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# Hemicerebellectomy and motor behaviour in rats. III. Kinematics of recovered spontaneous locomotion after lesions at different developmental stages

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The locomotion of rats with a right hemicerebellectomy (HCb) performed in adulthood was compared by means of kinematic analysis with the locomotion of rats with a similar lesion performed on the first postnatal day. The age at which the animals received cerebellar lesion made a significant difference with respect to the locomotor strategies utilized in adulthood. During stance, neonatal operated rats showed a clear hyperextension of both hindlimbs but not of the forelimbs. Their locomotor posture was characterized by spinal flexion with the head held lower than normal. During swing, they showed a tendency towards 'high stepping'. Their steps were regular and symmetrical but hypometric. Adult lesioned animals displayed a marked extensor hypotonia, ipsilateral to the lesion during stance and a relevant hyperflexion affecting both sides, during swing. Alteration of the interlimb coordination and modified sequence of steps were also observed. Thus, a highly asymmetrical, impaired and unstable locomotion was displayed by this group of animals. The present findings demonstrate the importance of the age-at-lesion factor in determining the motor strategies in the recovery of locomotor function after HCb in the rat. This evidence is discussed in the light of the widespread anatomical remodelling already demonstrated following neonatal, but not adult, HCb in rats.

## INTRODUCTION

The model of hemicerebellectomy (HCb) performed at different developmental stages has proved a fruitful way to study the relationship between recovery of motor function and developmental brain plasticity<sup>12,25,28</sup>. The age at which animals receive cerebellar lesions makes a significant difference in the behavioural outcome as adults. Rats with neonatal cerebellar lesions exhibit a slight extensor hypotonia contralateral to the lesion side, a normal-appearing locomotion and complex postural adjustments. Conversely, adult lesioned rats show a severe extensor hypotonia ipsilateral to the lesion, a wide-based, ataxic locomotion and severely disrupted coordination<sup>25</sup>.

Many reports indicate that early lesioning of cerebellar circuitry leads to a widespread remodelling of the

spared connections<sup>5,22</sup>. Aberrant ipsilateral cerebello-rubral<sup>26,31</sup> and cerebello-thalamic<sup>23</sup> projections develop that maintain the topographic specificity of the normal contralateral projection. An anomalous increase in crossed sensorimotor cortico-pontine<sup>6</sup> and diencephalo-olivary<sup>33</sup> projections has been also described. Furthermore, the absence of a hemicerebellum induces an increase of the ipsilateral spinal projection to Deiters' nucleus<sup>7</sup>. On the other hand, after adult HCb the remodelling of synapses is limited to motor cortex<sup>19</sup> and red nucleus<sup>34</sup> contralateral to the lesion. Therefore, in the proposed model the highest extent of functional recovery is exhibited by those animals that present the highest degree of neuronal remodelling.

Which function is mediated by these remodelled circuitries? In other words, can the remodelled circuitries sustain the reinstatement of the disrupted function or do they determine the development of new motor strategies, that although abnormal, might be behaviourally adaptive? The present study aimed to analyze the ki-

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nematic characteristics of spontaneous locomotion in rats HCbed at birth or in adulthood.

Among the different motor behaviours, the study of locomotion is particularly useful to assess the influences of the newly formed cerebellar circuitries. In fact, although based on a spinal generator, locomotion is heavily controlled by supraspinal centres directly influenced by the cerebellum<sup>14</sup>. Furthermore, this performance appears to be an 'ensemble repertoire' in which central and peripheral neural components aggregate and integrate themselves, creating a behavioural complex highly modifiable both by learning processes and by neurological events. So, spontaneous walking encompasses the neuronal 'history' of the animal with all degrees of variability, from the most minute to the most disrupting.

To study the influences of the supraspinal systems on locomotion, kinematic analyses are particularly effective because mechanical and electrical muscle activity is translated into coordinated movements such that limb trajectories represent the motor strategies applied. Therefore, the precise characterization of locomotion in the two experimental groups allows us to define the strategies employed, determining if rats HCbed neonatally or as adults use normal patterns in the recovery of spontaneous locomotion or if novel locomotor patterns are used. These results will further elucidate the relationship between developmental brain plasticity and recovery of function. In fact, the animals of the two experimental groups, though identically lesioned, have an organization of the spared cerebellar circuitries profoundly different such that the influences of the remodelled projections on the recovered function can be inferred.

## MATERIALS AND METHODS

In this study 34 rats were used: out of these, 12 were operated 24 h after birth (neonatal operate group), 11 were operated at the 90th postnatal day (adult operate group), and 11 were intact adult animals, used as controls. The lesioned rats were the same animals whose motor development and general motor performances have been described previously<sup>25,28</sup>. Surgery was done under deep anaesthesia by cooling over ice in pups and by injection of Nembutal (40 mg/kg, i.p.) in adults. After removal of the right half of the occipital bone, the cerebellum was bisected midsagittally. The right side of the cerebellum was then aspirated through a small glass pipette. After recovery from the anaesthesia, pups were returned to their mothers until weaning; thereafter, animals were housed alone or two per cage, as were all adult operates. Although behavioural testing had been

given regularly throughout the postoperative period, the measurements reported here were taken entirely in the fourth postoperative month, when no changes were seen in symptomatology, indicating a stable neurological and behavioural state.

Besides the locomotor studies, we also tested each animal for posture, muscle tone, 'vestibular drop', supporting and righting reactions, and complex postural adjustments such as those required during locomotion on an elevated horizontal bar or during rearing. These observations allowed us both a more thorough analysis of motor behaviour and a 'clinical' control of the accuracy of the lesion placement.

### *Kinematic analysis*

Prior to cinematography, the rat's limbs were shaved and the lengths of the limb bones measured. The position of the following joints was inked: hip, knee, ankle and toe (hindlimbs), and shoulder, 'elbow', 'wrist' and toe (forelimbs). Walking patterns and joint angle trajectories were investigated during spontaneous over-ground locomotion. Animals were encouraged to walk straight by cookie rewards. According to the needs, the animal walked in front of a wall on which marking lines were plotted every two cm, or in front of a mirror, which gave a lateral view of the limbs not exposed to the direct view. The lines behind the rats were used for reference to measure optical deviations as well as the step lengths of each limb and then walking speed of the rat. Mirror images were used for quadrupedal analyses. Locomotor performances of the rats were recorded on tape made with a TELM-SCH video camera at 25 frames/s and their kinematics were examined by single frame analysis of the tapes. The angular trajectories of the joints were detected from the position of the landmarks over the joints. A source of error in joint angle analysis derives from the slippage of the skin over pivot points during stepping. For the hindlimb, the skin remains closely related to the underlying joints in all places indicated, except at the knee. Here, considerable slippage of the skin occurs during stepping and the ink mark cannot be used as a reference for determining the absolute excursion of the knee. According to previous reports<sup>36</sup>, a more precise definition of the knee angle can be obtained through triangulation from the known positions of the hip and ankle joints and the measured lengths of the femur and tibia. The application of this technique reduces errors to limits acceptable for our protocol. A similar approach was applied to elbow angle calculation. Joint angles were calculated in each frame and then plotted in time-series. Stick figure representations of limb position for each portion of the step cycle were also drawn.

### Interlimb coordination

To study step cycles, paw prints during walking were obtained while the rat walked on a sheet of white paper (the surface a bit rough and not slippery) after the right forepaw was soaked with green ink, the right hindpaw with red ink, the left forepaw with black ink, and the left hindpaw with blue ink.

We plotted gait diagrams describing the relative timing of swing and stance phases for all four limbs. To define the phases of step cycle, we mainly followed the accurate description by Gruner and coworkers<sup>15</sup>. Therefore, we divided the step cycle into four phases, two during stance, defined as E2 and E3, and two during swing, defined as F and E1. Starting from foot contact, E2 lasts until the minimum knee angle is reached. The E3 phase lasts from that point to lift off. From lift off to minimum knee angle during swing is defined as the F phase, and the E1 phase lasts from this point until foot placement. Foot liftoff was chosen to divide stance and swing phases. The only difference between our phase division and that by Gruner is the event chosen to divide stance and swing phases. Because hip angles were not measured in our study, we followed the traditional Phillipson's schema<sup>29</sup> that indicates foot lift off as the event separating stance and swing phases. As for the forelimbs, since no event clearly distinguished E2 from E3, the stance phase of the forelimb was considered as a whole.

