Cerebellar involvement in cognitive flexibility

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ABSTRACT

The aim of the present study was to investigate whether the cerebellar structures are involved in functions requiring cognitive flexibility abilities. The flexibility of the hemicerebellectomized and control animals in learning a four-choice learning task, adapting to ever-changing response rules was investigated. While in the initial phase of the task both experimental groups exhibited similar performances, only the control animals significantly improved their performance as the sessions went by. The lack of improvement in lesioned animals' performance rendered their responses particularly defective in the final phases of the task, when conversely intact animals performed best, exploiting their "learning to learn" ability. The findings demonstrate the defective influence of the cerebellar lesion on the acquisition, not the execution, of new responses. The results underline the crucial role of the cerebellum in mediating cognitive flexibility behaviors.

1. Introduction

Of the many cognitive functions controlled by the cerebellum, the ability to link a context with the appropriate response represents a cerebellar specificity (Thach, 1997, 2007). When this linkage is built the occurrence of the context (represented by a certain input reaching the cerebellum) triggers the appropriate response through the cerebellar areas. The implication is that through practice an experiential context automatically evokes a certain action plan. The specific cerebellar contribution is the context-response linkage and the shaping of the response through trial and error learning. Learning to associate a context with a response is useful in specific situations, but it has to be generalized to conditions slightly different from those present during training; otherwise, any context variation would prevent learning from ever being expressed. However, too much generalization is undesirable, because a learned response would be maladaptive if expressed in an inappropriate context. Thus, the interplay between applying a learned response in a given context and modifying it to adapt to a different situation is an important adaptive property that allows optimizing performances. Accordingly, cognitive flexibility (CF) allows animals and humans to adjust their behavior to environmental changes, that is, to learn

how to link a changing context to a novel behavior. Besides context-response linkage, properties of flexibility involve detection of novelty, use of working memory, performance monitoring, response inhibition, and selection or decision making (Dalley, Cardinal, & Robbins, 2004; Wolpaw & Carp, 2006). There is increasing evidence that in its different forms CF is mediated by the medial prefrontal cortex (Birrel & Brown, 2000; Miller, 2000) and the orbitofrontal cortex (Boulougouris, Dalley, & Robbins, 2007). While medial prefrontal cortex is involved in switching general rules, strategies or attentional sets (Birrel & Brown, 2000; Brown & Bowman, 2002; Dias, Robbins, & Roberts, 1996, 1997; Ragozzino, Detrick, & Kesner, 1999), the orbitofrontal cortex has a role in stimulus-reinforcement associations. Also cholinergic depletion of basal forebrain affects flexibility in adapting to changing response rules in serial learning tasks (Cabrera, Chavez, Corley, Kitto, & Butt, 2006; De Bartolo et al., 2008).

Because of the large number of anatomo-functional connections between the cerebellum and the prefrontal cortex (Middleton & Strick, 2001), it can be speculated that these areas interact in planning, the former by permitting acquisition of new efficient competencies and the latter by providing flexible shifting among already acquired and stored solutions (Bellebaum & Daum, 2007; Frith, Friston, Liddle, & Frackowiak, 1991; Hyder et al., 1997; Pochon et al., 2001; Spence, Hirsch, Brooks, & Grasby, 1998). Therefore, it is consistent to hypothesize that the cerebellum has a role in cognitive flexibility (CF). To investigate this hypothesis hemicerebellectomized (HCbed) and intact rats were tested in dai-

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ly sessions using a four-choice serial task in which the sequence of correct choices changed every day (De Bartolo et al., 2008). This serial learning task requires CF in that animals have to adapt to changing response sequences because reinforcement contingencies are modified daily. As the correct sequence was unpredictable and different every day, the task required a continuous and efficient change in response. Facing daily changing sequences addressed the question of whether it was possible to forget previously learned correct choices and acquire new ones in the presence of a cerebellar lesion.

Hemicerebellectomy (HCb) was chosen as experimental model of the cerebellar lesion because provoking less disrupting motor effects than a complete cerebellectomy (Federico, Leggio, Mandolesi, & Petrosini, 2006; Manni & Dow, 1963; Molinari, Petrosini, & Gremoli, 1990) and it allows executing tasks requiring locomotor performances (traveling down the alley and traverse the doors), as required by the present task. Furthermore, it has been repeatedly demonstrated that in rats even a unilateral cerebellar lesion is able to affect a large range of cognitive functions (Colombel, Lalonde, & Caston, 2004; Leggio et al., 2000; Mandolesi, Leggio, Graziano, Neri, & Petrosini, 2001; Mandolesi, Leggio, Spirito, Federico, & Petrosini, 2007; Mandolesi, Leggio, Spirito, & Petrosini, 2003; Molinari, Grammaldo, & Petrosini, 1997; Petrosini, Molinari, & Dell'Anna, 1996).

Since no definite indication is present in the literature as for any behavioral lateralization of cerebellar structures (Colombel et al., 2004), the unilateral cerebellar lesion was performed in all lesioned animals on the right side, on the analogy of the quoted studies.

2. Materials and methods

2.1. Subjects and experimental groups

Adult male Wistar rats were used in the present research. They were housed two animals to a cage and maintained on a standardized dark/light schedule (12 h), following the guidelines for ethical conduct developed by the European Communities.

Council Directive of 24 November 1986 (86/609/EEC).

The animals were randomly assigned to the two experimental groups. Data were collected from eight HCbed animals that performed the task for the first time after the cerebellar lesion (H group) and from eight intact rats used as controls (C group).

