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Research report

Is there an optimal age for recovery from motor cortex lesions? I. Behavioral and anatomical sequelae of bilateral motor cortex lesions in rats on postnatal days 1, 10, and in adulthood

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Abstract

Rats were given bilateral lesions of the motor cortex on the day of birth (P1), tenth day of life (P10), or in adulthood. They were trained on several motor tasks (skilled forelimb reaching, beam traversing, tongue extension), general motor activity, and a test of spatial learning (Morris water task). Although all lesion groups were impaired at skilled reaching, the P10 group was less impaired than either of the other two lesion groups. Furthermore, on the other motor tests the P10 group did not differ from controls whereas both P1 and adult groups were impaired. Only the P1 lesion group was impaired at the acquisition of the Morris water task. Anatomical analyses revealed that the P1 and P10 rats had smaller brains than the other two groups as well as having a generalized decrease in cortical thickness. Dendritic analysis of layer III pyramidal cells in the parietal cortex revealed a decrease in apical arbor in the lesion groups and an increase in the basilar arbor of the P1 and adult lesion animals. The P1 and adult operated groups showed an increase in spine density in the basilar dendrites of layer V pyramidal cells. Finally, analysis of the pattern of corticospinal projections revealed that the P1 animals had a markedly wider field of corticospinal projection neurons than any of the other groups. The widespread anatomical changes in all lesion groups versus the relatively better behavioral recovery after P10 lesions suggests that day 10 represents an optimal period for adapting to brain damage and subsequent brain reorganization.

Theme: Neural basis of behavior

Topic: Neural plasticity

Keywords: Recovery of function; Motor cortex; Corticospinal

1. Introduction

This study is one of a series examining the behavioral and anatomical effects of neonatal decortication, hemidecortication, or restricted lesions in rats of different ages (for reviews, see Refs. [11,20]). The present study compared the effects of bilateral removal of motor cortex in neonatal, infant, and adult rats on a broad range of behavioral and anatomical measures. It has been shown that rats with unilateral motor cortex lesions on the first few days after birth have better skilled forelimb reaching movements than adults with similar lesions (e.g., Refs. [2,3,7,8,17,33]). The better functional outcome in the neonatal group is correlated with an augmentation of the ipsilateral corticospinal pathway from the normal hemisphere. The questions we asked here was whether there were changes in the corticospinal connectivity after bilateral lesions and whether there was an age-dependent difference in recovery after bilateral motor cortex lesions. It was our expectation that if there were changes in connectivity within the lesion hemispheres, then this might produce what Teuber [28] referred to as crowding. That is, there would be an interference with the control of other behaviors not normally affected by lesions of the motor cortex. We therefore chose a broad battery of behavioral tasks that included tests sensitive to motor cortex injury in adulthood (skilled forelimb reaching; beam traversing; tongue extension; see Refs. [18,31]) as well as tests that

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we knew were unaffected by motor cortex lesions in adulthood (general activity, spatial learning) but that were extremely sensitive to the effects of cortical lesions early in life (for a review, see Ref. [20]).

In the current study we chose to compare the effects of bilateral lesions on postnatal day 1 to those of animals with lesions on postnatal day 10 or in adulthood. The anatomical analysis in our previous studies of rats with neonatal motor cortex lesions was limited to an investigation of cortical thickness and corticospinal connectivity. In this study we expanded our analysis to look at the changes in dendritic morphology in the parietal cortex adjacent to the lesions. We chose to measure pyramidal neurons in area Par1 for several reasons. Brains with lesions on the first few postnatal days show considerable distortion in cortical architecture and, particularly in Golgi-stained sections it is difficult to distinguish frontal cortical regions. Neurons in Par1 are much easier to identify reliably, thus ensuring that we are measuring comparable neurons in the different groups. Furthermore, in previous studies we had found large changes in these neurons after midline frontal lesions in both infant and adult rats (e.g., Refs. [12,14,15]). Finally, because preliminary results had shown us that there was an expansion of the corticospinal projection neurons into the parietal cortex of rats with P1 motor cortex lesions, we anticipated that there might be differences in the cell morphology in the parietal cortex of the P1 animals.

2. Materials and methods

2.1. Subjects

This study used 42 rats, derived from the Charles River Long-Evans strains, which were divided into four groups: control (six M, 11 F), and bilateral motor cortex lesions performed at postnatal day 1 (P1; four M, six F), postnatal day 10 (P10; four M, three F), and at adulthood (Ad; five M, five F). Half of the control animals were age-matched with the infant operates, the other half being age-matched with the adult operates. The animals were group housed with same sex littermates in stainless steel hanging cages on a 12:12 h light–dark schedule throughout the experiments. The animals were on ad lib food throughout except during the food reaching task. Animals were cared for under the rules and provisions of the Canadian Council on Animal Care.

2.2. Surgical procedures

Adult rats (90 days of age at surgery) were anesthetized with sodium pentobarbital (60 mg/kg for males, 40 mg/kg for females). Cortical removal was achieved by removing the bone over the motor cortex from 2 mm lateral to the midline to the sagittal ridge and from +2 anterior to −3 relative to bregma. After retraction of the dura, the neocortex exposed was removed by aspiration. This included Zilles' [39] areas Fr1, the lateral part of Fr2, the posterior part of Fr3, and FL. Following hemostasis the scalp wound was closed with wound clips. Control animals were anesthetized, the skin incised, and closed with wound clips.

The neonatal animals were anesthetized by cooling them in a Thermatron cooling chamber until their rectal body temperatures were in the range of 18–20°C. The bone over the motor neocortex area was removed with iris scissors, and motor decortication was achieved as in the adult rats. We have shown previously that on postnatal day 1, the frontal cortex lies more anterior relative to bregma than it does in adulthood, but that the relation between the bregmoidal junction and the underlying cortex is constant after about postnatal day 5 [10]. Thus, the lesion coordinates were adjusted accordingly. On day 1 the lesion was made from about +1.5 to −1 whereas on day 10 it was made from about +2 to −2, relative to the bregmoidal junction. The scalp wound was sutured with silk thread as soon as the operation was complete. The normal control animals were anesthetized, the skin incised, and then closed with silk suture. The control animals were littermates of the operated animals. Behavioral training began about 3 months after surgery.

2.3. Retrograde dyes

At the completion of the behavioral tests, 14 rats (four control, three P1, three P10, four adult) were anesthetized with sodium pentobarbital and placed in a stereotaxic apparatus. By blunt dissection, an aperture to the spinal cord was made through the neck, the spinal cord was exposed at the cervical enlargement, and the rats were given two 1-μl injections of a 5% solution of True Blue at approximately C8-T1. The injections were made unilaterally into the gray matter. The rats were killed 14 days after the True Blue injections. They were deeply anesthetized and intracardially perfused with a solution of 0.9% saline and 10% formalin. The brains were removed and weighed, and then placed into a 30% sucrose formalin solution for 48 h before being cut frozen at 40 μm. Every tenth and eleventh section was mounted to make two complete sets of sections through the entire brain. One section was kept for fluorescence microscopy and one set was stained with Cresyl Violet.

2.4. Anatomical methods

At the completion of behavioral testing (about 4 months postoperative), the animals were given an overdose of sodium pentobarbital and transcardially perfused with 0.9% saline. The brains were removed and weighed before being immersed whole in 20 ml of Golgi-Cox solution for 14 days. The brains were then placed in a 30% sucrose

solution for 2 days and cut on a vibratome at 200 μm and developed using a procedure described by Gibb and Kolb [6]. Layer III and layer V pyramidal cells in Zilles' [30] area Par1 were traced using a camera lucida drawing tube, magnified at 250×, that was attached to the microscope. Both the basilar and apical fields were drawn for layer III but because many of the apical dendrites were incomplete for the layer V cells, only the basilar dendrites were drawn. In order to be included in the data analysis, the dendritic trees of pyramidal cells had to fulfill the following criteria: (a) the cell had to be well impregnated and not obscured with blood vessels, astrocytes, or heavy clusters of dendrites from other cells; and (b) the dendritic branching had to appear to be largely intact and visible in the plane of section. The cells were analyzed by using the concentric ring procedure of Sholl [26]. The number of intersections of dendrites with a series of concentric spheres at 20-μm intervals from the center of the cell body was counted for each cell. Statistical analyses were performed by averaging across all cells per hemisphere. An estimate of mean total dendritic length (in μm) can be made by multiplying the mean total number of intersections by 20.