Strides in which blurring of the image occurred during the most rapid phase of flexion were discarded. The duration of the total stride, stance and swing durations for all four limbs, and intervals between successive limb touchdowns were calculated. Other measures included vertical and horizontal displacements of the limbs and the number of continuous alternating step cycles.

### Limb angular displacements during the step cycle

The caudal-most position of the limb during the step cycle was measured as angle  $\beta$ ; it was calculated as the angle between the limbline from the greater trochanter of the femur (or head of humerus for the forelimb) to the end of the metatarsophalangeal (or metacarpophalangeal) joint and the vertical axis passing through the greater trochanter (or head of humerus). The active excursion of the limb during swing phase was measured as the total angular displacement (angle  $\phi$ ) from the limb's most posterior extension (end of E3 phase) to its most forward position (at the end of E1 phase). The forward displacement of the limb (angle  $\alpha$ ) was calculated by subtracting angle  $\beta$  from angle  $\phi$ .

### Statistical analysis

Metric unit results of neonatal and adult operate animals as well as of controls were first tested for ho-

moscedasticity of variance and then compared using ' $p \times q$ ' or ' $p \times q \times z$ ' analyses of variance (ANOVAs). Since the cell frequency was unequal, Winer's<sup>38</sup> model of ANOVA with unequal cell frequency was used. When significant differences were found with the overall analyses, post-hoc comparisons among groups were assessed with Duncan's multiple range or Tukey's tests.

### Histological controls

After completion of behavioural testing, animals were deeply anaesthetized with Nembutal and perfused with saline followed by 10% buffered formalin. The precise description of the lesions has been reported elsewhere<sup>25,28</sup>. Although there was some variation in the extent of cerebellar ablation among animals, all hemicerebellar lesions involved the right cerebellar cortex, the white matter, and the cerebellar nuclei of the right side (Fig. 1). In all cases reported here, the left side of the cerebellum and extracerebellar structures were completely spared, except for the dorsal cap of the right Deiters' nucleus which in some cases was slightly affected. The extent of right fastigial lesion, in some cases

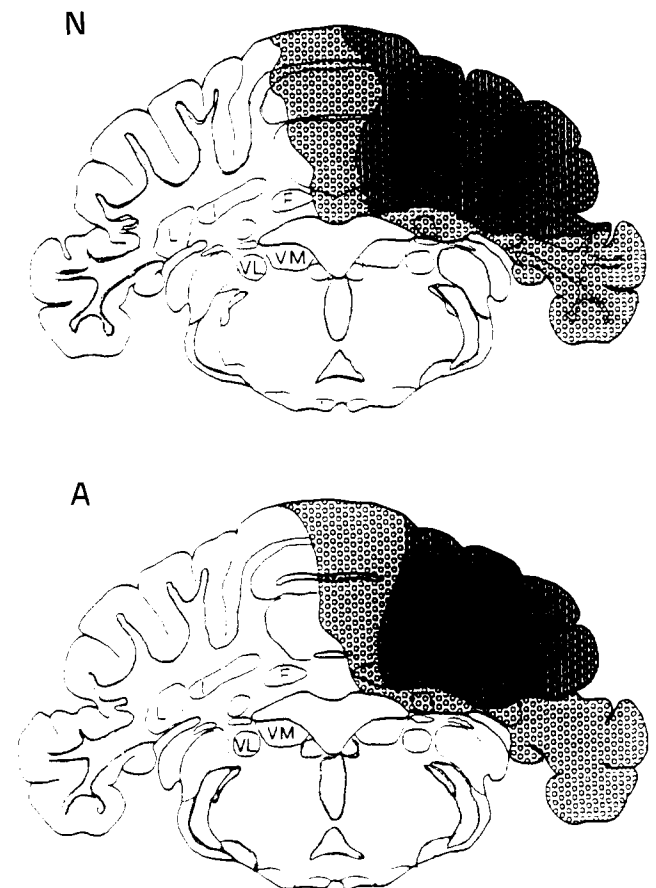


Fig. 1. Reconstructions of minimal (stripes) and maximal (empty circles) lesion damage in neonatal (N) and adult (A) HCbed groups. L, lateral cerebellar nucleus; I, interpositus nucleus; F, fastigial nucleus; VL, lateral vestibular nucleus; VM, medial vestibular nucleus.

not complete, was always greater than 60% of nucleus size. The variability in the extent of the floccular and vermal lesion was considered non-influencing because in all cases these structures were functionally disconnected because of the ablation of the cerebellar peduncles and deep nuclei of the right side.

RESULTS

The gait used by the unlesioned rats was the lateral sequence gait, namely the diagonal couplet or the single foot forms<sup>8</sup>. According to these forms the sequence of the lift off of the limbs is: left forelimb (LF), right hindlimb (RH), right forelimb (RF), left hindlimb (LH). In the usual gait of an unlesioned rat, the body is supported in sequence by different numbers of feet in combination. Thus, a walking rat commonly has three feet on the ground for an instant followed by two and then again three, then two, and so on (formula 22 introduced by Hildebrand<sup>16</sup>). Seldom normal rats display forms of gait which alternate support by two, three and four feet (formula 3 by Hildebrand<sup>16</sup>).

Qualitative description of locomotor behaviour

Neonatal operate animals

Except during the most challenging tasks, the neonatally HCbed animals appeared almost normal when observed as adults. During standing, a slight extensor hypotonia was observed in the left limbs, contralateral to the lesion. During stepping, very little ataxia was seen. The animals tended to hyperextend both hindlimbs so that the spine was flexed and the head was generally lower to the ground than normal. The characteristic cerebellar 'high stepping' or hyperflexion during swing was also displayed. An unduly hard placement of the feet on the ground provoked a clearly audible locomotion. We never observed rats dragging a hindpaw. The gait of these animals was usually smooth in appearance and suitable to achieve high speeds. The neonatally HCbed rats were able to walk and run without any directional bias and to rapidly turn. When placed on an uneven surface, they could avoid small obstacles and perform simple motor tasks. During grooming, they were able to rear on the hindlimbs and maintain an upright posture. Nevertheless, as it has