2.2. Surgery

The rats were anesthetized with an i.p. solution of ketamine (90 mg/kg) and xylazine (15 mg/kg). A craniotomy was performed over the right hemicerebellum. The dura was excised and the right cerebellar hemisphere and hemivermis were ablated by suction. Care was taken not to lesion extra-cerebellar structures. The cavity was filled with sterile gel foam, the wound edges were sutured, and the animals were allowed to recover from anesthesia and surgical stress. Testing was performed three weeks after the HCb, when no change in cerebellar symptomatology was observed. Animals were submitted to behavioral testing only if they exhibited stable motor symptomatology consistent with a cerebellar lesion. Animals of the same age, sex and weight of the hemicerebellectomized rats were used as Control animals on the analogy to the previously quoted papers analyzing cognitive functions in the presence of hemicerebellectomy (Leggio et al., 2000; Mandolesi et al., 2001, 2003, 2007; Molinari, Grammaldo, et al.,1997; Petrosini et al., 1996).

2.3. Neurological assessment

Three weeks after the cerebellar lesion HCbed rats displayed a slight extensor hypotonia ipsilateral to the lesion that resulted in

a slight tendency to body tilt to the right. The rhythmic head bobbing observed immediately after the lesion was no more present. Animals' gait was wide-based and slightly ataxic. During locomotion HCbed rats tended to lower their center of gravity, which led them to collapse on their bellies. In spite of such a motor symptomatology, no HCbed animal exhibited such critical akinetic symptoms as to impede reliable behavioral testing.

2.4. Apparatus

The apparatus was placed in a lab that was uniformly illuminated by means of a masked neon ceiling lamp (40 W). It consisted of a straight white wooden alley (cm $150 \times 40 \times 40$) subdivided into five compartments (30 cm long) by four pale gray panels (Fig. 1A). Each panel had two unidirectional swinging doors (height 10 cm, width 8 cm). Each door could be locked using a pivot put in the vertical wall behind the door. If the animal pushed the door when it was unlocked, it opened and allowed access to the next compartment. If the animal pushed the door when it was locked, it only opened about 2 cm. The small split allowed the rat to introduce its muzzle, but prevented it from going through the door. This trick permitted us to obtain sure proof that they were attempting to open the "wrong" door. The entire apparatus was covered by a transparent Plexiglas cover. The fourth panel introduced the fifth and final compartment which contained the reward, i.e., a piece of Purina chow. To motivate the animal further to reach the reward, the final compartment was darkened using a black cover.

2.5. Pre-training

Before the experiments began the animals were food-deprived, but had free access to water. They were weighed once a day and were kept at 80–85% of their ad lib weight throughout the experiment. At the end of testing no significant difference was found in the body weight of the animals in the experimental groups (mean values recorded on the last day of testing: C group: 471 ± 52 g; HCbed group: 462 ± 52 g).

On the first pre-training day, pairs of animals were allowed free exploration of the apparatus, from which the inner panels had been removed. The final part of the apparatus, which would become the rewarded compartment, had many pieces of Purina chow scattered on the floor. On the second pre-training day, a single rat was placed in the apparatus, which was, again, without the inner panels. The trial ended when the animal reached the final part of the apparatus and took the reward. On the third pre-training day, a single rat was
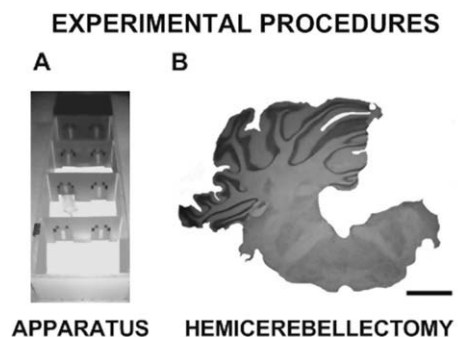

Fig. 1. (A) Apparatus of the four-choice serial learning task. Note the compartments subdivided by the panels with two unidirectional swinging doors. The last compartment was darkened by a black cover. The rat was traversing the second panel by passing through the left "correct" door to reach the third compartment. (B) Nissl-stained coronal section through cerebellum and brain stem in a HCbed rat. Note the total absence of the right hemicerebellum and the sparing of any extra-cerebellar structure. Scale bar: 2 mm.

again placed in the apparatus. The first and fourth panel had been replaced and both doors were unlocked. Thus, the first and last compartments were closed, while the central area was still an open space. One piece of Purina chow was put on the floor of the last compartment. The trial ended when the animal reached the reward and took it. On the fourth pre-training day, a single rat was placed in the apparatus in which all panels had been replaced and both doors unlocked. The trial ended when the animal reached the reward and took it. Testing sessions began the next day.

2.6. Testing procedures

To be sure that the two groups were equated for within-session performance the animals of both groups were tested in a pre-test session formed by 12 trials. A two-way ANOVA (group × trial) on the total errors failed to reveal any significant group effect ($F_{1,14}$ = 1.67; p n.s.), while trials ($F_{1,154}$ = 6.67; p = 0.00001) were significant. The interaction was also not significant ($F_{1,154}$ = 0.98; p n.s.).

On such a basis, after the pre-test session, each rat underwent a 12-trial session a day for nine consecutive days. At the beginning of the trial, the rat was placed in the starting compartment facing the panel with the doors. In each trial, the goal was to reach the fifth compartment and collect the reward by going through the open doors and making no attempt to force open the closed ones. Each animal was given a prearranged sequence of open doors (e.g., right–right–left–right), which remained stable for all 12 trials of a session and changed every session; thus, each animal was tested in nine different sequences. The only sequence never used was four doors open on one side at the same time, e.g., right–right–right–right. A trial ended when the rat reached the last compartment.

At the end of each trial, the rats were put back in their cages for 60 s. During this interval, experimenters replaced the reward. At the end of each session, the apparatus was cleaned with alcohol to prevent the rats from sniffing other animals' pathways.

At the end of testing, each animal had undergone 108 trials in which the number of correct openings of the right or left doors was completely balanced.

