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Cerebellum and procedural learning: evidence from focal cerebellar lesions

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Summary

The aim of the present study was to investigate the influence of focal cerebellar lesions on procedural learning. Eight patients with cerebellar lesions and six control subjects were tested in a serial reaction-time task. A four-choice reaction-time task was employed in which the stimuli followed (or not) a sequence repeated 10 times, with the subjects aware (or not) of the item sequence. Learning was manifested by the reduction in response latency over the sequential blocks. Acquisition of declarative knowledge of the sequence was also tested. Reaction times displayed by patients with cerebellar lesions, even though they tended to be longer than those of control subjects in all testing conditions, significantly differed from control subjects only when the stimuli were presented in sequence. The reaction times in sequential trials were still statistically significant when simple motor response times were taken into account. Cerebellar patients were also significantly impaired in detecting and repeating the sequence. On the other hand, when the sequence was learned before testing, motor performances were significantly improved in all subjects. These data indicate that cerebellar lesions induce specific impairment in the procedural learning of a motor sequence and suggest a role of the cerebellar circuitry in detecting and recognizing event sequences.

Keywords: implicit memory; lesion studies; serial reaction-time task; man

Abbreviations: RT = reaction time

Introduction

Numerous lines of research utilizing different methodologies such as chronic recordings and lesions in monkeys (Thach *et al*., 1992; Nixon and Passingham, 1996), classical conditioning in rabbits (Yeo *et al*., 1985) and vestibulo-ocular reflex adaptation in monkeys (Lisberger, 1984), strongly indicate a cerebellar role in motor learning. A number of studies in healthy human subjects report a decrement in cerebellar activation during progressive acquisition of a motor skill. A PET analysis of the modifications in regional cerebral blood flow during learning of a complex sequence of movements demonstrated a significant increment of blood flow in the right anterior cerebellar lobe which was not related to motor parameters, such as frequency and velocity of finger movements (Setz *et al*., 1990). More recently, in a functional MRI study, Flament *et al.* (1994) reported high cerebellar activation during a motor task subjects were hardly able to learn, while the same task, once learned, provoked very low activation in the cerebellar regions. Similar results were reported in a PET study (Jenkins *et al*., 1994) indicating marked activation of the cerebellum during the first phases of learning a new sequence. Thus, functionally related activity in lateral cerebellum is more linked to the novelty of the motor task than to its complexity. This indicates a specific role of the lateral cerebellum in acquiring novel motor tasks.

One of the many different tests used to analyse procedural learning in humans is Nissen and Bullemer's (1987) 'serial reaction-time task', in which the subject has to give a motor response to visual stimuli presented at random or in sequence. The difference betwen the reaction times recorded in random and in sequence conditions represents an index of learning. By using a modified version of this serial reaction task, Pascual-Leone *et al.* (1993) compared the performances of a group of parkinsonian patients with those of a group of patients affected by cerebellar atrophy. While parkinsonian

patients were able to demonstrate procedural and, with some difficulty, also declarative learning, patients with cerebellar lesions did not display any improvement with task repetition, thus indicating that their procedural learning was impaired. According to Pascual-Leone *et al.* (1993), procedural learning requires that the sequence of stimulus positions be collected into a 'working memory buffer' and that each new position be compared to the previous ones (Baddeley, 1992). These functions are probably controlled by the prefrontal cortex in association with basal ganglia and cerebellum (Salmon and Butters, 1995). According to this hypothesis, connections between basal ganglia and prefrontal cortex are necessary to control access to, and output from, the buffer, and the cerebellum is involved in the timing and on-line comparison of actual movements with the information present in the buffer.

To further analyse the relationship between the cerebellum and procedural learning, we studied procedural learning abilities in a selected group of patients with unilateral focal cerebellar lesions. Studies in patients with focal lesions are easier to interpret than similar investigations in patients with degenerative diseases. In fact, in the latter group the systemic nature of the insult can induce widespread malfunctioning of the CNS, thus the cause–effect relationship between cerebellar atrophy and the symptoms observed should be questioned. On the other hand, MRI evidence in patients with focal lesions allows exclusion of extra-cerebellar damage thus providing a more precise correlation between lesion site and functional deficits presented. Furthermore, the analysis of patients with unilateral cerebellar lesions also allowed us to address the problem of lateralization in the cerebellar influence on procedural learning. In fact, while it is well-known that, in man, motor deficits are ipsilateral to the lesioned hemicerebellum, at present no reports deal with the problem of cerebellar lateralization in procedural learning.

Methods

Subjects

The present study was carried out on eight patients with focal cerebellar lesions (Fig. 1), five with lesions in the left cerebellar hemisphere and three with lesions in the right cerebellar hemisphere and six healthy control subjects recruited from relatives of the patients or laboratory staff. All patients underwent a stringent selection process including MRI study to exclude the presence of any other neurological pathology and hydrocephalus, as well as cognitive deterioration. The extents of the cerebellar lesions, as evaluated on MRI, have been plotted on two reference sections taken from the Kretschmann and Weinrich (1992) atlas (*see* Fig. 1). All subjects were evaluated by the Wechsler Adult Intelligence Scale; scores did not differ significantly between groups [mean ± SD of scores: subjects with right cerebellar lesions, 117.66 ± 10.01; subjects with left cerebellar lesions, 103.2 ± 9.09; control subjects, 113.5 ± 10.94; one-way ANOVA, $F(2,11) = 2.30$].

