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Research report

Effects of early midline cerebellar lesion on cognitive and emotional functions in the rat

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Abstract

Midline lesion of the cerebellum was performed in young 10-day-old DA/HAN strained (pigmented) rats. Once adults, the lesioned animals were subjected to a series of behavioral tests and their performances were compared with those of control (nonlesioned) rats. Compared with controls, the spontaneous motor activity of the lesioned rats was higher, they showed persevering behavior and did not pay attention to environmental distractors. In anxiety and social discrimination tests, disinhibition tendencies were obvious, which suggested that the animals were less dependent on the context. These abnormalities were most likely due to early midline lesion of the cerebellum and not to a deficit in maternal care before weaning, since the dams took care of the lesioned and control pups similarly. From these results, it can be concluded that the cerebellar vermis is involved in motor control, attentional capabilities and emotional behavior. Given that the lesioned rats observed in this study presented obvious autistic-like symptoms, and since a number of autistic subjects have cerebellar deficits and, particularly, a hypoplasia of vermal lobules, our results may strengthen the idea that the cerebellar vermis is involved in autism, as already suggested in the guinea pig (Caston J, et al. Eur J Neurosci 1998;10:2677–2684).

Keywords: Cerebellum; Motor activity; Attention; Anxiety; Emotional behavior; Neophobia; Autism; Rat

1. Introduction

Many studies have demonstrated that the cerebellum is involved not only in the regulation of motor skills, but also in more complex integrated functions [34,37,44,55], such as classical conditioning [4,41,54,75], learning of motor skills [10,32,35,46,61], spatial processing and spatial learning [22,45,56], habituation of exploration behavior [21] and of the acoustic startle response [42], and perception of time [57], as well as in a variety of learning procedures [5,7,51,65]. The cerebellum is also involved in integrated autonomic functions [27,49,53,58,67], and there is growing evidence that it is implicated in motivations [11] and emotional behavior [30]. Indeed, electrical stimulation of the medial cerebellar structures (vermis and fastigial nuclei) elicited fear manifestations [3,73], and massive cerebellar degeneration, such as that seen in staggerer and lurcher mutant mice, is associated with fear decrease in the former [31] and an increased endocrine response to stress in the latter [26]. Besides, transgenic mice overexpressing bcl-2 in neurons are characterized by an increased number of neurons in different parts of the central nervous system, including the cerebellum [52,76,77], and by a fear decrease [61]. It has also been found that the cerebellum is implicated in autism, a neurodevelopmental pathology characterized by socio-emotional alterations, since a number of autistic patients show cerebellar abnormalities [2,15,19,20, 60,66,74] and, particularly, a hypoplasia of vermal

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lobules VI and VII [12,15,19,20,29,62]. Moreover, in the GS strain guinea pig, characterized by a hypoplasia of vermal lobules VI and VII, and by alterations of neurons in the fifth layer of the cerebral cortex [47], autistic-like symptoms have been demonstrated [11].

All these studies clearly demonstrated the involvement of the cerebellum in cognitive and emotional functions. However, the relative part of the vermis and of the cerebellar hemispheres in these functions is poorly documented. Lesion of the midline of the cerebellum retarded long-term habituation of the acoustic startle response [43], and impaired posture and equilibrium [25], but did not abolish motor skill acquisition [33]. Moreover, rats with midline lesions have defects in visuo-motor abilities as demonstrated in the visible platform test of the Morris water maze, but no deficit of spatial memory in spontaneous alternation testing [33].

Given that the effects of a cerebellar lesion depend on the age of the animals when lesioned [1] and since, in the aforementioned experiments, lesions were carried out in adults, the aim of the present study was to examine the effects, on the adult behavior, of a midline cerebellar lesion done in the young rat, at 10 days of age, when the cerebellum is still immature. We examined the spontaneous motor activity of the animals and their cognitive and emotional functions: attentional capabilities, anxiety-like behavior, and social discrimination abilities. Moreover, to know whether the eventual deficits of the lesioned animals were due to the lesion per se or, at least in part, to deficit of maternal care, we investigated interactions between pups and dams following the operation.

2. Materials and methods

2.1. Animals

The animals used in this study were DA/HAN strained (pigmented) rats, born in the laboratory. They were housed four per cage in standard conditions: 12 h light (08.00–20.00 h)–12 h dark (20.00–8.00 h), 20–22°C, food and water delivered ad libitum.

Pregnant females were isolated in a cage a few days before delivery. The day of birth (day 0) was known by inspection of the cages twice daily. Pups were kept with their dam until 1 month of age. After weaning, they were separated according to their sex and reared four per cage until adulthood.

2.2. Operation

Ablation of the cerebellar vermis was done at 10 days of age. About one-half of the animals of a litter were operated on, while the others were not. All the animals were deeply anesthetized by ether. The rats to be lesioned were secured in a modified stereotaxic apparatus, the skin of the head cut, and a small hole drilled in the sagittal plane, just posterior to lambda. The dura was carefully incised and the vermal lobe gently removed by suction; the skin was then sutured. All the animals (the lesioned as well as the nonlesioned) were kept in a cage without sawdust on a soft sheet of paper and were heated by the way of an incandescent lamp (60 W) placed 40 cm above the cage until they were fully awake. After that, they were replaced in their cage with their dam until the age of 1 month. Then, they were separated according to their sex and reared four per cage until the experiments.

As a whole, 41 animals were obtained from eight litters. Twenty-five were lesioned, while the 16 others were not. Only four lesioned rats died during the days following the operation. The 21 rats that survived were kept in a good shape until adulthood.

2.3. Experimental protocols

2.3.1. Evolution of weight

All the animals were weighed every 1 days from the age of 4 days to 1 month, and then every week until 12 weeks of age.

2.3.2. Maternal behavior

In order to know whether the dams took care of the operated and nonoperated pups similarly, the maternal behavior was quantified at days 11, 12, 14 and 16; i.e. for the lesioned animals, 1, 2, 4 and 6 days after the operation, respectively.

In a first step, the behavior of the animals was filmed during a 10-min period between 20:00 and 22:00 h, that is during their active phase. The interactions between the dam and the pups were quantified outside the nest (the interactions within the nest could not be quantified with accuracy and were therefore not studied): (1) the number of times the dam interacted with a pup (sniffing, grooming, carrying a pup from one place to another; Mann–Whitney U-test used to compare the interactions between dams and intact and lesioned pups; (2) the number of retrievings (depending on the number of pups that were outside the nest and on the number of times they went out of the nest during the 10-min observation period; the ratio 'number of retreivings/number of possible retrievings' was calculated, the possible retrievings being the number of times the dam had the opportunity to retrieve a pup to the nest); the χ^2 test was used to compare the ratios between intact and lesioned pups; (3) the number of times the pups went out of the nest and returned to the nest by themselves was also quantified; Mann–Whitney U-test was used to compare the behavior of intact and lesioned pups.

In a second step, which began 10 min after the end of the first step, all the pups were taken off the nest by the experimenter and grouped at the opposite corner of the cage. Then, during a 20-min period, the behavior of the dam and of the pups was filmed and the following items were quantified: (1) the number of retrievings; (2) the time taken to retrieve the first pup; (3) the number of pups that returned to the nest by themselves and time taken by these pups to reach the nest. All these observations were compared between intact and lesioned pups according to the Mann–Whitney U-test.