2.7. Behavioral parameters

The following parameters were analyzed: *time* (s) spent to complete each trial; *total errors*, that is, the attempts to force open the closed door of a panel during a trial (in each trial this parameter ranged from the worst value of four to the best value of zero); *perseverative errors*, that is, the number of errors made at the same door in consecutive trials; *right/left errors*, that is, the number of errors considering their side to determine whether there was a side bias; *correct sequences*, that is, the longest sequence of correct choices (in each trial this parameter ranged from the worst value of zero to the best value of four); *learning velocity*, that is, the slopes of the linear interpolation (β) in each session calculated for each animal on times (β_t) and on the number of errors (β_e) in the 12 trials of a session; *error-free trials*, that is, the number of trials in which no attempts were made to force open the closed doors.

2.8. Open-field testing

To analyze differences in general activity levels and emotionality of the rats belonging to the two experimental groups, open-field activity was measured. The apparatus consisted of a circular box (diameter 140 cm) delimited by a wall 30 cm high. The floor was painted pale gray and divided into sections by black lines (Mandolesi et al., 2003). During the session, which lasted 6 min, each rat

was allowed to move in the empty open field. The following parameters were analyzed: as emotional parameters, number of defecation boluses and motionless time; as motor parameters, total distance (m) traveled in the arena and percentage of total distance traveled in a 20 cm peripheral annulus.

2.9. Histological controls

When behavioral testing was completed the lesioned animals were deeply anesthetized and perfused intracardially with saline followed by 10% buffered formalin. The extent of the cerebellar lesions was determined from Nissl-stained 50 μm frozen sections. Lesioned animals were included in the present study if they had received a right HCb with total ablation of deep nuclei. In all animals included in the present research the left side of the cerebellum and all extra-cerebellar structures were completely spared, except for the dorsal cap of Deiters' nuclei, which in some cases was slightly affected. Variability in the extent of the floccular and vermian lesions was not taken into consideration, because in all cases these structures were functionally disconnected due to ablation of the cerebellar peduncles and deep nuclei of the right side (Fig. 1B).

2.10. Statistical analysis

Metric unit results were compared using one-way, two-way (with group as between-subject factor and phase (or side) as within-subject factor), or three-way (with group as between-subject factor and session and trial as within-subject factors), followed by multiple comparisons using Duncan's test.

Results of the nine sessions were averaged (or summed as in the case of perseverative errors and the error-free trials) in three groups of three sessions to analyze the behavioral results in the initial (1–3rd sessions), middle (4–6th sessions) and final (7–9th sessions) phases of the task.

3. Results

3.1. Times

The two experimental groups spent different times in performing the task as revealed by a two-way ANOVA (group × phase). This analysis showed significant group ($F_{1,14}$ = 14.82; p = 0.0017) and phase ($F_{2,28}$ = 8.20; p = 0.0015) effects. The interaction was also significant ($F_{2,28}$ = 16.0; p = 0.000001). *Post-hoc* comparisons showed significant group differences in all phases (initial phase: H vs. C p = 0.048; middle phase: p = 0.00013; final phase: p = 0.00013). Furthermore, whereas C animals significantly (p = 0.000001) reduced their times as the phases went by, H animals did not decrease their times to complete the task.

To analyze learning within trials and sessions, the times spent by the two groups of animals in the twelve trials of the first, fifth and ninth sessions were compared by means of a three-way ANOVA (group × session × trial). Group ($F_{1,14}$ = 11.19; p = 0.0048), session ($F_{2,28}$ = 25.40; p = 0.000001) and trial ($F_{11,154}$ = 3.73; p = 0.000001) effects were highly significant. The first-order interaction group × session ($F_{2,28}$ = 9.12; p = 0.0008), as well as the second-order interaction ($F_{22,308}$ = 2.56, p = 0.0001) were also significant.

Interestingly, while C animals exhibited significant learning throughout the 12 trials of all three sessions (one-way ANOVAs: 1st session: $F_{11,77}$ = 3.70; p = 0. 0003; 5th session: $F_{11,77}$ = 4.72; p = 0.00001; 9th session: $F_{11,77}$ = 6.66; p = 0.00001), H animals showed no learning in any session (1st session $F_{11,77}$ = 0.69; p n.s.; 5th session: $F_{11,77}$ = 0.66; p n.s.; 9th session: $F_{11,77}$ = 1.64; p n.s.) (Fig. 2A).

3.2. Errors

Although both groups of animals made a similar number of errors in the initial phase, from the middle phase onward the two groups differed significantly. A two-way ANOVA (group × phase) revealed significant group ($F_{1,14}$ = 10.40; p = 0.006) and phase ($F_{2,28}$ = 6.90; p = 0.0036) effects. The interaction was also significant ($F_{2,28}$ = 14.03; p = 0.000001). *Post-hoc* comparisons showed no significant group difference in the initial phase, whereas the middle (p = 0.00021) and final (p = 0.00013) phases were characterized by significantly more errors in the H animals than in the C animals. Once more, while C animals progressively learned the task diminishing the errors (p = 0.0002), H animals maintained unvaried performances during the whole task.

To analyze learning within trials and sessions, the errors made by the two groups of animals in the twelve trials of the first, fifth and ninth sessions were compared by means of a three-way ANOVA (group × session × trial). Group ($F_{1,14}$ = 11.12; p = 0.0049), session ($F_{2,28}$ = 18.47; p = 0.000001) and trial ($F_{11,154}$ = 15.30; p = 0.000001) effects were highly significant. The first-order interactions group × session ($F_{2,28}$ = 8.50; p = 0.0012) and group × trial ($F_{11,154}$ = 2.73, p = 0.002) were significant, whereas the interaction trial × session ($F_{22,308}$ = 1.44, p n.s.), and the second-order interaction ($F_{22,308}$ = 0.49, p n.s.) were not significant. It is noteworthy that although C animals exhibited a significant learning throughout the 12 trials of all three sessions taken into account (one-way ANOVAs: 1st session: $F_{11,77}$ = 4.96; p = 0.00001; 5th session: $F_{11,77}$ = 4.82; p = 0.00001; 9th session: $F_{11,77}$ = 10.52; p = 0.000001), H animals showed a significant learning only in the 1st session ($F_{11,77}$ = 2.52; p = 0.009) and no learning in the 5th ($F_{11,77}$ = 0.73; p n.s.) and 9th ($F_{11,77}$ = 1.64; p n.s.) session (Fig. 2B). These data

indicate that the deficit of the H group was characterized by impaired shifting of responses, a behavior that requires forgetting the previously correct sequence in order to acquire a new one.