Cerebellar pathologies consisted of ischaemic or haemorrhagic ictus and surgical ablations caused by arteriovenous malformations or tumours. Experimental and control groups were matched for age, sex, and education level [subjects with right cerebellar lesions: mean age 54.66 years (range 32–75 years), educational level 10.33 years (range 5–13 years); subjects with left cerebellar lesions: mean age 38 years (range 20–48 years), educational level 12.2 years (range 8–18 years); control subjects: mean age 42.5 years (range 26–68 years), educational level 11.83 years (range 8–18 years)]. All subjects were right-handed.

Motor impairment of patients with cerebellar lesions was quantified by using a modified version of the motor deficit scale proposed by Appollonio *et al*. (1993), which ranges from zero (absence of any deficit) to 42 (presence of all deficits to the highest degree). Patients included in the present study presented deficit scores (mean ± SD) of 4.4 ± 3.41 in the group with left cerebellar lesions and 8.5 ± 6.46 in the group with right cerebellar lesions; thus both groups presented a low level of motor impairment. One-way ANOVA failed to demonstrate any significant difference between right and left groups [$F(1,6) = 1.05$]. Experimental procedures were approved by the ethical committee of the Catholic University of Rome, and informed, written consent was obtained from each subject according to the declaration of Helsinki.

Experimental apparatus

The serial reaction-time task was administered on a Macintosh Performa personal computer, which controlled stimulus presentation and reaction times, and stored data on-line. The subject sat facing a video screen on which a bar with four empty squares appeared. During the task an asterisk appeared in one of the four squares. To perform the task the subject was instructed to put four fingers (but not the thumb) of the left or right hand on the C, V, B and N keys on the keyboard and to press the key corresponding to the asterisk position that appeared on the screen. The subject was asked to respond as quickly and accurately as possible. When the subject pressed the correct key, the asterisk disappeared and after an interval of 500 ms it appeared again in a new position. Conversely, when the subject pressed an incorrect key, a short buzz was elicited and the asterisk position did not change. Asterisk positions changed in a pseudorandom pattern or according to a pre-established sequence. The only limitation on randomness was that the asterisk did not appear in the same position twice in a row.

Study design

Each subject was tested in five different experimental paradigms; the details are described separately. The succession of the different experiments was fixed. Experiment 1 was based on the alternation of random and serial eight-item sequences and was designed to test procedural learning

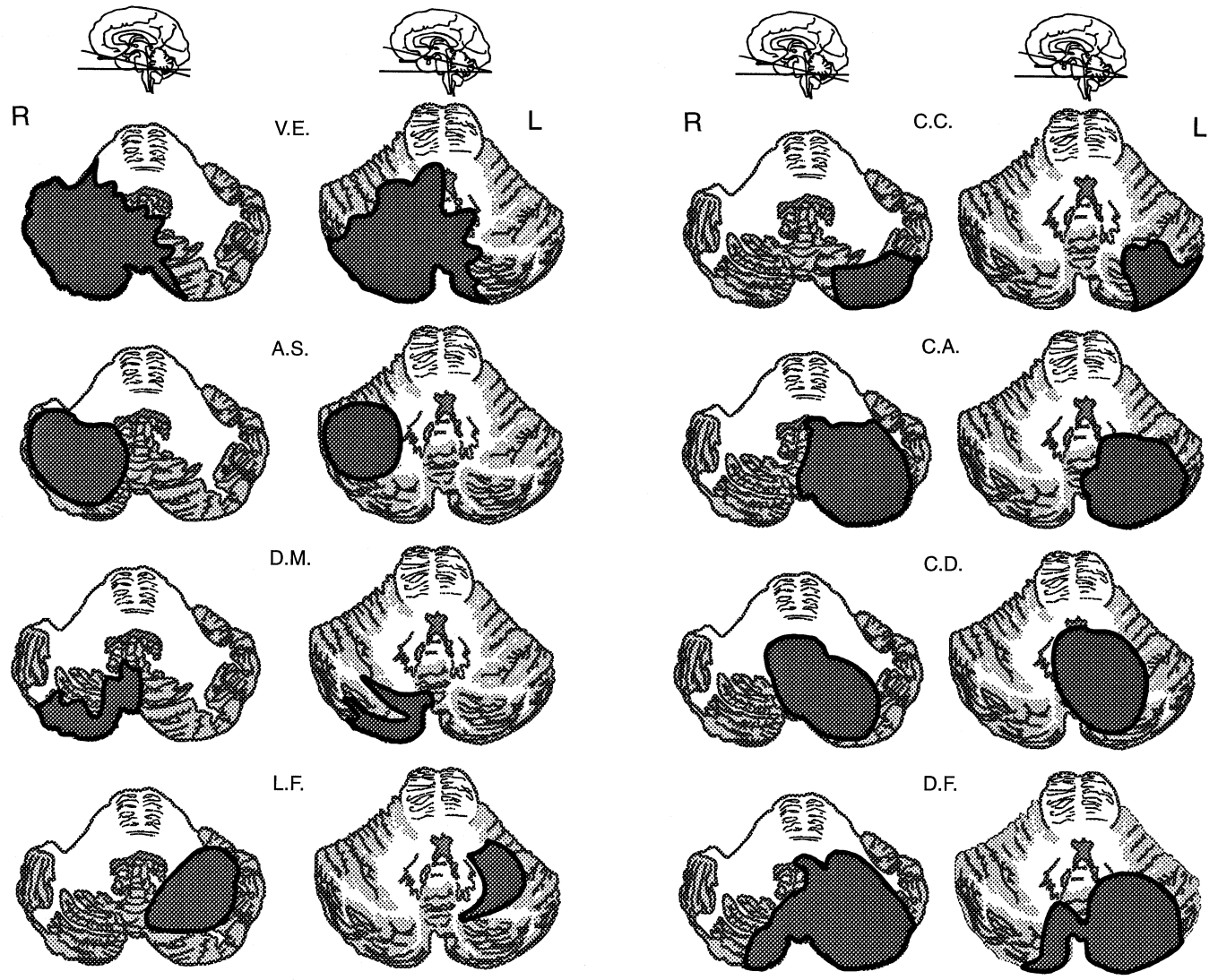

Fig. 1 Topography of the cerebellar lesions in the eight patients included in the study. R = right; L = left.