Cells were chosen by locating area Par1 and every cell meeting the criteria above was then drawn until there were 10 drawn for each area per hemisphere. Statistical analyses were performed by averaging across all cells per hemisphere. Spine density was measured on the layer V neurons only. A terminal tip segment and a secondary basilar branch were measured. A terminal tip was the distal 40 μm of a third order branch. A secondary branch was a 40 μm segment beginning with the proximal stump of the secondary branch. Spine-density measures were made from a segment greater than 10 μm in length, and usually about 40 μm in length. The dendrite was traced at 1000× using a camera lucida drawing tube, and the exact length of the dendritic segment was calculated. Spine density was expressed as the number of spines per 10 μm. Because we did not attempt to correct for spines hidden beneath or above the dendritic segment, the spine-density values likely underestimated the actual density of the dendritic spines. As for the dendritic drawing, the cytologist was blind to the group assignment of individual rats.

Cortical thickness was measured by projecting the Golgi-Cox stained sections on a Zeiss DL 2 POL petrographic projector set at magnification 20×. Measurements made with a plastic millimeter ruler were taken at three points at each of the following planes proceeding from rostral to caudal: plane 1, first section with caudate putamen visible, measures in Zilles' [30] areas Gu, Par1, Fr2; plane 2, center of anterior commissure, measures in Par2, Par1, Fr1; plane 3, first hippocampal section, measures area Gu Par1, Fr1; plane 4, posterior commissure, measures areas Te1, Oc2L, RSA; plane 5, most posterior hippocampal section, measures Te1, Oc1B, Oc2ML. Mean thickness at each plane was calculated by averaging across each of the three measurement locations. We note parenthetically that although early cortical lesions do alter cortical thickness, the general pattern of cortical cytoarchitecture is largely maintained in the Golgi-stained sections. Thus, we are confident that we are measuring the same tissue in the animals with early lesions as those with the later lesions.

2.5. Behavioral methods

The behavioral tests for this experiment included a measure of forepaw use, hindlimb placing, tongue extension, locomotor activity and spatial navigation (i.e., Morris water task). Behavioral training began about 3 months after surgery.

2.5.1. Forelimb reaching task

Forepaw use was measured with a procedure that was adapted from the method devised by Whishaw et al. [36]. Each animal was food-deprived to 85% body weight for the training and testing. The animals were placed in the test cages (10×18×10 cm high) with floors and fronts constructed of 2-mm bars, 9 mm apart edge to edge. A 4-cm wide and 5-cm deep tray, containing chicken feed pellets, was mounted in the front of each box. The rats were required to extend a forelimb through the gap in the bars, grasp and retract the food. The tray was mounted on runners and was retracted 0.5 cm from the cage so that the rats could not scrape the food into the cage. If the animal attempted to rake the pellet out of the tray, the pellet would fall irretrievably through the gap. An attempt was scored only when the rat reached into the tray and touched the food. If it reached into the tray without touching a pellet, no attempt was scored. Animals were trained for 20–30 min per day for a minimum of 10 days, by which time their performance had reached asymptote [27,37]. Once trained, the rats received a 5-min reaching test with each paw during which time they were videotaped and then scored later. In order to try to control for training effects on the brain, all animals continued to practice reaching for about 4 months. Some of this training was concurrent with training on the other behavioral measures.

2.5.2. Beam walking

Hindlimb placement was measured by forcing animals to traverse a narrow elevated runway to obtain access to Fruit Loops, as described by Kolb and Whishaw [18]. The wooden runway was 200 cm in length, 5 cm wide, and elevated 38 cm above a counter. The rats were trained to run from one end of the beam to the other to obtain access to the reward. Once the animals had learned to run rapidly from one end to the other, they were videotaped on three consecutive trials. The tapes were later analyzed to determine the presence of any abnormalities in foot placement. Because intact control rats nearly always place the paws flat on the runway, a fault was scored each time an

animal's hindfoot was placed over the edge of the runway or if it slipped off the edge of the runway.

2.5.3. Tongue extension

Each rat was tested to see how far it could extend its tongue beyond the wire mesh front of its home cage [34]. The last 2 cm of a clear plastic millimeter ruler was smeared with a slurry of chocolate-chip cookies and water and placed against the wire of the cage. The rats, which had been previously exposed to the food, licked the ruler through the wire bars of the cage front, and the area cleared of cookie mixture on the ruler indicated how far the rat could extend its tongue.

2.5.4. Locomotor activity

Activity was measured in a bank of 18-wire photocell cages using a procedure described by Whishaw et al. [32]. The individual cages were 40 cm long, 25 cm deep, and 18 cm high, with two parallel horizontal infrared beams 1 cm above the floor, 12 cm from each end of the cage, and perpendicular to the long axis of the cage. The beam breaks, registered incrementally, by a computer, were summed into 5-min time bins during the locomotor tests. The animal activity score was the mean of the daily sum over a 4-day test period.

2.5.5. Morris water task

Spatial navigation was assessed in a swimming pool [22] and was based on a procedure devised by Kolb and Whishaw [18]. Rats were trained to swim to a Plexiglas platform hidden 1.5 cm beneath the surface of water in a circular pool (diameter, 150 cm; height 45 cm). Milk powder was dissolved in the water to render the platform invisible. The rats began their search from one of four locations at the perimeter of the pool; the order of starting locations was randomly assigned. A group of four trials from each of the starting locations constituted a trial block. Rats were given four trials per day for 10 consecutive days with an intertrial interval of approximately 5 min. If on a particular trial a rat found the platform, it was permitted to remain on the platform for 10 s. A trial was terminated if a rat failed to find the platform after 90 s. At the end of a trial, the rat was returned to a holding cage, and approximately 5 min elapsed before beginning the next trial. The latency to find the platform was timed by an experimenter standing by the pool's edge. The route traversed by each rat was traced using pencil and paper by a second observer. An error was scored if the rat's path deviated from a 20 cm wide channel going directly from the start point to the platform. The hidden platform was kept in a constant location for all 10 trial blocks.

2.6. Statistical methods

Analyses of variance (ANOVAs) were used for all measures and Fisher's LSD ($P<0.05$) was used for post hoc evaluations. Sex differences were assessed for each measure using a two-way ANOVA but were only significant for brain and body weights, and are thus reported separately for these measures. There were no differences in the performance of the infant control animals, who began behavioral training about 100 days of age, and the adult control animals, who began training at about 200 days of age. The two control groups therefore were combined for all statistical analyses.

3. Anatomical results

The lesions removed the intended targets in both the adult and infant rats but there were obvious differences in the brains across the three lesion groups (Fig. 1). The lesion extent in the brains of rats with P1 lesions was more variable than in the other two groups, especially in the medial extent. Six of the 10 P1 rats had unilateral damage to Zilles' [30] area Cg1. Two of the seven P10 rats had similar damage but none of the adult operates did. The corpus callosum and external capsule were markedly shrunken in both of the young operated groups and the ventricles were clearly enlarged. Finally, the intact posterior cortex was visibly thinner in the injured hemisphere in the infant, but not the adult, lesion groups.

3.1. Brain weights

Because the fixation procedure for Golgi-Cox embedding and retrograde labeling are different, the brain weights are not comparable. We therefore report here only the data for brains fixed for Golgi-Cox, which was the majority of the brains. Because brain weight is sexually dimorphic (male brains are heavier), a two-way ANOVA for sex and lesion was conducted. This showed a significant main effect of sex, $F(1,28)=6.96$, $P=0.014$, lesion, $F(3,28)=19.67$, $P<0.0001$, but not an interaction, $F(3,28)=0.69$, $P=0.565$.