3.3. Perseverative errors

A two-way ANOVA (group × phase) on the perseverative errors revealed a significant group effect ($F_{1,14}$ = 11.02; p = 0.005), while the phase effect was not significant ($F_{2,28}$ = 0.67; p n.s.). The interaction was significant ($F_{2,28}$ = 4.31; p = 0.023). *Post-hoc* comparisons indicated significant differences between groups as the phases went by (Fig. 3A). Once more, while C animals progressively diminished the perseverative errors (p = 0.043), H animals maintained unvaried performances during the whole task.

3.4. Right/left errors

Because of the presence of a unilateral cerebellar lesion it seemed necessary to determine whether there was a side bias. Thus, the number of errors made at the right and left doors was analyzed in both groups of animals. A two-way ANOVA (group × side) revealed a significant group effect ($F_{1,14}$ = 8.86; p = 0.01), whereas side effect ($F_{1,14}$ = 2.0; p n.s.) and interaction ($F_{1,14}$ = 0.4; p n.s.) were not significant, indicating there was no side prevalence in either group (Fig. 3B).

3.5. Learning velocities

3.5.1. β_t Analysis

The learning velocity calculated on times to complete the task of the C animals was significantly higher to that of the H animals
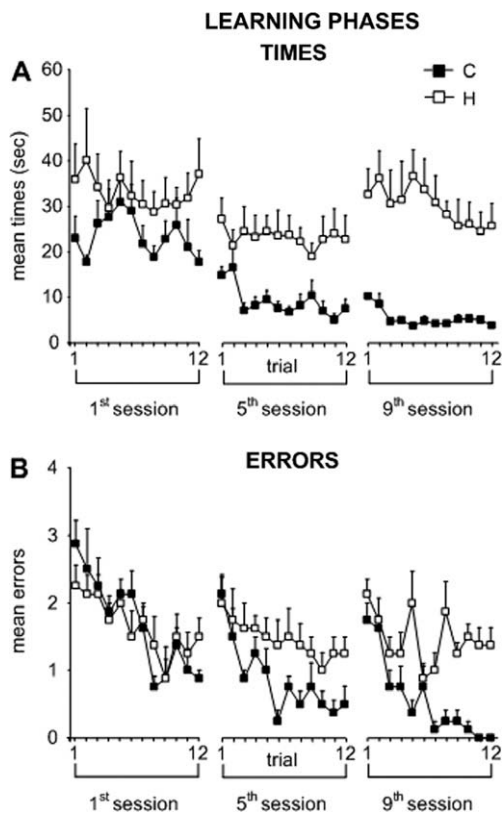

Fig. 2. Times (A) and errors (B) displayed by Control (C) and HCbed (H) groups in the 12 trials of the 1st, 5th and 9th session are depicted. In this and in the following figures vertical bars indicate SEM.
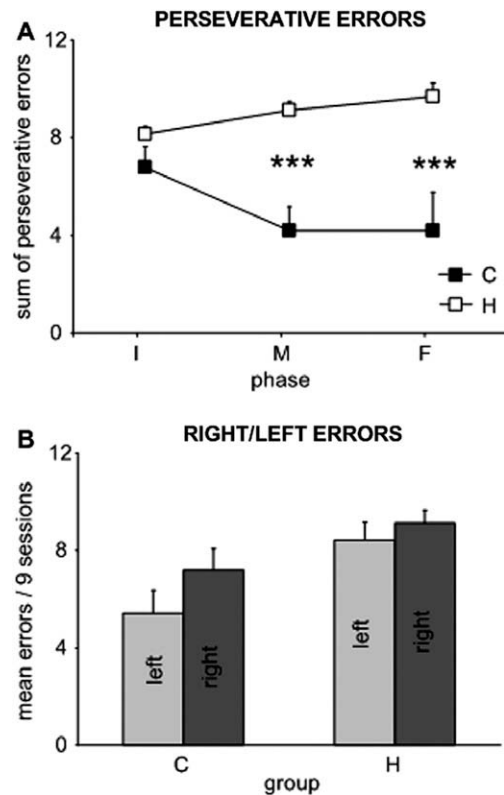

Fig. 3. Performance of control (C) and HCbed (H) groups in the serial learning task. Perseverative errors (A) and right/left errors (B) are depicted. Initial (1–3rd sessions), middle (4–6th sessions) and final (7–9th sessions) phases of the task are indicated. Asterisks indicate *post-hoc* comparisons between groups. ***p < 0.0001.

($F_{1,14}$ = 5.63; p = 0.032) as revealed by a two-way ANOVA (group × phase). While the session effect was not significant ($F_{2,28}$ = 1.58; p n.s.), the interaction ($F_{2,28}$ = 5.17; p = 0.012) was significant. *Post-hoc* comparisons indicated significant differences between groups as the phases went by (Fig. 4A). Once more, while C animals progressively enhanced their learning velocity (p = 0.0079), H animals maintained unvaried performances during the whole task.

3.5.2. β_e Analysis

In all phases the learning velocity calculated on errors of the C animals was superior to that of the H animals ($F_{1,14}$ = 20.14; p = 0.00051) as revealed by a two-way ANOVA (group × phase). Neither the session effect ($F_{2,28}$ = 0.12; p n.s.) nor the interaction ($F_{2,28}$ = 1.04; p n.s.) were significant since both groups exhibited stable learning velocities as the phases went by (Fig. 4B).