2.3.3. Spontaneous motor activity

The spontaneous motor activity was studied on an actisystem (Apelex, France), the platform of which was $35 \times 35 \text{cm}^2$. The motor activity level was given in arbitrary units. It was recorded every minute for 15 min, 5 min after the animal had been placed at the center of the platform. Recordings were made late in the evening (20:00–22:00 h), during the active phase of the animals. After each experiment, the platform was cleaned with a alcoholic solution to mask the olfactory cues. Comparison of motor activity between intact and lesioned rats was performed according to the Mann–Whitney U-test.

2.3.4. Attentional capabilities

The experiment was conducted in an experimental box ($60 \times 60 \times 40$ cm^3), on the floor of which five different items were placed. It was split into two sessions of 15 min each, separated by a 30-min interval. During the first session of the experiment (ES1), the animals explored freely the objects in a silent room. During the second session (ES2), 5 min after the animal had been put in the experimental box in which the five items were placed differently to create a novel environment, the rat was subjected to an intermittent noise (rock music at about 80 dB) of different durations being delivered at random. The total durations of the noisy and the silent periods were 3 and 7 min, respectively. The two sessions of the experiment were recorded on a video tape and the following data collected: (1) motion and motionless durations; (2) exploration duration; (3) number of items visited. Moreover, during the second session of the experiment, the number of items visited during the 3-min noisy period and during the 7-min silent period was counted separately. Useful comparisons between intact and lesioned rats were made according to the Mann–Whitney U-test.

2.3.5. Anxiety-like behavior

2.3.5.1. Elevated + maze. The elevated + maze was a cross maze with two closed and two open arms. It was located 50 cm above the floor. Each arm was 50 cm in length and 10 cm in width, the walls of the closed arms being 40 cm in height. The open arms were divided by a fictive line in two parts of equal length: a proximal part and a distal part. Previous studies showed that anxious mice spent more time in closed arms than in open arms [50], and that anxiolytics increased significantly the number of entries in open arms, whereas anxiogenic drugs reduced it [59]. Three parameters were quantified: number of entries in open and closed arms, respectively (an entry was considered as effective when the animal had its four paws in the considered arm); time spent in the open and closed arms, respectively; time spent in the distal part (the most anxiogenic one) of the open arms. The test lasted 10 min and was recorded on a video tape. Intra- and intergroup comparisons were made according to Wilcoxon and Mann–Whitney U-tests, respectively.

2.3.5.2. Burying behavior. This defensive behavior [72] consists of burying an electrode in sawdust once the rat has touched it and received an electric shock. The experimental device consisted of a cage similar to the housing cage of the animal and containing a 1-cm thick layer of sawdust. An electrode passing through a wall of the cage was positioned 1 cm above the surface of sawdust and connected to a 50 Hz a.c. 35 V generator. The current intensity was adjusted with a variable resistor in series with the generator and set at 85 mA. For 1 h before the test, the rat was put in the cage from which the electrode was removed, for the purpose of familiarization. Then, it was taken off and replaced in its housing cage for 15 min. During this time, the electrode was positioned into the experimental cage. The rat was replaced in it and, after a latency time, its attention was driven towards the electrode; the animal went close to the electrode, touched it and received an electric shock as revealed by its behavior. Usually, after the shock, the animal buried the electrode in sawdust. The behavior of the rat from the time it was placed into the experimental cage was recorded on a video tape for 30 min and the following items were quantified: latency time to touch the electrode; number of animals that moved sawdust; number of animals burying the electrode in sawdust. Latency times in control and lesioned rats were compared according to the Mann–Whitney U-test and the numbers of intact and lesioned animals moving sawdust or burying the electrode were compared according to the χ^2 test.

2.3.6. Social discrimination

The aim of this experiment was to test whether a rat prefers to visit its own territory or to explore the territory of an unknown rat when both territories were accessible. The apparatus consisted of two wooden boxes ($35 \times 35 \times 35$ cm^3), a known (K) one and an unknown (U) one, and of a starting box (S) of smaller dimensions which could communicate with K and U. The floor of K and U compartments was covered with sawdust. In a first step, the doors between the starting box and the experimental compartments K and U were closed, and the rat to be tested ('K' rat) was placed for 24 h in K, while another rat ('U' rat) was placed for 24 h in U. During this time, food and water were delivered ad libitum. Then, both rats were removed from their experimental territory and the K rat was placed into the starting box for 2 min. After that, the doors isolating the starting box from the experimental compartments K and U were removed, and the animal could therefore enter its own territory (K) or the unfamiliar territory (U). Its behavior was recorded on a video tape for 15 min. The time spent in the known and the unknown compartments as well as the number of entries in the unknown compartment were quantified in the following conditions: K and U compartments were the territories of control rats or of lesioned rats; K compartment was the territory of a control rat (or of a lesioned rat) and U compartment was the territory of a lesioned rat (or of a control rat). Useful comparisons were made according to the Mann–Whitney U-test. As in females social discrimination could be biased by the estrous cycle, the experiments were done only in males. Moreover, both K and U rats belonged to different litters to be sure that they were strangers.

2.4. Control of the lesion

After completion of the behavioral experiments, the lesioned animals were administered an overdose of chloral hydrate and perfused intracardially; first, with physiological saline; then, with 4% paraformaldehyde and 0.3% glutaraldehyde in phosphate buffer. Then, the brains were removed, placed in the same fixative solution for 10 days, and examined under the operating microscope to estimate the extent of the lesion. All the brains with wrong or incomplete lesions were discarded and the results not included in this study. Among 21 brains that were controlled, six were discarded. The results concerning the lesioned rats are therefore given in 15 animals (six males and nine females) which were perfectly lesioned (Fig. 1A). The control group contained 16 rats (nine males and seven females). Ten brains (eight among the lesioned group and two controls) were prepared for histological control. Frozen sections, 20 μm thick, stained with cresyl violet were examined under the light microscope.