Follow-up one-way ANOVAs for males only found a significant lesion group effect, $F(3,9)=11.77$, $P=0.002$. Both the P1 and P10 males had significantly lighter brains compared to control and adult lesioned animals which did not differ (see Table 1). There was also a significant effect of lesion group for the females, $F(3,19)=14.41$, $P<0.0001$. All of the lesion groups had significantly lighter brains than the female control group. The P1 group was the lightest and differed from both the P10 and adult animals.

3.2. Cortical thickness

As Fig. 2 demonstrates, all of the lesion groups had decreased cortical thickness compared to the control animals at the two most anterior planes. The two infant lesion groups had a thinner cortex throughout the entire

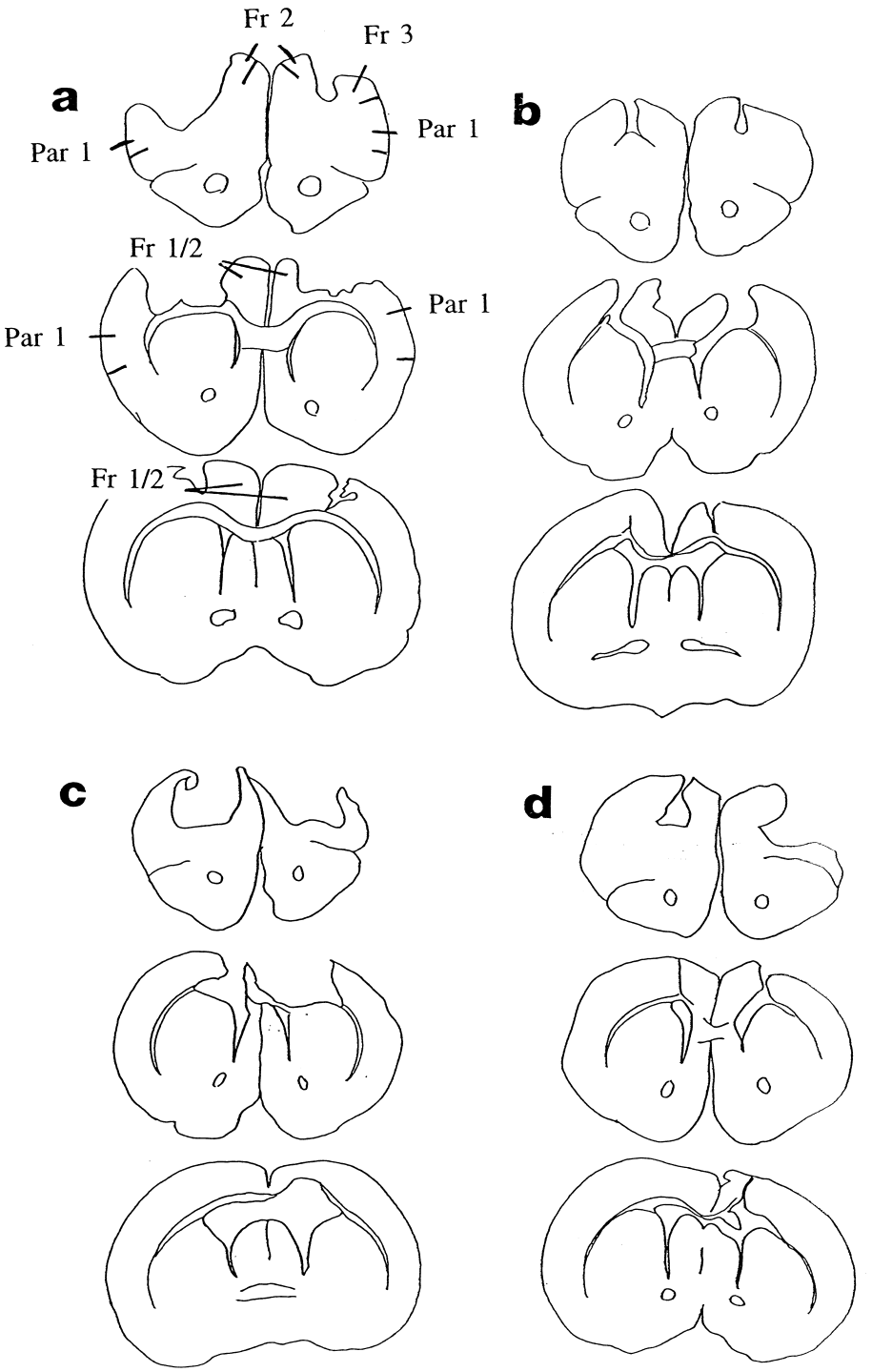

Fig. 1. Serial drawings of Golgi-Cox stained-coronal sections through the brain of representative rats with bilateral lesions of the motor cortex as adults (A), on postnatal day 10 (B), or postnatal day 1 (C and D). There was more variance in the P1 operates, which is illustrated in the range between the large (C) and small (D) examples.

extent of the brain although the effect was somewhat bigger in the P1 brains.

A two-way, repeated measures ANOVA with lesion group and plane as factors showed significant main effects of the lesion group, $F(3,27)=20.74$, $P<0.0001$, and the repeated factor plane, $F(4,12)=37.53$, $P<0.0001$, as well as a significant interaction, $F(12,108)=5.44$, $P<0.0001$. Follow-up one-way ANOVAs by plane revealed significant (P values<0.001) effects of the lesion group at all the planes except plane 4: plane 1, $F(3,27)=17.9$; plane 2, $F(3,27)=14.6$; plane 3, $F(3,27)=8.6$; plane 4, $F(3,27)=1.9$; and plane 5, $F(3,27)=6.9$.

Table 1
Summary of brain weights by lesion group[a]

Group[b]	Sex	
	Male	Female
Control	2.19±0.02	2.07±0.04[d]
P10 lesion	1.84±0.08[c,d]	1.84±0.03[c,d]
P1 lesion	1.81±0.04[c,d]	1.60±0.07[c,d,e]
Adult lesion	2.08±0.05	1.90±0.07[c]

[a] Note: numbers refer to means±S.E.M. of brain weights in g.
[b] Sample size for each group is as follows: control, males $n=4$, females $n=11$; P10, males $n=2$, females $n=2$; P1, males $n=2$, females $n=5$; adult, males $n=5$, females $n=5$.
[c] Differs significantly from the corresponding control group, $P<0.05$.
[d] Differs significantly from the corresponding adult lesion group, $P<0.05$.
[e] Differs significantly from the corresponding postnatal day 10 group, $P<0.05$.

3.3. Dendritic arborization

The effects of the lesions varied by the measure of dendritic arborization (see Fig. 3). For the apical fields of layer III, all lesion groups showed reduced dendritic length compared to the control group. In contrast, for the layer III basilar field there was a significant increase in dendritic length in the adult and P1 operate groups but no change in the P10 group. Curiously, however, for the basilar fields of layer V, all the lesioned groups showed reduced branching compared to the control animals. Thus, there was an effect of lesion on all measures but it was only the layer III basilar measure that dissociated the lesion groups from one another.

Repeated measures ANOVAs (group×intersection ring) were conducted for each of the cell measures. For the apical layer III measure there was a significant main effect of group, $F(3,62)=15.6$, $P<0.0001$, and ring intersection, $F(15,930)=97.8$, $P<0.0001$, as well as of the interaction,

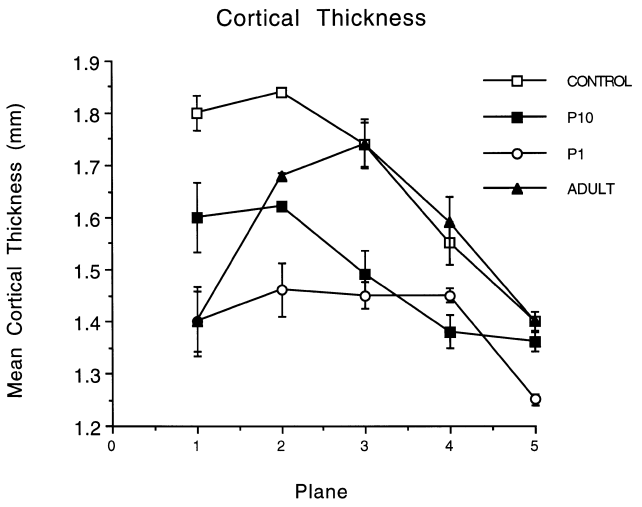

Fig. 2. Summary of cortical thickness at five planes of section. Except for the planes of lesion (1 and 2), rats with adult motor cortex lesions had cortex of normal thickness whereas rats with P10 or P1 lesions had thinner cortex across the cortical mantle.

