

Exposure to an Enriched Environment Accelerates Recovery from Cerebellar Lesion

Francesca Foti · Daniela Laricchiuta · Debora Cutuli ·
Paola De Bartolo · Francesca Gelfo ·
Francesco Angelucci · Laura Petrosini

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Abstract The exposure to enriched environments allows the maintenance of normal cognitive functioning even in the presence of brain pathology. Up until now, clinical and experimental studies have investigated environmental effects mainly on the symptoms linked to the presence of neuro-degenerative diseases, and no study has yet analyzed whether prolonged exposure to complex environments allows modifying the clinical expression and compensation of deficits of cerebellar origin. In animals previously exposed to complex stimulations, the effects of cerebellar lesions have been analyzed to verify whether a prolonged and intense exposure to complex stimulations affected the compensation of motor and cognitive functions following a cerebellar lesion. Hemicerebellectomized or intact animals housed in enriched or standard conditions were administered spatial tests. Postural asymmetries and motor behavior were also assessed. Exposure to the enriched environment almost completely compensated the effects of the hemicerebellectomy. In fact, the motor and cognitive performances of the enriched hemicerebellectomized animals were similar to those of the intact animals. The plastic changes induced by enhanced mental and physical activity seem to provide the development of compensatory responses against the disrupting motor and cognitive consequences of the cerebellar damage.

Keywords Hemicerebellectomy · Cerebellar compensation · Postural and locomotor behavior · Spatial tests

Introduction

Cognitive enrichment plays an important role in maintaining cognitive performance even in the presence of brain damage [1–3]. It has been reported that even though cognitive enrichment does not prevent the onset of neuro-degenerative diseases, it may provide protection against the expression of clinical symptoms [4–8]. Thus, environmental complexity fosters the development of neuroplasticity properties that allow normal motor and cognitive functioning even in the presence of brain pathology. Up until now, the experimental studies on the behavioral effects of environmental enrichment have been performed in models of dementia-like neurodegeneration [9–13]. However, no study has yet addressed the question as to whether prolonged exposure to complex environments beneficially affects the clinical expression and compensation of deficits of cerebellar origin.

Cerebellar damage elicits motor disorders in muscle coordination, balance and muscle strength [14, 15] as well as significant impairments in a variety of cognitive, emotional, and affective functions [16–18]. In both humans and animals, most motor symptoms of cerebellar origin following surgical ablation or stroke injury gradually and efficiently compensate over time. In the compensated state, faceted cognitive impairment, which affects memory, attention, visuo-spatial abilities, and executive functions, is present together with stable motor symptomatology characterized by some ataxic, dysmetric, and asthenic symptoms [17, 19–26]. Whereas, the pharmacological treatment of cerebellar compensation is largely unsatisfactory, physical

F. Foti · D. Laricchiuta · D. Cutuli · P. De Bartolo · F. Gelfo ·
F. Angelucci · L. Petrosini
IRCCS Santa Lucia Foundation,
Rome, Italy

F. Foti · D. Laricchiuta · D. Cutuli · P. De Bartolo ·
L. Petrosini (✉)
Department of Psychology, University “Sapienza” of Rome,
Via dei Marsi 78,
00185, Rome, Italy
e-mail: laura.petrosini@uniroma1.it

therapy and neuro-rehabilitation have been found of some efficacy, although the basis of the effects of these treatments is still unknown [27–29]. In this regard, it is important to analyze whether compensation of cerebellar symptoms may be positively influenced by previous exposure to an enriched environment. In other words, does exposure to complex stimulation may accelerate compensation of cerebellar impairments? To clarify this issue, we studied whether early and long-lasting exposure to an enriched environment affects the motor and cognitive symptoms evoked by a hemicerebellectomy (HCb). The beneficial effects of the exposure to an enriched environment can be demonstrated only if the previously enriched hemicerebellectomized (HCbed) animals exhibit an accelerated compensation of motor and cognitive impairments in comparison to standard-housed HCbed animals.

Materials and Methods

Animals

Forty adult male Wistar rats (250–300 g; Harlan, Italy) were used in the present research. The animals were kept under standard conditions (temperature, 22°C±2; relative humidity, 60%±10) with food and water ad libitum on a 12/12-h dark/light cycle (light on between 0700 and 1900 hours). Rats were assigned to four experimental groups. The first group comprised enriched-housed hemicerebellectomized rats (EH group), the second group comprised standard-housed hemicerebellectomized rats (SH group), the third group comprised enriched-housed control rats (EC), and the fourth group comprised standard-housed control rats (SC group). The animals were maintained according to the guidelines for ethical conduct developed by the European Communities Council Directive of November 24, 1986 (86/609/EEC). All efforts were made to minimize pain or discomfort of the animals.

Housing Conditions

On the 21st postnatal day, an even number of male littermates of the same dam were randomly assigned to one of two experimental groups. The first group was reared in enriched conditions and the other in standard conditions.

The enriched rats were housed in groups of ten animals in a large cage (100×50×80 cm) with an extra level constructed of galvanized wire mesh and connected by ramps to create two interconnected levels. The cage contained wood shavings, a running wheel, a shelter (a house-shaped toy with a concave opening in which the rat could enter), colored plastic toys (red or green small balls, little bells, jingle noise-maker playthings, and ropes), and small objects (transparent rat igloo, colored

bricks, cubes, tunnels, a mirror, and platform). Throughout the enrichment period, the shelter and running wheel were kept in the cage but the toys and constructions were changed twice a week. Once a week, the feeding boxes and water bottles were moved to different areas of the cage to encourage explorative behaviors. Furthermore, each enriched animal was handled daily for at least 10 min.

The rats reared in standard conditions were pair-housed in a standard cage (42×26×18 cm) containing wood shavings but no objects. Feeding boxes and water bottles were kept in the same position. These animals received the usual care provided by the animal facilities staff but no particular or prolonged manipulation. This procedure did not result in impoverished rearing because the standard animals were accustomed to human contact. Both groups of animals received the same type of food.

