (Molinari, Grammaldo, & Petrosini, 1997; Petrosini, Molinari, & Dell-Anna, 1996). However, the extent of cerebellar damage in the other studies was much greater than the focal lesions of the present study, and the nature of the T-maze task was also different. The lack of a statistically significant difference between lesioned and sham rats in T-maze performance may be attributed to the nature of the task itself. That is, delayed spatial alternation is predominantly a working memory task. The spatial component of the T-maze task is negligible in comparison with the requirements of invisible platform learning on the MWM. The T maze simply requires discrimination between left and right, whereas the water maze demands visuospatial mapping to navigate a broad and much less defined area. Not only is there the obvious difference in complexity between the two tasks, but T-maze learning is also devoid of salient visual cues, thus relying more on retention of information from trial to trial than any sort of visuospatial strategy. Recent research (Belzung, Chapillon, & Lalonde, 2001) investigating the spatial abilities of lurcher mice with cerebellar mutations found that mutated mice were not impaired in left–right discrimination on a T-maze version of the MWM, despite impairment on the radial arm maze. In the present experiment, comparisons between water-maze Day 5 and Day 13 in both experiments indicated no significant differences, arguing against a prominent memory component in lateral cerebellar contributions to hidden platform learning.

Of course, working memory is different than reference memory. Working memory requires the retention and continued manipulation of recently perceived information and is reflected in learning across trials on any given day (i.e., retaining and manipulating information acquired in the previous trial), whereas longer term memory is necessary for progress across days. Delayed spatial alternation is much more dependent on working memory than is water maze learning because of the shorter intertrial intervals and lack of visual cues associated with the T-maze task. Thus, if the significant contributions of the lateral cerebellar deep nuclei do not include reference memory or working memory, ablation of this region might not be expected to affect T-maze learning to a large degree. Gross impairment of the cerebellum has been shown to affect spatial working memory (Goldowitz & Koch, 1986; Lalonde, 1987; Lalonde & Botez, 1986; Pellegrino & Altman, 1979), but the effects of selective lesions of the deep nuclei have not been examined to date. On the other hand, if the lateral nuclei were more involved in processing of visuospatial information, lesions of these nuclei would be expected to impair water maze learning much more than T-maze learning, a prediction that is supported by the present results. Further research is necessary to clarify these issues.

The identification of a cerebellothalamicprefrontal projection pathway (Middleton & Strick, 1994) was crucial in identifying the existence of a physiological substrate for a cerebellar contribution to cognition. Although the functional significance of this pathway remains to be determined, as within the context of visuospatial learning, it can serve as a useful conceptual model in driving experimental research. The results of the present study suggest that the contribution of the cerebelloprefrontal pathway to spatial working memory is minimal because the cerebellar lesions did not impair T-maze performance. Disruptions in prefrontal activity have been shown to significantly weaken T-maze performance when a similar paradigm to that which was used in the present study is utilized (Romanides, Duffy, & Kalivas, 1999; Verma & Moghaddam, 1996). If the lateral cerebellar deep nuclei do, in fact, affect prefrontal functioning via the mediodorsal thalamus, why would the lesions of the lateral nuclei in the current study not also
impair working memory tasks mediated by the thalamocortical pathway? To answer this question, it is important to identify exactly what information the cerebellum is providing during the learning process. In studying the ability of rats to learn the MWM through observation, Leggio, Molinari, Neri, Mandolesi, and Petrosini (2000) proposed that the rats “did not learn a ‘what’ or a ‘where,’ but a ‘how’” (p. 2324). That is, cerebellar lesions did not necessarily affect the ability of the rats to learn the platform location via observation, but rather impaired the observer rats’ ability to learn effective search strategies. The important component of T-maze learning is the “where” (left or right) rather than the “how” because it does not necessitate the implementation of any sort of search strategy. Thus, it seems plausible that cerebellar lesions would impair water maze learning more than T-maze learning.

A surprising result of the present experiment involved the effect of simple exposure to the T-maze apparatus (T-maze control) as compared with the sedentary and T-maze pretrained groups in enhancing subsequent water maze learning. In both lesioned and sham rats, the T-maze control group was intermediate between sedentary control and T-maze-trained groups, with no significant difference between them (i.e., T-maze control vs. sedentary control and T-maze control vs. T-maze pretrained). This pattern suggests that mere exposure to the T-maze had a mild effect in enhancing water maze learning. One possible explanation for this pattern is that T-maze control rats were engaged in some type of learning and/or sensorimotor enhancement that improved subsequent water maze performance. A large body of research literature has demonstrated the effect of enriched environments in enhancing subsequent learning following traumatic brain injury, including spatial learning on the MWM (e.g., Briones, Therrien, & Metzger, 2000; Hamm, Temple, O’Dell, Pike, & Lyeth, 1996; Passineau, Green, & Dietrich, 2001). Correspondingly, sensorimotor stimulation has been shown to accelerate recovery after damage to the cerebellum (Auveray, Caston, Reber, & Stelzl, 1989; Caston et al., 1995, 1999; Zion, Auveray, Caston, Reber, & Stelzl, 1990). However, the proposal that learning or environmental novelty alone contributed to enhanced water maze performance in the present study seems implausible because (a) exposure to the T maze was relatively brief, as compared with the amount of time the rats are typically housed in enriched environments; (b) the T maze was not an enriched environment relative to those provided in the aforementioned studies; and (c) no behaviors were being reinforced and, hence, no structured learning paradigm was in place. An additional point of interest arises when looking at the qualitative nature of the learning pattern within lesioned T-maze control rats (see Figure 5). That is, T-maze control rats performed identically to T-maze-trained rats until the platform was moved on Day 4, at which point the pattern mirrored that of the sedentary group. The purpose of moving the platform on Day 4 was to ensure that the rats were integrating visual and spatial information rather than simply learning taxonomic or kinesthetic search patterns. Perhaps motor activity enhances the kinesthetic or memory dimension of visuospatial learning rather than visuospatial processing, which is why the T-maze control rats were unable to adjust to the new platform position as well as the T-maze-trained rats.