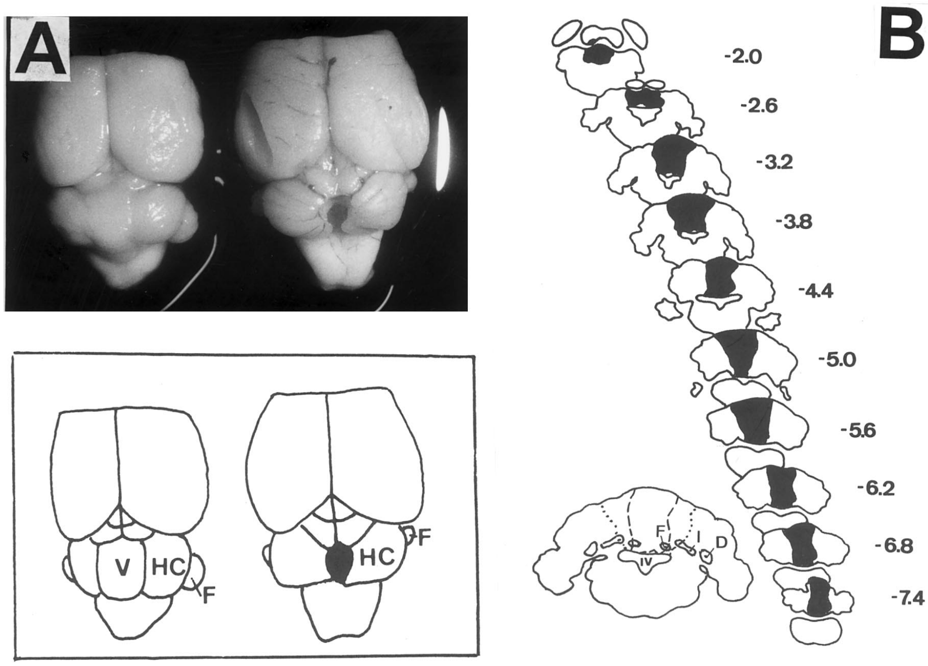

Fig. 1. (A) Cerebellum of a control (left side) and a lesioned (right side) rat. CH, Cerebellar hemisphere; F, flocculus; V, vermis. In the lesioned rat, the vermis is completely extirpated. (B) Reconstruction of transverse sections of the cerebellum and the brain stem showing in one rat the parts of the cerebellum (filled in black) which were removed by suction. Figures on the right indicate the anteroposterior coordinates with respect to the De Groot system. At the bottom of the figure, on the left, is depicted a transverse section at the −3.8 anteroposterior coordinates in the eight brains examined, showing the less extensive (broken line) and the most extensive (dotted line) lesion. F, Fastigial nuclei; I, interpositus nuclei; D, dentate nuclei; IV, fourth ventricle.

3. Results

3.1. Histological controls

The histological controls demonstrated that in the eight cases (among 15) examined, the vermal lobe was completely removed and the fastigial nuclei destroyed on both sides. Moreover, the brain stem exhibited no sign of lesion (Fig. 1B), which could be correlated with the lack of vestibular and vegetative disorders.

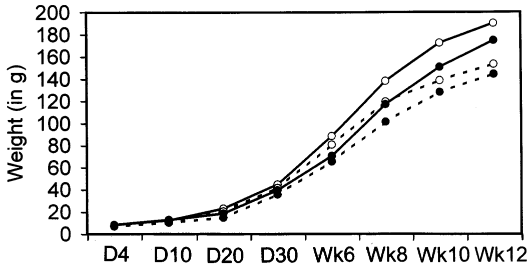

Fig. 2. Evolution of weight from the 4th day to the 12th week of male (full lines) and female (dotted lines) control (open circles) and lesioned (full circles) rats. Error bars have been omitted for clarity.

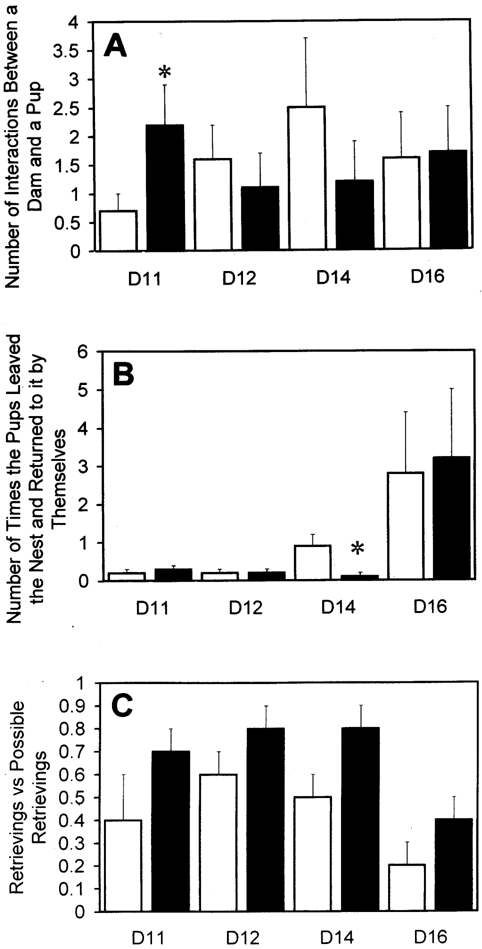

Fig. 3. Interactions (means $\pm$ S.E.M.) between dams and control (open histograms) and lesioned (full histograms) pups. D11, D12, D14, D16, age of the pups in days. * Significant difference between lesioned and control rats at $P<0.05$.

3.2. Evolution of weight

Fig. 2 depicts the evolution of weight of male and female intact and lesioned rats from 4 days to 12 weeks. For both sexes, weights of the lesioned animals were always lower than those of intact ones. However, the differences were never significant (at 12 weeks: $U[6, 9]=25.5$, $P>0.05$, for males; $U[7, 9]=12.5$, $P>0.05$, for females).

3.3. Maternal behavior

The number of interactions of a dam with an intact pup and a lesioned pup was not significantly different except for the 11th day (i.e. the day after the operation); at this day, the dam took more care of the lesioned pups than of the intact ones ($U[8, 8]=10$, $P<0.05$) (Fig. 3A). The total number of pups that went out of the nest and returned to it by themselves increased by day 16 both in intact and lesioned groups, and the difference between them was not significant, except for day 14 when the lesioned pups still did not move easily, while the intact pups began to do so ($U[8, 8]=12$, $P<0.05$) (Fig. 3B). The number of retrievings versus the number of possible retrievings decreased abruptly at day 16 both in intact and lesioned animals (because the pups moved enough to return to the nest by themselves), and the difference between the two groups was never significant (χ^2 between 0.11 and 0.35, $P>0.05$) (Fig. 3C).

When the pups were taken off the nest by the experimenter and grouped at the opposite corner of the cage, the behavior of the dams towards the pups was similar whether they were lesioned or intact, and at day 16, when the pups were sufficiently moving by themselves, no difference was noted between intact and lesioned animals. Indeed, the mean latency needed to retrieve the first pup was similar, whatever the day, for intact and lesioned pups ($U[8, 8]=20$–31, $P>0.05$) (Fig. 4A), as was the percentage of retrievings ($U[8, 8]=23$–28.5, $P>0.05$) (Fig. 4B). At day 16, the number of intact and lesioned pups that returned to the nest by themselves was similar (57.9 and 57.1%, respectively: $U[8, 8]=28.5$, $P>0.05$), but the mean time taken by the pups to reach the nest was greater in the lesioned group (193.8 ± 101.8 s) than in the intact one (53.4 ± 17.8 s), while the difference was not significant ($U[8, 8]=29$, $P>0.05$).

3.4. Spontaneous motor activity

In control as well as in lesioned rats, the evolution of the cumulated motor activity was a linear function of time during the first 8–10 min of recording. This means that, for both groups, motor activity was similar during the first, the second, … the eighth or the tenth minute. From this time, the activity per minute decreased slowly, showing a habituation process. From the first minute of observation, the spontaneous motor activity of the lesioned animals (85.8 $\pm$ 9.4) was greater than that of controls (54.1 $\pm$ 7.2) (U[15, 16] = 35.5, $P < 0.01$) (Fig. 5). Such a difference persisted throughout the 15-min observation period and at this time, the cumulated motor activity was still greater in the lesioned animals (1016.0 $\pm$ 63.1) than in the control ones (798.5 $\pm$ 45.7) (U[15, 16] = 45, $P < 0.01$).