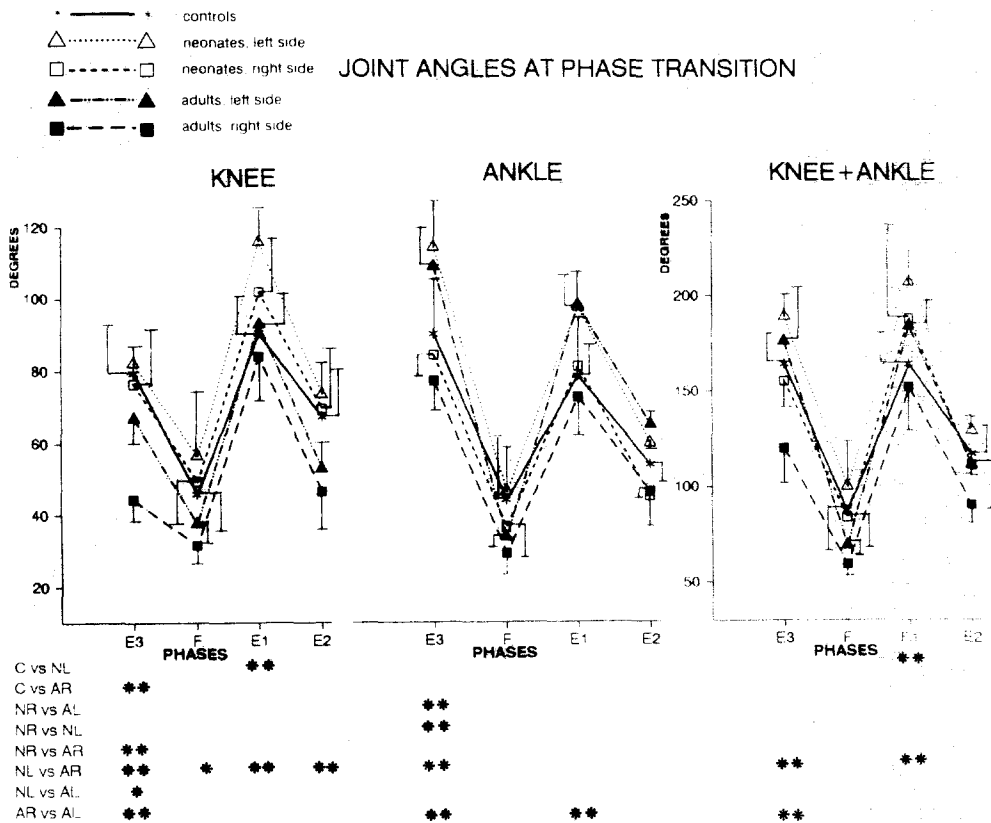


Fig. 2. Mean joint angles at phase transition in the hindlimbs for single joints (knee, ankle) and for the sum of these two joints in the three experimental groups. E3, F, E1, E2: transition points between subsequent step phases valuated at the last frame of each phase indicated. For definition of step phases see text. Post-hoc comparisons between groups are made using Tukey's test (\* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ). C, controls; NL, neonatal operates — left side; NR, neonatal operates — right side; AL, adult operates — left side; AR, adult operates — right side. Vertical bars indicate standard deviations.

been reported elsewhere<sup>25,28</sup> demanding tasks, such as crossing narrow paths, betrayed signs of motor deficiency: the animals often did not succeed in stepping on the bridge, often slipping and occasionally falling.

#### Adult operate animals

Adult HCbed animals displayed many abnormal movements and postures. One of the most prominent deficits was a marked extensor hypotonia ipsilateral to the lesion resulting in an asymmetrically crouched posture with a body tilt to the lesion side. The gait of these animals was markedly ataxic, characterized by oscillatory movements, staggering, a wide-based support, and occasional falls. The adult operate animals made exaggerated extension movements during the support phase and hyperflexion movements during swing phase. Even slight spontaneous shifts of position evoked a series of oscillations, with a rhythmic bobbing of head and body. In motion these animals tended to veer to the lesion side and they often lost their balance. To compensate, the rats leaned against any supporting surface. To change direction, the right hindlimb was used as a pivot, remaining flexed under body weight. These deficits induced a locomotion stunted and very often interrupted by brief periods of stopping so that it was difficult to observe more than 6–7 consecutive steps. Rearing was rarely observed. The adult operate animals met with difficulties in crossing narrow paths. They fell from the bridge or clung on it upside down in a four-legged support.

#### Quantitative description

##### Neonatal operate animals

For the neonatal operates, quantitative analysis of joint kinematics has been performed by calculating the

mean values of knee and ankle joint angles at phase transition (Fig. 2). The overall pattern displayed by both joints exhibited a tendency towards the hyperextension when compared to controls, more evident in E3 and E1 phases. It is worth noting that greatest differences with respect to controls were exhibited by joint angles of left hindlimbs. Highly significant differences among groups were revealed by  $3 \times 2 \times 4$  (treatment  $\times$  side  $\times$  phase) ANOVAs (Table I).

Figs. 3 and 4 exemplify the time course of joint angles of fore- and hindlimbs during stepping in representative animals.

By calculating the degree of range of motion from the end of E3 phase to the end of F phase as the difference in the height of the toe at the apogee of the trajectory measured as a percentage of the overall distance from the ground to the hip at the hip's highest point during the cycle, both hindlimbs displayed a tendency toward 'high stepping' during the swing phase (Fig. 5). In fact, the mean values resulted of  $26\% \pm 4$  in control animals, while in neonatal HCbed animals were of  $43\% \pm 9$  and  $36\% \pm 8$  for the left and right hindlimbs respectively.

During stance, there was little step-to-step variability in the joint angle values across or within animals. The tendency towards hyperextension in both hindlimbs was further confirmed by comparing the mean values of the sums of the three joint angles of a hindlimb during the stance phase for a single step in the neonatal HCbed animals vs. the control rats. A highly significant hyperextension was evidenced in the right ( $F_{1,21} = 37.58$ ;  $P = 0.00003$ ) as well as in the left hindlimb ( $F_{1,21} = 28.42$ ;  $P = 0.0001$ ) of neonatal HCbed rats. As for forelimbs, the tendency towards hyperextension displayed by the wrist joints was counterbalanced by hyperflexion of the elbow joints (Fig. 3), so that the mean

TABLE I

#### Joint angles at phase transition

Statistical comparisons among groups by ' $p \times q \times z$ ' ANOVAs.

	Knee			Ankle			Sum		
	FD	F	P	FD	F	P	FD	F	P
Treatment	2.30	16.47	0.00001	2.28		n.s.	2.29	9.41	0.0007
Side	1.30	5.8	0.022	1.28	41.6	0.00001	1.29	19.91	0.0001
Phase	3.90	180.7	0.00000	3.84	185.9	0.00000	3.87	188.12	0.00000
$p \times z$	6.90	3.2	0.006	6.84	2.24	0.046			
$p \times q \times z$				6.84	2.86	0.013			
Tukey C vs. N			n.s.			n.s.			n.s.
Tukey C vs. A			0.001			n.s.			n.s.
Tukey A vs. N			0.0001			n.s.			0.0005
Tukey R vs. L			0.0001			0.0001			0.0002

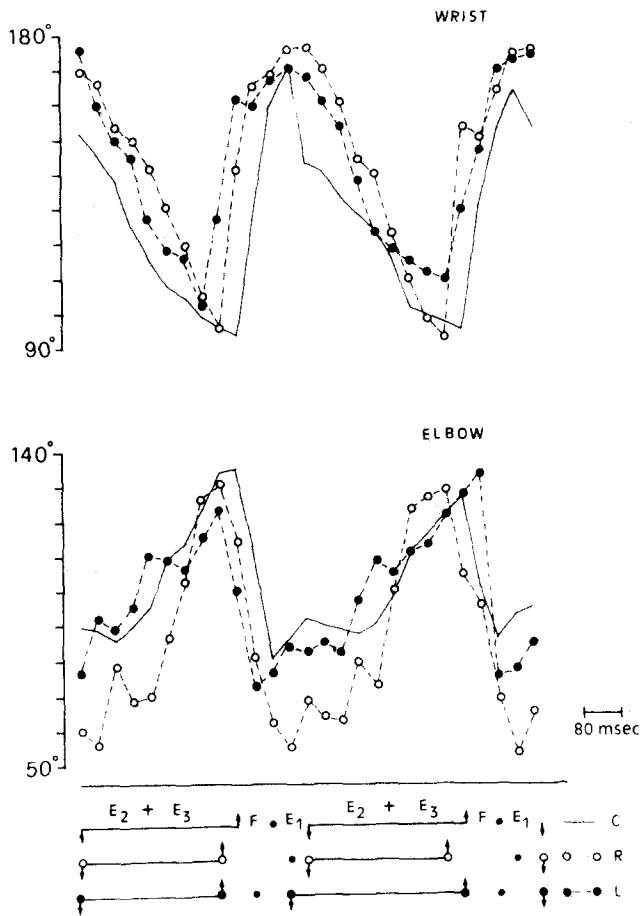


Fig. 3. Forelimb joint angles of a selected neonatal HCbed rat during step cycles. F, E1, E2, E3: step cycle phases. C, control values; R, right side; L, left side.

values of the sum of joint angles of the forelimbs were not significantly different from control ones.

The overall features of joint angles of hind- and forelimbs during stepping can account for the typical posture of the neonatal HCbed rats characterized by spinal flexion with the animal's head held lower than normal. Stick diagrams of the limb positions during stepping (Figs. 6, 7) indicate that the successive positions were acquired rather smoothly.