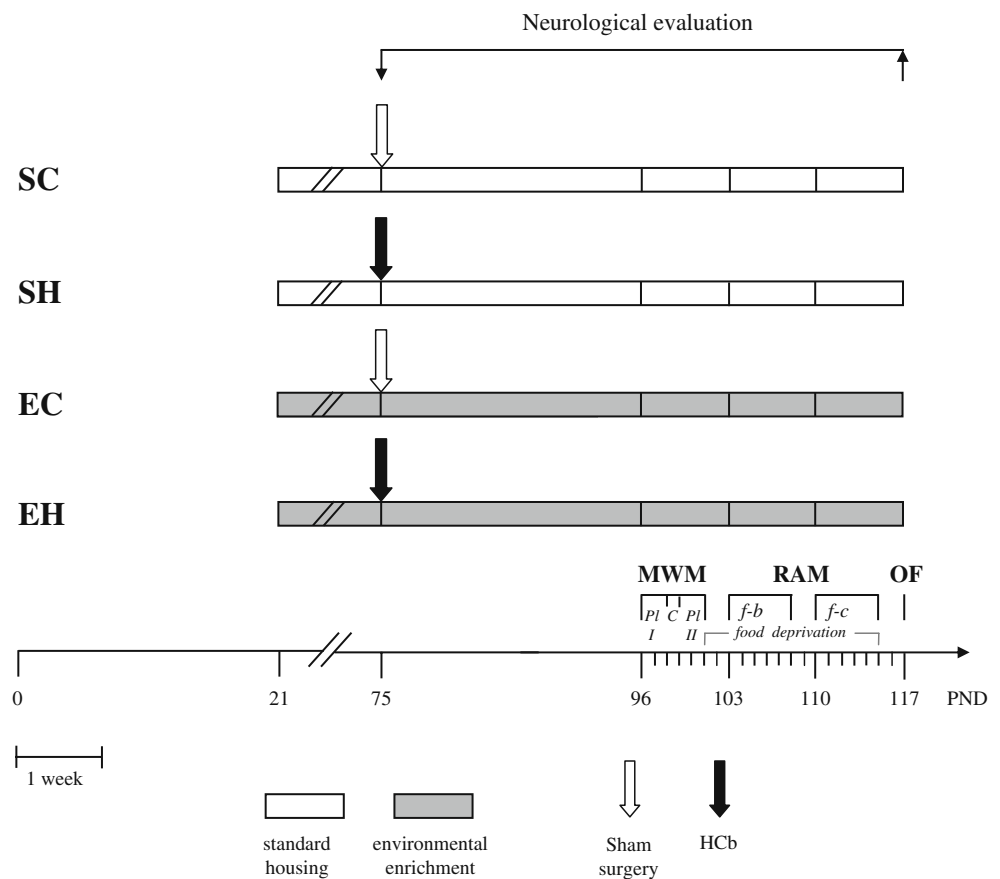
Surgery

On the 75th postnatal day, ten enriched- and ten standard-housed rats received a right HCb. Rats were anesthetized with Zoletil 100 (Tiletamine and Zolazepam: 50 mg/kg i.p.—Virbac s.r.l., Milan, Italy) and Rompun (Xylazine: 10 mg/Kg i.p.—Bayer s.p.a., Milan, Italy). A craniotomy was performed over the right hemicerebellum. The dura was excised, and the right cerebellar hemisphere and hemivermis as well as the fastigial, interpositus, and dentate cerebellar nuclei of the right side were ablated by suction. Care was taken not to lesion the 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. The control animals belonging to the sham surgery groups were anesthetized to perform the craniotomy over the cerebellar structures, but neither excision of meningeal membranes nor cerebellar ablation was performed. The wound edges were then sutured and the animals were allowed to recover from anesthesia and surgical stress. They were maintained in their respective housing conditions for the entire testing period.

Experimental Groups

Four of the hemicerebellectomized rats died during surgery or behavioral testing. Furthermore, data obtained from two lesioned animals that completed the behavioral testing were discarded because lesion verification indicated lesion incorrectness. Thus, the EH and SH groups were comprised of seven lesioned animals each. The EC and SC groups were comprised of ten sham-lesioned rats. Postural evaluation started at 24 h after HCb, and it was performed at variable time intervals to the end of the behavioral testing. Three weeks after surgery, the animals were behaviorally tested in the Morris water maze (MWM), radial arm maze (RAM), and open field (OF) (Fig. 1).

Fig. 1 Diagram describing the behavioral procedure and the global timing of the experimental design of the four groups since animals' birth to the 117th postnatal day (PND). Enrichment period, surgery, neurological evaluation, and behavioral testing are indicated. *MWM* Morris water maze (*PI I* place I, *C* cue, *PI II* place II, phases). *RAM* radial arm maze (*f-b* full-baited, *f-c* forced-choice, procedures), *OF* open field



Neurological Evaluation

Postural symptoms, locomotor handicaps and complex behavior deficits were assessed by means of a behavioral rating scale described elsewhere in detail [30, 31]. Neurological evaluations were scored by an examiner unaware of housing conditions the animals were submitted to. Presence or absence of head and body tilt, hyperflexion, or hyper-extension of fore- and hind-limbs in relation to the trunk, ankle extra-rotation, hypotonia, eye nystagmus, head oscillations (bobbing), and tremor were evaluated. Some characteristics of locomotion, namely, wide-based, collapsing on the belly, steering, circling, pivoting, and falling to the side, were also analyzed. Finally, complex motor skills, such as ascending a ladder and suspension on a wire as well as vestibular drop reaction and rearing behavior, were assessed. Video records were taken throughout the entire testing cycle and were used to supplement direct behavioral observations. A score from 0 (complete absence of deficit) to 2 (presence of the symptom to the highest degree) was assigned to each symptom according to its degree of severity, as described in Table 1.

Morris Water Maze

The rats were placed in a circular white pool (diameter 140 cm) located in a normally equipped laboratory room,

uniformly lit by four neon lamps (40 W each). Extra-maze spatial cues were on the walls and held in constant spatial relations throughout the experiments. The pool was filled with 24°C water (60 cm deep), made opaque by the addition of 2 l of milk. An escape platform (diameter 10 cm) submerged 2 cm below or elevated 2 cm above the water level was placed in the middle of one cardinal quadrant located 30 cm from the pool walls. Testing was performed between 0900 and 1700 hours. The rat was released into the water from randomly varied starting points and was allowed to swim around to find the platform. If this did not occur within 120 s, the experimenter guided it there. At the end of each trial the rat was left on the platform for 30 s. Each rat was submitted to two sessions of four trials per day, with a 3-min inter-trial and 4-h inter-session intervals. In the first four sessions, the platform was hidden in the northwest quadrant (place I); in the next two sessions, the platform was kept visible in the northeast quadrant (cue phase); in the final four sessions, the platform was hidden in the northeast quadrant (place II) [32–34]. The MWM protocol we used allowed to investigate numerous components of spatial function. The place I analyzed first the general procedures such as the inhibiting non-adaptive behaviors, as scrabbling at pool walls, then the sequence of navigational strategies (spatial procedural learning) put into action to explore the pool and to find the platform as well as the ability of building a spatial map

Table 1 Behavioral rating scale for postural symptoms, locomotor handicaps and complex behavior deficits

Postural symptoms									
Score	Body tilt	Head tilt	Nystagmus	Limb hyper-extension	Limb hyperflexion	Ankle extra-rotation	Head bobbing	Hypotonia	Tremor
0	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
1	Slight	Slight	<20 beats/min	Slight	Slight	Slight	Occasionally present	Slight	Slight
2	Marked	Marked	>20 beats/min	Marked	Marked	Marked	Repeatedly present	Marked	Marked
Locomotor handicaps									
Score	Wide base	Collapse on the belly	Circling	Steering	Side falls	Pivoting	Hyperactivity		
0	Absent	Absent	Absent	Absent	Absent	Absent	Absent		
1	Slight tendency	Slight tendency	Occasionally present	Occasionally present	Occasionally present	Rarely present	Slight		
2	Markedly present	Markedly present	Repeatedly present	Compulsively present	Repeatedly present	Present	Marked		
Complex behavior deficits									
Score	Ascending a ladder	Descending a ladder	Suspension on a wire (s)	Rearing	Vestibular drop				
0	Successful	Successful	>10	Repeatedly present	Present without directionality				
1	Only a few steps	Only a few steps	<10	Occasionally present	Present with side prevalence				
2	Failed	Failed	Absent	Absent	Absent				

(spatial memory) by using extra-maze cues to localize the hidden platform. The cue phase analyzed the development of a stimulus–response (platform/reaching) associative learning and the employment of the procedural knowledge acquired during place I. The place II analyzed the abilities of remodeling the spatial map by exploiting the intra-maze information acquired in the cue phase, in the presence of procedural strategies already gained, providing even information about plastic properties of spatial learning processes.

The rats' trajectories in the pool were monitored by a video camera mounted on the ceiling. The resulting video signal was relayed to a monitor and to an image analyzer (Ethovision, Noldus, Wageningen, The Netherlands).

The following behavioral parameters were considered in analyzing performances in the three MWM phases separately: successes, that is, successful escapes in finding the platform; latencies to reach the platform, total distance swum in the entire pool, distance traveled in a 20-cm peripheral annulus, mean swimming velocity. Furthermore, navigational strategies put into action in reaching the platform were classified in five main categories, regardless the platform was reached or not: circling, that is, swimming in a 20-cm peripheral annulus, with inversion of swimming direction and counterclockwise and clockwise turnings in

the peripheral sectors of the pool; extended searching, that is, swimming around the pool in all quadrants, visiting the same areas more than once; restricted searching, that is, swimming in some pool quadrants, not visiting some tank areas at all; restricted circling, that is, reaching the platform swimming only in the peripheral annulus; direct finding, that is, swimming towards the platform without any foraging around the pool. Two researchers who were unaware of the individual specimen's group assignment categorized the swimming trajectories drawn by the image analyzer. They attributed the dominant behavior in each trial to a specific category. Categorization was considered reliable only when their judgments were consistent.

Radial Arm Maze

The apparatus consisted of a central platform (diameter, 30 cm) from which eight arms (12.5 cm wide × 60 cm long) radiated like the spokes of a wheel. A food well (diameter, 5 cm; 2 cm deep) was located at the end of each arm [35]. A 40 W red light bulb provided the only source of illumination in the testing room. Testing was performed between 0900 and 1700 h. Starting from the habituation phase and throughout the entire RAM testing, rats were

food-restricted to decrease their weight by 20%. The habituation phase, during which the rat was allowed to freely explore the maze (in which a piece of Purina chow had been placed in each arm) for 10 min a day, was carried out 2 days before the full-baited maze procedure.