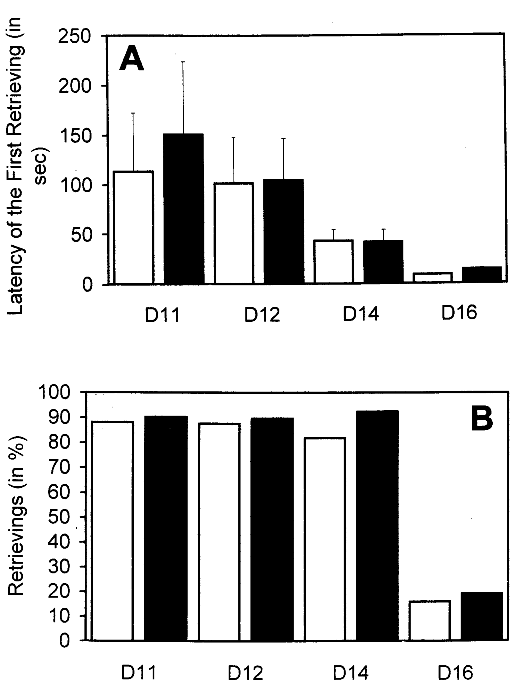

Fig. 4. Retrieving behavior when all the pups were taken off the nest. D11, D12, D14, D16, age of the pups in days. Open histograms, control pups; full histograms, lesioned pups.

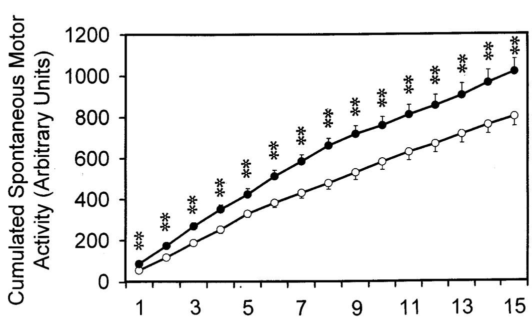

Fig. 5. Cumulated spontaneous motor activity (means $\pm$ S.E.M.) during 15 min (abscissae) in control (open circles) and lesioned (full circles) rats. ** Significant difference between control and lesioned rats at $P < 0.01$.

3.5. Attentional capabilities

From comparison of the behavior of the animals during the first exploration session without noise (ES1) and the second exploration session with noise (ES2), it can be stated that the number of items visited was not significantly different during the two experimental conditions, both in control (11.4 $\pm$ 1.9 during ES1 and 9.7 $\pm$ 1.7 during ES2: U[16, 16] = 96, $P > 0.05$) and in lesioned animals (13.0 $\pm$ 1.9 during ES1 and 12.5 $\pm$ 1.7 during ES2: U[15, 15] = 91, $P > 0.05$) (Fig. 6A). Duration of the exploration period was always shorter during ES2 than during ES1, but the difference was significant only in control animals (U[16, 16] = 74, $P < 0.05$) (Fig. 6B). Therefore, the mean time spent by control rats around an item was shorter during ES2 (2.6 $\pm$ 0.2 s) than during ES1 (4.4 $\pm$ 0.7 s) (U[16, 16] = 72.5, $P < 0.05$), while it was not significantly different in lesioned animals (3.1 $\pm$ 0.3 s during ES2 and 4.3 $\pm$ 0.3 s during ES1: U[15, 15] = 68, $P > 0.05$) (Fig. 6C). In control rats, the exploration frequency (number of items visited per minute of walking) was significantly higher during ES2 (30.1 $\pm$ 3.1) than during ES1 (20.5 $\pm$ 2.3) (U[16, 16) = 64, $P < 0.05$). In lesioned rats, it was similar during ES2 (21.0 $\pm$ 3.3) and ES1 (21.8 $\pm$ 3.2) (U[15, 15] = 92.5, $P > 0.05$) (Fig. 6D). From these results, it can be concluded that noise elicited during ES2 altered the attentional capabilities of control rats towards the item much more than it did in lesioned animals. In other words, control rats focused their attention towards the environmental noise that disrupted their exploration behavior, while lesioned animals seemed to ignore noise to a great extent and, consequently, their exploration behavior was similar whether noise was present or absent. In that way, they demonstrated some persevering behavior. An alternative explanation would be that in a noisy environment, the lesioned animals had difficulties in shifting attention, which agrees with the observation of Courchesne et al. in cerebellar and autistic patients.

During ES2, the number of items visited during the 3 min that the noise was elicited was greater than during 3 min of silence, both in control (U[16, 16] = 35, $P < 0.01$) and lesioned animals (U[15, 15] = 48, $P < 0.01$) (Fig. 6E), showing that noise enhanced the activity of the rats whether lesioned or not. However, in controls, the number of items visited during the ES2 7-min silent period was lower than during the ES1 7-min silent period (U[16, 16] = 74, $P < 0.05$), while it was not in lesioned animals (U[15, 15] = 71, $P > 0.05$) (Fig. 6F). This shows that during the internoise interval, the exploration behavior was altered in control but not in lesioned rats, suggesting that noise had post-effects which were greater in control than in lesioned rats.