In the neonatal operates, a pawprint analysis demonstrated the presence of steps of reduced length. As clarified by limb angular displacement analysis, these hypometric steps were due to the reduction of both angle  $\beta$  (caudal-most limb position) and angle  $\phi$  (total excursion of the step) (Fig. 8). A  $3 \times 2$  (group  $\times$  side) ANOVA revealed a significant effect on hindlimb angular displacements of treatment ( $F_{2,62} = 139.23$ ;  $P < 0.001$ ) and side ( $F_{1,62} = 87.54$ ;  $P < 0.001$ ). The interaction was also significant ( $F_{2,62} = 42.04$ ;  $P < 0.001$ ). Post-hoc comparisons made by Duncan's test are described in Fig. 8. No significant differences were found when comparing forelimb angular displacements.

The analysis of the interlimb coordination provides further details on the locomotion of neonatal operate rats. During walking at normal speed, the neonatal operate rats lifted the LF, with a swing phase of normal duration, which started in a position slightly advanced from normal, as demonstrated by the reduction of the  $\beta$  angle. The limb was then put forward and wrist extension created an increased  $\alpha$  angle. The limb was then loaded, and after about 0–40 ms of quadrupedal support, the RH was lifted starting from a position characterized by a reduced  $\beta$  angle. The duration of the swing phase of this limb was normal or increased, although the total excursion ( $\phi$  angle) was always reduced, indicating a slow movement. The limb was then loaded, maintaining a knee angle more extended than

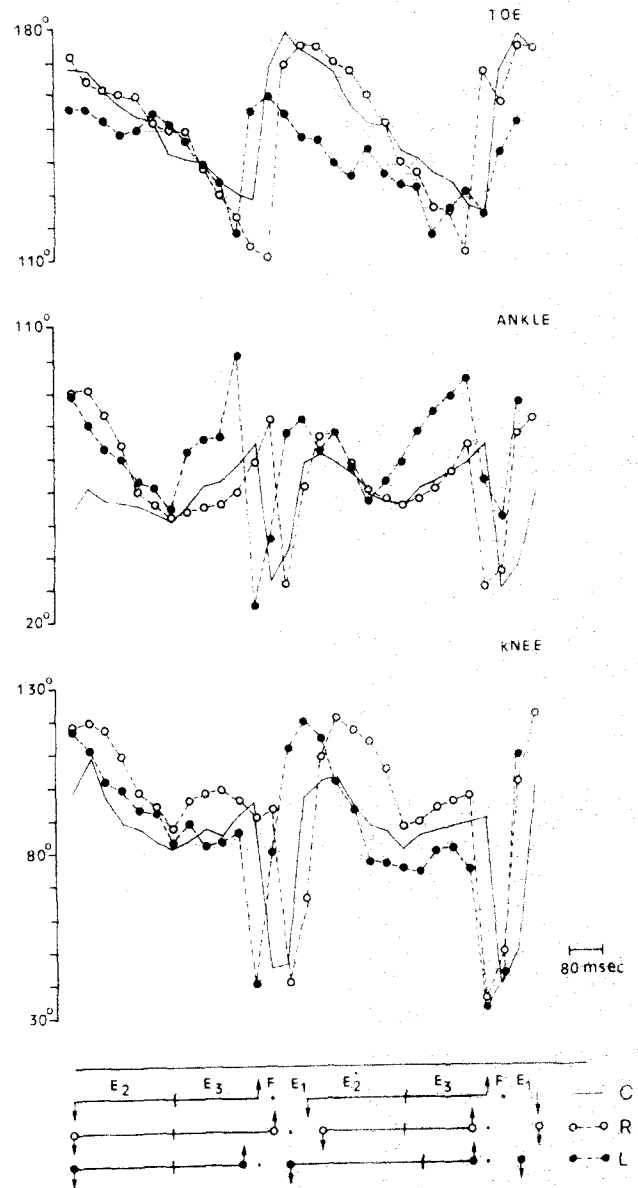


Fig. 4. Hindlimb joint angles of a selected neonatal HCbed rat during step cycles. Abbreviations as in Fig. 3.

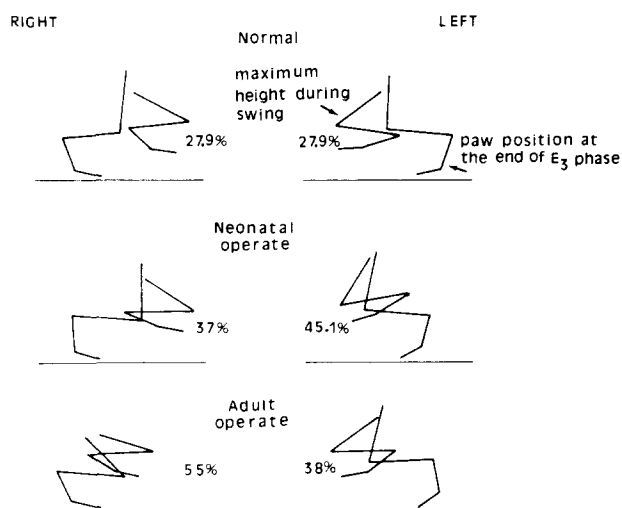


Fig. 5. Percentage of hindlimb flexion during swing phase in HCbed rats. Representative specimens of the three experimental groups are depicted.

normal. With a delay of about 0–40 ms, the RF was lifted off with a swing phase characterized by a reduced  $\beta$  angle and an increased  $\alpha$  angle and by an extended

#### KINEMATIC ANALYSIS OF STEP CYCLE IN HEMICEREBELLECTOMIZED RATS

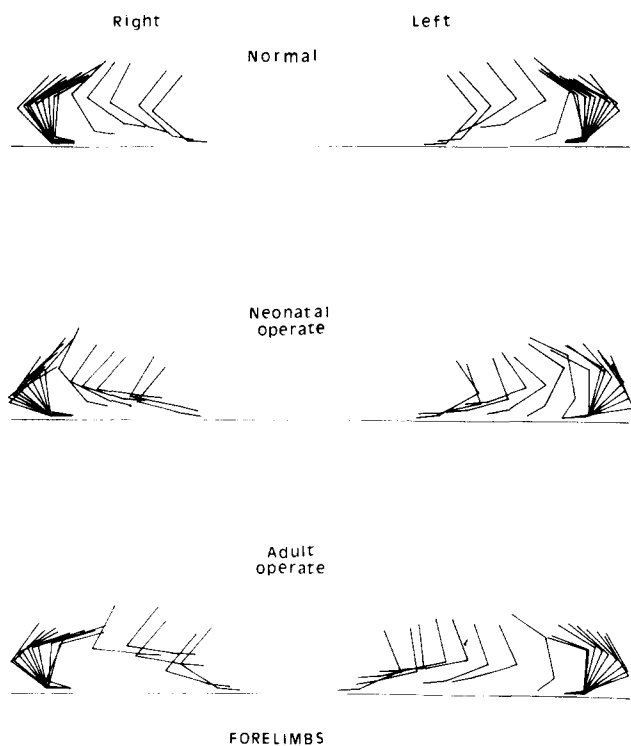


Fig. 6. Stick diagrams of the temporal sequence of forelimb positions during exemplar stepping in a normal animal and in selected neonatal and adult HCbed rats.