Full-Baited Maze Procedure All maze arms were baited with a piece of Purina chow prior to each session. The rat was placed on the central platform. The task goal was to collect the eight rewards. This aim could be reached through a maximal number of 16 entries. Once all the eight rewards were baited or the allowed 16 visits were made the animals were removed by the maze and put in their cages. Any RAM session was comprised only one trial. The animals were submitted to two sessions a day for five consecutive days. The inter-session interval was 4 h. The apparatus between subjects was cleaned with a 70% solution of ethanol.

The following parameters were considered: total errors (number of re-visited arms divided by total number of visits, correct and incorrect, $\times 100$); mean spatial span (the longest sequence of correctly visited arms displayed in each session); perseverations (sum of consecutive entries in the same arm or in a fixed sequence of a maximum of three tray arms in the ten sessions. Perseverations on more than three trays were never observed); number of 45° angles (entries in adjacent arms); 45° angle span (the longest sequence of 45° angles made during each session).

Forced-Choice Procedure All animals were submitted to the forced-choice paradigm 48 h after the preceding protocol ended. In the first phase, only four arms (for example, arms 1, 3, 4, and 7) were opened and baited; the others arms remained closed. The baited arms were separated by different angles to prevent the animal from reaching the solution by adopting a stereotyped pattern. The rat was allowed to explore the open arms. Then, it spent 60 s in its cage before being returned to the maze. In the second phase, the rat was allowed free access to all eight arms, but only the four previously closed arms were baited. This task was repeated for five consecutive days with a different configuration of arms closed each day to avoid any fixed search pattern.

The parameter considered was working memory errors, considered as re-entries into already visited arms. In the second phase, this parameter was further broken down into two error subtypes: across-phase errors, defined as entries into an arm entered during the first phase; and within-phase errors, defined as re-entries into an arm visited earlier in the same session.

Open Field

The apparatus consisted of a circular arena (diameter, 140 cm) delimited by a 30-cm high wall. A 40 W red light

bulb provided the only source of illumination in the testing room. Testing was performed between 0900 and 1700 h. Forty-eight hours after the preceding RAM test, each rat was gently placed in the periphery of the arena facing toward the center of the arena and allowed to move freely in the empty open field. The starting point was the south point for all animals. The baseline level of activity was measured during a 6-min period. All testing was recorded by a video camera; the signal was relayed to a monitor and to the previously described image analyzer. The apparatus between subjects was cleaned with a 70% solution of ethanol. The following emotional and motor parameters were analyzed: number of defecation boluses, motionless time, rearings, total distance (in cm) traveled in the arena, percentage of the distance traveled in exploring a 20-cm peripheral annulus (peripheral distance), percentage of the total distance traveled in exploring a central area of 35 cm of radius, and central area crossings, that is the number of entries into the central area of 35 cm of radius.

Histological Controls

When the behavioral testing was finished, the HCbed animals were deeply anesthetized and transcardially perfused with saline followed by 4% buffered formalin. The extent of the cerebellar lesion was determined from Nissl-stained 50- μ m frozen sections. Animals were included in the present study if they had received a complete right HCb with total ablation of deep nuclei (Fig. 2a). In all cases reported here, the left side of the cerebellum and all extra-cerebellar structures were spared, except for the dorsal cap of the right Deiters' nucleus which in some cases was slightly affected. The variability in the extent of the floccular and vermian lesions was considered not influencing, because in all cases these structures were functionally disconnected due to the ablation of the cerebellar peduncles and deep nuclei of the right side. To verify variability of the extent of cerebellar lesion, Nissl-stained coronal sections at the same representative levels from bregma (−10.04, −11.60, and −13.24 mm) were selected for each animal. Lesion boundaries were then drawn on schematic drawings of corresponding atlas tables [36] and the areas ablated computed by the software ImageJ (version 1.42q).

Statistical Analysis

The data were first tested for normality (Shapiro–Wilk's test) and homoscedasticity (Levene's test). All data presented as the mean \pm SEM were analyzed by one-, two- or three-way analyses of variance (ANOVAs). The two-way ANOVAs were performed by applying either the mixed model for independent variable (group) and repeated measures (day) or the model for two independent variables

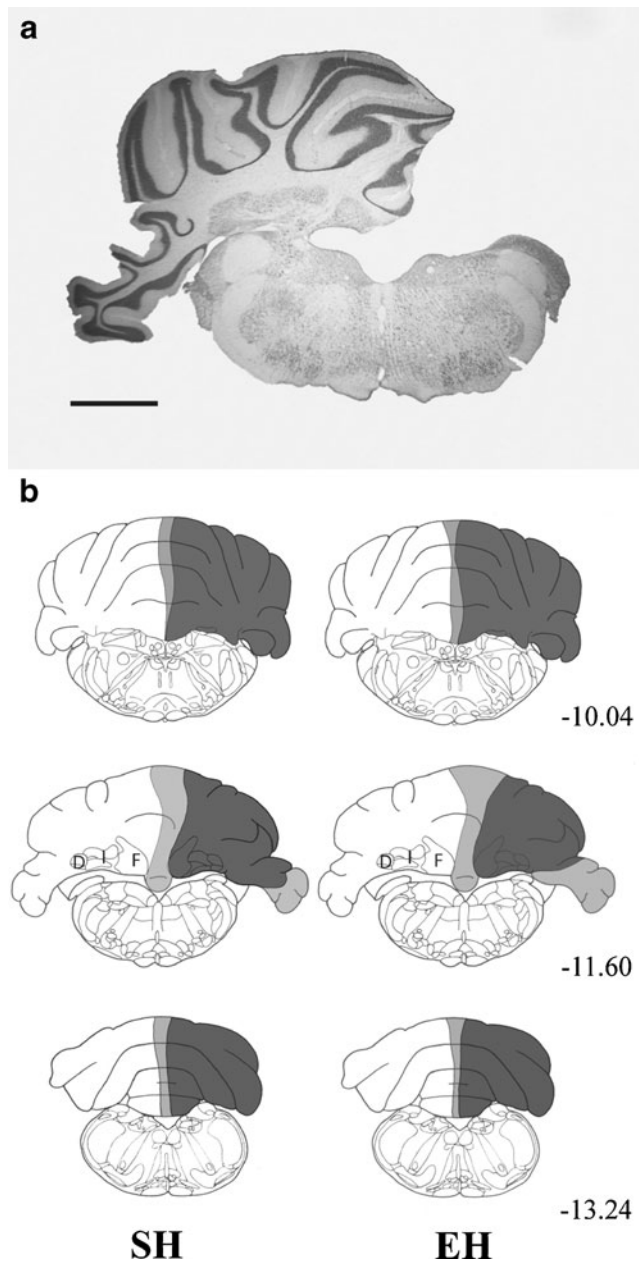


Fig. 2 **a** Nissl-stained coronal section through cerebellum and brain stem in a HCbed rat. Note the total absence of the right hemi-cerebellum and the sparing of any extra-cerebellar structure. Scale bar, 2 mm. **b** Schematic drawings of the cerebellum and brainstem at -10.04, -11.60, and -13.24 mm from bregma illustrating the reconstruction of minimal (dark gray) and maximal (light gray) extension of the lesion in SH and EH groups. Note that the ablations of the right cerebellar hemisphere totally include the ipsilateral deep nuclei (*F* fastigial, *I* interpositus, *D* dentate, nuclei). Deep nuclei are conversely spared on the left side

(housing × lesion). Three-way ANOVAs (housing × lesion × session or strategy) were also performed. These analyses were followed by Newman–Keuls test. All analyses were performed by using Statistica 7.0 for Windows and the significance level was established at $p \leq 0.05$.