acquisition. The effect of sequence length on procedural learning was subsequently tested in Experiment 2, in which a 10-item sequence was used. The ability to acquire an item sequence through only visual input was evaluated in Experiment 3. Then the influence of previously acquired declarative knowledge of the sequence was evaluated in Experiment 4. Finally, in Experiment 5 motor abilities of all subjects were tested in a simple motor reaction-time task.

Data analysis

We computed reaction times and response accuracy in each trial. The reaction time (RT) was calculated as the latency between appearance of the stimulus on the screen and pressing of the key, regardless of the correctness of the key pressed. The progressive reduction of RT during repetition of sequence blocks was considered an index of procedural learning. Response accuracy was evaluated as the percentage of incorrect key-presses during single blocks of each trial. Procedural learning was evaluated independently of absolute values of RT, by calculating the ratios between motor performances in each block and motor performances in random conditions and expressing them as percentages of the median of RTs of the last random block (RT%). The percentage of sequence items correctly repeated verbally at the end of each experiment was considered an index of the declarative knowledge gained during the different tasks.

Statistical analysis

Metric units of the results of each group were first tested for homoscedasticity of variance and then compared using analyses of variance.

Experiment 1: procedural learning acquisition

Methods

Six blocks of 80 stimulus-response pairs were given. Although in blocks 1 and 6 asterisk presentation was random, in blocks

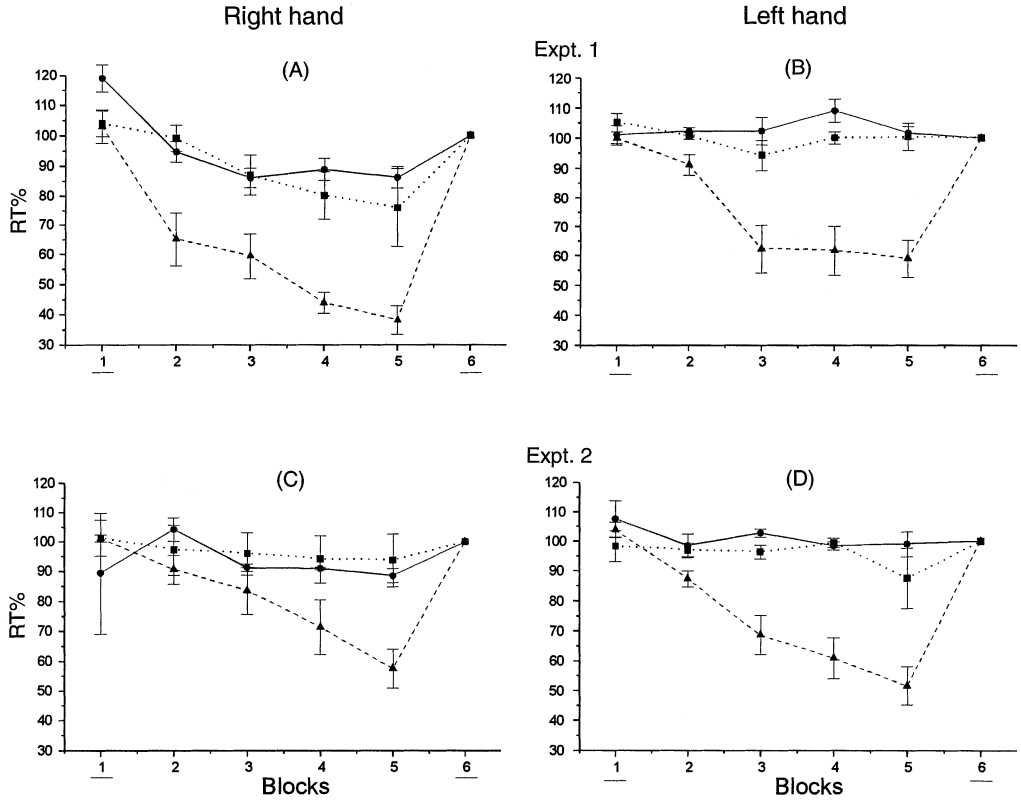

Fig. 2 Reaction times (RT%) are expressed as percentages of the last random block (block 6). (**A**) Experiment 1, right hand; (**B**) Experiment 1, left hand; (**C**) Experiment 2, right hand; (**D**) Experiment 2, left hand. Underlining (blocks 1 and 6) indicates random blocks; vertical bars show standard errors. Squares = patients with cerebellar lesions on right; circles = patients with cerebellar lesions on left; triangles = controls.

2–5 an eight-item sequence (for the right hand, NBVCNBNC; for the left hand, VCVNCBVN) of stimuli was repeated 10 times in each block. The subject was not informed of the existence of the repeating pattern. To verify whether the subject had gained declarative knowledge of the sequence presented, he/she was asked, at the end of the six blocks, whether asterisk presentation was patterned or not. If the answer was affirmative, the subject was invited to reproduce the sequence. The degree of declarative knowledge gained was evaluated by calculating the percentage of sequence items correctly reproduced.