#### KINEMATIC ANALYSIS OF THE STEP CYCLE IN HEMICEREBELLECTOMIZED RATS

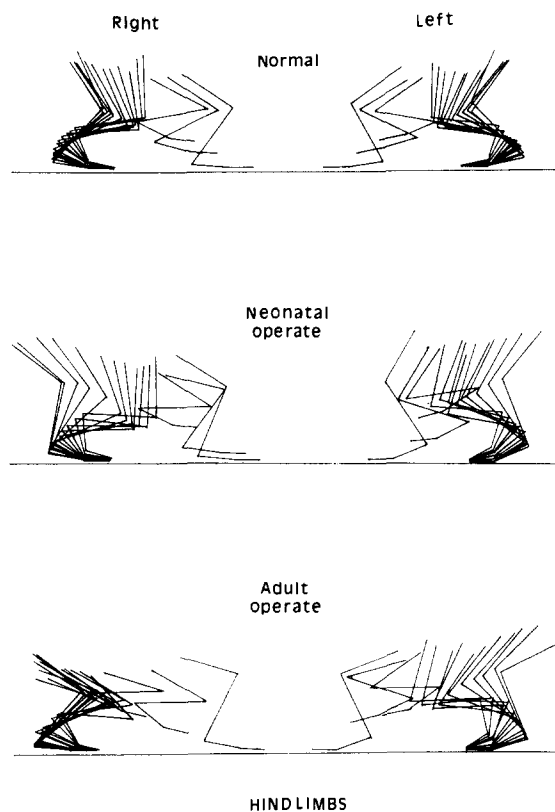


Fig. 7. Stick diagrams of the temporal sequence of hindlimb positions during exemplar stepping in a normal animal and in selected neonatal and adult HCbed rats.

wrist angle. After an inconstant period of quadrupedal support lasting 0–160 ms, the LH was lifted off to accomplish a hypometric step, as demonstrated by its  $\beta$  and  $\phi$  angles smaller than normal. At the end of swing phase of the LH, after 0–40 ms of quadrupedal support the LF was lifted off. And the step cycle started again.

In summary, the neonatal operates exhibited a gait in which two sequences of foot placement (number 1 and 20 by Hildebrand<sup>16</sup>) are mixed (Fig. 9). In fact, the foot formula based on alternating quadrupedal and tripodal support (number 1 by Hildebrand<sup>16</sup>) was observed only occasionally, because of the frequent skipping of the quadrupedal support period, more often before forelimb lifting off. Therefore, a footfall sequence based only on tripodal support (formula 20 by Hildebrand<sup>16</sup>), that requires more precise postural adjustments, was frequently observed.

These animals were also able to increase the speed of their gait either by eliminating the periods of quadrupedal support or by reducing the duration of stance phases. The gait used in this situation was again a mixture of two sequences of foot placement, in which a tripodal support was mixed with a bipedal support on

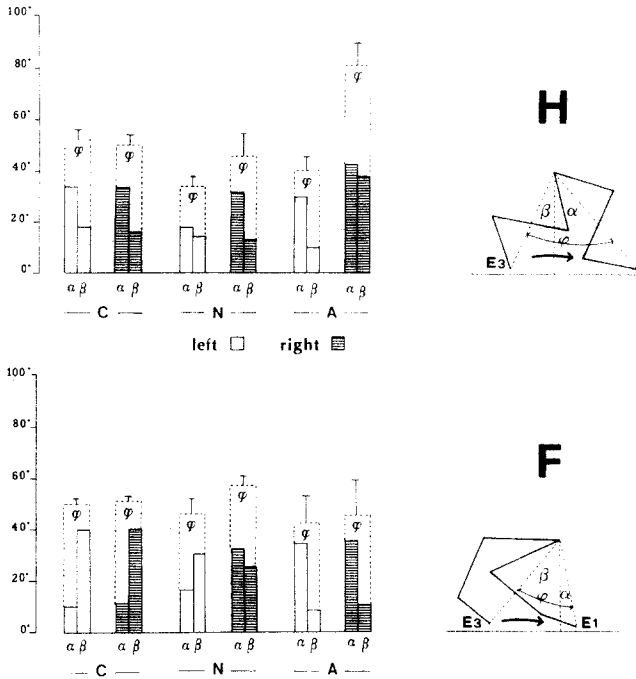


Fig. 8. Angular displacements of hind- (H) and forelimbs (F) during locomotion in controls (C), neonatal (N) and adult (A) HCbed rats. The most extended backward position (end of E3 phase) immediately before paw lift-off and the most extended forward position (end of E1 phase) immediately after paw contact are indicated.  $\beta$ , posterior angular displacement;  $\alpha$ , anterior angular displacement;  $\phi$ , ( $\beta + \alpha$ ), total angular displacement. In neonatal and adult operate groups  $\phi$  angles of left and right hindlimbs significantly differ from controls. No significant difference was found in forelimbs.

opposite sides (formula 22 by Hildebrand<sup>16</sup>), although this latter form of support, being rather unstable, was often skipped.

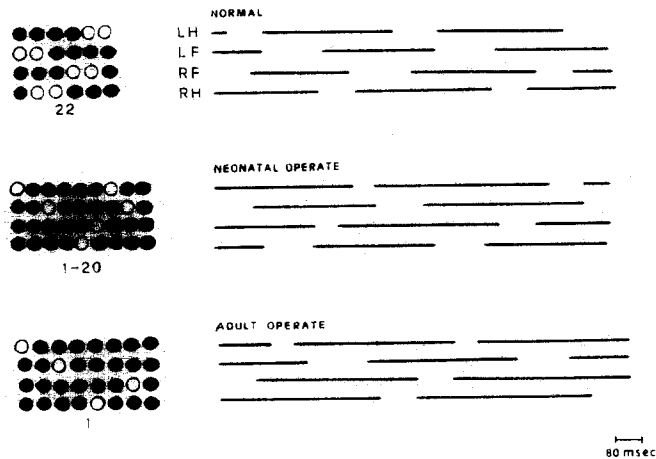


Fig. 9. Footfall diagram of typical step cycles for normal, neonatal operate and adult operate rats. Digits on the left side indicate the walking footfall formulas according to Hildebrand<sup>16</sup>. The initials L, R, H and F stand for the left, right, hind and fore feet. Dark circles indicate feet supporting weight; open circles, unweighted feet.

Adult operate animals

Quantitative evaluation of hindlimb joint kinematics at phase transition demonstrates a tendency towards the hyperflexion, particularly evident on the right side (Fig. 2, Table I).

The time course of joint excursions during stepping is described in Figs. 10 and 11. In these diagrams, the angular displacements in left and right limb joints in a representative animal during step cycle are shown.

In the stance phase, an excessive flexion of the proximal joints of the right limbs (responsible for the body tilt to the lesion) was accompanied by a tendency to the extension of ankle and wrist angles and to the flexion of knee and elbow angles of the left limbs (Figs. 2, 10, 11). By comparing the mean values of the sums of the three joint angles of a hindlimb during the stance phase for a single step in the adult HCbed animals vs. the control rats, a highly significant hyperflexion was evidenced in the RH ( $F_{1,20} = 262.36; P = 0.00001$ ) while on the LH no significant differences were present ( $F_{1,20} = 0.93; P = n.s.$ ).

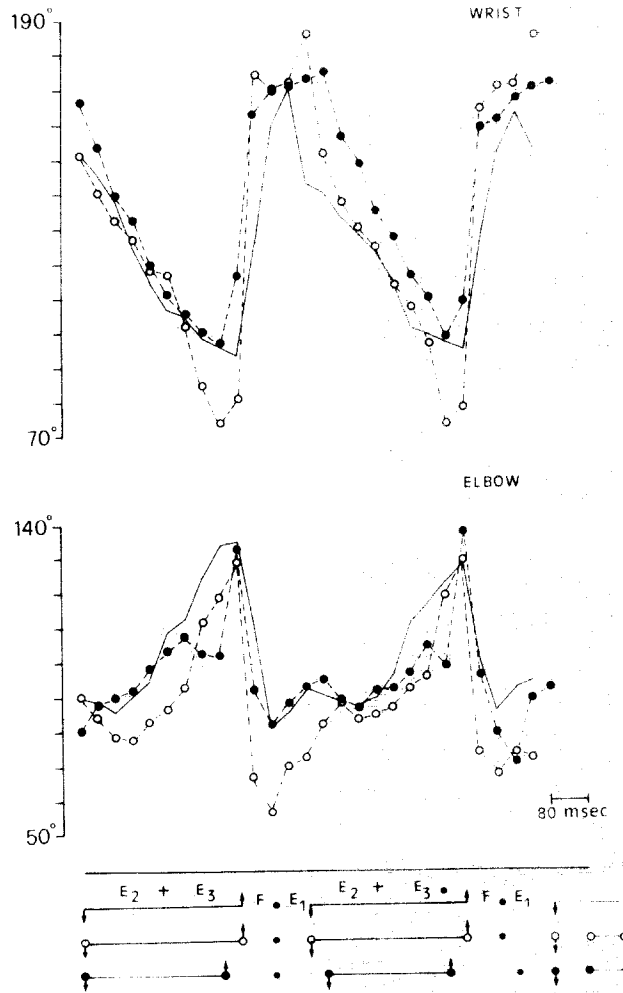


Fig. 10. Forelimb joint angles of a selected adult HCbed rat during step cycles. Abbreviations as in Fig. 3.