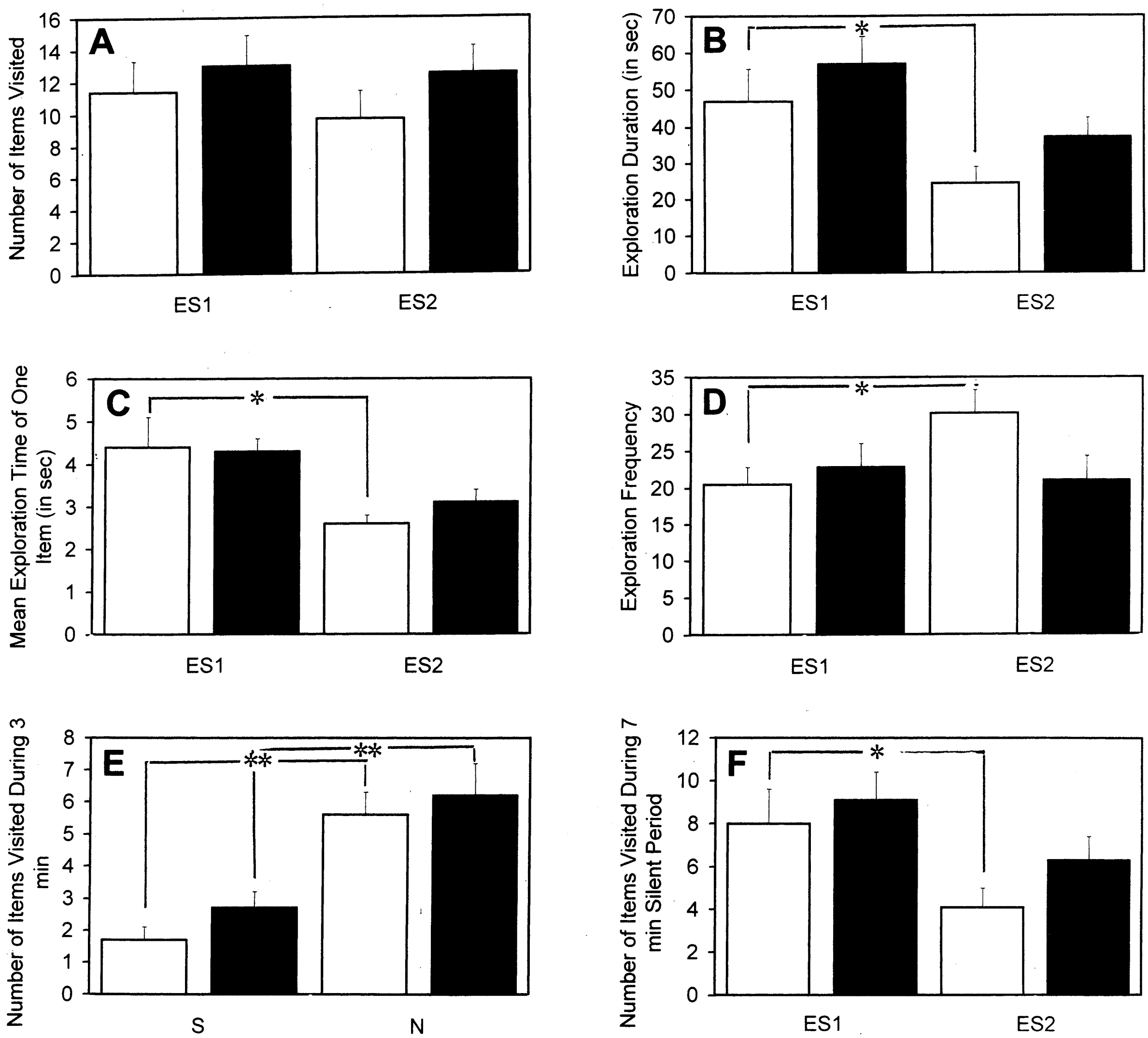

Fig. 6. Attentional capabilities in control (open histograms) and lesioned (full histograms) rats. ES1, First exploratory session (10 min) without noise; ES2, second exploratory session (10 min) with noise for 3 min. In E, S and N mean silent period and noisy period, respectively. *, ** Significant difference at $P<0.05$ and $P<0.01$, respectively.

3.6. Anxiety-like behavior

3.6.1. Elevated +maze

The control rats less often entered the open arms (2.6 ± 0.5) than the closed arms (5.9 ± 0.8) ($W[12]=3.5$, $P<0.001$), while the lesioned rats did not (4.2 ± 1.2 and 6.9 ± 1.2, respectively: $W[11]=16$, $P>0.05$) (Fig. 7A). However, the total number of entries into the open and the closed arms of the elevated + maze was not significantly different in lesioned (11.1 ± 1.9) and control (8.5 ± 1.0) animals. The time spent in the open arms was always shorter than the time spent in the closed arms whatever the animals ($W[16]=5$, $P<0.001$ in control rats; $W[15]=6$, $P<0.001$ in lesioned rats) (Fig. 7B). However, the time spent in the open arms was greater in lesioned rats (88.7 ± 26.4 s) than in control rats (59.4 ± 17.3 s) while, due to the great interindividual variability, the difference was not significant ($U[15, 16]=104$, $P>0.05$). Moreover, the time spent in the distal area (the most anxiogenic one) of the open arms was greater in the lesioned animals (36.5 ± 14.2 s) than in controls (13.7 ± 4.4 s) (Fig. 7B), the difference being significant ($U[15, 16]=45$, $P<0.01$).

3.6.2. Burying behavior

The time elapsing from the moment the animal was placed into the experimental cage and the moment it touched the electrode and received an electric shock was quite similar in control (101.3 ± 32.4 s) and lesioned (108.5 ± 60.7 s) rats ($U[15, 16]=97$, $P>0.05$) (Fig. 8A). Once they have received the electric shock, the percentage of rats moving the sawdust was greater in the lesioned group (73.3%) than in the control group (43.8%), while the difference was not significant

($\chi^2 = 2.78$, $P > 0.05$) (Fig. 8B). However, the percentage of rats burying the electrode was significantly greater in the lesioned group (46.7%) than in the control one (6.3%) ($\chi^2 = 6.61$, $P < 0.05$) (Fig. 8C). The control rats were prostrated in the corner of the cage opposite to the electrode.

3.7. Social discrimination

The number of entries into the unknown compartment was similar in control (3.2 ± 0.7) and lesioned (3.0 ± 0.9) rats. However, the time spent in each of the two compartments by control and lesioned rats was quite different. Indeed, control animals spent in the unknown compartment one-half the time they spent in the known one; on the contrary, lesioned rats spent in the unknown compartment twice the time they spent in the known one (Fig. 9). It can be observed that the lesioned rats were less interested by a known area than the control rats ($U[6, 13] = 9$, $P < 0.01$) and more interested by an unknown area than controls ($U[6, 13] = 7$, $P < 0.01$) (Fig. 9).

4. Discussion

The results of this study obviously show that the behavior of rats with midline lesion of the cerebellum at 10 days of age was different in many aspects from that of control animals. Indeed, their spontaneous motor activity was greater than that of controls. This fully agrees with previous studies demonstrating that the activity level of lurcher mutant mice, whose cerebellar

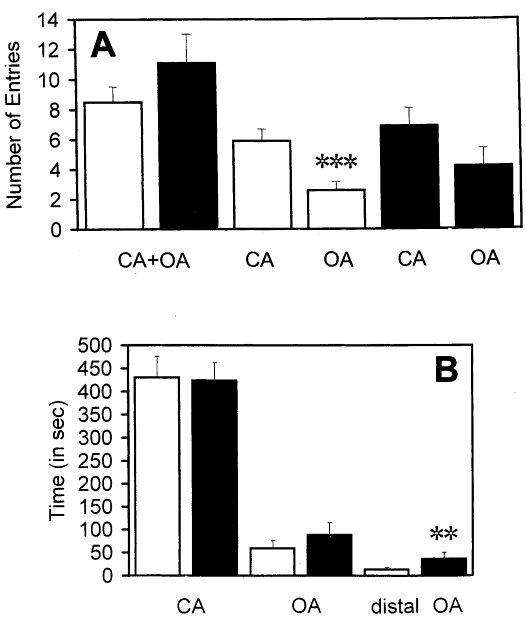

Fig. 7. Elevated + maze test. Mean number of entries ± S.E.M. (A), and mean time ± S.E.M. spent in the closed arms and the open arms of the elevated + maze (B) by control (open histograms) and lesioned (full histograms) rats. CA, Closed arms; OA, open arms. Significant difference at $P < 0.01$ (**) and $P < 0.001$ (***).