Results

Verification of Cerebellar Lesion

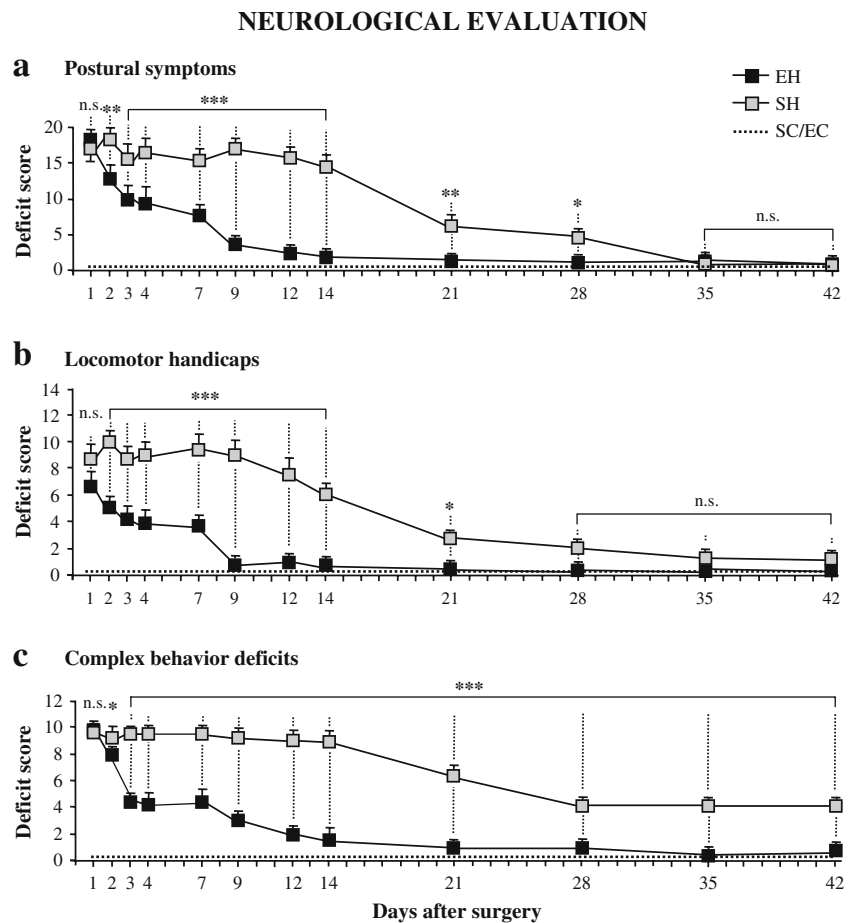
The mean percentages of cerebellar ablation were calculated for both SH and EH groups (at -10.04 mm from bregma, SH=49.9%±0.16; EH=50.3%±0.16; at -11.60 mm from bregma, SH=45.6%±0.35; EH=44.4%±0.37; at -13.24 mm from bregma, SH=50.4%±0.22; EH=49.6%±0.19). One-way ANOVA on the mean percentages of ablation revealed no significant differences between groups ($F_{1, 12}=0.32$; $p=0.58$) (Fig. 2b).

Neurological Evaluation

Figure 3 depicts the time courses of compensation of the cerebellar symptoms of the HCbed groups (EH and SH) together with the baseline of the postural evaluation of sham-operated control groups (EC and SC). All animals were evaluated by means of the rating scale described in Table 1. However, since it evaluated symptoms and asymmetries, the sham-operated animals obtained always scores of 0 (complete absence of deficits). This kind of evaluation prevented the inclusion of the scores obtained by the sham-operated groups in the ANOVAs.

As shown in Fig. 3, dramatic effects of housing conditions were observed on postural, locomotor and complex behaviors of the two groups of lesioned animals. Twenty-four hours after the HCb, both EH and SH groups exhibited postural and motor impairments of the same severity. From the second post-operative day onward, the EH group exhibited significantly less impaired posture (Fig. 3a), locomotion (Fig. 3b) and complex behaviors (Fig. 3c) compared to the SH group. As for the postural symptoms, a two-way ANOVA (group × day) revealed significant group ($F_{1, 12}=64.1$; $p<0.00001$) and day ($F_{11, 132}=70.6$; $p<0.00001$) effects. Interaction was also significant ($F_{11, 132}=14.7$; $p<0.00001$). *Post hoc* comparisons showed that the two HCbed groups were similarly impaired 24 h after the lesion but obtained significantly different scores from the 2nd to the 28th day (Fig. 3a). As for the locomotor handicaps, a two-way ANOVA (group × day) revealed significant group ($F_{1, 12}=71.4$; $p<0.00001$) and day ($F_{11, 132}=41.7$; $p<0.00001$) effects. Interaction was also significant ($F_{11, 132}=8.4$, $p<0.00001$). Once again, *post hoc* comparisons showed that the two HCbed groups were similarly impaired 24 h after the lesion but obtained significantly different scores from the 2nd to the 21st day (Fig. 3b). The two HCbed groups were significantly different even in putting complex behaviors into action. A two-way ANOVA (group × day) revealed significant group ($F_{1, 12}=993.1$; $p<0.00001$) and day ($F_{11, 132}=93.2$; $p<0.00001$) effects. Interaction was also significant ($F_{11, 132}=19.8$; $p<0.00001$). Interestingly, *post hoc* comparisons showed that 6 weeks after the cerebellar lesion SH animals

Fig. 3 Neurological evaluation of **a** postural symptoms, **b** locomotor handicaps, and **c** complex behavior deficits in enriched hemicerebellectomized (EH; $n=7$) and standard-housed hemicerebellectomized (SH; $n=7$) rats together with the baseline of the evaluation of enriched sham-operated (EC; $n=10$) and standard-housed sham-operated (SC; $n=10$) control groups. Abscissa: time in days after surgery. Ordinate: degree of severity of symptoms evaluated according to the rating scale described in Table 1. Data are presented as means \pm SEM. Asterisks indicate the significance level of the *post hoc* comparisons between groups: * $p<0.05$; ** $p<0.005$; *** $p<0.001$



did not yet reach the same level of compensation of the complex behaviors of the EH group (Fig. 3c).

As a whole, these findings provide evidence of the beneficial effects of housing in an enriched environment on all facets of motor performance in the presence of a cerebellar lesion.

Morris Water Maze

The ANOVAs for all parameters are reported in Table 2. All experimental groups explored the pool and found the platform with latencies progressively lower, as the sessions went by. While in place I both groups of enriched animals displayed a significantly higher number of successes (EC=3.8 \pm 0.2; EH=3.9 \pm 0.2) in comparison to the non-enriched groups (SC=3.7 \pm 0.3; SH=3.5 \pm 0.3), and in the cue and place II phases all animals succeeded in mastering the task with a 100% of successes.

Figure 4a shows no differences in latency values among groups except in the place II, where the SH displayed the highest mean latencies in comparison to the remaining groups (*post hoc* comparisons: SH vs. EH, SC, or EC: $p\leq 0.0005$). Similarly, when total distances swam to reach the platform were analyzed in the three MWM phases, EH, SC, and EC animals traveled similar distances, while SH

animals swam the longest distances (*post hoc* comparisons: SH vs. EH, SC, or EC: at least $p<0.05$) (Fig. 4b).

By analyzing the percentages of distance traveled in the peripheral annulus, we observed that while during place I EH and SH groups displayed higher percentages than the un-lesioned groups (*post hoc* comparisons: EH or SH vs. SC or EC: at least $p<0.05$), in place II EH group interestingly exhibited a percentage of peripheral traveling not different from SC group (Fig. 4c).

In place I, EC group displayed the highest velocity values (*post hoc* comparison: EC vs. EH, SH, or SC: at least $p<0.0005$) while EH group displayed velocity values not different from SH and SC groups. The SH group that in place I swam significantly more quickly than SC (*post hoc* comparison: $p<0.01$) in place II swam as quickly as EC group. In place II, the velocities of EH group did not differ from those of SC group, but were significantly lower than those of EC and SH groups (*post hoc* comparison: EH or SC vs. EC or SH: at least $p<0.05$) (Fig. 4d).