Results

By analysing the performances of the three groups of subjects using the right hand as RT% it is possible to show that all groups exhibited slightly longer RT values in the first block than those they displayed in block 6, and that in the sequential blocks (2–5) RTs progressively decreased in all groups, although at a different rate (Fig. 2A). A 3 × 6 ANOVA (group × block) revealed significant group [$F(2,11) = 15.46$, $P < 0.001$] and block effects [$F(5,55) = 41.83$, $P < 0.001$]. The interaction was also significant [$F(10,55) = 6.37$, $P < 0.001$]. One-way ANOVAs revealed that in all groups RT% reductions were significant [for subjects with right cerebellar lesions $F(5,10) = 5.03$, $P < 0.05$; for subjects with left cerebellar lesions $F(5,20) = 13.82$, $P < 0.001$; for control subjects $F(5,25) = 41.65$, $P < 0.001$]. In Fig. 3A the same data are plotted as absolute values and the general pattern is maintained with statistical differences even more significant. A 3 × 6 ANOVA (group × block) revealed significant group [$F(2,11) = 23.07$, $P < 0.001$] and block effects [$F(5,55) = 37.14$, $P < 0.0001$]. The interaction was also significant [$F(10,55) = 3.29$, $P < 0.01$].

Although a learning effect was displayed by all three groups, the decrement in RT% was more marked in control subjects. A 3 × 4 ANOVA performed by comparing performances of the three groups in blocks 2–5, i.e. blocks with a sequential presentation of asterisks, revealed significant group [$F(2,11) = 16.22$, $P < 0.001$] and block effects [$F(3,33) = 15.44$, $P < 0.001$]. The interaction was also significant [$F(6,33) = 2.62$, $P < 0.05$]. One-way ANOVAs revealed significant sequential block effects in control subjects [$F(3,15) = 11.32$, $P < 0.001$] and patients with right cerebellar lesions [$F(3,6) = 4.93$, $P < 0.05$], but not in patients with left cerebellar lesions [$F(3,12) = 2.81$].

Statistical analyses failed to reveal any significant differences in response accuracy, either within or between groups [3 × 6 ANOVA: group $F(2,11) = 0.91$; block $F(5,55) = 1.04$; interaction $F(10,55) = 1.04$].

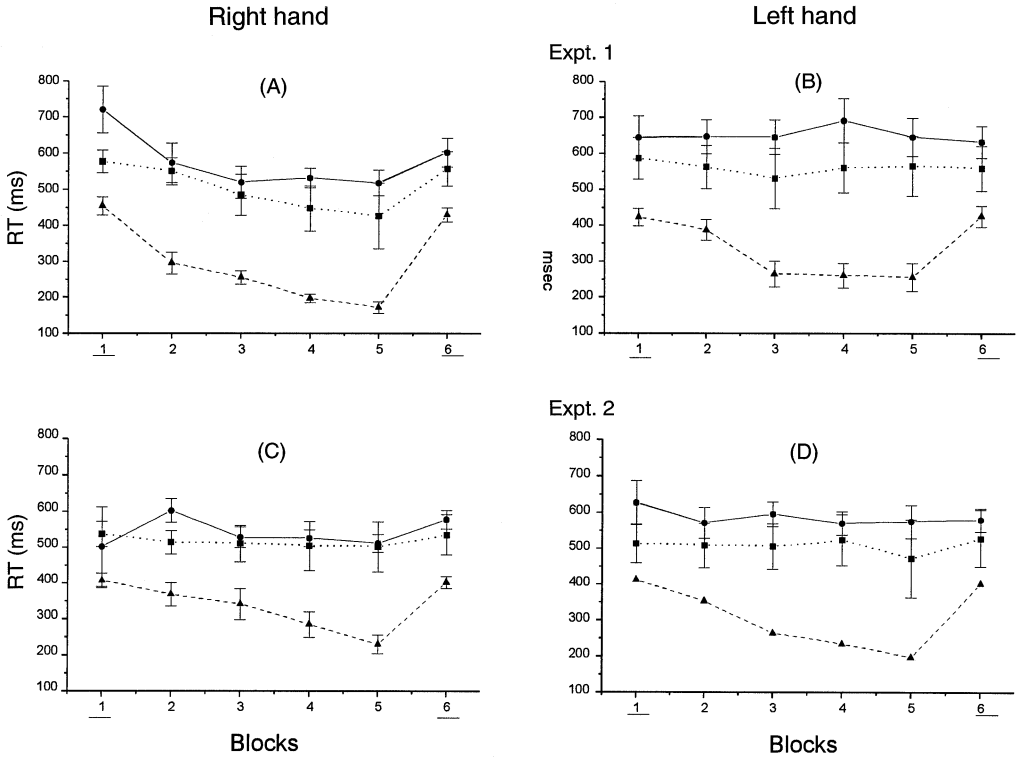

Fig. 3 Reaction times in milliseconds. (**A**) Experiment 1, right hand; (**B**) Experiment 1, left hand; (**C**) Experiment 2, right hand; (**D**) Experiment 2, left hand. Underlining (blocks 1 and 6) indicates random blocks; vertical bars show standard errors. Squares = patients with cerebellar lesions on right; circles = patients with cerebellar lesions on left; triangles = controls.