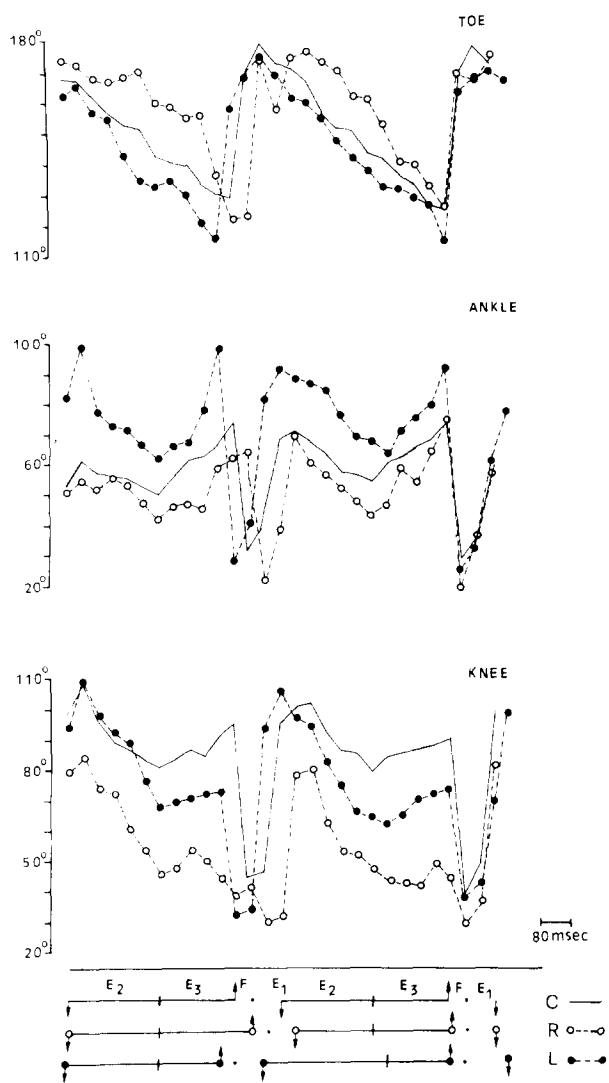


Fig. 11. Hindlimb joint angles of a selected adult HCbed rat during step cycles. Abbreviations as in Fig. 3.

During swing phase, a characteristic of adult operates was the hyperflexion of joint angles (Fig. 5). The mean percentage of hindlimb flexion during swing phase, calculated as already described for neonatal HCbed rats, reached the  $58\% \pm 5$  of stance value in RH and  $35\% \pm 7$  in the LH. This was achieved through a marked reduction not only of ankle angles, as in the neonatal operate group, but also of knee angles (Fig. 11). The sharply asymmetrical joint angles between sides caused the body to jerk up and down instead of remaining horizontal, resulting in the waddling gait of these animals.

Stick diagrams of the limb positions during stepping showed that the different limb positions during step phases were acquired through highly irregular trajectories, more evident in hind- than in forelimbs and on the right side more than on the left side (Figs. 6, 7).

As regards limb angular displacements, a highly ir-

regular pattern was exhibited by the adult operates (Fig. 8). In the RH, the feature most commonly observed was an increase of  $\beta$  and  $\alpha$  angles, creating a hypermetric step, although hypometric steps with reduction of  $\beta$  and  $\alpha$  angles were also occasionally seen. For the LH, angular displacements were less affected, with the  $\beta$  angle normal or slightly reduced, leading to normal or slightly hypometric steps. For the forelimbs, a general reduction of  $\beta$  angles was present bilaterally, while  $\alpha$  angles showed a great variability with normal or increased values. Therefore, the resulting step lengths of the LF and RF were extremely different, creating a highly unstable walking. In fact, these animals showed an extremely simplified gait characterized by a sequence of foot placement based on alternating quadrupedal and tripedal support (formula 1 by Hildebrand<sup>16</sup>) (Fig. 9), the most stable gait able to compensate for the extreme variance and instability of the limb movements. Furthermore, as clarified by paw print analysis, the differences in stride length between sides induced modifications of the paw contact sequence. In fact, the normal sequence (LF→RH→RF→LH→LF) was periodically modified by skipping the RH swing phase, so that LF lift off was followed by RF lift off (LF→RF→LH→LF).

Despite these compensatory strategies, the gait was still very unstable and continuous walking lasted for very short periods. In fact, the animals stopped every few cycles in a state of quadrupedal support that allowed a kind of 'resetting to zero' of the different components affecting gait stability.

These animals were not able to successfully increase their locomotion speed.

## DISCUSSION

The present study analyzes the locomotor strategies present in rats after HCb according to the time of lesioning. Quantitative kinematic analysis allowed us to clarify the specific disturbances in the locomotor control in the absence of a hemicerebellum and to determine whether functional recovery of locomotion after HCb at different developmental stages involves the recovery of lost functions or the appearance of new strategies.

In *adult HCbed rats* the basic scheme of locomotion was maintained and the locomotor pattern was characterized by the following aspects: (1) a marked ipsilateral extensor hypotonia during stance, inducing a pronounced asymmetry between sides in the degree of limb extension, responsible for the sharply tilted locomotion; (2) a clear hyperflexion on both sides, but

prevalent on the lesion side during swing; (3) a relevant lack of movement smoothness, especially present on the lesion side; (4) an alteration of interlimb coordination, characterized by an extreme variability in the timing of the different step phases among limbs and by a modified sequence of steps with an occasional skipping of the RH swing; (5) a compounding of small errors into a complete loss of balance.

Taken together, these features depict a locomotor pattern closely related to the asymmetry in the motor system elicited by the unilateral lack of cerebellar function<sup>25</sup>. On one hand, the severe extensor hypotonia ipsilateral to the lesion can be related to the lack of cerebellar input to the thalamo-cortico-spinal pathway, leading to a reduced fusimotor discharge ipsilateral to the cerebellar lesion<sup>10,13</sup>. On the other hand, the bilateral hyperflexion during swing phase could result from the lack of cerebellar control over reticulo- and rubro-spinal pathways. In fact, while a hemicerebellum influences through the rubro-spinal system only the ipsilateral spinal cord, bilateral effects can be mediated by the reticulo-spinal system because of its bilateral organization<sup>18,30</sup>. Both reticulo-<sup>30</sup> and rubro-spinal<sup>2,27</sup> neurons have a facilitatory influence on flexor muscles during swing. Therefore, the marked hyperflexion ipsilateral to the lesion could be the result of the interplay between the lack of cerebellar control on rubro-spinal cells and the asymmetrical cerebellar control on the reticulo-spinal pathways. Contralaterally, only a small degree of hyperflexion was present as a result of the persistence of cerebellar control on rubro-spinal neurons and the asymmetrical impairment of reticulo-spinal system.

It is widely accepted that the suppression of the cerebellar role as comparator between central motor commands and sensory feed back provokes errors of force, velocity and timing of muscle contraction during locomotion<sup>17</sup>. In our experimental model the cerebellar adjustment of the timing of locomotor movements is lost *on the lesion side* as shown by movements decomposed into sequential constituents made with errors, indicating that muscles acting on joints normally moving together lose their synergies. The prolonged stance, with an excessive  $\beta$  angle, and the prolonged swing, with an excessive  $\alpha$  angle, ipsilateral to the lesion can be interpreted as the result of a prolonged action of agonist muscles and a delayed action of antagonists. In fact, this pattern has already been demonstrated electrophysiologically in other paradigms of closely patterned interplay between extensors and flexors in the absence of the cerebellum<sup>17</sup>.