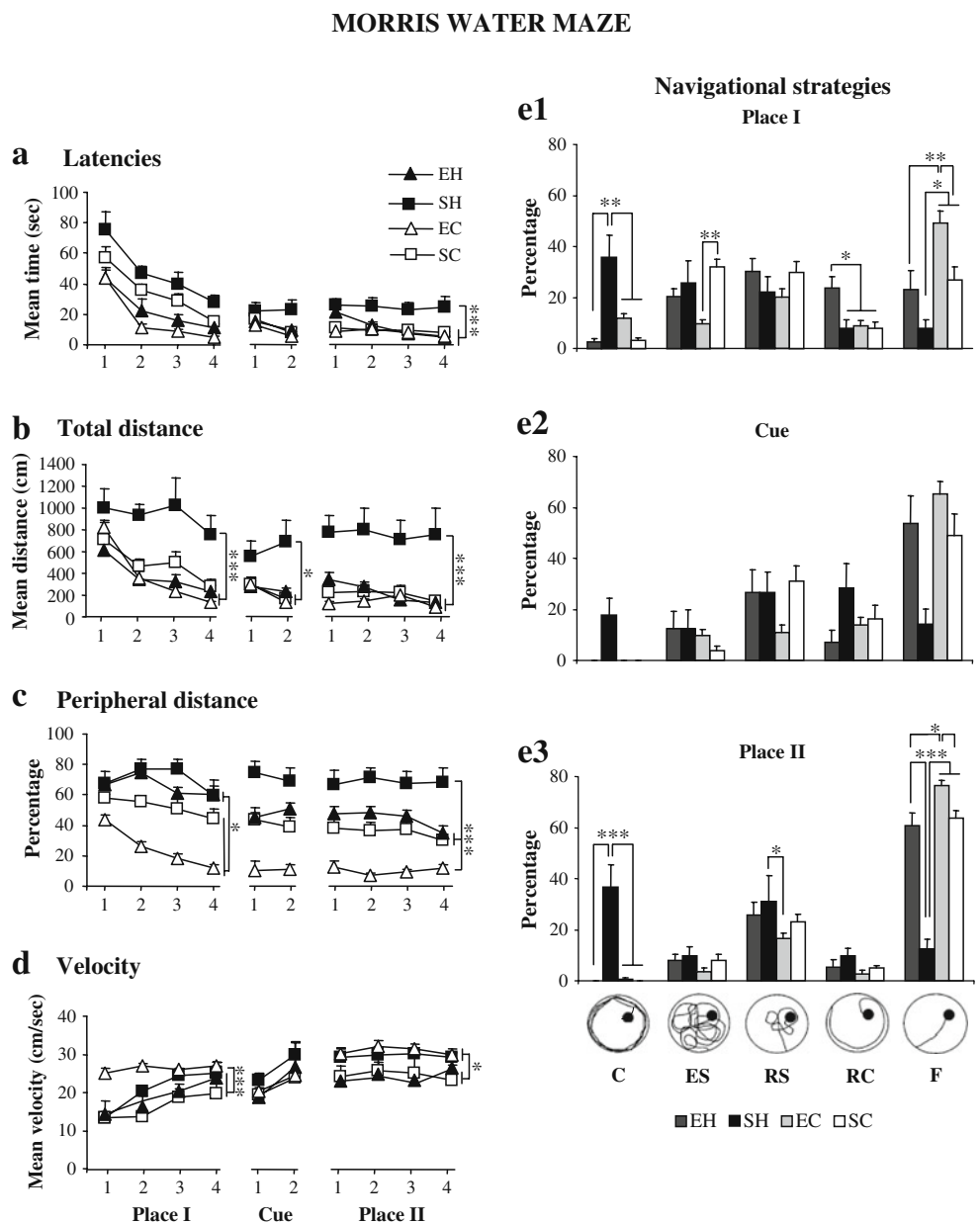
An analysis of the explorative strategies the animals put into action in performing the task revealed interesting differences among groups and phases (Fig. 4e). In place I phase, SH animals were unable to orientate their bodies to reach the platform and consequently swam in circles around the

Table 2 Statistical comparisons (three-way ANOVAs) of behavioral responses in the MWM

Parameters	Housing		Lesion		Session		H×L		H×S		L×S		H×L×S	
	<i>F</i> (<i>df</i> , 1 and 30)	<i>p</i>	<i>F</i> (<i>df</i> , 1 and 30)	<i>p</i>	<i>F</i> (<i>df</i> , 3 and 90)	<i>p</i>	<i>F</i> (<i>df</i> , 1 and 30)	<i>p</i>	<i>F</i> (<i>df</i> , 3 and 90)	<i>p</i>	<i>F</i> (<i>df</i> , 3 and 90)	<i>p</i>	<i>F</i> (<i>df</i> , 3 and 90)	<i>p</i>
Place I														
Successes	7.24	<0.05	0.23	n.s.	12.51	<0.00001	3.24	n.s.	0.91	n.s.	0.33	n.s.	0.74	n.s.
Latencies	39.16	<0.0001	8.85	<0.01	46.75	<0.00001	1.15	n.s.	0.99	n.s.	0.03	n.s.	0.62	n.s.
Total distance	18.44	<0.0005	8.13	<0.01	16.17	<0.00001	8.65	<0.01	2.45	n.s.	1.87	n.s.	0.10	n.s.
Peripheral distance	13.37	<0.001	45.27	<0.00001	16.97	<0.00001	6.75	<0.01	3.95	<0.01	6.55	<0.0005	1.76	n.s.
Velocity	18.22	<0.0005	2.20	n.s.	30.13	<0.0001	35.90	<0.00001	3.44	<0.05	5.87	<0.005	1.75	n.s.
Strategies	0	n.s.	0	n.s.	8.34	<0.00001	5.65	<0.05	6.54	<0.0001	6.18	<0.0005	6.09	<0.0005
Cue														
Successes	0.03	n.s.	0.03	n.s.	0.03	n.s.	2.28	n.s.	2.28	n.s.	2.28	n.s.	0.03	n.s.
Latencies	5.11	<0.05	5.20	<0.05	9.84	<0.005	1.48	n.s.	0.34	n.s.	2.44	n.s.	1.46	n.s.
Total distance	6.07	<0.05	7.36	<0.05	2.15	n.s.	5.12	<0.05	2.50	n.s.	6.81	<0.01	0.75	n.s.
Peripheral distance	26.46	<0.00005	42.08	<0.00001	0.23	n.s.	0.37	n.s.	3.34	n.s.	0.11	n.s.	0.24	n.s.
Velocity	0.71	n.s.	2.26	n.s.	26.94	<0.00005	1.84	n.s.	0.07	n.s.	1.67	n.s.	0.16	n.s.
Strategies	0.47	n.s.	0.07	n.s.	22.74	<0.00001	0.47	n.s.	6.15	<0.0005	3.79	<0.01	2.27	n.s.
Place II														
Successes	0.46	n.s.	0.46	n.s.	0.75	n.s.	2.88	n.s.	2.16	n.s.	2.16	n.s.	0.75	n.s.
Latencies	11.28	<0.005	18.38	<0.0005	6.0	<0.001	7.65	<0.01	2.12	n.s.	2.13	n.s.	1.96	n.s.
Total distance	15.91	<0.0005	18.20	<0.0005	2.04	n.s.	9.43	<0.0005	0.23	n.s.	1.49	n.s.	0.82	n.s.
Peripheral distance	27.49	<0.00005	50.40	<0.00001	1.99	n.s.	0.005	n.s.	0.30	n.s.	0.93	n.s.	2.82	<0.05
Velocity	0.03	n.s.	1.30	n.s.	4.65	<0.005	23.87	<0.00005	3.45	<0.05	3.63	<0.05	2.76	<0.05
Strategies	0.06	n.s.	0.06	n.s.	75.93	<0.00001	2.07	n.s.	15.41	<0.00001	18.21	<0.00001	7.88	<0.00001

Fig. 4 Effects of hemiserebellectomy and environmental enrichment on the Morris Water Maze performance analyzed in the three phases. Latencies (a), total distance (b) peripheral distance, (c) and swimming velocity (d) are depicted. Asterisks at the right side of the graphs indicate *post hoc* comparisons between groups: * $p < 0.05$; *** $p < 0.0005$. In (e), navigational strategies exhibited in the three MWM phases are shown.

Asterisks inside the graphs indicate the *post hoc* comparisons between groups: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0005$. The circular figurines under the graphs illustrate the typical explorative patterns of the five main navigational strategies. The black-filled circles indicate platform position. C circling strategy, ES extended searching, RS restricted searching, RC restricted circling, F finding. Vertical bars indicate SEM. In this and the following figures: EH enriched hemicerebellectomized animals, SH standard-housed hemicerebellectomized animals, EC enriched sham-operated controls, SC standard-housed sham-operated controls



periphery. Then, they displayed higher percentages of circling and lower percentages of finding in comparison to the remaining groups. Conversely, EH group exhibited percentages of circling and finding not different to SC and highest percentages of restricted circling. EC group displayed the highest percentages of Finding since this first phase (Fig. 4e1). In the cue phase, no significant difference among groups in the five navigational strategies was found (Fig. 4e2). In the place II phase, while SH group persisted in displaying high percentages of strategies of peripheral exploration (circling and restricted circling) as well as low percentages of finding, EH group increased its percentages of finding, as the SC group, and dramatically decreased the percentage of restricted circling (Fig. 4 e3).