When the subjects performed the same task using the left hand, group differences became even more evident (Fig. 2B). Only control subjects displayed a significant reduction in RT% during sequential blocks, and patients with cerebellar lesions did not display any improvement. A 3 × 6 ANOVA (group × block) revealed significant group [$F(2,11) = 19.45$, $P < 0.001$] and block effects [$F(5,55) = 8.43$, $P < 0.0001$]. The interaction was also significant [$F(10,55) = 10.20$, $P < 0.001$]. One-way ANOVAs revealed a significant sequential block effect only in control subjects [$F(5,25) = 19.95$, $P < 0.001$]; they failed to reveal any significant block repetition effects in patients with right [$F(5,10) = 17.89$] or left cerebellar lesions [$F(5,20) = 1.25$]. Plotting data as absolute values did not alter the pattern or the significance of the statistics (Fig. 3B). A 3 × 6 ANOVA (group × block) revealed significant group [$F(2,11) = 15.53$, $P < 0.001$] and block effects [$F(5,55) = 6.67$, $P < 0.0001$].

Statistical analyses failed to reveal any significant differences in response accuracy, either within or between groups [3 × 6 ANOVA: group $F(2,11) = 0.16$; block $F(5,55) = 2.19$; interaction $F(10,55) = 0.5$].

Experiment 2: effect of sequence length on procedural learning

Methods

Serial reaction-time task complexity can be varied by modifying the sequence length. This might affect procedural learning as well as recognition performance. In fact, the degree of procedural learning is inversely related to the length of the repeating sequence in normal subjects and parkinsonian patients (Pascual-Leone *et al*., 1993). On the other hand, in patients with cerebellar degeneration no correlation between sequence length and degree of procedural learning has been reported (Pascual-Leone *et al*., 1993). Thus the effect of sequence length on procedural learning was tested by using a sequence of 10 elements (for the right hand, VNBCBNVCBN; for the left hand, BCBVNBVCBN). The alternation of random and sequential blocks and evaluation of declarative knowledge were the same as those in Experiment 1.

Results

The results of this section emphasize the differences already observed between groups. With longer item sequences, RT% decreased only in the control group, regardless of the hand used (Fig. 2C and D). Although a 3 × 6 ANOVA performed on data obtained with the right hand revealed a significant block effect [$F(5,55) = 3.61$, $P < 0.05$], it did not reach statistical significance as a group effect [$F(2,11) = 3.03$]. The interaction was not significant [$F(10,55) = 1.76$]. Further analyses performed by 2 × 6 ANOVA, comparing the performances of control subjects and patients with cerebellar lesions, revealed significant between-group differences

[$F(1,12) = 6.23$, $P < 0.05$]. One-way ANOVAs revealed a significant block effect only in control subjects [$F(5,25) = 9.36$, $P < 0.001$] and failed to reveal any effect in patients with right [$F(5,10) = 0.53$] or left cerebellar lesions [$F(5,20) = 0.59$].

Plotting the same data as absolute values (Fig. 3C) showed a similar general trend. A 3×6 ANOVA (group $\times$ block) revealed a significant group effect [$F(2,11) = 19.79$, $P = < 0.01$], while the block effect was not significant [$F(5,55) = 2.21$].

Statistical analyses failed to reveal any significant differences in response accuracy, either within or between groups [3×6 ANOVA: group $F(2,11) = 0.66$; block $F(5,55) = 2.08$; interaction $F(10,55) = 0.92$].

A 3×6 ANOVA performed on left hand results revealed significant group [$F(2,11) = 20.98$, $P < 0.001$] and block [$F(5,55) = 12.69$, $P < 0.001$] effects. The interaction was also significant [$F(10,55) = 8.36$, $P = 0.0001$]. One-way ANOVAs revealed a significant block effect only in control subjects [$F(5,25) = 25.67$, $P < 0.0001$] while in patients with right [$F(5,10) = 0.95$] and left cerebellar lesions [$F(5,20) = 1.24$] no significant effects were revealed.

Plotting the same data as absolute values (Fig. 3D) showed a similar general trend. A 3×6 ANOVA (group $\times$ block) revealed significant group [$F(2,11) = 13.65$, $P < 0.01$] and block [$F(5,55) = 11.03$, $P < 0.0001$] effects.

Statistical analyses failed to reveal any significant difference in response accuracy between groups [3×6 ANOVA: $F(2,11) = 1.19$].

The percentages of items recalled in the correct sequence at the end of the six blocks by control subjects and patients with cerebellar lesions, in Experiments 1 and 2, are shown in Fig. 4A. The patients are clearly impaired in their ability to reproduce sequence items, regardless of hand used and sequence length. A 3×4 ANOVA revealed significant group effect [$F(2,11) = 32.12$, $P < 0.0001$], while task [$F(3,33) = 2.84$] and interaction [$F(6,33) = 1.49$] did not reach statistical significance.