3.6. Correct sequences

The longest sequences of correct choices displayed in the final (12th) trials of the sessions belonging to the initial, middle and final phases by the animals of two experimental groups were compared by means of a two-way ANOVA (group × phase). This analysis revealed a significant group effect ($F_{1,14}$ = 27.96; p = 0.00011) while phase ($F_{2,28}$ = 0.20; p n.s.) and interaction ($F_{2,28}$ = 1.47; p n.s.) were not significant (Fig. 4C).

3.7. Error-free trials

When the error-free trials were considered the pattern of performance observed in the two groups was further confirmed. A two-way ANOVA (group × phase) revealed significant group ($F_{1,14}$ = 19.28; p = 0.0006) and phase ($F_{2,28}$ = 56.15; p = 0.012) effects. Interaction was also significant ($F_{2,28}$ = 5.49; p = 0.009). *Post-hoc* comparisons showed no significant differences between groups in the initial phase, whereas C animals performed a significantly higher number of error-free trials in the middle and final phases than H animals (Fig. 4D). Once more, while C animals progressively improved their performance (p = 0.0002), H animals maintained unvaried performance during the whole task.

3.8. Open-field activity

To analyze differences in rats' general motor activity levels and emotionality, open-field activity was measured. All animals exhibited a comparable level of anxiety, as indicated by the absence of significant differences in the number of defecation boluses (one-way ANOVA: $F_{1,14}$ = 0.09; p n.s.) (Fig. 5A). Although a true freezing behavior was never observed, not significantly different length motionless periods (one-way ANOVA: $F_{1,14}$ = 0.30; p n.s.) were observed in the two groups (Fig. 5B). Conversely, while no significant difference was found between groups in the total distance traveled within the arena ($F_{1,14}$ = 2.81; p n.s.) (Fig. 5C), the H group displayed higher percentages of peripheral traveling ($F_{1,14}$ = 7.71; p = 0.0014) (Fig. 5D).

4. Discussion

The present findings demonstrate that cerebellar circuits play a crucial role in mediating cognitive flexibility. In fact, cerebellar lesions severely impaired the animals' behavior in adapting to changing sequences in a four-choice learning task. Such impairment in the task acquisition cannot be ascribed to severely impaired motor functions or discriminative abilities, or to low levels of motivation. In spite of the postural and motor deficits provoked by the cerebellar lesion, H animals exhibited efficient locomotor function as well as preserved motivation inside the OF arena and in the alley of the flexibility task. In agreement with previous reports (Mandolesi et al., 2003) the explorative pattern of the
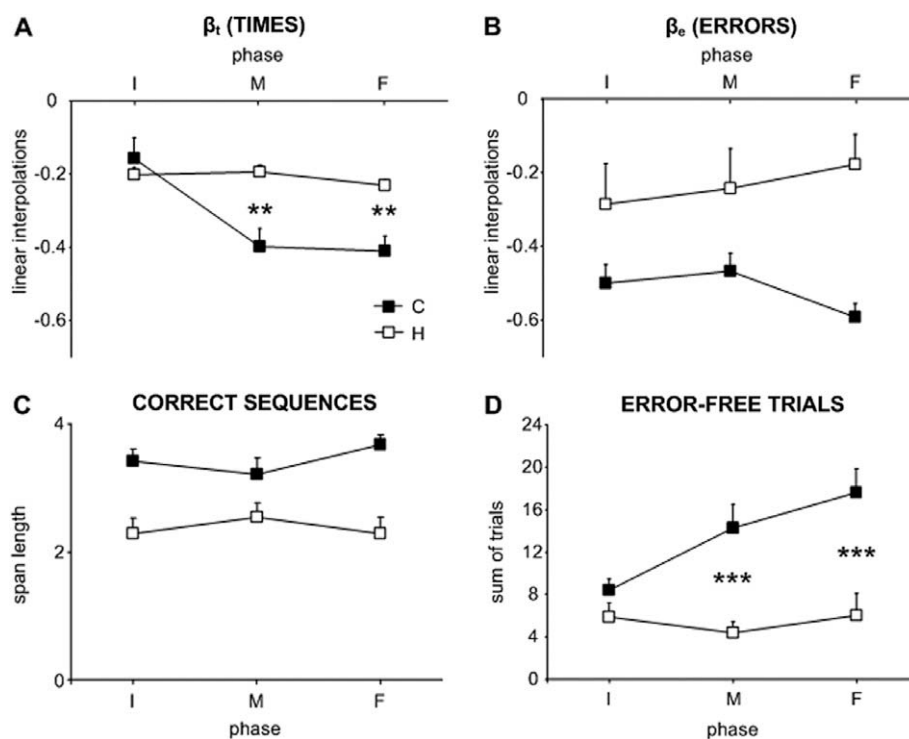