Summing up, EH animals learned MWM task similarly to SC animals, with the only difference of a more evident peripheral exploration in the first phase of the task. It is important to underline that even in the initial Place I the EH rats did not exhibit the compulsive circling typically displayed by HCbed animals. It is possible that the early recovery of postural and locomotor deficits depicted in Fig. 3 contributed to the improved performances of EH animals in the MWM.

Radial Arm Maze

Full-Baited Procedure The EH, EC and SC animals significantly reduced their errors as the sessions went by (one-way ANOVA, EH: $F_{9, 54} = 2.4$; $p < 0.05$; EC: $F_{9, 81} =$

5.5; $p < 0.00001$; SC: $F_{9, 81} = 2.5$; $p < 0.05$) unlike the SH ($F_{9, 54} = 1.3$; $p = 0.3$) (Fig. 5a). A three-way ANOVA (housing \times lesion \times session) on total errors revealed significant housing ($F_{1, 30} = 82.7$; $p < 0.00001$), lesion ($F_{1, 30} = 54.6$; $p < 0.00001$) and session ($F_{9, 270} = 4.6$; $p < 0.00005$) effects; some first-order interactions were significant (housing \times session: $F_{9, 270} = 2.4$; $p < 0.05$; lesion \times session: $F_{9, 270} = 1.9$; $p = 0.05$).

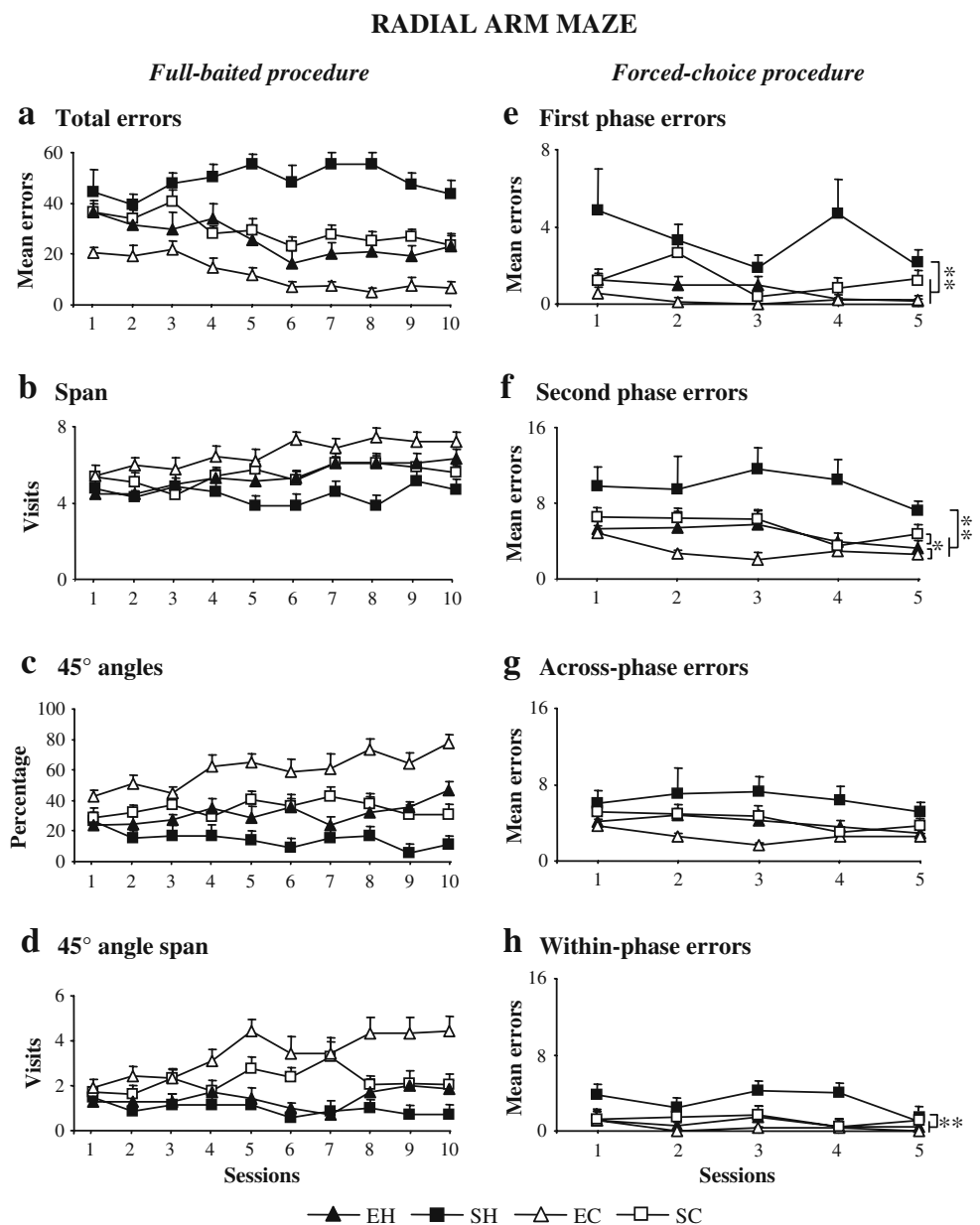
EH, EC and SC groups progressively lengthened their spatial span (one-way ANOVA, EH: $F_{9, 54} = 2.1$; $p < 0.05$; EC: $F_{9, 81} = 4.1$; $p < 0.0005$; SC: $F_{9, 81} = 2.1$; $p < 0.05$), unlike the SH group ($F_{9, 54} = 0.8$; $p = 0.6$). A three-way ANOVA (housing \times lesion \times session) on spatial span values revealed significant housing ($F_{1, 30} = 41.6$; $p < 0.00001$), lesion ($F_{1, 30} = 45.1$; $p < 0.00001$), and session ($F_{9, 270} = 4.3$; $p < 0.00005$)

effects; the first-order interaction housing \times session ($F_{9, 270} = 2.1$; $p < 0.05$) was also significant (Fig. 5b).

Perseverative errors were present mainly in the SH group ($X = 5.43 \pm 1.63$) and almost lacking in the remaining groups (EH: $X = 1.29 \pm 0.52$; EC: $X = 0.62 \pm 0.26$; SC: $X = 0.35 \pm 0.13$). Given the scattered occurrence of perseverations, we analyzed perseverative errors not considering session effect. A two-way ANOVA (housing \times lesion) revealed significant housing ($F_{1, 30} = 7.1$; $p < 0.05$) and lesion ($F_{1, 30} = 15.6$; $p < 0.0005$) effects. Also, interaction was significant ($F_{1, 30} = 9.2$; $p < 0.01$) (*post hoc* comparisons: SH vs. EH, SC, or EC: at least $p < 0.0005$).

To analyze the procedural strategies used to explore the maze, we considered the number of 45° angles made in visiting the eight arms that is the entries in adjacent arms. A

Fig. 5 Effects of hemispherectomy and environmental enrichment on the Radial Arm Maze performance. Total errors (a), span (b), 45° angles (c), and 45° angle span (d) in the full-baited procedure are depicted. Working memory errors in first (e) and in second (f) phases of the forced-choice procedure are depicted. The working memory errors of second phase are distinguished in across- (g) and within- (h) phase errors. The asterisks at the right side of the graphs indicate the *post hoc* comparisons between groups: * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0005$. Vertical bars indicate SEM



three-way ANOVA (housing \times lesion \times session) on 45° angles revealed significant housing ($F_{1, 30}=27.9$; $p<0.00001$), lesion ($F_{1, 30}=37.6$; $p<0.00001$), and session ($F_{9, 270}=2.3$; $p<0.05$) effects; the first-order interaction housing \times session was also significant ($F_{9, 270}=4.9$; $p<0.00001$) (Fig. 5c).

To determine whether a chaining strategy was used, the 45° angle span was analyzed. A three-way ANOVA (housing \times lesion \times session) on 45° angle span revealed significant housing ($F_{1, 30}=8.6$; $p<0.01$), lesion ($F_{1, 30}=31.4$; $p<0.00001$), and session ($F_{9, 270}=2.2$; $p<0.05$) effects; some first-order interactions were significant (housing \times session: $F_{9, 270}=2.7$; $p<0.01$; lesion \times session: $F_{9, 270}=2.8$; $p<0.005$) (Fig. 5d).