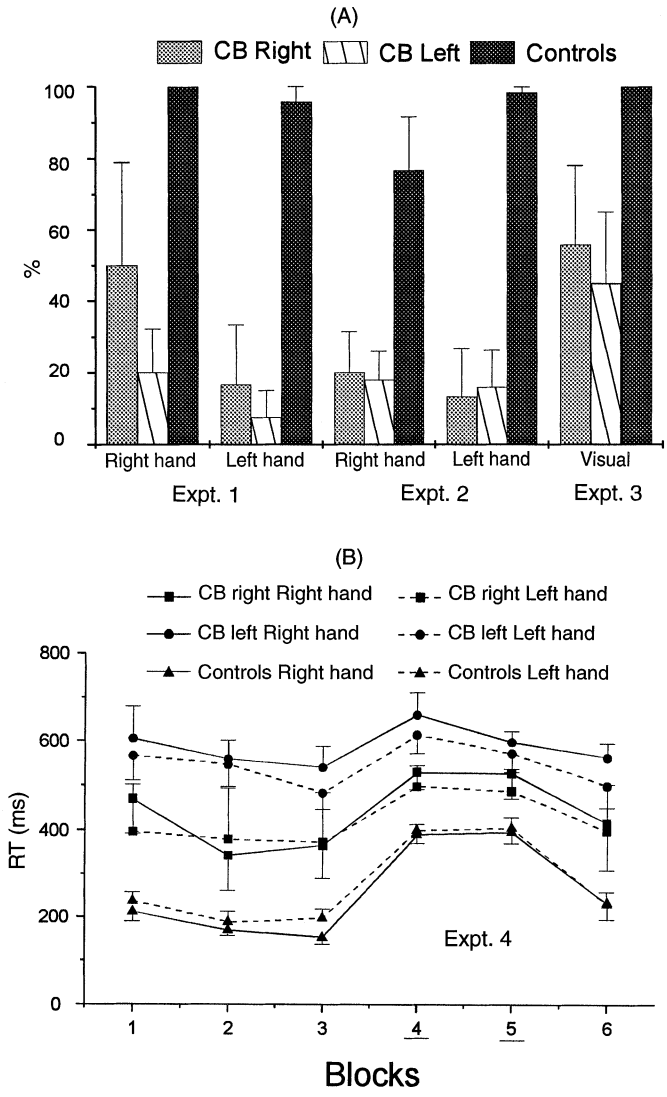

Fig. 4 (**A**) Percentage of sequence items reproduced in Experiments 1–3. (**B**) Reaction times (mseconds) in Experiment 4. Underlining indicates random blocks. Vertical bars show standard errors. CB = patients with cerebellar lesions (on right/left as indicated).

Experiment 3: detection and reproduction of the item sequence on visual input

This task differed from the others because no motor response was required. The subject was invited to watch the screen on which asterisks appeared at 250-ms intervals. There were six blocks of 80 items; in blocks 1 and 6, asterisk presentation was random, while in blocks 2–5 it was sequential, with the items patterned in a sequence of eight elements (BNCNVBCN). At the end of the last block the subject was asked whether asterisk presentation was sequential or not and then he/she had to verbally reproduce the sequence. This approach allowed analysis of the ability to detect and reproduce the asterisk sequence in a task not requiring any motor performance.

Results

Control subjects not only recognized the presence of the sequence without difficulty, they also reproduced it completely. Conversely, clear deficits were present in both groups of patients with cerebellar lesions. On average, patients with right cerebellar lesions were able to repeat only 60%, and patients with left cerebellar lesions 40%, of the sequence items (Fig. 4A). One-way ANOVAs revealed that both patients with right [$F(1,7) = 9.24$, $P < 0.05$] and left [$F(1,9) = 9.28$, $P < 0.05$] cerebellar lesions differed significantly from control subjects.

Experiment 4: effect of declarative knowledge of the sequence on procedural learning.

To evaluate the use of declarative knowledge in performing the task, subjects were first asked to memorize the 10-digit sequence that would be used. To accomplish this, the four response keys were numbered (1–4) and the subjects were taught the numerical sequence of the asterisk positions (For the right hand, 4231324321; for the left hand, 4143432434). The test began only when the subject was able to repeat the whole numerical sequence verbally without mistakes. Then, six blocks of 100 asterisk positions were presented: in blocks 1–3, asterisk presentation followed the previously learned sequence, and the subjects were informed of this; in block 4, the presentation was random but subjects were informed that it would be sequential; in block 5, the presentation was random and the subjects were informed; finally, in block 6 the asterisk presentation reproduced the previously learned sequence, but the subjects were not informed. To ascertain whether the information was retained, subjects had to repeat the numerical sequence verbally before and after each block.

Results

Control subjects used the previously acquired declarative knowledge efficiently, and thereby markedly improved their performances (Fig. 4B). In fact, all control subjects displayed reduced RTs in blocks 1–3 and 6 with the memorized sequence; they showed a rebound towards longer RTs in random blocks 4 and 5, detected the erroneously announced random sequence in block 4 and recognized the sequence in block 6. No difference was found when the task was performed with the other (right or left) hand. One-way ANOVAs demonstrated the statistical significance of block variables in the trials performed either with the right [$F(5,25) = 38.12$, $P < 0.0001$] or the left hand [$F(5,25) = 21.09$, $P < 0.0001$].

Although patients with cerebellar lesions exhibited significantly longer RTs than control subjects, they maintained the same general trend with shorter RTs in sequential blocks than in random ones (Fig. 4B). This trend was present regardless of which hand was used. A $3 \times 6 \times 2$ ANOVA (group $\times$ block $\times$ hand) revealed significant group [$F(2,11) = 19.74$, $P < 0.001$] and block effects [$F(5,55) = 4.68$, $P < 0.01$], but the effect of the hand used did not reach statistical significance [$F(1,11) = 0.042$].

Experiment 5: motor RT

In the last task, subjects were required to perform a simple motor reaction in response to the asterisk presentation. In this task, the asterisk position was kept stable; therefore there was no sequence to follow and no need to predict the asterisk position. Subjects were told which finger to use, that the asterisk would appear 30 times in the given position and that they had to press the corresponding key on the keyboard. Thirty items were presented for each finger, for a total of 120 items for each hand.