Fig. 4. Performance of control (C) and HCbed (H) groups in the serial learning task. (A) β_t; (B) β_e; (C) the longest correct sequences made by the two experimental groups; D: sum of the error-free trials. Initial (1–3rd sessions), middle (4–6th sessions) and final (7–9th sessions) phases of the task are indicated. Asterisks indicate *post-hoc* comparisons between groups. **p < 0.001; ***p < 0.0001.
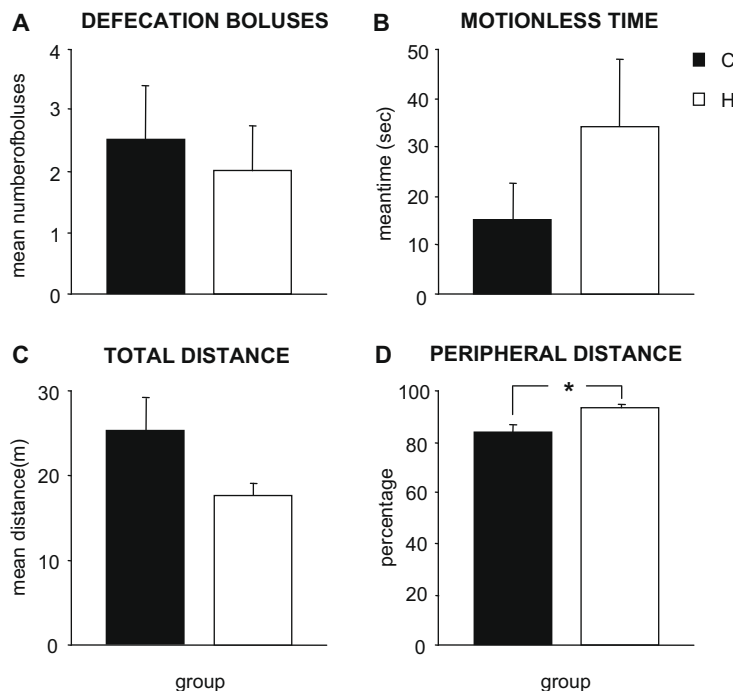
Fig. 5. Performance of Control (C) and HCbed (H) groups in the open-field task. (A) mean number of defecation boluses; (B) motionless time spent throughout the task; (C) total distance traveled in the arena; (D) distance traveled in the peripheral annulus of the arena. $^*p < 0.01$.

HCbed animals in the OF arena was characterized by long exploration of the outer annulus. This finding could be interpreted as a sign of the impaired explorative strategies displayed by the lesioned animals only if it was excluded that it indicated increased anxiety levels. Indeed, when the level of emotionality was assessed by counting the number of defecation boluses in the OF test (Mandolesi et al., 2003; Whimbey & Denenberg, 1967), it appeared to be similar in both groups of animals. The absence of freezing behavior, which is considered indicative of a high-stress state (Whimbey & Denenberg, 1967), was a further sign of very low anxiety levels. These observations fit with analogous reports of reduced anxiety in other models of cerebellar damage, such as *nervous* mutant mice, characterized by a loss of Purkinje cells, and *staggerer* mice, whose cerebellar cortex is almost completely degenerated (Lalonde & Botez, 1985; Lalonde, Manseau, & Botez, 1988).

In the initial phase of cognitive flexibility test both experimental groups exhibited a similar number of total errors, of perseverative errors and of error-free trials, indicating that all animals were able to perform the task regardless of the presence of the lesion. Nevertheless, although control animals significantly improved their performance as the sessions went by diminishing total and perseverative errors, increasing learning velocity, lengthening the sequence of correct responses, and enhancing the number of error-free trials, lesioned animals showed no performance improvement. In fact, their number of total and perseverative errors remained the same throughout the task; learning velocity not only did not increase, it even tended to diminish in the final phase; and their error-free trials did not increase and their sequence of correct responses did not lengthen, as the sessions went by. Even the analysis of error distribution within trials and sessions demonstrated that HCbed animals significantly reduced their errors only in the twelve trials of the initial session and that they failed to decrease them in the successive sessions, unlike the Control animals. Furthermore, differently from intact animals, HCbed animals did not improve their performances from the first to the last session. Even the times spent to complete the

twelve trials of the first, fifth and ninth session did not exhibit any progressive reduction in HCbed animals at odds with those displayed by Control animals. On the whole, the lack of improvement in HCbed animals' performance rendered their responses particularly defective in the final phases of the task, when intact animals performed best, exploiting their "learning to learn" ability.

This defective influence on the acquisition, not the execution, of new sequences completely fits with previous results. In spatial paradigms HCbed rats displayed severe deficits in acquiring efficient navigational strategies (Petrosini, Leggio, & Molinari, 1998; Petrosini et al., 1996) and put into action only the preoperatively learned explorative strategies. Thus, they failed to shift their behavior according to modified contexts (Leggio et al., 1999). Furthermore, HCbed rats exhibited an inflexible use of the procedures. They showed neither worsening nor learning in the radial maze (Mandolesi et al., 2001) and in the open-field task (Mandolesi et al., 2003).

To sum up, the HCbed animals displayed great difficulty in facing tasks with changing responses and, paradoxically, this was not because of their motor symptoms. It has to be underlined that these effects were observed three weeks after the surgery and it remains to be determined whether the results could be different in the advanced stages of recovery, for example, months after the cerebellar ablation.

The CF task adopted in the present research was previously employed in the paper by De Bartolo and colleagues (2008) to analyze the flexibility abilities of cholinergically depleted rats. An optimal performance in this task required detecting the correct sequence of open doors in the first day/session and remembering it for the twelve trials of the session. In the successive sessions, the general rule remained obviously constant: throughout all sessions four doors were open; in the same panel only one door was opened; the doors were closed by unidirectional panels, so it was forbidden to come back to the preceding compartment; the fifth compartment was always rewarded; the sequence of

open doors was maintained constant for the whole session. However, the sequence of open doors was daily changed, thus the stimulus/response associations had to be modified from session to session. It was, in fact, incongruous to form a "stable" reference frame, because what was correct in one session was no longer correct in a later session. The record of the correct choices made in one session had to be canceled from memory and updated in the next session. Thus, the processing needed to acquire the task in all twelve trials of a session was different from the processing needed to deal with the ever-changing sequences of the nine sessions. While the daily task tapped mainly mnesic functions, acquisition of new sequences, which were unpredictable and different every day, required efficient response switching tapping thus flexibility behaviors. In the presence of cerebellar lesions it was very difficult to forget the previously correct sequence and acquire a new one. This cerebellar deficit resulted in progressively flattened learning curves within trials as the sessions went by. Along these same lines the HCbed animals exhibited marked perseverative tendencies that greatly disrupted performances that necessitated a response shift. Perseverations are distinctive symptoms, characterized by prefrontal dysfunction, observed in human and experimental pathologies (Hauser, 1999). They are also elicited by cerebellar damage (Schmahmann, 2004). This finding supports the view that cerebellar lesions might provoke "frontal-like" cognitive deficits and fits with clinical reports of severe problems in initiation/perseveration and in cognitive planning in cerebellar patients (Apollonio, Grafman, Schwartz, Massaquoi, & Hallett, 1993; El-Awar et al., 1991; Grafman et al., 1992; Hauser, 1999; Molinari, Leggio, et al., 1997; Schmahmann & Sherman, 1997). The lack of flexibility in changing behavior in the presence of cerebellar lesion might be due to impairment in planning intentional strategies, that is, in the ability to access and use different strategies effectively to change behavior in accordance with the changed context. This hypothesis is tempting because it allows speculation that the cerebellum and prefrontal cortex interact in planning actions and responses, the former by permitting acquisition of efficient responses and the latter by providing flexibility among different solutions already acquired and stored (Botez-Marquard, Bard, Léveillé, & Botez, 2001).