Forced-Choice Procedure In the forced-choice paradigm with selectively baited arms, any regular search pattern was discouraged by the irregularity and variation of the baited arms distribution session by session. Thus, success in visiting only rewarded arms depended on remembering the already visited arms, rather than putting into action specific search patterns. This feature of the protocol rendered possible to distinguish procedural from working memory components. A three-way ANOVA (housing \times lesion \times session) on working memory errors of the first phase in which only four arms were opened revealed significant housing ($F_{1, 30}=31.4$; $p<0.00001$), lesion ($F_{1, 30}=15.5$; $p<0.0005$), and session ($F_{4, 120}=2.6$; $p<0.05$) effects; first-order interaction housing \times lesion was also significant ($F_{1, 30}=5.5$; $p<0.05$; *post hoc* comparisons: EH vs. SH $p<0.001$; EH vs. EC $p=0.3$; EH vs. SC $p=0.3$; SH vs. EC or SC at least $p<0.0005$) (Fig. 5e).

A three-way ANOVA (housing \times lesion \times session) on working memory errors of the second phase when all eight arms were opened revealed significant housing ($F_{1, 30}=45.2$; $p<0.000001$), lesion ($F_{1, 30}=28.8$; $p<0.00001$), and session ($F_{4, 120}=2.4$; $p=0.05$) effects; first-order interaction housing \times lesion was also significant ($F_{1, 30}=4.9$; $p<0.05$). In fact, performance of EH group was better than that of SH group (*post hoc* comparisons: $p<0.001$), it was worse than that of EC group ($p<0.05$) and did not differ from that of SC group (Fig. 5f). The errors of the second phase were further divided in across-phase and within-phase errors. A three-way ANOVA (housing \times lesion \times session) on across-phase errors revealed significant housing ($F_{1, 30}=30.6$; $p<0.00001$) and lesion ($F_{1, 30}=21.2$; $p<0.0001$) effects. The other main effect and all interactions were not significant (Fig. 5g). A similar three-way ANOVA (housing \times lesion \times session) on within-phase errors revealed significant housing ($F_{1, 30}=52.1$; $p<0.00001$), lesion ($F_{1, 30}=30.0$; $p<0.00001$), and session ($F_{4, 120}=5.9$; $p<0.0005$) effects. Some first-order interactions were also significant (housing \times lesion: $F_{1, 30}=11.8$; $p<0.005$ (*post hoc* comparisons: EH vs. SH $p<0.001$; EH vs. EC $p=0.1$; EH vs. SC $p=0.2$;

SH vs. EC or SC at least $p<0.0005$); lesion \times session: $F_{4, 120}=2.6$; $p<0.05$). The second-order interaction was also significant ($F_{4, 120}=3.1$; $p<0.05$) (Fig. 5h).

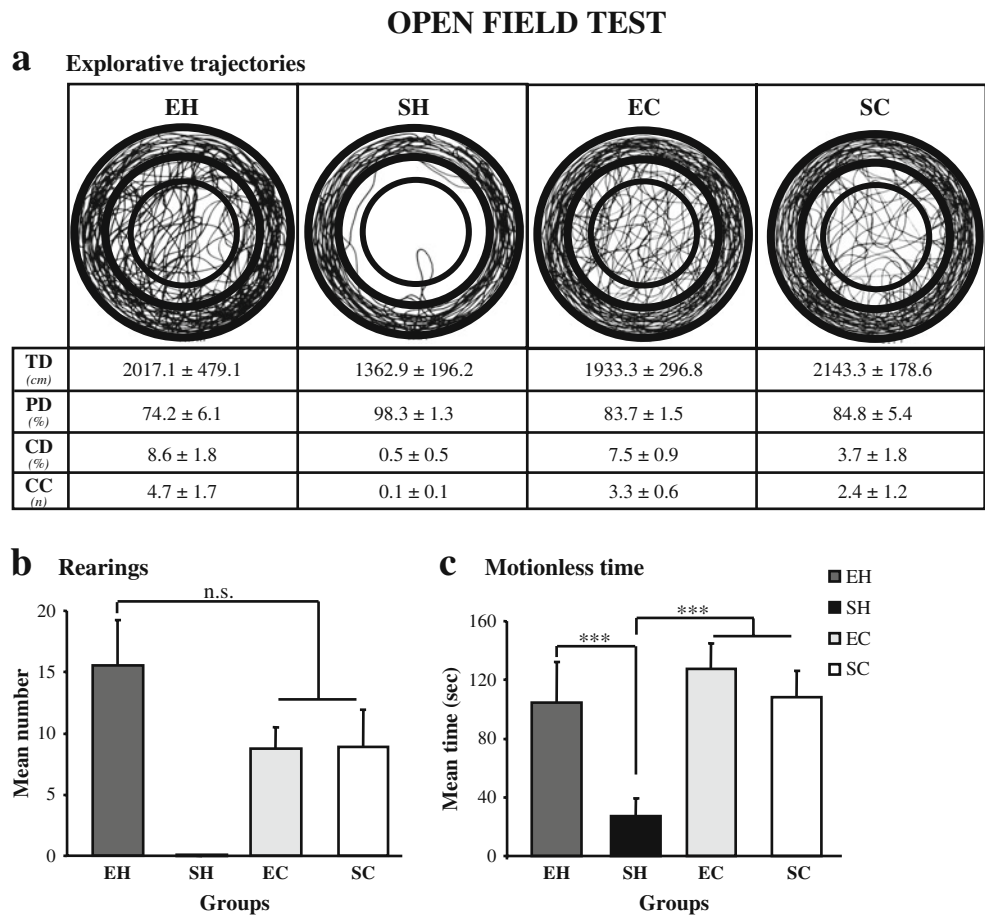
Summing up, in the forced-choice paradigm of RAM task EH animals behaved as SC animals in most parameters considered and even in some parameters reached the optimal performances of EC animals.

Open Field

When exploring the OF arena, all animals traveled similar total distances as revealed by a two-way ANOVA (housing \times lesion). This analysis failed to reveal any significant effect on housing ($F_{1, 30}=0.5$; $p=0.5$) or lesion ($F_{1, 30}=1.3$; $p=0.3$) factors. Also, interaction was not significant ($F_{1, 30}=2.1$; $p=0.2$). The percentages of the distance traveled in exploring a peripheral annulus (peripheral distance) were significantly different among groups. A two-way ANOVA (housing \times lesion) revealed significant housing effect ($F_{1, 30}=8.7$; $p<0.01$) and interaction housing \times lesion ($F_{1, 30}=7.3$; $p<0.05$). Lesion effect was not significant ($F_{1, 30}=0.2$; $p=0.6$). *Post hoc* comparisons revealed that the EH animals' percentages of peripheral distance were not significantly different from those of the un-lesioned SC and EC animals and significantly lower than those of the SH animals ($p<0.005$) that traveled almost exclusively in the peripheral annulus (Fig. 6a). The percentage of peripheral distance displayed by SH animals was significantly ($p=0.05$) higher than that of SC group. This behavior of SH group could be related to defective exploration as well as to increased anxiety levels. The total distances traveled in the central area were affected by housing condition, as revealed by a two-way ANOVA (housing \times lesion; housing effect: $F_{1, 30}=17.0$; $p<0.0005$; lesion effect: $F_{1, 30}=0.5$; $p=0.5$; interaction: $F_{1, 30}=2.2$; $p=0.1$) (Fig. 6a). The same pattern was observed when central area crossings were considered (two-way ANOVA: housing effect: $F_{1, 30}=6.9$; $p<0.05$; lesion effect: $F_{1, 30}=0.2$; $p=0.6$; interaction: $F_{1, 30}=3.1$; $p=0.09$). Rearing behavior was computed on data exhibited by EH, EC, and SC groups, since SH animals displayed no rearing at all because of the severity of their postural impairment (Fig. 6b). One-way ANOVA revealed that EH group exhibited a number of rearings not significantly different from both un-lesioned SC and EC groups ($F_{2, 24}=1.7$; $p=0.2$).