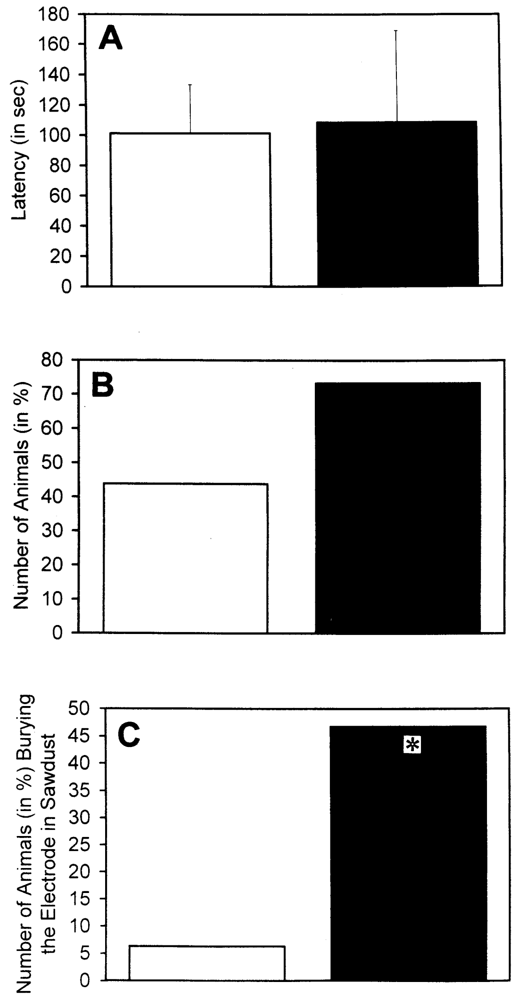

Fig. 8. Burying behavior. (A) Mean (± S.E.M.) latency time (s) needed to touch the electrode and to receive the electric shock; (B) number of animals (in percentage) that moved sawdust; (C) number of animals (in percentage) burying the electrode in sawdust. Open histograms, control rats; full histograms, lesioned rats. * Significant difference at $P < 0.05$.

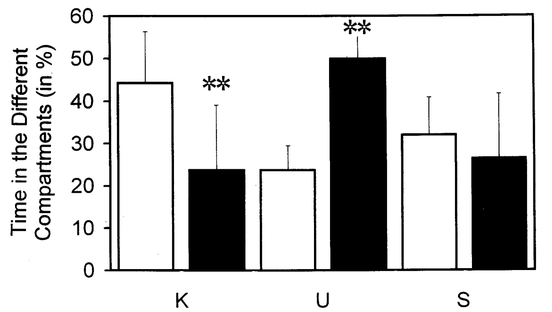

Fig. 9. Social discrimination. Mean (± S.E.M.) time (%) spent by control (open histograms) and lesioned (full histograms) rats in the known (K), the unknown (U) compartments and in the starting box (S). ** Significant difference at $P < 0.01$.

cortex is almost completely degenerated, was greater than that of controls (Caston, unpublished results). This can be explained by a lack of inhibition normally exerted by the cerebellum on motor structures. While the exploration behavior was not altered by early midline lesion of the cerebellum, a result consistent with those obtained in rats cerebellectomized in the adulthood [21] and nervous mutant mice whose cerebellar Purkinje cells and neurons of the deep cerebellar nuclei are degenerated [36], noise elicited during exploration altered the exploration behavior much more in control than in lesioned rats. An environmental perturbation therefore had a greater effect in control than in lesioned animals. This can be explained by the fact that the latter did not pay attention to environmental distractors. Such a result, which has been found in the guinea pig with midline abnormalities [11], suggests that the lesioned rats develop a persevering behavior, as is the case in some cerebellar mutant mice [38–40], which may result from a lack of inhibition. This can alternatively be explained by difficulties of lesioned rats in shifting attention as it has been demonstrated to be the case in cerebellar and autistic patients (Courchesne et al., 1994).

Moreover, ablation of the cerebellar vermis at 10 days of age influenced the emotional behavior of the adult rats and their interest towards social stimuli. In the elevated + maze, lesioned animals entered equally the closed arms and the open arms, while control rats did not. This cannot be explained by a difference in motor activity between lesioned and control animals, since the total number of entries into the open and the closed arms was similar in both groups. Moreover, the lesioned rats spent much more time in the distal part of the open arms (that is the more anxiogenic area of the apparatus) than controls. Given that it is known that the number of entries and the time spent in the open arms are increased by anxiolytic agents and reduced by anxiogenic drugs [59], it can reasonably be assumed that ablation of the vermal lobe of the cerebellum decreased anxiety.

In spite of the appearance, results of the burying behavior can be interpreted in the same way. Indeed, this behavior is decreased by anxiolytics [70,71] and can therefore be considered as essentially anxiogenic in nature. Since the burying frequency was much greater in lesioned rats than in controls, one could conclude that the former were more anxious than the latter. However, we had intentionally chosen to deliver an electric shock of high intensity that inhibits the behavior of control rats. Indeed, control animals were prostrated in the corner of the cage opposite to the electrode for a long time. Only some of them moved the sawdust, this behavior being not directed towards the electrode (except for one rat). On the contrary, the lesioned rats were not prostrated after the electric shock and about one-half of them buried the electrode in sawdust. Given that the behavior of the lesioned animals was more goal-directed than that of controls, it can be thought that their discrimination capabilities were better than those of the latter and that their anxiety or fear was lower than that of controls. An alternative hypothesis would be that the nociceptive sensitivity of the lesioned rats was lower than that of controls and, consequently, the experienced pain was less than in controls.

The results of social discrimination demonstrated that the lesioned rats were much more neophilic than controls since they preferred to visit an unknown compartment than their own one. Such a neophilic behavior can be interpreted in terms of decreased anxiety.

These conclusions agree perfectly with the fact that mutant mice whose cerebellar cortex is almost completely degenerated, such as staggerer [64] and lurcher [8], are much more neophilic and less anxious than nonmutants [31,38]. Similar observations were found in transgenic mice overexpressing bcl-2 in neurons [61], mice which are characterized by supernumerary neurons in the central nervous system, including the cerebellum [76,77]. Therefore, it seems that alterations of the cerebellum are associated with a reduced emotional behavior, as mentioned earlier [30], and more generally with a disinhibition. Such a disinhibition alters the selectivity of the information and, consequently, the behavior of the animals becomes less dependent on the context. Such an independence towards the context is typical of autistic infants, particularly the attention control abnormalities, the abnormally reduced responsiveness to environmental stimuli [6,9,13,14,16–18,24,48,68,69] and the persevering behavior as demonstrated by stereotypes [23,28,63]. Such symptoms are due neither to a loss of weight of the lesioned rats, since their weight was not significantly different from that of controls, nor to a deficit in maternal care, given that the dams took care of the lesioned and control animals similarly. Similar symptoms have been previously reported in guinea pigs with abnormalities of the cerebellar vermis [11], and a number of autistic patients have cerebellar deficits [2,60,74], particularly a hypoplasia of lobules VI and VII [12,15,19,20,29,62]. Our results may strengthen the idea that the cerebellar vermis is involved in autism. Such autistic-like symptoms would not be due to the vermal lesion per se, but to disruption of a circuitry including the cerebellum, the amygdala and the hypothalamus [37].

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References

[1] Auvray N, Caston J, Reber A, Stelz T. Role of the cerebellum in the ontogenesis of the equilibrium behavior in the young rat: a behavioral study. Brain Res 1989;505:291–301.

[2] Bauman ML, Kemper TL. Histoanatomic observations of the brain in early infantile autism. Neurologia 1985;35:866–74.