Speculating on the specific contribution of the cerebellum to cognition, Thach (1996) stated: "the cerebellum may link a behavioral context to a motor response". The present findings suggest that the cerebellum has an important role in monitoring incoming sensory information and in providing online adaptation of both motor and non-motor functions to perform contextually relevant behaviors (Bower, 2002; Ito, 2002; Schmahmann, 2004; Thach, 2007).

Even if cerebellar subjects are still able to put into action fixed and already acquired responses, their rapidly changing response patterns (Thach, 1996, 1997, 1998) are notoriously impaired. As cerebellar lesions impair rapidly alternating movements without preventing the slow execution of the same movements, they appear to affect behavioral responses requiring rapidly changing adaptations, but do not prevent the acquisition of the original correct response.

Analogously with the term "dysmetria of thought", advanced by Schmahmann and colleagues (Schmahmann, 1991, 2004; Schmahmann & Caplan, 2006; Schmahmann & Pandya, 1997) to define the nature of the cognitive impairments following cerebellar lesions, it is possible to describe the impaired cognitive flexibility pattern displayed in the presence of cerebellar lesions as a sort of "cognitive dysdiadochokinesis".

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References

Apollonio, I. M., Grafman, J., Schwartz, M. S., Massaquoi, S., & Hallett, M. (1993). Memory in patients with cerebellar degeneration. *Neurology, 43*, 1536–1544.

Bellebaum, C., & Daum, I. (2007). Cerebellar involvement in executive control. *Cerebellum, 6*, 184–192.

Birrel, J. M., & Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. *Journal of Neuroscience, 20*, 4320–4324.

Botez-Marquard, T., Bard, C., Léveillé, J., & Botez, M. I. (2001). A severe frontal–parietal lobe syndrome following cerebellar damage. *European Journal of Neurology, 8*, 347–353.

Boulougouris, V., Dalley, J. W., & Robbins, T. W. (2007). Effects of orbitofrontal, infralimbic and prelimbic cortical lesions on serial spatial reversal learning in the rat. *Behavioral Brain Research, 179*, 219–228.

Bower, J. M. (2002). The organization of cerebellar cortical circuitry revisited: Implications for functions. *Annals of the New York Academy of Sciences, 978*, 135–155.

Brown, V. J., & Bowman, E. M. (2002). Rodent models of prefrontal cortical function. *Trends in Neurosciences, 25*, 340–343.

Cabrera, S. M., Chavez, C. M., Corley, S. R., Kitto, M. R., & Butt, A. E. (2006). Selective lesions of the nucleus basalis magnocellularis impair cognitive flexibility. *Behavioral Neuroscience, 120*, 298–306.

Colombel, C., Lalonde, R., & Caston, J. (2004). The effects of unilateral removal of the cerebellar hemispheres on spatial learning and memory in rats. *Brain Research, 1004*, 108–115.

Dalley, J. W., Cardinal, R. N., & Robbins, T. W. (2004). Prefrontal executive and cognitive functions in rodents: Neural and neurochemical substrates. *Neuroscience and Biobehavioral Reviews, 28*, 771–784.

De Bartolo, P., Leggio, M. G., Mandolesi, L., Foti, F., Gelfo, F., Federico, F., et al. (2008). Environmental enrichment mitigates the effects of basal forebrain lesions on cognitive flexibility. *Neuroscience, 154*, 444–453.

Dias, R., Robbins, T. W., & Roberts, A. C. (1996). Dissociation in prefrontal cortex of affective and attentional shifts. *Nature, 380*, 69–72.

Dias, R., Robbins, T. W., & Roberts, A. C. (1997). Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: Restriction to novel situations and independence from "on-line" processing. *Journal of Neuroscience, 17*, 9285–9287.

El-Awar, M., Kish, S., Oscar-Berman, M., Robitaile, Y., Schut, L., & Freedman, M. (1991). Selective delayed alternation deficits in dominantly in herited olivopontocerebellar atrophy. *Brain and Cognition, 16*, 121–129.

Federico, F., Leggio, M. G., Mandolesi, L., & Petrosini, L. (2006). The NMDA receptor antagonist CGS 19755 disrupts recovery following cerebellar lesions. *Restorative Neurology and Neuroscience, 24*, 1–7.

Frith, C. D., Friston, K., Liddle, P. F., & Frackowiak, R. S. (1991). "Willed action" and prefrontal cortex in man a study with PET. *Proceedings Biological sciences, 244*, 241–246.

Grafman, J., Litvan, I., Massaquoi, S., Stewart, M., Sirigu, A., & Hallett, M. (1992). Cognitive planning deficit in patient with cerebellar atrophy. *Neurology, 42*, 1493–1496.

Hauser, M. D. (1999). Perseveration, inhibition and the prefrontal cortex: A new look. *Current Opinion in Neurobiology, 9*, 214–222.

Hyder, F., Phelps, E. A., Wiggings, C. J., Labar, K. S., Blamire, A. M., & Shulman, R. G. (1997). Willed action: A functional MRI study of the human prefrontal cortex during a sensorimotor task. *Proceedings of the National Academy of Sciences USA, 94*, 6989–6994.