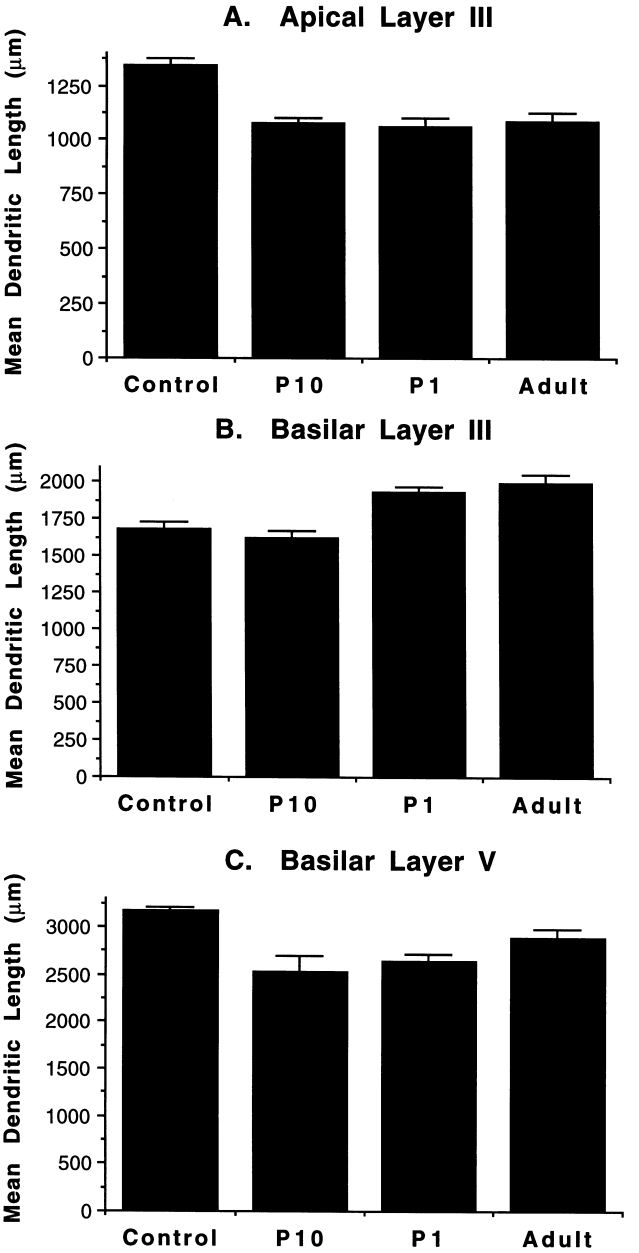

Fig. 3. Summary of total dendritic length for the apical and basilar dendritic trees of layer III and the basilar tree of layer V pyramidal neurons in Zilles' area Par1.

$F(45,930)=2.2$, $P<0.0001$. The interaction reflected the fact that the control group had more dendritic arbor distal to the cell body than did the other three groups (Fig. 4). For the basilar layer III measure there also was a significant main effect of group, $F(3,62)=7.5$, $P<0.001$, and ring intersection, $F(9,432)=435.5$, $P<0.0001$, as well as of the interaction, $F(27,432)=1.7$, $P<0.01$. This interaction is reflected in the differential response of the adult and P1 operates that is shown in Fig. 3. The increased dendritic arbor in those two groups was found in the more distal segments. Finally, for the basilar layer V measure there also was a significant main effect of group, $F(3,62)=$

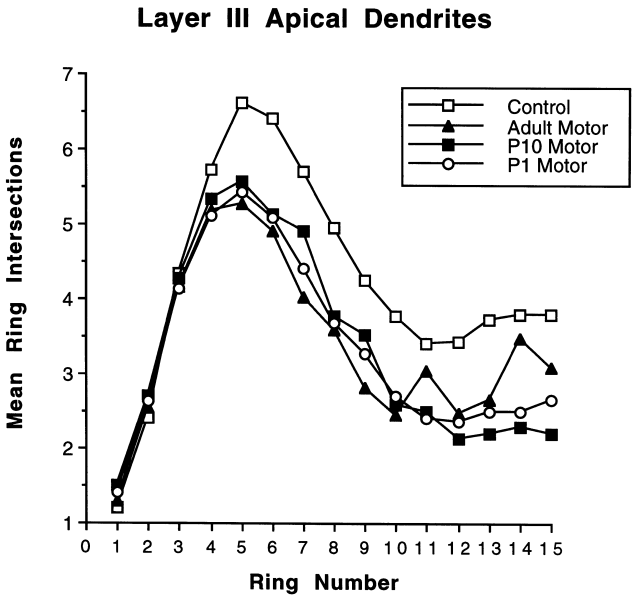

Fig. 4. Summary of Sholl ring intersections on the apical dendrites of layer III pyramidal cells at different distances from the cell body (rings were 20 μm apart). There is no lesion effect near the cell body but all lesion groups show a reduction in dendritic extent at the more distal rings.

10.9, $P<0.0001$, and ring intersection, $F(15,930)=2086.7$, $P<0.0001$, as well as of the interaction, $F(45,930)=8.5$, $P<0.0001$. Once again, the interaction reflected a decrease in the distal dendritic arbor in the lesion groups relative to the control group, much as shown in Fig. 4 for the layer III cells.

3.4. Spine density

Spine density was measured only on the layer V basilar branches. Compared to the control and P10 animals there was an increase in spine density on the terminal tip for the both the P1 and adult lesioned animals (see Table 2). One-way ANOVAs showed an effect of lesion group for spine density on the terminal tips, $F(3,52)=5.56$, $P=0.002$, but not for spines on the oblique branches, $F(3,52)=2.5$, $P=0.07$. Post hoc tests showed that lesions at both P1 and in adulthood resulted in increased spine density on the terminal and oblique branches compared to the control and P10 animals.

Table 2
Summary of dendritic spine density on basilar branches in layer V of area Par1[a]

Group[b]	Terminal	Oblique
Control	9.3±0.17	8.9±0.20
P10 lesion	9.5±0.29	7.9±0.21
P1 lesion	10.4±0.38[c]	9.2±0.27[d]
Adult lesion	10.2±0.17[c]	9.3±0.19[d]

[a] Note: numbers represent mean±S.E.M. for the spine density at the terminal tip and on an oblique branch.
[b] Sample size for each group is as follows: control, $n=13$; P10, $n=5$; P1, $n=5$; adult $n=10$.
[c] Differs significantly from the control group, $P<0.05$.
[d] Differs significantly from the animals lesioned at postnatal day 10, $P<0.05$.

3.5. Retrograde tracing

In order for a dye injection to be considered successful, three conditions were required: (a) labeled cells were present in the cortex; (b) the site of dye injection in the spinal cord was located histologically, and (c) the dye injection was found to be largely unilateral. In total, nine injections met these criteria (two control, three P10, two P1, two adult).

The control rats had cells labeled in layer V of three cortical locations contralateral to the injections: the forelimb representations in the motor cortex; the anterior cingulate region; and Zilles' [30] parietal area 2 (Fig. 5). In some rats there was also label ipsilateral to the injection, particularly in the forelimb area, although this label was light compared to the contralateral side. Rats with lesions in adulthood or on day 10 had essentially identical patterns of labeling, although there was obviously no label in the damaged area. There was little ipsilateral label visible. Thus, the total labeled area was reduced in these animals. In contrast, the rats with P1 lesions had an expanded area of labeling that included most of the parietal cortex and little of the remaining motor cortex. The extent of expansion of labeling was proportional to the lesion extent: the larger the lesion, the larger the field of corticospinal projections. The lesion shown in Fig. 5 is actually the largest lesion and this animal had the most extensive area of corticospinal projections. The labeled cells were not only more diffusely located in the P1 rats, but the labeled cells also occupied a more restricted band in layer V, as illustrated in Fig. 6.