Results

Both control subjects and patients with cerebellar lesions managed the task very easily and although patients with cerebellar lesions displayed slightly longer RTs than control subjects, the difference failed to reach statistical significance in most trials. Only patients with left cerebellar lesions using their left hands displayed significantly longer RTs than control subjects [$F(1,9) = 36.22$, $P < 0.001$]. In any case, in this simple motor task, the mean RTs of patients with cerebellar lesions never exceeded 250 ms, while in sequential trials mean RTs ranged from 450 to 700 ms.

By taking into account the RTs obtained in Experiment 5 (by covarying the RTs obtained in Experiments 1 and 2 in the sequential blocks for RTs obtained in Experiment 5) with a two-way ANCOVA (group $\times$ block), it was possible to confirm the significance already described for the group effect [in Experiment 1 $F(2,10) = 14.14$ ($P < 0.01$) for the right hand and $F(2.10) = 4.87$ ($P < 0.05$) for the left hand; in Experiment 2 $F(2,10) = 11.61$ ($P < 0.01$) for the right hand and $F(2,10) = 4.86$ ($P < 0.05$) for the left hand] and for block effect [in Experiment 1 $F(3,33) = 17.39$ ($P < 0.0001$) for the right hand and $F(3,33) = 3.90$ ($P < 0.05$) for the left hand; in Experiment 2 $F(3,33) = 5.34$ ($P < 0.01$) for the right hand and $F(3,33) = 7.88$ ($P < 0.001$) for the left hand].

Discussion

The main results of the present study are as follows: unilateral cerebellar lesions severely impair procedural learning of a visuo-motor task, regardless of hand used and degree of post-lesional motor disturbance; focal cerebellar damage clearly affects the detection of a sequence and the acquisition of declarative knowledge about it; these deficits are present regardless of the side of the cerebellar lesion. Thus, unilateral cerebellar lesions affect procedural learning of both hands.

Pascual-Leone *et al.* (1993) reported a lack of procedural learning in patients affected by cerebellar degeneration in a task similar to that employed in the present research. The main difference between Pascual-Leone's study and ours is the aetiology of the cerebellar pathology, i.e. atrophy versus focal unilateral damage. Studying cognitive functions in patients with focal lesions provides various advantages than similar studies in atrophic patients. First, cerebellar lesions can be precisely defined on MRI scans and the lack of MRI evidence of any extracerebellar damage makes the lesion selectivity highly probable. In addition, the use of patients with unilateral cerebellar damage allows examination of the problem of lateralization. Although cerebellar efferents are lateralized and cerebellar motor deficits are mainly ipsilateral, surprisingly, in our experimental protocol, patients with unilateral cerebellar lesions were defective in learning the

task with both hands. Recent PET studies (van Mier *et al*., 1994, 1995), in normal right-handed subjects performing procedural motor learning tasks with right or left hands separately, are consistent with the present data. Marked bilateral cerebellar activation was observed, regardless of the hand used. Left cerebellar activation was more evident during procedural learning, and decreased once the procedure was learned. Conversely, in the right cerebellum two activation areas were revealed, one at the level of the dentate nucleus and another slightly below it, both present during learning and execution of already learned procedures. In our study, the difference between patients with right or left cerebellar lesions did not reach statistical significance in any of the tests employed; nevertheless, it is important to stress that patients with left cerebellar lesions always performed worse than right cerebellar ones, thus supporting PET findings of bilateral activation with left prevalence of cerebellar structures during procedural learning.

It is always possible in any study on procedural learning in patients with cerebellar lesions that motor deficits affecting motor components of the task may obscure the significance of concomitant cognitive effects. To overcome this pitfall, patients included in the present study were not very impaired in their motor abilities so that, when a simple motor reaction was tested in response to asterisk presentation (Experiment 5), their RTs did not, in general, differ significantly from those of the control subjects.

By comparing RTs obtained in trials with random or patterned sequences, it was possible to analyse the effect of repeating a sequence in the very same subject. In Experiment 1, using right hands, control subjects as well as patients with cerebellar lesions displayed an RT reduction in sequential blocks, with a clear improvement in the last random block. This finding indicates that the response facilitation in blocks 2–5 resulted from the repetition and subsequent learning of the sequence and not from a mere motor 'practice' effect. The difference between control subjects and patients with cerebellar lesions was most clear when they used their left hands: control subjects displayed clear-cut learning and patients with cerebellar lesions exhibited RTs not influenced by presence/absence, or repetition of, sequential blocks, indicating the absence of any procedural learning. When there were 10 elements in the sequence (Experiment 2), the performances of patients with cerebellar lesions were severely impaired, displaying flattened curves with no significant differences in RTs of random or sequential blocks, for either hand. The hypothesis that this lack of improvement could be due to a ceiling effect in performing faster finger responses is ruled out by the fact that RTs in the simple motor task of Experiment 5 were significantly shorter than those displayed in tasks with procedural demands (Experiments 1 and 2). Furthermore, by analysis of covariance, the RTs obtained in Experiments 1 and 2 with those obtained in Experiment 5, it was possible to demonstrate that the significance of the difference between random and sequential blocks, like that between patients with cerebellar lesions and control subjects, was unaffected by differences in the execution of the visuomotor components of the task.

How can the bimanual alteration in procedural learning be interpreted? First, the fact that learning during a serial reaction-time task results in bilateral activation of the cerebral cortex (Grafton *et al*., 1995) should be considered. Several studies on cognitive abilities of patients with cerebellar lesions have focused on the role of the cerebellum in indexing and ordering activation of different functional modules (Pascual-Leone *et al*., 1993; Silveri *et al*., 1994, 1997). Following this line of thought, since procedural learning requires integration between cortical hemispheres, a unilateral cerebellar lesion will affect learning regardless of which side of the body performs the motor task. On the other hand, taking into account the importance of the cerebellar circuits for detecting event sequences (Braitenberg *et al*., 1997), it is possible that the procedural impairment of patients with cerebellar lesions depends on difficulties in detecting the sequential pattern. In any case, bilateral processing of sensory information is still required, and thus a unilateral lesion would still affect performances bilaterally.