Ito, M. (2002). Hopes for cerebellar research in the 21st century. *Cerebellum, 1*, 93–94.

Lalonde, R., & Botez, M. I. (1985). Exploration and habituation in nervous mutant mice. *Behavioral Brain Research, 17*, 83–86.

Lalonde, R., Manseau, M., & Botez, M. I. (1988). Spontaneous alternation and exploration in staggerer mutant mice. *Behavioral Brain Research, 27*, 273–276.

Leggio, M. G., Molinari, M., Neri, P., Graziano, A., Mandolesi, L., & Petrosini, L. (2000). Representation of actions in rats: The role of cerebellum in learning spatial performances by observation. *Proceedings of the National Academy of Sciences USA, 97*, 2320–2325.

Leggio, M. G., Neri, P., Graziano, A., Mandolesi, L., Molinari, M., & Petrosini, L. (1999). Cerebellar contribution to spatial event processing: Characterization of procedural learning. *Experimental Brain Research, 127*, 1–11.

Mandolesi, L., Leggio, M. G., Graziano, A., Neri, P., & Petrosini, L. (2001). Cerebellar contribution to spatial event processing: Involvement in procedural and working memory components. *European Journal of Neuroscience, 14*, 2011–2022.

Mandolesi, L., Leggio, M. G., Spirito, F., Federico, F., & Petrosini, L. (2007). Is the cerebellum involved in the visuo-locomotor associative learning? *Behavioral Brain Research, 184*, 47–56.

Mandolesi, L., Leggio, M. G., Spirito, F., & Petrosini, L. (2003). Cerebellar contribution to spatial event processing: Do spatial procedures contribute to formation of spatial declarative knowledge? *European Journal of Neuroscience, 18*, 2618–2626.

Manni, E., & Dow, R. S. (1963). Some observations of the effects of cerebellectomy in the rat. *Journal of Comparative Neurology, 121*, 189–194.

Middleton, F. A., & Strick, P. L. (2001). Cerebellar projections to the prefrontal cortex of the primate. *Journal of Neuroscience, 21*, 700–712.

Miller, E. K. (2000). The prefrontal cortex and cognitive control. *Nature Reviews Neuroscience, 1*, 59–65.

Molinari, M., Grammaldo, L. G., & Petrosini, L. (1997). Cerebellar contribution to spatial event processing: Right/left discrimination abilities in rats. *European Journal of Neuroscience, 9*, 1986–1992.

Molinari, M., Leggio, M. G., Solida, A., Corra, R., Misciagna, S., Silveri, M. C., et al. (1997). Procedural learning is impaired in cerebellar patients. *Brain, 120*, 1753–1762.

Molinari, M., Petrosini, L., & Gremoli, T. (1990). Hemicerebellectomy and motor behaviour in rats. II. Effects of cerebellar lesion performed at different developmental stages. *Experimental Brain Research, 82*, 483–492.

Petrosini, L., Leggio, M. G., & Molinari, M. (1998). The cerebellum in the spatial problem solving: A co-star or a guest star? *Progress in Neurobiology, 56*, 191–210.

Petrosini, L., Molinari, M., & Dell'Anna, M. E. (1996). Cerebellar contribution to spatial event processing: Morris water maze and T-maze. *European Journal of Neuroscience, 8*, 1882–1896.

Pochon, J. B., Levi, R., Poline, J. B., Crozier, S., Lehèricy, S., Pillon, B., et al. (2001). The role of dorsolateral prefrontal cortex in the preparation of forthcoming actions: An fMRI study. *Cerebral Cortex, 11*, 260–266.

Ragozzino, M. E., Detrick, S., & Kesner, R. P. (1999). Involvement of the prelimbic–infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. *Journal of Neuroscience, 19*, 4585–4594.

Schmahmann, J. D. (1991). An emerging concept. The cerebellar contribution to higher function. *Archives of Neurology, 48*, 1178–1187 [comment in (1992), 49, 1229–1230].

Schmahmann, J. D. (2004). Disorders of the cerebellum: Ataxia, dysmetria, of though, and the cerebellar cognitive affective syndrome. *Journal of Neuropsychiatry and Clinical Neurosciences, 16*, 367–378.

Schmahmann, J. D., & Caplan, D. (2006). Cognition, emotion and the cerebellum. *Brain, 129*, 290–292.

Schmahmann, J. D., & Pandya, D. N. (1997). The cerebrocerebellar system. *International Review of Neurobiology, 41*, 31–60.

Schmahmann, J. D., & Sherman, J. C. (1997). Cerebellar cognitive affective syndrome. *International Review of Neurobiology, 41*, 433–440.

Spence, S. A., Hirsch, S. R., Brooks, D. J., & Grasby, P. M. (1998). Prefrontal cortex activity in people with schizophrenia and control subjects. Evidence from positron emission tomography for remission of "hypo frontality" with recovery from acute schizophrenia. *British Journal of Psychiatry, 172*, 316–323.

Thach, W. T. (1996). On the specific role of the cerebellum in motor learning and cognition: Clues from PET activation and lesion studies in man. *Behavioral and Brain Sciences, 19*, 411–431.

Thach, W. T. (1997). Context-response linkage. *International Review of Neurobiology, 41*, 599–611.

Thach, W. T. (1998). What is the role of the cerebellum in motor learning and cognition? *Trends in Cognitive Sciences, 2*, 331–337.

Thach, W. T. (2007). On the mechanism of cerebellar contributions to cognition. *Cerebellum, 6*, 163–167.

Whimbey, A. E., & Denenberg, V. H. (1967). Two independent behavioral dimensions in open-field performance. *Journal of Comparative Physiology and Psychology, 63*, 500–504.

Wolpaw, J. R., & Carp, J. S. (2006). Plasticity from muscle to brain. *Progress in Neurobiology, 78*, 233–263.