*On the contralateral side*,  $\alpha$  and  $\beta$  angles were normal or slightly reduced, such that a strongly asymmetrical pattern was present with hypermetric steps ipsilateral

and hypometric steps contralateral to the lesion. This disruption of interlimb coordination fits with electrophysiological results indicating a similar effect on locomotion after unilateral cooling of cerebellar cortex in cats<sup>35,36</sup>.

Adult rats which had undergone a *neonatal HCb* presented a skillful locomotion without falls and with a great facility to change velocity. Nonetheless, kinematic analyses showed that many components were altered: (1) during stance, a clear hyperextension of both hindlimbs was present. Since the forelimbs were not hyperextended, the locomotor posture of this experimental group was extremely characteristic, with the spine flexed and the head lower to the ground than normal; (2) during swing, a tendency towards hyperflexion was present in both hindlimbs, more so contralateral to the lesion; (3) the locomotor pattern was characterized by regular and symmetrical but hypometric steps.

Therefore, in spite of the unilateral lesion of the cerebellum, these animals exhibited only slight asymmetries in the locomotor performances. A possible explanation lies in the remodelling of the spared cerebellar circuitries that neuroanatomically characterized the neonatal operates. Many studies have evidenced that, following a neonatal HCb, neuronal circuits develop that allow the spared hemicerebellum to be connected bilaterally with the descending motor systems<sup>5-7,22-26,28,33</sup>. In fact, both cerebello-cortico-ponto-cerebellar<sup>6,23</sup> and cerebello-rubro-olivo-cerebellar<sup>26,31,33</sup> loops of both sides are connected with the spared hemicerebellum which can thus exert its influence on ipsi- and contralateral movements. The hyperactivity of extensor muscles observed in this group of animals during stance can be attributed to enhanced discharge of vestibulo-spinal neurons that are known to have a bilateral facilitatory influence on extensor motoneurons<sup>17</sup>. Tentatively, this modified activity in the vestibular nuclear complex might be interpreted as a consequence of the enhanced spinal afferents to the Deiters' nucleus ipsilateral to the lesioned hemicerebellum because of the rerouting of the spino-cerebellar bundle<sup>7</sup>. In our opinion, this vestibular hyperactivity cannot be simply due to the lack of the cerebellar Purkinje cell inhibition on vestibular neurons. In fact, in the adult operates, the lack of cerebellar inhibition, not accompanied by spino-cerebellar rerouting, did not elicit an extensor hyperactivity.

This relation between enhanced bilaterality of the spared circuits after asymmetrical brain lesions and the maintenance of a higher degree of symmetry in the motor performances has been described in other experimental models. In cats, a neonatal hemispherectomy provokes an enhanced bilaterality of the cortico-

descending pathways accompanied with mild or absent postural and locomotor changes, in contrast with the highly asymmetrical behavioural outcome of the adult hemispherectomy, where no neuronatomical remodelling has been observed<sup>4</sup>. Furthermore, unilateral motor cortex lesions performed neonatally in rats allow sparing of reaching movements which seems promoted by the spared contralateral neocortex through an augmented ipsilateral corticofugal pathway<sup>37</sup>. All the above-mentioned data, though not a direct proof of the functional role played by the postlesional remodelled circuits, strongly support the hypothesis of a strict relationship between neuronal plasticity and postlesional behaviour.

By comparing the motor strategies displayed by the two experimental groups during locomotion, some considerations can be drawn. Although the basic components of the movements were present in both groups, they were programmed into two different behavioural strategies. The *adult HCbed animals*, besides the irregular nature of their locomotion, exhibited features characteristic of specific steps of ontogenetic development of spontaneous walking. In fact, they utilized a gait based on alternating tripedal and quadrupedal support, the same sequence utilized by young puppies<sup>16</sup> at developmental stages in which cerebellar function is immature. Interestingly, this form of gait also characterizes the locomotion of ancestral tetrapods<sup>16</sup>. These animals have short and weak legs and usually a wide stance<sup>16</sup>. Therefore, in view of the peculiar phylogenetic history of the cerebellum, the comparison with the hyperflexed, hypotonic and abducted limbs of the adult HCbed specimens appears to us very teasing. Also, in turning, adult HCbed rats utilized a strategy based on pivoting typical of a specific period of locomotor development<sup>28</sup>. Taken together, these data suggest that the lesion of a hemicerebellum in adulthood induces the loss of sophisticated patterns of walking and provokes the reappearance of developmentally 'old' patterns present when cerebellar control was lacking.

In contrast, *neonatal operates* displayed a locomotor strategy characterized by hyperextension of both hindlimbs during stance and by hypometric but symmetrical steps. This gait, resembling a 'tip-toe' gait, was unique to neonatal operates, being absent either in the normal adult or in the infant. This feature is, in our opinion, related to the already discussed specific anatomical organization of cerebello-motor circuits. Therefore, in neonatal operates, the postlesional motor strategies represent the development of new function as consequence of the new anatomical organization.

It can be argued<sup>32</sup> that any postlesional remodelling of circuits, besides regeneration of cut axons, will pro-

duce an anatomical organization that, by definition, is different from normal and therefore can never result in the precise reinstatement of the lost function. Nevertheless, many reports<sup>3,4,25,37</sup>, as well as the present study, indicate that in some experimental models a high extent of postlesional plasticity is associated with functionally advantageous behavioural outcomes. This gap can be bridged in the light of the present results. In our model, neuroanatomical remodelling does not allow the reinstatement of the lost locomotor pattern, but induces the appearance of a locomotor strategy that, though novel, appears to be extremely efficient. We are aware that just because of their novelty, the aberrant projections do not necessarily elicit advantageous behaviours<sup>1</sup>. In fact, some reports support models where growth processes may exaggerate an imbalance in already damaged systems, resulting in maladaptive behaviours rather than in recovery of function<sup>9,20</sup>. Taken together, these evidences indicate that many factors are involved in determining aberrant circuit activity and therefore it is difficult to predict the functional properties of a specific remodelled circuit. However, by analyzing the models in which postlesional plasticity has been hypothesized as the basis of functional recovery, some considerations can be advanced<sup>24</sup>. It appears that for a functional recovery, remodelled circuits must permit a flow of information as similar as possible to that utilized by the damaged system. This characteristic is particularly present in the circuits that are the contralateral homologue of the lesioned ones. In fact, in many models<sup>4,25,37</sup>, when the plastic changes are sustained by these structures, their behavioural outcome is highly functional. Alternatively, when remodelling of synapses is prevalent in non-homologue structures, the behavioural outcome is often greatly impaired<sup>11</sup>, suggesting that the 'quality' of the information flow is profoundly different from normal.

Finally, another aspect cannot be discarded. When relocation of function does occur, there is always a cost associated with it. When an infant lesion induces the relocation of some functions, some compromises often occur in those brain areas which take over the functions of the injured structures, in terms of crowding of functions<sup>21</sup>. Therefore, neuronal remodelling might have positive as well as negative functional effects. In this light, it is possible to interpret the contralateral signs of cerebellar deficiency showed by our neonatally HCbed rats. The extensor hypotonia affecting the left limbs resulted in a tilted posture in neonatal operates that could be due to a crowding of functions in the spared hemicerebellum, which in these animals, because of neuronal remodelling, controls ipsi- and contralateral motor systems.

In conclusion, the present study demonstrates reappearance of old motor strategies in lesioned animals in the absence of neuronal plasticity and the development of new ones in face of intense neuronal remodelling. These data clarify some aspects of the functional recovery and of the effects of the aberrant circuits on postlesional behaviour that are of interest for a better comprehension of the different factors that regulate recovery of function after brain trauma.