4. Behavioral results

4.1. Reaching task

Bilateral motor cortex lesions at any age had an adverse effect on the animals' ability to reach for food in the free choice condition. A summary of the reaching performance with the preferred forelimb is shown in Fig. 7.

If an animal did not learn to reach, it was excluded from the ANOVA. One out of 17 control animals, 0 of 7 postnatal day 10, 5 of 11 postnatal day 1, and 6 of 10 adult lesioned animals did not reach using either forelimb, even with daily training for 4 months. The mean hit percent for the successfully reaching animals was as follows: control, 68%; P10, 38%; P1, 24%; and, adult lesion, 24%. A one-way ANOVA on the reaching hit percent for these

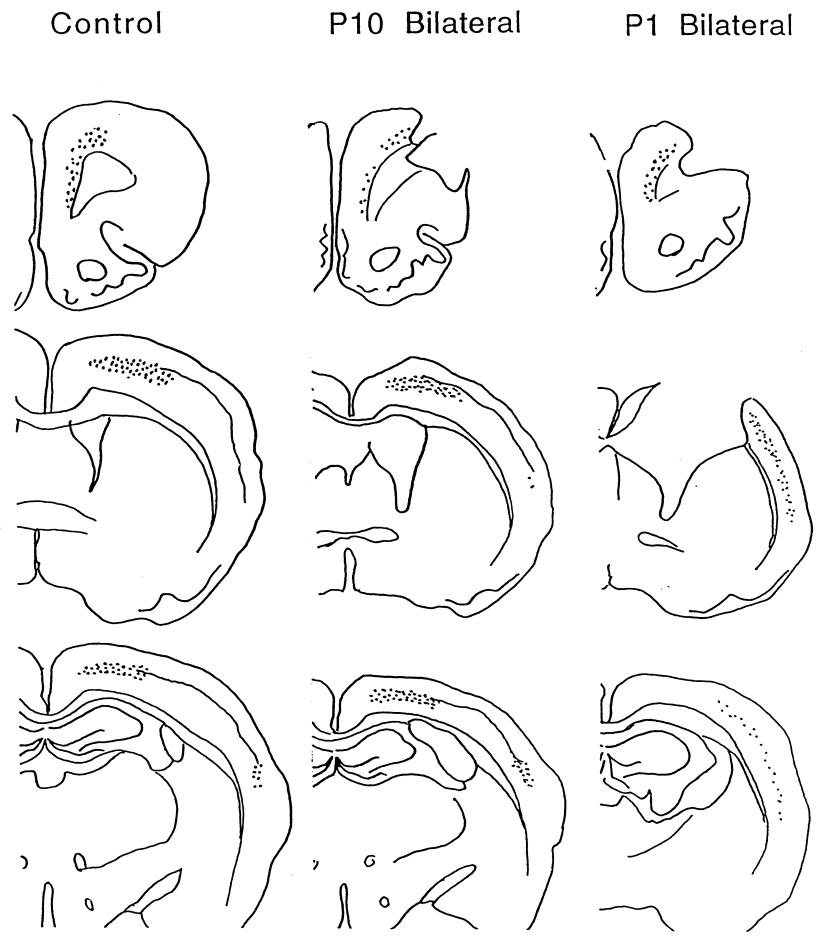

Fig. 5. Illustrations of the pattern of retrograde labeling in the cortex of rats receiving unilateral injections of True Blue in the spinal cord. The pattern of label is similar in the control and P10 rats (as well as the adult lesion rats that are not illustrated) but there is a marked expansion of the field of cortical projection neurons in the P1 group. Each dot represents a retrogradely labeled neuron.

animals showed a significant effect of the lesions, $F(3,28)=30.7$, $P<0.0001$, with the post hoc tests revealing significantly lower hit percents for all the lesioned animals compared to the control animals and a difference between the P10 and the adult and P1 groups.

An alternative way to assess the effects of the lesions on reaching is to include all the animals in the data set and to assign a hit percent of 0% to those who failed to reach criterion. The new mean hit percent for each of the groups is as follows: control, 63%; P10, 38% (no change), P1, 13%, and adult lesion, 10%.

4.2. Beam walking

When the rats were initially placed upon the beam they made many placing errors but within a few trials they ran across with very few misplaced steps. There was sex difference in performance with males making more slips than the females. Both male and female rats with motor cortex lesions at postnatal day 1 made many more placing errors while traversing the beam (see Fig. 8); male rats with adult lesions were also impaired.

A two-way ANOVA on the total number of slips revealed a significant main effect of lesion group, $F(3,35)=8.3$, $P=0.0003$, and sex, $F(1,35)=4.9$, $P=0.034$, but no interaction, $F(3,35)=1.6$, $P=0.20$. A follow-up one-way ANOVA showed no significant overall effect of the lesion group for females, $F(3,20)=2.0$, $P=0.142$, but the post hoc test showed significantly more slips by the P1 lesion group compared to the controls (see Fig. 8). For the males, both the P1 and adult lesion groups had significantly more slips than both the control and P10 animals, $F(3,15)=7.1$, $P=0.004$.

4.3. Tongue extension

In previous studies (e.g., Ref. [17]), rats with bilateral motor cortex lesions showed significant differences from control animals in their ability to extend their tongues to lick off food. This study found that rats with P1 and adult