As widely demonstrated (Schacter, 1985; Squire, 1992; Grigsby, 1994), systems of both procedural and declarative learning can work on a parallel and independently of each other. Thus, impairment of one system is not necessarily coupled with impairment of the other. Along these lines, it seems interesting to recall that, although a number of paradigms are reported in the literature in which procedural learning is spared and declarative memory is severely defective, the mirror paradigm, in which a procedural deficit is linked to relative sparing of a declarative one, is rather unusual (Schacter, 1985, 1987; Heindel *et al*., 1989; Deweer *et al*., 1993; Pascual-Leone *et al*., 1993; Butters *et al*., 1994).

Our results and those of Pascual-Leone *et al.* (1993) indicate that patients with cerebellar lesions are clearly impaired in acquiring a sequence, regardless of the means of presentation (visuomotor or visual). The possible use of declarative knowledge to improve procedural learning was tested in Experiment 5. Our findings are somewhat different from those of Pascual-Leone *et al.* (1993), who reported that declarative knowledge of the sequence did not result in shortening RTs of their patients with cerebellar atrophy. Conversely, in our study, patients with focal cerebellar lesions were very competent in making use of their previously acquired knowledge of the sequence, significantly improving their performances. This observation suggests that patients with focal cerebellar lesions are more affected in detecting a sequence than in performing it. These different findings could be due to differences in the selectivity or severity of the cerebellar lesion. While the severity of the procedural impairment in the patients of Pascual-Leone *et al*. (1993) did not allow for speculation about the level of cerebellar contribution to learning, our data suggest that the role of the cerebellum in procedural learning might be more crucial in detecting index and order of events than in planning the execution of indexed and ordered events. This emphasis on

the sensory function of the cerebellum is in line with recent MRI or PET data reporting cerebellar activation during acquisition and discrimination of sensory information (Gao *et al*., 1996) or during observation of movements (Decety *et al*., 1994).

Recently, a further dissociation of different forms of procedural memory has been added to the classical dissociation of procedural and declarative memory. This arises from clinical observations of patients with progressive degenerative brain diseases. For example, Parkinson's disease patients are selectively impaired in skill learning components of the fragmented pictures test and mirror reading, but not in pursuit-rotor tracking (Bondi and Kaszniak, 1991; Roncacci *et al*., 1996). Huntington's disease patients are impaired in tasks involving skill learning but not in those involving priming (Knopman and Nissen, 1991). Alzheimer's patients are unimpaired in pursuit-rotor learning, but impaired in lexical priming (Bondi and Kaszniak, 1991). This dissociation can be explained by the hypothesis that different forms of procedural memory may be dependent on distinct neuroanatomical systems (Perani *et al*., 1993, Pascual-Leone *et al*., 1996). According to Saint-Cyr *et al*. (1988), procedural learning depends on the establishment of heuristic strategies through the action of a circuit involving the neostriatum, particularly the caudate nucleus, and the prefrontal cortex. Our results, in accordance with the proposal of Grafman *et al.* (1992), indicate that the cerebellum has to be added to these structures. In fact, increasing knowledge about the connectivity, behavioural function and neural activation of the cerebellum in animals and man requires inclusion of cerebellar networks in the list of areas responsible for procedural learning. Along these lines, it is important to note that recent experimental data obtained testing spatial performances of hemicerebellectomized rats in the Morris Water Maze (Petrosini *et al*., 1996), demonstrate clear deficits in the procedural aspects of the task, aspects which are normally prerequisits for correct performance of the task and for learning its declarative component. Another interesting analogy between these two very different experimental settings is that, just as patients with cerebellar lesions are able to detect and repeat a sequence once it has been learned through a declarative strategy, hemicerebellectomized rats are also able to show an adequate procedural response once they have learned the appropriate response through declarative components. Both observations suggest the cerebellum may be one of the sites of formation and use, but not of storage, of procedural strategies. As suggested by Squire and Zola-Morgan (1988), the neocortex is perhaps the most attractive site for long-term storage of declaratively and procedurely acquired knowledge.

In normal subjects it is possible to impair procedural learning in serial RT tasks by requiring them to perform another task simultaneously, such as tone counting, demonstrating that a certain attentional level is required for learning to occur (Nissen and Bullemer, 1987). Recently, difficulties in attention shifting have been described in patients with cerebellar lesions (Courchesne *et al*., 1994). Although it is not possible to exclude the possibility that the procedural learning impairment might partially derive from a reduction in attention resources, analysis of the present results, reports of normal attentional processes in patients with cerebellar degeneration (Dimitrov *et al*., 1996), as well as recent PET data demonstrating that attention interference affects declarative but not implicit learning (Grafton *et al*., 1995), support the hypothesis of direct involvement of cerebellar circuits in procedural learning.

In conclusion, the present data, concerning patients with focal cerebellar lesions, demonstrate procedural deficits which are not correlated with the degree of motor impairment and are present regardless of the side of cerebellar damage and the somatic side involved in the motor execution of the procedure. Finally, the severity of the difficulty in detecting a sequence, with respect to defects in performing it, points toward a prevalent role of the cerebellar circuitry in detecting and recognizing event sequences rather than in planning and executing them.

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