Another possible explanation for the moderate enhancement of MWM performance following simple exposure to the T maze is that exercise—alone—had some beneficial effect in ameliorating the observed visuospatial deficit. Research has, in fact, shown a beneficial effect of exercise on spatial learning and neurogenesis (Anderson et al., 2000; Fordyce & Farrar, 1991; Fordyce & Werner, 1993; Van Praag, Kempermann, & Gage, 1999). However, the amount of aerobic exercise necessary to achieve this effect is substantial, typically requiring weeks or months of exercise on a running wheel or treadmill prior to spatial learning assessment. It is likely that the moderate enhancement of MWM learning observed in the T-maze control rats in the present study resulted from a combination of motor activity and increased sensorimotor stimulation from repeated exposure to a “novel” stimulus (i.e., T maze). Both components have been shown to facilitate changes in brain morphology that underlie cognitive enhancement in both cerebellar and extracerebellar regions. Environmental stimulation increases the number of spines on cerebellar Purkinje cells (Black, Isacs, Anderson, Alcantara, & Greenough, 1990), the number of parallel and climbing fiber synapses per Purkinje cell (Anderson, Alcantara, & Greenough, 1996; Kleim et al., 1998), dendritic arborization of stellate cells (Kleim et al., 1997), number of hippocampal cells (Kempermann, Kuhn, & Gage, 1997), and dendritic arborization in cortical pyramidal neurons (Uylings et al., 1978). The facilitative effects of combined motor activity and sensorimotor stimulation in the T-maze control rats in the present study could be operating at the level of surviving cerebellar neurons and/or intact extracerebellar regions (e.g., prefrontal cortex). The added learning component of T-maze training provided additional neural stimulation that would likely produce even greater morphological change above and beyond that of the control conditions.

Global Implications

Much research implicating the cerebellum in visuospatial abilities identifies a hippocampal and/or parietal system as mediating the impairment in water maze learning. There has been enough evidence over the years to trace some degree of functional connectivity among the hippocampus, cerebellum, posterior parietal cortex, and prefrontal cortex to provide the anatomic substrate that would allow the development of a model of network impairment should any one structure be injured. The hippocampus has long been labeled an integral structure in such abilities. A number of studies have demonstrated a learning deficit on the MWM following ablation of the hippocampus (e.g., Kolb et al., 1984; Morris et al., 1982), as well as afferent pathways (e.g. Schenk & Morris, 1985; Skelton & McNamara, 1992; Wig & Bilkey, 1994). Similarly, lesions of parietal cortex produce a profound impairment in water maze learning (Crowne et al., 1992; Dimattia & Kesner, 1988; Kolb et al., 1983, 1994). The limbic system, including the hippocampus, has a large degree of connectivity with the cerebellum (Anand, Malhotra, Singh, & Dua, 1959; Harper & Heath, 1973; Snider & Maiti, 1976). The consequences of cerebellar lesions, therefore, could potentially have broad consequences affecting multiple brain centers involved in visuospatial processing.

The words timing and synchronization are often used in describing the motoric contributions of the cerebellum, based in large part on the behavioral symptoms of patients with cerebellar cortical trauma or degenerative disease, including characteristic gait and limb ataxia as well as loss of coordination. However, research has also suggested an important role of the cerebellum in the timing of cognition and learning (Ivry, 1993, 1997; Ivry & Keele, 1989) as well as hallmark cognitive deficits of schizophrenia (Andreasen,
O’Leary, Arndt, et al., 1995. Andreasen, O’Leary, Cizadlo, et al., 1995, Andreasen et al., 1996; Crespo-Facorro et al., 1999). That is, the cerebellum can be characterized as modulating the temporal activation of multiple brain regions involved not just in the performance of the specific movement, but also in the learning process. Cerebellar insult would therefore likely interfere with both the motor and cognitive components of the task performance and acquisition across numerous brain regions. Each brain region involved in visuospatial processing can be conceptualized as a node within a network of neural involvement, with each node providing unique information to the overall process. The cerebellum modulates the synchronization or cooperative activation of all nodes involved, such that damage to the cerebellum would alter the fluidity of the system as a whole. The pretraining component of the present experiment may provide “cognitive exercise” by strengthening the connectivity between remaining nodes, just as physical rehabilitation following injury focuses on improving coordination and strength of remaining muscle groups. Although such a model is simplistic, it warrants further investigation given the growing body of evidence for cerebellar involvement in spatial navigation and cognition.

References