No differences among groups were found as for defecations (two-way ANOVA: housing effect: $F_{1, 30}=1.0$; $p=0.3$; lesion effect: $F_{1, 30}=1.6$; $p=0.2$; interaction: $F_{1, 30}=2.3$; $p=0.1$). Finally, when the motionless time was considered, a two-way ANOVA (housing \times lesion) revealed significant housing ($F_{1, 30}=25.3$; $p<0.00005$) and lesion

Fig. 6 Effects of hemispherectomy and environmental enrichment on the Open Field performance. In (a), the explorative trajectories traveled by EH, SH, EC, and SC rats in exploring the arena are reported. Total distances (*TD*), percentages of peripheral distance (*PD*), of central distance (*CD*), and central crossings (*CC*) are also indicated. Rearrings (b) and motionless time (c) displayed by the four experimental groups are illustrated. In (b–c), asterisks inside the graphs indicate the *post hoc* comparisons between groups: *** $p < 0.0005$. Vertical bars indicate SEM



($F_{1, 30} = 29.1$; $p < 0.0001$) effects. Also, interaction was significant ($F_{1, 30} = 8.9$; $p < 0.01$) (Fig. 6c).

Discussion

Thus far, clinical and experimental studies have examined the factors that stabilize and improve the cognitive symptoms following cortical neuro-degenerative damage [4, 9, 37]. We wondered whether environmental complexity might promote motor and cognitive functioning also in the presence of cerebellar pathologies.

The present results demonstrate that long-term exposure to an enriched environment exerts beneficial effects on motor, cognitive and emotional symptoms of cerebellar origin. Exposure to complex stimulations almost completely compensated the HCb effects and rendered the performances of EH animals similar to those of intact animals. It is noteworthy that the outstanding compensation of the EH animals was not due to a less severe initial impairment. In fact, both EH and SH groups exhibited similar initial values in almost all parameters of the postural and cognitive testing. Namely, housing in complex situations accelerated compensation of postural and locomotor deficits of cere-

bellar origin; it improved both working and long-term memory; it allowed rapid transition from one competence to another (e.g., from procedural to mnemonic competence); it ameliorated functions linked to declarative (localizatory) memory; it improved exploratory strategies; and it decreased anxiety levels.

First of all, we considered the possibility that the improvements observed in the enriched lesioned animals were due to changes in motivation rather than to specific effects on cognitive and motor functions. However, the environmental enrichment did not appear to have affected motivational components by modifying the salience of reward/punishment and explorative tendencies. In fact, regardless of whether or not they were lesioned or enriched, all animals promptly climbed onto the MWM platform, consumed the food pellets in RAM and searched throughout the environments (MWM pool, radial maze, or OF arena).

A primary feature of HCb symptomatology is the maximum degree of severity of the postural and locomotor impairment present one day after the lesion, which decreases progressively as time goes by. The time course of the compensation of the motor cerebellar symptoms was favorably influenced by the previous exposure to enriched

environment. As repeatedly reported in the literature about the behavioral effects of the exposure to enriched environments [9, 38–44], rearing in enriched conditions promotes physical activity by encouraging exploratory movements to forage the large cages, fine manipulation skills linked to the inspection of the repeatedly renewed objects, and general motor activity in using running wheels. All motor components are beneficially influenced by enrichment condition. In the present research, the complex behaviors requiring limb coordination, eumetric movements, muscle strength and subtle balance control were particularly well-compensated in EH animals. Consistently, in rats recovered from motor effects of cerebellar ablations the complex skills were more affected than postural and locomotor behaviors by the treatment with NMDA antagonists that disrupted the compensated state [29, 30].

The present results on the effects of environmental enrichment on motor deficits of cerebellar origin completely fit with data obtained in other models of brain pathologies. Exposure to an enriched environment ameliorated motor coordination deficits and spatial learning in the female mice of a murine model of the Rett's syndrome, an autistic spectrum developmental disorder characterized by severe behavioral and neuropathological deficits [39, 45, 46]. Furthermore, research on the R6/1 transgenic mice model of Huntington's disease (HD) demonstrated that exposure of HD mice to an enriched environment delayed the onset and progression of disease [41, 47–49]. Furthermore, environmental enrichment improved behavioral performances in models of dementia-like neurodegeneration [10–12, 50].

The enhanced stimulations provided by the enriched environment influenced not only motor but also cognitive function, in accordance with clinical studies reporting that more physical exercise and cognitive engagement in youth are associated with maintenance of memory function in midlife [51, 52].

In the MWM, the EH animals exhibited improved mnesic and mapping abilities (hidden platform) as well as increased attention to contextual cues (visible platform). Moreover, they exhibited reduced peripheral swimming and efficient navigational strategies, as indicated by the high percentage of finding strategy they used to reach the platform. In general, the spatial behavior severely impaired by cerebellar lesions in the MWM [33, 34, 53–56] was thoroughly recovered by EH animals that behave as intact rats. One possible explanation for the improved functional recovery in EH rats could take into account their improved motor performances as well as improved abilities of visuo-motor coordination. In fact, EH animals were able to orientate their bodies in the pool because of their accelerated postural recovery. However, the tuned navigational strategies the EH animals exhibited in the phases in

which the platform was hidden (places I and II) seem to support even improved cognitive performances.

In the RAM, the EH group made fewer errors and perseverations and more entries in consecutive arms than the SH group in the full-baited procedure and performed significantly less errors than the SH rats even in the forced-choice procedure. Thus, depending on the context the EH animals reorganized their strategies and shifted from using spatial procedures to applying mnesic competencies. The present data fit with the improved mnesic and procedural performances displayed by enriched intact [57] or cholinergically depleted animals [12].

In the OF, the EH group traveled the peripheral sectors not differently from the un-lesioned EC and SC groups. This kind of explorative pattern is in line with the reduced percentages of Circling displayed by EH, EC, and SC rats in the MWM. Furthermore, EH and EC animals similarly traveled and crossed the central area of the arena. Noteworthy, a pattern featured by increased central traveling is retained to be linked to reduced anxiety levels [58, 59]. Thus, it is possible that the ameliorative effects of the environmental enrichment we observed on various parameters of the behavioral tasks were related to diminished levels of anxiety of animals exposed to a complex environment [60]. Accordingly, the prolonged experience of enrichment may contribute to the improved performance of EH animals not only potentiating the cognitive competencies but also by reducing the anxiety levels. Even the motionless time displayed by EH, EC and SC animals overlapped. The number of rearings exhibited by EH animals markedly differed from that of SH animals. Typically, HCbed animals show almost no rearings because they have difficulty keeping their balance standing on their hind-limbs [61]. Notably, the EH animals produced the same number of rearings as the un-lesioned animals, once again indicating their almost complete compensation of the HCb motor deficits.

Summing up, besides symmetrical and coordinated postural and locomotor behaviors, the EH rats exhibited advanced working memory and planning activities, an almost complete absence of perseverations, rapid acquisition and flexible use of efficient explorative strategies and of procedural abilities. These functions are typically mediated by the frontal and cerebellar circuits. Extensive anatomo-functional connections [62] support the interplay between the cerebellar and frontal areas that interact in executive control of voluntary behavior [63–65]: the cerebellum permits acquisition of efficient procedural competencies and the frontal cortex provides flexibility among already acquired and stored solutions [66–68]. Previously, we demonstrated that in rats HCb provokes lack of flexibility in changing strategy, impossibility to inhibit patently wrong responses [69–71], deficits due to impairment in adapting behavior to context. The evidence

that the EH animals displayed no “frontal-like” deficits as perseverations and lack of attention to novel stimuli, as well as no deficient working memory, could be an index of an efficient use of cerebello-frontal networks. One of the most extensively replicated findings in enriched rodents is the modulation of neurotrophin expression [48, 72–74] retained related to the improved performances of the enriched animals [75]. We recently demonstrated that rats enriched with the very same protocol used in the present research exhibited marked up-regulation of the levels of BDNF in the frontal cortex and cerebellum [76, 77]. Although speculative, the relation between the increased expression of neurotrophins in the cerebello-frontal areas and the improved abilities related to these areas in the enriched animals is noteworthy.

To our knowledge, this is the first attempt at demonstrating that early and prolonged exposure to an enriched environment strikingly improves compensation of deficits of cerebellar origin. Further studies are needed to discover the mechanisms through which exposure to complex stimulations may represent an endogenous device to ameliorate motor and cognitive performance in the presence of brain damage. The molecular changes underlying experience-dependent synaptic plasticity should be investigated in future research to develop new therapeutic approaches not only for brain disorders in general but also for cerebellar pathologies in particular.

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Conflicts of interest All the authors have approved the manuscript that is enclosed and fully disclose all conflicts of interest.

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