[3] Bernston GG, Torello MW. The paleocerebellum and the integration of behavioral function. Physiol Behav 1982;10:2–12.

[4] Berthier ME, Moore JW. Cerebellar Purkinje cell activity related to the classically conditioned nictating membrane response. Exp Brain Res 1986;63:341–51.

[5] Bianki VL. Conditioned reflexes to light and sound in fishes deprived of the cerebellum. Zh Vyssh Nervn Deyat Im I P Pavlova 1962;12:962–8.

[6] Bryson SE, Wainwright-Sharp JA, Smith IM. Autism: a developmental spatial neglect syndrome? In: Enns JT, editor. The Development of Attention: Research and Theory. Amsterdam: Elsevier North-Holland, 1990:405–27.

[7] Buchtel HH. Visual learning deficits following cerebellar damage in rats. J Comp Physiol Psychol 1970;72:296–305.

[8] Caddy KWT, Biscoe TJ. Structural and quantitative studies on the normal C3H and lurcher mutant mouse. Philos Trans R Soc Lond 1979;287:167–201.

[9] Casey BJ, Gordon CT, Mannheim GB, Rumsey J. Dysfunctional attention in autistic savants. J Clin Exp Neuropsychol 1993;15:933–46.

[10] Caston J, Jones N, Stelz T. Role of preoperative and postoperative sensorimotor training on restoration of the equilibrium behavior in adult mice following cerebellectomy. Neurobiol Learn Mem 1995;64:195–202.

[11] Caston J, Yon E, Mellier D, Godfrey HP, Delhaye-Bouchaud N, Mariani J. An animal model of autism: behavioral studies in the GS guinea pig. Eur J Neurosci 1998;10:2677–84.

[12] Ciesielski KT, Allen PS, Sinclair BD, Pabst HF, Yanofsky R, Ludwig R. Hypoplasia of cerebellar vermis in autism and childhood leukemia. In: Proceedings of the 5th Internation Child Neurol Congress. New York: Karger, 1990. pp. 650.

[13] Ciesielski KT, Courchesne E, Elmasian R. Effects of focused selective attention tasks on event-related potentials in autistic and normal individuals. Electroencephalogr Clin Neurophysiol 1990;75:207–20.

[14] Courchesne E, Akshoomoff N, Townsend J. Recent advances in autism. Curr Opin Pediatr 1990;2:685–93.

[15] Courchesne E, Hesselink JR, Jernigan TL, Yeung-Courchesne R. Abnormal neuroanatomy in a nonretarded person with autism. Unusual findings with magnetic resonance imaging. Arch Neurol 1987;44:335–41.

[16] Courchesne E, Kilman BA, Galambos R, Lincoln AJ. Autism: processing of novel auditory information assessed by event-related brain potentials. Electroencephalogr Clin Neurophysiol 1984;59:238–48.

[17] Courchesne E, Lincoln AJ, Kilman BA, Galambos R. Event-related brain potential correlates of the processing of novel visual and auditory information in autism. J Autism Dev Disord 1985;15:55–76.

[18] Courchesne E, Townsend J, Akshoomoff N, Saitoh O, Yeung-Courchesne R, Lincoln A, James H, Haas R, Schreibman L, Lau L. Impairment in shifting attention in autism and cerebellar patients. Behav Neurosci 1994;108:848–65.

[19] Courchesne E, Townsend J, Saitoh O. The brain in infantile autism. Neurologia 1994;44:214–28.

[20] Courchesne E, Yeung-Courchesne R, Press GA, Hesselink JR, Jernigan TL. Hypoplasia of cerebellar vermal lobules VI and VII in autism. N Engl J Med 1988;318:1349–54.

[21] Dahhaoui M, Caston J, Lannou J, Avenel S. Role of the cerebellum in habituation exploration behavior in the rat. Physiol Behav 1992;52:339–44.

[22] Dahhaoui M, Lannou J, Stelz T, Caston J, Guastavino JM. Role of the cerebellum in spatial orientation in the rat. Behav Neural Biol 1992;58:180–9.

[23] Damasio AR, Maurer RG. A neurological model of autism. Arch Neurol 1978;35:777–86.

[24] Dawson G, Finley C, Phillips S, Galpert L, Lewy A. Reduced P3 amplitude of the event-related brain potential: its relationship to language ability in autism. J Autism Dev Disord 1988;18:493–504.

[25] Dow RS, Kramer RE, Robertson LT. Disorders of the cerebellum. In: Joynt RF, editor. Clinical Neurology, vol. Vol 3. New York: Lippincott, 1991:1–143.

[26] Frederic F, Chautard T, Brochard R, Chianale C, Wollman E, Oliver C, Delhaye-Bouchaud N, Mariani J. Enhanced endocrine response to novel environment stress and endotoxin in Lurcher mutant mice. Neuroendocrinology 1997;66:341–7.

[27] Ghelarducci B, Salamone D, Simoni A, Sebastiani L. Effects of early cerebellar removal on the classically conditioned bradycardia of adult rabbits. Exp Brain Res 1996;111:417–23.

[28] Golse B. L'Autisme infantile. Prat Méd 1986;42:7–25.

[29] Hashimoto T, Tayama M, Murakawa K, Yoshimoto T, Miyazali M, Harada M, Kuroda Y. Development of brainstem and cerebellum in autistic patients. J Autism Dev Disord 1995;25:1–18.

[30] Heath RG, Dempesy OW, Fontana CJ, Fitzjarrell AT. Feedback loop between cerebellum and septal-hippocampal sites: its role in emotion and epilepsy. Biol Psychol 1980;15:541–6.

[31] Herrup K, Mullen RJ. Regional variation and absence of large neurons in the cerebellum of the staggerer mouse. Brain Res 1979;172:1–12.

[32] Jones N, Stelz T, Batini C, Caston J. Effects of lesion of the inferior olivary complex in learning of the equilibrium behavior in the young rat during ontogenesis. I. Total lesion of the inferior olive by 3-acetylpyridine. Brain Res 1995;697:216–24.

[33] Joyal CC, Meyer C, Jacquart G, Mahler P, Caston J, Lalonde R. Effects of midline and lateral cerebellar lesions on motor coordination and spatial orientation. Brain Res 1996;739:1–11.

[34] Lalonde R. Cerebellar contributions to instrumental learning. Neurosci Biobehav Rev 1994;18:161–70.

[35] Lalonde R, Bensoula AN, Filali M. Rotorod sensorimotor learning in cerebellar mutant mice. Neurosci Res 1995;2:423–6.

[36] Lalonde R, Botez MI. Exploration of a hole-board matrix in nervous mutant mice. Brain Res 1985;343:356–9.

[37] Lalonde R, Botez MI. The cerebellum and learning processes in animals. Brain Res Rev 1990;15:325–32.

[38] Lalonde R, Botez MI, Boivin D. Object exploration in staggerer mutant mice. Physiol Behav 1987;41:115–7.

[39] Lalonde R, Botez MI, Joyal CC, Caumartin M. Motor abnormalities in lurcher mutant mice. Physiol Behav 1992;51:523–5.