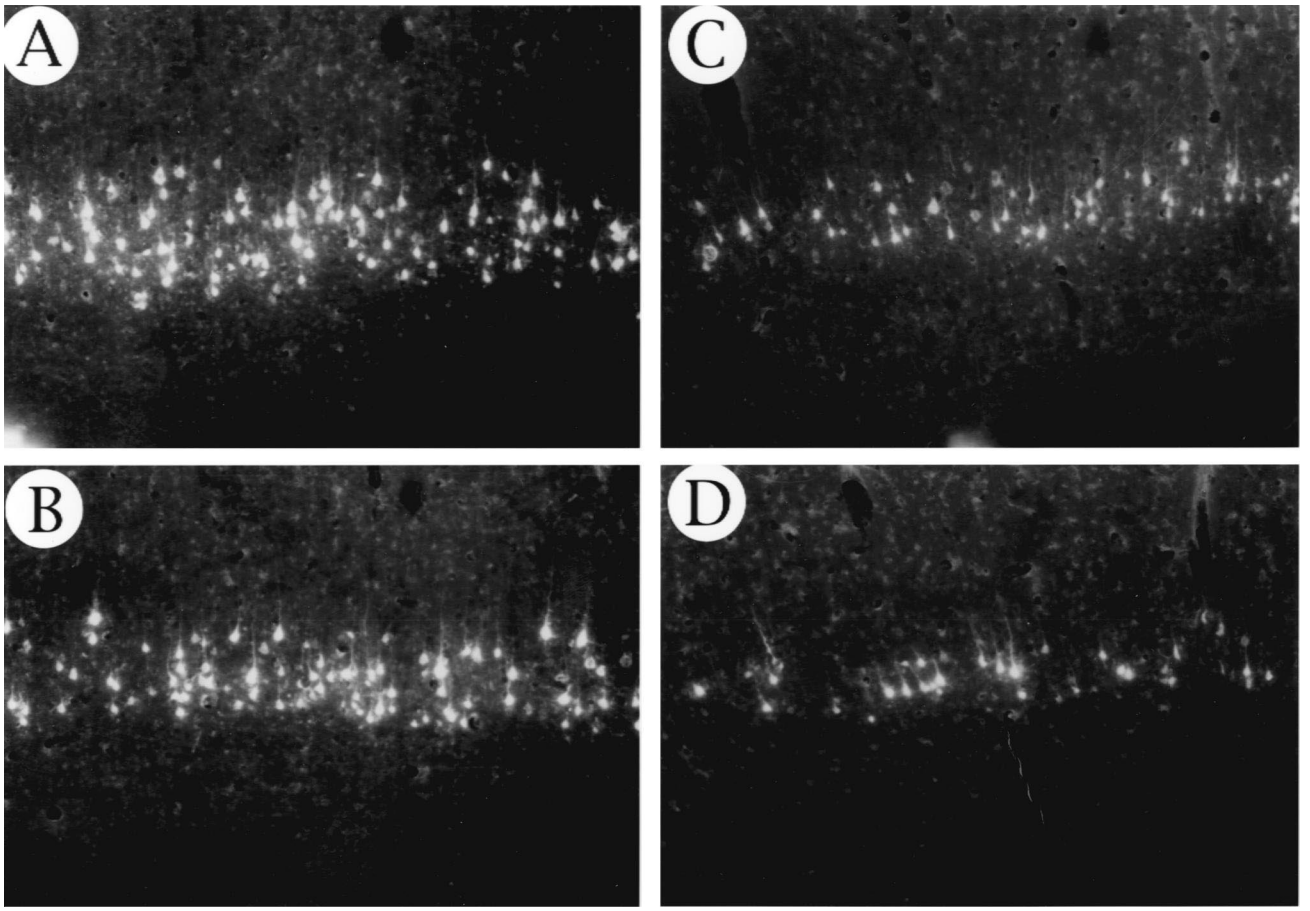

Fig. 6. Photomicrograph (250×) showing examples of the layer V retrogradely labeled neurons from two control (A, B) and two P1 lesion (C, D) rats. The layer of projection cells is distinctly narrower in the P1 brains.

lesions were impaired at tongue extension compared to control animals. The P1 animals were also impaired in comparison to the P10 rats.

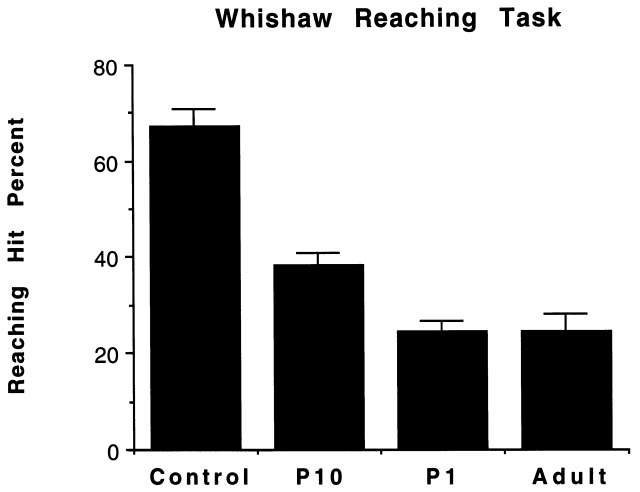

Fig. 7. Summary of reaching performance. All motor cortex lesion groups were impaired but the P10 rats were significantly less impaired than the other two groups.

One-way ANOVAs revealed a significant difference based on lesion group for both the average tongue extension measure, $F(3,39)=8.1$, $P=0.0003$, and the longest tongue extension measure, $F(3,39)=5.9$, $P=0.002$. The pattern of results were similar for the two measures in that

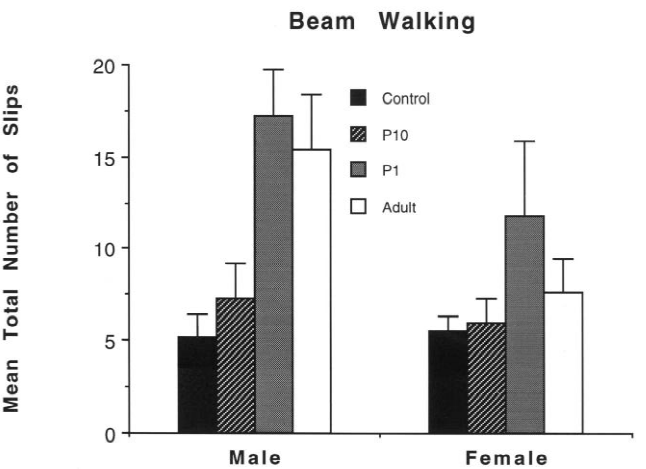

Fig. 8. Summary of total foot faults by rats on the beam-traversing task. The P1 and adult groups differed from the other groups.

Table 3
Summary of tongue extension and locomotor activity[a]

Group[b]	Behavioral task		
	Tongue extension, average	Tongue extension, longest	Locomotor activity, total
Control	11.0±0.36	12.1±0.34	457.4±25.8
P10 lesion	9.8±0.99	11.7±1.26	424.3±42.8
P1 lesion	7.7±0.65[c,d]	9.0±0.68[c,d]	736.4±143.3[c,d,e]
Adult lesion	8.1±0.72[c]	9.3±0.87[c,d]	313.9±25.9

[a] Note: numbers refer the mean±S.E.M. The unit of measure for tongue extension is the distance in mm, and for locomotor activity it is the total number of beam crossings.
[b] Sample size for each group: control $n=17$; postnatal day 10 lesion, $n=6$; postnatal day 1 lesion, $n=10$; adult lesion, $n=10$.
[c] Differs significantly from the control group, $P<0.05$.
[d] Differs significantly from the postnatal day 10 lesion group, $P<0.05$.
[e] Differs significantly from the adult lesion group, $P<0.05$.

both the P1 and adult animals did worse than the controls and the P10 animals (see Table 3).

4.4. Locomotor activity

Bilateral lesions at postnatal day 1 significantly increased the locomotor activity in comparison to all of the other groups (see Table 3). The ANOVA showed a significant effect for lesion group, $F(3,39)=5.8$, $P=0.002$.

4.5. Morris water task

The control rats in this study performed much like those described in detail elsewhere [27]. When initially placed in the milk tank, the normal control rats swam over a wide area until they accidentally bumped into the hidden platform. Performance improved rapidly on successive trials until it reached asymptote around 5 s by trial block 5 (20 trials).

Rats with bilateral motor cortex lesions on postnatal day 1 showed a marked impairment at the task over the 10 trial blocks compared to all other groups (see Fig. 9). Rats with adult lesions showed some initial impairment on the latency measure and an impairment on the total number of errors. There was no obvious motor impairment in swimming behavior, nor in swimming speed, although the latter was not explicitly measured. In any event, there is little reason to suspect that the deficit in water task learning was due to a motor difficulty. Rather, the error measure indicates that in contrast to the control animals, the P1 animals did not learn to swim directly to the platform. Inspection of the swim paths indicated that they learned a looping strategy whereby they swam parallel to the tank wall with a trajectory that insured that they would eventually hit the platform. The mean errors (±S.E.) were as follows: control, 5.1±0.5; adult lesion, 8.1±1.0; P10 lesion, 4.2±0.9; and, P1 lesion, 18.7±4.

A two-factor, repeated measures (trial) ANOVA revealed a significant main effect for lesion group, $F(3,39)=12.0$, $P<0.0001$, a significant main effect over trials,

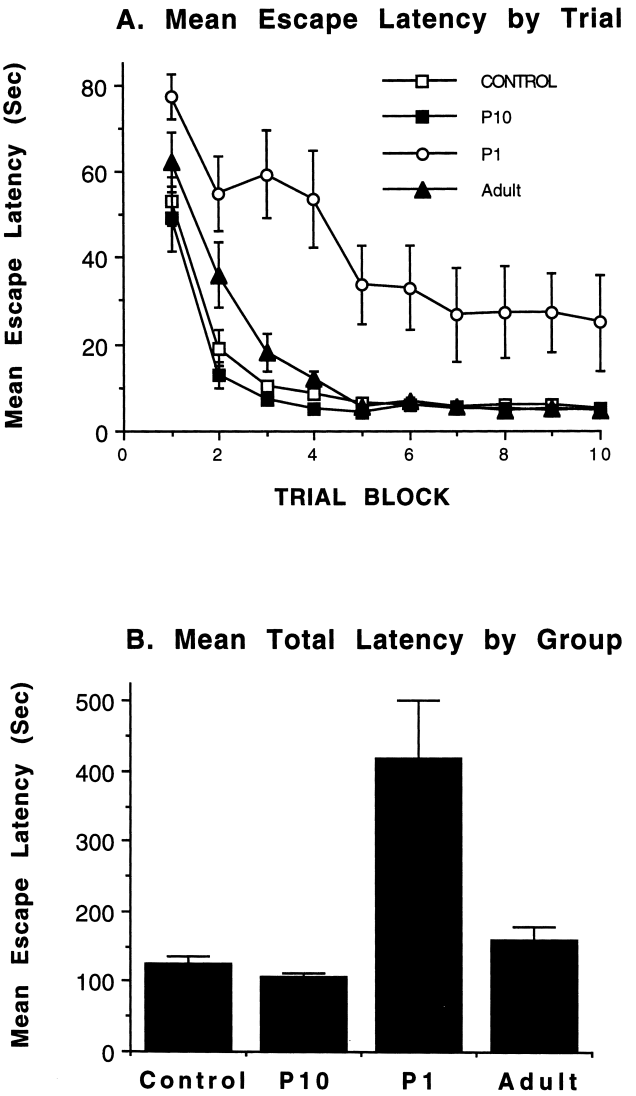

Fig. 9. Summary of the Morris water task performance. The top graph illustrates the escape latency across the 10 trial blocks. The bottom graph summarizes the total escape latency summarized over the 10 trial blocks.