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#### REFERENCES

- Almli, C.R. and Finger, S., Toward a definition of recovery of function. In S. Finger, T.E. Levere, C.R. Almli and D.G. Stein (Eds.), *Brain Injury and Recovery. Theoretical and Controversial Issues*, Plenum Press, New York, London, 1988, pp. 1–14.
- Arshavsky, Y.I., Orlovsky, G.N. and Perret, C., Activity of rubrospinal neurons during locomotion and scratching in the cat, *Behav. Brain Res.*, 28 (1988) 193–199.
- Bregman, B.S. and Goldberger, M.E., Infant lesion effect: II. Sparing and recovery of function after spinal cord damage in newborn and adult cats, *Dev. Brain Res.*, 9 (1983) 119–135.
- Burgess, J.W. and Villablanca, J.R., Recovery of function after neonatal or adult hemispherectomy in cats. II. Limb bias and development, paw usage, locomotion and rehabilitative effects of exercise, *Behav. Brain Res.*, 20 (1986) 1–18.
- Castro, A.J., Projections of the superior cerebellar peduncle in rats and the development of new connections in response to neonatal hemicerebellectomy, *J. Comp. Neurol.*, 178 (1978) 611–628.
- Castro, A.J. and Mihailoff, G.A., Corticopontine remodelling after cortical and/or cerebellar lesions in newborn rats, *J. Comp. Neurol.*, 219 (1982) 112–123.
- Castro, A.J. and Smith, D.E., Plasticity of spinoventricular projections in response to hemicerebellectomy in newborn rats, *Neurosci. Lett.*, 12 (1979) 69–74.
- Cohen, A.H. and Gans, C., Muscle activity in rat locomotion: movement analysis and electromyography of the flexors and extensors of the elbow, *J. Morphol.*, 146 (1975) 177–196.
- Finger, S. and Almli, C.R., Brain damage and neuroplasticity: mechanisms of recovery or development?, *Brain Res. Rev.*, 10 (1975) 177–186.
- Gilman, S., The nature of cerebellar dyssynergia, *Mod. Trends Neurol.*, 5 (1970) 60–79.
- Goldberger, M.E., Partial and complete deafferentation of cat hindlimb: the contribution of behavioural substitution to recovery of motor function, *Exp. Brain Res.*, 73 (1988) 343–353.
- Gramsbergen, A. and Ijkema-Paassen, J., The effects of early cerebellar hemispherectomy in the rat: behavioral, neuroanatomical and electrophysiological sequelae. In S. Finger and C.R. Almli (Eds.), *Early Brain Damage*, Vol. 2, Academic Press, New York, 1984, pp. 155–175.
- Granit, R., *Receptors and Sensory Perception*, Yale University Press, New Haven London, 1955.
- Grillner, S., Control of locomotion in bipeds, tetrapods and fish. In J.M. Brookhart and V.B. Mountcastle (Eds.), *The Nervous System: Handbook of Physiology*, Vol. II/2, Bethesda, MD, 1981, pp. 1179–1236.
- Gruner, J.A., Altman, J. and Spivack, N., Effects of arrested cerebellar development on locomotion in the rat. Cinematographic and electromyographic analysis, *Exp. Brain Res.*, 40 (1980) 361–373.
- Hildebrand, M., Analysis of tetrapod gaits: general considerations and symmetrical gaits. In R.M. Herman, S. Grillner, P.S.G. Stein and D.G. Stuart (Eds.), *Neural Control of Locomotion: Advances in Behavioural Biology*, Vol. 18, Plenum, New York, 1976, pp. 203–236.
- Ito, M., *The Cerebellum and Neural Control*, Raven, New York, 1984, pp. 1–580.
- Jones, B.E. and Yang, T.Z., The efferent projections from the reticular formation and the locus coeruleus studied by anterograde and retrograde axonal transport in the rat, *J. Comp. Neurol.*, 242 (1985) 56–92.
- Keller, A., Arissian, K. and Asanuma, H., Formation of new synapses in the cat motor cortex following lesions of the deep cerebellar nuclei, *Exp. Brain Res.*, 80 (1990) 23–33.
- Kolb, B., Holmes, C. and Whishaw, I.Q., Recovery from early cortical lesions in rats. III. Neonatal removal of posterior parietal cortex has greater behavioural and anatomical effects than similar removals in adulthood, *Behav. Brain Res.*, 26 (1987) 119–137.
- Levere, N.D., Gray-Silva, S. and Levere, T.E., Infant brain injury: the benefit of relocation and the cost of crowding, In S. Finger, T.E. Levere, C.R. Almli and D.G. Stein (Eds.), *Brain Injury and Recovery. Theoretical and Controversial Issues*, Plenum, New York, 1988, pp. 133–150.
- Lim, K.H. and Leong, S.K., Aberrant bilateral projections from the dentate and interposed nuclei in albino rats after neonatal lesions, *Brain Res.*, 96 (1975) 306–309.
- Molinari, M., Bentivoglio, M., Granato, A. and Minciocchi, D., Increased collateralization of the cerebellothalamic pathway following neonatal hemicerebellectomy, *Brain Res.*, 372 (1986) 1–10.
- Molinari, M., Petrosini, L. and Dell'Anna, M.E., Interrelationships between neuroplasticity and recovery of function after cerebellar lesions in rats, *Brain Dysf.*, 5 (1992) 169–183.
- Molinari, M., Petrosini, L. and Gremoli, T., Hemicerebellectomy and motor behaviour in rats. II. Effects of cerebellar lesion performed at different developmental stages, *Exp. Brain Res.*, 82 (1990) 483–492.
- Naus, C.G., Flumerfelt, B.A. and Hryciyshyn, A.W., Topographic specificity of aberrant cerebellorubral projections following neonatal hemicerebellectomy in the rat, *Brain Res.*, 309 (1984) 1–15.
- Orlovsky, G.N., Activity of rubrospinal neurons during locomotion, *Brain Res.*, 46 (1972) 99–112.
- Petrosini, L., Molinari, M. and Gremoli, T., Hemicerebellectomy and motor behaviour in rats. I. Development of motor function after neonatal lesion, *Exp. Brain Res.*, 82 (1990) 472–482.
- Phillipson, M., L'Autonomie et la centralization dans le système nerveux des animaux, *Trav. Lab. Physiol. Inst. Solvay*, 7 (1905) 1–208.
- Shimamura, M., Kogure, I. and Wada, S., Reticular neuron activities associated with locomotion in thalamic cats, *Brain Res.*, 231 (1982) 51–62.
- Song, W.J. and Murakami, F., Ipsilateral interpositorubral projection in the kitten and its relation to post-hemicerebellectomy plasticity, *Dev. Brain Res.*, 56 (1990) 75–85.

- 32 Steward, O., Lesioning induced neuroplasticity and the sparing or recovery of function following early brain damage. In C.R. Almli and S. Finger (Eds.), *Early Brain Damage*, Academic Press, New York, 1984, pp. 59–77.
- 33 Swenson, R.S. and Castro, A.J., Plasticity of mesodiencephalic projections to the inferior olive following neonatal hemicerebellectomy in rats, *Brain Res.*, 244 (1982) 169–172.
- 34 Tsukahara, N., Synaptic plasticity in the mammalian central nervous system, *Annu. Rev. Neurosci.*, 4 (1981) 351–379.
- 35 Udo, M., Matsukawa, K., Kamei, H. and Oda, Y., Cerebellar control of locomotion: effects of cooling cerebellar intermediate cortex in high decerebrate and awake walking cats, *J. Neurophysiol.*, 44 (1980) 119–134.
- 36 Yu, J. and Eidelberg E., Recovery of locomotor function in cats after localized cerebellar lesions, *Brain Res.*, 273 (1983) 121–131.
- 37 Whishaw, I.Q. and Kolb, B., Sparing of skilled forelimb reaching and corticospinal projections after neonatal motor cortex removal or hemidecortication in the rat: support for the Kennard doctrine, *Brain Res.*, 451 (1988) 97–114.
- 38 Winer, B.J., *Statistical Principles in Experimental Design*, McGraw-Hill, New York, 1962, pp. 1–907.