[40] Lalonde R, Manseau M, Botez MI. Exploration and habituation in Purkinje cell degeneration mutant mice. Brain Res 1989;479:201–3.

[41] Lavond DG, Hembree T, Thompson RF. Effect of kainic acid lesions of the cerebellar interpositus nucleus on eyelid conditioning in the rabbit. Brain Res 1985;326:179–82.

[42] Leaton RN, Supple WF Jr. Cerebellar vermis: essential for long-term habituation of the acoustic startle response. Science 1986;232:513–5.

[43] Leaton RN, Supple WF Jr. Medial cerebellum and long-term habituation of acoustic startle in rats. Behav Neurosci 1991;105:804–16.

[44] Leiner HC, Leiner AL, Dow RS. Cognitive and language functions of the human cerebellum. Trends Neurosci 1993;16:444–7.

[45] Le Marec N, Dahhaoui M, Stelz T, Bakalian A, Delhaye-Bouchaud N, Caston J, Mariani J. Effect of cerebellar granule cell depletion on spatial learning and memory and in an avoidance conditioning task: studies in postnatally X-irradiated rats. Dev Brain Res 1997;99:20–8.

[46] Le Marec N, Lalonde R. Sensorimotor learning and retention during equilibrium test in Purkinje cell degeneration mutant mice. Brain Res 1997;768:310–6.

[47] Lev-Ram V, Valsamis M, Masliah E, Levine S, Godfrey HP. A novel non-ataxic guinea pig strain with cerebrocortical and cerebellar abnormalities. Brain Res 1993;606:325–31.

[48] Lincoln AJ, Courchesne E, Harms L, Allen M. Contextual probability evaluation in autistic, receptive developmental language disorder and control children: ERP evidence. J Autism Dev Disord 1993;23:37–58.

[49] Lisander B, Martner J. Integrated somatomotor, cardiovascular and gastrointestinal adjustments induced from cerebellar fastigial nucleus. Acta Physiol Scand 1975;94:358–67.

[50] Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 1987;92:180–5.

[51] Malyukova IV. Influence of the neocerebellum extirpation on situational conditioned reflexes in dogs. Zh Vyssh Nervn Deyat Im I P Pavlova 1963;13:1052–8.

[52] Martinou JC, Dubois-Dauphin M, Staple JK, Rodrigez I, Frankowski H, Missotten M, Albertini P, Tabalot D, Catsicas S, Pietra C, Huarte J. Overexpression of BCL-2 in transgenic mice protects neurons from naturally occuring cell death and experimental ischemia. Neuron 1994;13:1017–30.

[53] Martner J. Cerebellar influences on autonomic mechanisms: an experimental study in the cat with special reference to the fastigial nucleus. Acta Physiol Scand Suppl 1975;425:1–42.

[54] McCormick DA, Thompson RF. Cerebellum: essential involvement in the classically conditioned eyelid response. Science 1984;223:296–9.

[55] Middleton A, Strick PL. Anatomical evidence for cerebellar and basal ganglia involvement in higher cognitive function. Science 1994;266:458–61.

[56] Molinari M, Grammaldo LG, Petrosini L. Cerebellar contribution to spatial event processing: right/left discrimination abilities in rats. Eur J Neurosci 1997;9:1986–92.

[57] Monfort V, Chapillon P, Mellier D, Lalonde R, Caston J. Timed active avoidance learning in lurcher mutant mice. Behav Brain Res 1998;91:165–72.

[58] Moruzzi G. Paleocerebellar inhibition of vasomotor and respiratory carotid sinus reflexes. J Neurophysiol 1940;3:20–32.

[59] Pellow S, Chopin P, File SE, Bridey M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.

[60] Ritvo ER, Freeman BJ, Scheibel AB, Duong T, Robinson H, Guthrie D, Ritvo A. Lower Purkinje cell counts in the cerebella of four autistic subjects: initial findings of the UCLA-NSAC Autiopsy Research Report. Am J Psychiatry 1986;143:862–6.

[61] Rondi-Reig L, Lemaigre-Dubreuil Y, Martinou JC, Delhaye-Bouchaud N, Caston J, Mariani J. Fear decrease in transgenic mice overexpressing bcl-2 in neurons. Neuroreport 1997;8:2429–32.

[62] Saitoh O, Courchesne E, Egaas B, Lincoln AJ, Schreibman L. Cross-sectional area of the posterior hippocampus in autistic patients with cerebellar and corpus callosum abnormalities. Neurologia 1995;45:317–24.

[63] Sauvage D, Hameury L, Darves-Bomoz JM. Nosographie de l'autisme. Discussion critique des classifications actuelles. Presse Méd 1993;22:1449–54.

[64] Sax DS, Hirano A, Shofer RJ. Staggerer, a neurological murine mutant. Neurologia 1968;18:1093–100.

[65] Schneiderman-Fish B, Baisden RH, Woodruff ML. Cerebellar nuclear lesions in rats: subsequent avoidance behavior and ascending anatomical connections. Brain Res 1979;166:27–38.

[66] Sears LL, Finn PR, Steinmetz JE. Abnormal classical eyeblink conditioning in autism. J Autism Dev Disord 1994;24:737–51.

[67] Tong G, Robertson LT, Brons J. Climbing fiber representation of the renal afferent nerve in the vermal cortex of the cat cerebellum. Brain Res 1993;607:65–75.

[68] Townsend J, Courchesne E. Parietal damage and narrow 'spotlight' spatial attention. J Cogn Neurosci 1994;6:220–32.

[69] Townsend JH, Courchesne E, Egaas B. Slowed orienting of covert visual–spatial attention in autism: specific deficits associated with cerebellar and parietal abnormalities. Dev Psychopathol 1996;8:563–84.

[70] Treit D. A comparison of anxiolytic and nonanxiolytic agents in the shock-probe/burying test for anxiolytics. Pharmacol Biochem Behav 1990;36:203–5.

[71] Treit D, Pinel JPJ, Fibiger HC. Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. Pharmacol Biochem Behav 1981;15:619–26.

[72] Treit D, Pinel JPJ, Terlecki LJ. Shock intensity and conditioned defensive burying in rats. Bull Psychon Soc 1980;16:5–7.

[73] Watson PJ. Non motor functions of the cerebellum. Psychol Bull 1978;85:944–67.

[74] Williams RS, Hauser SL, Purpura DP, Delong R, Swisher CN. Autism and mental retardation: neuropathological studies performed in four retarded persons with autistic behavior. Arch Neurol 1980;37:749–53.

[75] Yeo CH, Hardiman MS, Glickstein M. Discrete lesions of the cerebellar cortex abolish the classical conditioned nictating membrane response in the rabbit. Behav Brain Res 1984;13:261–6.

[76] Zanjani HS, Vogel MW, Delhaye-Bouchaud N, Martinou JC, Mariani J. Increased inferior olivary neuron and cerebellar granule cell numbers in transgenic mice overexpressing the human bcl-2 gene. J Neurobiol 1994;32:502–16.

[77] Zanjani HS, Vogel MW, Delhaye-Bouchaud N, Martinou JC, Mariani J. Increased cerebellar Purkinje cell numbers in mice overexpressing a human bcl-2 transgene. J Comp Neurol 1996;374:332–41.