$F(9,27)=82.7$, $P<0.0001$, and a significant interaction, $F(27,351)=2.72$, $P<0.0001$ using mean escape latency per trial. The interaction is primarily the result of the atypical learning curve of the P1 animals. As a follow-up, one-way ANOVAs were conducted at each trial block. The P1 group had significantly longer latency than all of the other groups from trial block 2 on (see Fig. 9) and never did learn the task. Post hoc tests on the mean total latency confirmed this effect: P1 animals had longer latencies than all of the other groups.

A two-factor, repeated measures ANOVA on the mean number of errors revealed a similar pattern as the latency measure with significant main effects of lesion group, $F(3,38)=6.0$, $P=0.0329$, and trials, $F(9,27)=15.83$, $P=0.0001$. The interaction, however, did not quite reach significance, $F(27,342)=1.49$, $P=0.059$. Post hoc tests on the mean total number of errors showed the P1 group made significantly more errors overall than all of the other groups and that the adult lesion group made more errors than the P10 group who had the best performance on this measure.

5. Discussion

The results of this study suggest that recovery from bilateral motor cortex injury is age-dependent. Like injury elsewhere in the rat cortex, injury to the motor cortex around 10 days of age is associated with a better functional outcome than earlier injury or injury in adulthood. Thus, it is not simply that 'earlier is better', a view that is sometimes referred to as the 'Kennard Principle', but rather that 'certain times are better'. This idea is consistent with the speculations of Villablanca et al. [29] that there might be 'critical periods' during embryonic development that allow enhanced recovery after injury. In the rat this period appears to be around 10 days of age.

Rats with extensive bilateral motor cortex lesions in adulthood show poor recovery on a wide variety of skilled motor behaviors including skilled forelimb reaching, food manipulation, tongue protrusion, claw cutting, and beam traversing (e.g., current study; [17,18,34,35]). These deficits are largely restricted to the motor domain, however, as rats with bilateral adult motor cortex lesions perform as well as control animals on various tests of cognitive function such as the radial arm maze and the Morris water task (e.g., current study; [17]). Rats with bilateral lesions on postnatal days 1–4 also display severe motor deficits in adulthood but, in addition, these animals display more generalized deficits in cognitive functioning (e.g., current study; [17]), as well as hyperactivity. This hyperactivity response is also found in animals with prenatal or P1 medial frontal lesions and may reflect some nonspecific effect of perinatal frontal cortex injury [17,19]. In contrast to the effects of bilateral perinatal and adult motor cortex lesions, rats with similar lesions on postnatal day 10 show a selective deficit on skilled reaching, and this deficit is not as severe as that of the younger or older animals (current study).

The privileged outcome after cortical injury on postnatal day 10 versus the extremely poor outcome after similar lesions on postnatal day 1 is consistent with similar findings after damage to the medial frontal cortex, posterior parietal cortex, occipital cortex, and cingulate cortex at these two ages (for a review, see Ref. [11]). In each of these cases animals with P10 lesions have smaller brains and thinner remaining neocortex, yet they show considerable recovery of function and do not show the devastating sequelae of the lesions on P1. For example, in the current study the P10 brains weighed about 85% of control brains and the cortex was about 12% thinner. Nonetheless, the animals had remarkably selective impairments relative to adult operates whose brains weighed about 95% of control and whose posterior cortex did not differ from control. The good functional outcome of the P10 animals is presumably related in some way to the reorganization of neural circuits but the nature of this reorganization remains obscure. Rats with P10 motor lesions did not show abnormal patterns of corticospinal connectivity, a result that is parallel with our studies of cortical connectivity in rats with large P10 lesions to the medial frontal and motor cortex [16]. This absence of corticospinal reorganization contrasts with the effects of P1 lesions in which there are significant modifications of corticospinal and other cortical–brainstem projections (current study; [16]). Rats with P10 lesions also did not show compensatory changes in the dendritic arborization or spine density of cortical pyramidal neurons in adjacent parietal cortex. We cannot rule out the possibility that there may have been changes in dendritic arborization in other cortical regions, especially the anterior cingulate regions that have corticospinal projection neurons (e.g., Ref. [13]). We must point out, however, that in the current study the animals were trained extensively in the skilled reaching task. It is known that such training can increase dendritic arborization in motor cortex neurons (e.g., Ref. [38]). It is not known if such training influences parietal cortex neurons in normal animals. It is possible, however, that such training does affect parietal neurons. This leads to two interesting possibilities. First, it is possible that the neurons in the control brains have changed in response to the training and thus our postmortem analysis in these animals obscures possible lesion-induced compensatory changes. Second, it is also possible that the reason we found selective increases in the dendritic arborization and spine density of parietal neurons in the P1 and adult operates, but not in the P10 operates, is somehow related to an interaction of lesion and experience. These speculations can only be addressed by future studies in which both trained and untrained animals are examined.

The widespread anomalous corticospinal projections after the P1 lesions is consistent with previous studies showing extensive changes in the corticospinal projection

field of rats with unilateral cortex lesions (e.g., Refs. [5,33]). It is generally thought that such anomalous connections reflect a change in the selective elimination of exuberant axon projections [24] (see O'Leary [23] for a review). Thus, the anomalous corticospinal projections are conceptually similar to the anomalous projections of colossal neurons in cats with natural [25] or surgically-induced strabismus (e.g., Ref. [1]), or in experiments in which there are surgical manipulations of thalamocortical input during development (e.g., Refs. [4,33]).

The previous studies showing anomalous motor projections had suggested that this expanded corticospinal projection field was responsible for enhanced recovery of motor behaviors after unilateral perinatal motor cortex lesions but this is not likely in the current study. In fact, in spite of an increase in corticospinal projection fields in the P1 rats, there was not only no recovery of function on the motor tasks but there was also a large impairment on a cognitive task. This latter outcome implies that the anomalous corticospinal projection field may have been disruptive to the operations of the usual functions of the invaded regions, which is what Teuber [28] referred to as crowding. One prediction from this hypothesis is that there should be an anomalously located motor map located in the posterior cortex in the region of the expanded corticospinal projections and we have recently found this to be the case [9]. There is, of course, one other explanation for the worsened outcome after P1 lesions and that is that these animals may have had larger, or even different, lesions than the P10 animals. It seems likely that some of the P1 animals did, in fact, have larger lesions than the P10 animals. For example, 60% of the P1 rats had unilateral damage to the anterior cingulate region whereas only 30% of the P10 rats did. However, even when these animals are excluded from the analysis the difference between the outcome of the P1 and P10 lesions is maintained. Furthermore, the cingulate damage was always unilateral and unilateral anterior cingulate lesions produce virtually no difference on the Morris water maze performance, even when they are performed on the day of birth [21]. It thus seems unlikely that the spatial learning deficit can be ascribed to the cingulate damage. We note too, that the brain weights of the P1 and P10 operates were comparable, suggesting that the lesions were not wildly discrepant in size. We cannot be certain that exactly the same neurons were removed at the two ages, however, because neural migration is not complete at P1 so we may have either removed or hindered the migrational route of neurons destined for cortical regions beyond the motor cortex.

In conclusion, the results of the present study suggest that although there are wide ranging anatomical changes following motor cortex injury at all ages, rats with P10 lesions appeared to show better behavioral recovery. The puzzle posed by the current study, however, is just what the changes in brain morphology might be that support this privileged functional outcome.

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References

[1] N.E. Berman, B.R. Payne, Alterations in connections of the corpus callosum following convergent and divergent strabismus, Brain Res. 274 (1983) 201–212.
[2] A.J. Castro, Ipsilateral corticospinal projections after large lesions of the cerebral hemisphere in neonatal rats, Exp. Neurol. 46 (1975) 1–8.
[3] A.J. Castro, Limb preference after lesions of the cerebral hemisphere in adult and neonatal rats, Physiol. Behav. 18 (1977) 605–608.
[4] F. Chang, J.G. Steedman, R.D. Lund, The lamination and connectivity of embryonic cerebral cortex transplanted into newborn rat cortex, J. Comp. Neurol. 244 (1986) 401–411.
[5] C.J. D'Amato, S.P. Hicks, Normal development and post-traumatic plasticity of corticospinal neurons in rats, Exp. Neurol. 60 (1978) 557–569.
[6] R. Gibb, B. Kolb, A method for Golgi-Cox staining of Vibratome cut tissue, J. Neurosci. Methods 79 (1998) 1–4.
[7] S.P. Hicks, C.J. D'Amato, Motor-sensory cortex–corticospinal system and developing locomotion and placing in rats, Am. J. Anat. 143 (1975) 1–42.
[8] G. Kartje-Tillotson, E.J. Neafsey, A.J. Castro, Electrophysiological analysis of motor cortical plasticity after cortical lesions in newborn rat, Brain Res. 332 (1986) 103–111.
[9] J.A. Kleim, R.C. Ryder, A. Witt-Lajeunesse, P.M. VandenBerg, B. Kolb, Emergence of movement representations within adult parietal cortex following neonatal frontal cortex damage, submitted for publication.
[10] B. Kolb, Recovery from early cortical damage in rats. I. Differential behavioral and anatomical effects of frontal lesions at different ages of neural maturation, Behav. Brain Res. 25 (1987) 205–220.
[11] B. Kolb, Brain Plasticity and Behavior, Lawrence Erlbaum, Mahwah, NJ, 1995.
[12] B. Kolb, J. Cioe, D. Muirhead, Cerebral morphology and functional sparing after prenatal frontal cortex lesions in rats, Behav. Brain Res. 91 (1998) 143–155.
[13] B. Kolb, S. Cote, A. Ribeiro-da-Silva, A.C. Cuello, NGF stimulates recovery of function and dendritic growth after unilateral motor cortex lesions in rats, Neuroscience 76 (1977) 1139–1151.
[14] B. Kolb, R. Gibb, Environmental enrichment and cortical injury: behavioral and anatomical consequences of frontal cortex lesions in rats, Cereb. Cortex 1 (1991) 189–198.
[15] B. Kolb, R. Gibb, Sparing of function after neonatal frontal lesions correlates with increased cortical dendritic branching: a possible mechanism for the Kennard effect, Behav. Brain Res. 43 (1991) 51–56.
[16] B. Kolb, R. Gibb, D. van der Kooy, Cortical structure and connectivity after neonatal frontal cortex lesions in rats, Brain Res. 645 (1994) 85–97.
[17] B. Kolb, C. Holmes, Neonatal motor cortex lesions in the rat: absence of sparing of motor behaviors and impaired spatial learning concurrent with abnormal cerebral morphogenesis, Behav. Neurosci. 97 (1983) 697–709.
[18] B. Kolb, I.Q. Whishaw, Dissociation of the contributions of the prefrontal, motor, and parietal cortex to the control of movement in

the rat: an experimental review, Can. J. Psychol. 37 (1983) 211–232.
[19] B. Kolb, I.Q. Whishaw, Neonatal frontal lesions in the rat: sparing of learned but not species-typical behavior in the presence of reduced brain weight and cortical thickness, J. Comp. Physiol. Psychol. 95 (1981) 863–879.
[20] B. Kolb, I.Q. Whishaw, Plasticity in the neocortex: mechanisms underlying recovery from early brain damage, Prog. Neurobiol. 32 (1989) 235–276.
[21] B. Kolb, J. Zaborowski, I.Q. Whishaw, Recovery from early cortical damage in rats: 5. Unilateral lesions have different behavioral and anatomical effects than bilateral lesions, Psychobiology 17 (1989) 363–369.
[22] R.G.M. Morris, Spatial localization does not require the presence of local cues, Learn. Motivat. 12 (1981) 239–260.
[23] D.D. O'Leary, Development of connectional diversity and specificity in the mammalian brain by the pruning of collateral projections, Curr. Opin. Neurosci. 2 (1992) 70–77.
[24] D.D. O'Leary, B.B. Stanfield, Selective elimination of axons extended by developing cortical neurons is dependent on regional locale: experiments utilizing fetal cortical transplants, J. Neurosci. 9 (1989) 607–617.
[25] C.J. Shatz, Anatomy of interhemispheric connections in the visual system of Boston Siamese and ordinary cats, J. Comp. Neurol. 173 (1977) 497–518.
[26] D.A. Sholl, The Organization of the Cerebral Cortex, Methuen, London, 1956.
[27] R.J. Sutherland, I.Q. Whishaw, B. Kolb, A behavioural analysis of spatial localization following electrolytic, kainate-, or colchicine-induced damage to the hippocampal formation in the rat, Behav. Brain Res. 7 (1983) 133–153.
[28] H.-L. Teuber, Recovery of function after brain injury in man, in: Outcome of Severe Damage to the Central Nervous System (CIBA Foundation Symposium), Elsevier, Amsterdam, 1975.
[29] J.R. Villablanca, D.A. Hovda, G.F. Jackson, C. Infante, Neurological and behavioral effects of a unilateral frontal cortical lesion in fetal kittens, II. Visual system tests, and proposing a 'critical period' for lesion effects, Behav. Brain Res. 57 (1993) 79–92.
[30] I.Q. Whishaw, The decorticate rat, in: B. Kolb, R. Tees (Eds.), The Cerebral Cortex of the Rat, The MIT Press, London, 1990, pp. 239–267.
[31] I.Q. Whishaw, F. Haun, B. Kolb, Analysis of behavior in laboratory rodents, in: U. Windhorst, H. Johansson (Eds.), Modern Techniques in Neuroscience Research, Springer-Verlag, New York, 1999.
[32] I.Q. Whishaw, D. Fiorino, G. Mittleman, E. Casteneda, Do forebrain structures compete for behavioural expression? Evidence from amphetamine-induced behavior, microdialysis, and caudate-accumbens lesions in medial frontal cortex damaged rats, Brain Res. 576 (1992) 1–11.
[33] I.Q. Whishaw, B. Kolb, 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.
[34] I.Q. Whishaw, B. Kolb, Tongue protrusion mediated by spared anterior ventrolateral neocortex in neonatally decorticate rats: Behavioural support for the neurogenetic hypothesis, Behav. Brain Res. 32 (1989) 101–113.
[35] I.Q. Whishaw, B. Kolb, R.J. Sutherland, J. Becker, Cortical control of claw cutting in the rat, Behav. Neurosci. 97 (1983) 370–380.
[36] I.Q. Whishaw, R.B. O'Connor, S.B. Dunnett, The contributions of the motor cortex, nigrostriatal dopamine and caudate-putamen to skilled forelimb use in the rat, Brain 109 (1986) 805–843.
[37] I.Q. Whishaw, S.M. Pellis, B.P. Gorny, V.C. Pellis, The impairments in reaching and the movements of compensation in rats with motor cortex lesions: an endpoint, videorecording, and movement notation analysis, Behav. Brain Res. 42 (1991) 77–91.
[38] G.S. Withers, W.T. Greenough, Reaching training selectively alters dendritic branching in subpopulations of layer II–III pyramids in rat motor-somatosensory forelimb cortex, Neuropsychologia 27 (1989) 61–69.
[39] K. Zilles, The Cortex of the Rat, Springer-Verlag, Berlin, 1985.