ELSEVIER

Behavioural Brain Research 79 (1996) 1–13

BEHAVIOURAL BRAIN RESEARCH

Research report

Recovery from early cortical damage in rats, VII. Comparison of the behavioural and anatomical effects of medial prefrontal lesions at different ages of neural maturation

Bryan Kolb [a,*], Bruce Petrie [b], Jan Cioe [a,c]

[a] *Department of Psychology, University of Lethbridge, Lethbridge, AB T1K 3M4, Canada*
[b] *New Mexico Tech, Socorro, NM, USA*
[c] *Okanagan University College, Kelowna, BC, Canada*

Received 13 September 1993; revised 30 July 1995; accepted 31 October 1995

Abstract

Rats with removal of the medial prefrontal (mPFC) cortex at days 3, 6, 9, 15, or 30 were compared behaviourally and anatomically to littermate controls. In contrast to adult operates, mPFC lesions at all young ages led to the development of an abnormally thin cortical mantle. In addition, although there was an obvious cavity in brains examined in the early postoperative period, the brains of animals with lesions at day 9 or 15 had no lesion cavity in adulthood as part of the cortex appeared to regrow. The differential anatomical consequences of the lesions at days 9 and 15 was correlated with a differential behavioural outcome as well. Thus although rats in all young lesion groups showed a milder behavioural syndrome than rats with comparable lesions in adulthood, the functional outcome was best for animals with lesions at 9 days of age.

Keywords: Recovery of function; Prefrontal cortex; Kennard effect; Development

1. Introduction

In the course of examining the nature of behavioural sparing or recovery after damage to the frontal, motor, or parietal cortex of rats, we have found that damage at 1–5 days of age results in a small brain and miserable behavioural outcome. Indeed, neonatal animals with such lesions are often impaired at tasks that adults with similar lesions perform as well as normal control animals. In contrast, damage at 7–10 days of age allows significant sparing of function such that on some behavioural measures, which are normally adversely affected by similar lesions in adulthood, there is virtually normal behaviour in the animals [12,17–19,21–23,27,28]. Thus, it appears that there is something special about the 7–10 day old rat brain insofar as it is able to compensate for extensive damage to frontal or parietal cortex and can support surprisingly normal function. In contrast, there is also something special about the newborn rat's brain as cortical injury appears to have more severe effects than similar injury in adulthood. The question we addressed in the current study was whether the age-dependent effects of early cortical lesions in rats were a general phenomenon or were only observed after the relatively extensive removals made in our previous studies.

It is generally assumed that recovery is better after restricted lesions, but there has been no systematic study of the functional and anatomical effects of small cortical lesions at different times during development. Thus, in the present study we made lesions that were restricted to the midline prefrontal cortex (mPFC). Animals were given lesions at postnatal day 3, 6, 9, 15, or 30. They were tested in several behavioural tasks at which adult rats with comparable mPFC lesions are known to be impaired. These include the Morris water task [25], forepaw reaching [44], food hoarding [11], claw cutting [43], and swimming [26]. In addition, we measured adult body weight since we have consistently found male rats with large mPFC lesions at days 1–5 to be significantly smaller than older operates [12] at adulthood.

* Corresponding author. Fax: +1 (403) 329-2555;
E-mail: kolb@hg.uleth.ca

PII S0166-4328(95)00254-5

We asked several questions. First, are the deficits observed after small lesions comparable to those of animals with the complete removal of the PFC at the same age? Second, what effect do restricted mPFC lesions have upon brain development as measured by brain weight and cortical thickness? Third, is there still an age-related difference in the magnitude of behavioural and anatomical changes after such small lesions?

2. Materials and methods

2.1. Subjects

The study used 68 rats, derived from the Charles River Long–Evans strains, which were divided into six groups: control (9 M, 8 F), and frontal lesion performed at postnatal day 3 (P3; 5 M, 2 F), postnatal day 6 (P6; 5 M, 3 F), postnatal day 9 (P9; 3 M, 5 F), postnatal day 15 (P15; 5 M, 3 F), as well as postnatal day 30 (P30; 5 M, 5 F). The remaining ten animals were given lesions on postnatal day 9 and then sacrificed immediately after surgery or at various intervals over the following week. The surviving animals were housed individually in stainless steel hanging cages and maintained on ad lib water and 12 h/12 h light/dark schedule throughout the experiments. The animals were on ad lib food throughout except during the food reaching task. All frontal-operated rats were tested in all behavioural tasks in the order described below. The control rats were all tested in the water task but for the other tasks only 8 rats (4 M, 4 F) were studied. Testing began when the animals were about 120 days old. It was completed when the animals were about 200 days old.

2.2. Surgical and anatomical procedures

2.2.1. Juveniles (P30)

The animals were anesthetized with sodium pentobarbital (females, 45 mg/kg; males, 60 mg/kg). The frontal neocortex was exposed by removing the skull with rongeurs from the bregmoidal junction anteriorly to the frontal bone suture, and laterally about 2 mm from the midline on each side. Care was taken to leave the dorsal sagittal sinus and the anterior cerebral artery intact. After retraction of the dura, the mPFC neocortex was removed by aspiration with the aid of a surgical microscope. Following hemostasis the scalp wound was closed with wound clips.

2.2.2. P15 rats

The animals were given 50 mg/kg sodium pentobarbital that had been diluted to 32 mg/cm^3. It was our experience that it was very difficult to anesthetize animals appropriately unless the anesthetic was diluted. Even so, animals were very slow to recover from the anesthetic.

2.2.3. Infants (P3, P6, P9)

The 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 frontal bone was removed by cutting it with iris scissors, and frontal decortication was achieved as in the adult rats. One difficulty was that the size of the glass pipette was the same for all infants and so was larger relative to brain size for the young animals compared to the older ones. Thus, it is likely that owing to the small size of the brain relative to the pipette, the lateral extent of the lesions may have been greater in the P3, and perhaps P6 animals, than in the older animals. The animals were 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 with at least 2 controls coming from litters operated at each age.

2.3. Anatomical procedures

After all behavioural testing was complete the rats were weighed, given an overdose of sodium pentobarbital and intracardially perfused with 0.9% saline followed by 10% formalin in saline. The brains were removed, weighed, and placed in 30% sucrose formalin for at least 48 h before being cut frozen at 40 μm; every tenth section was saved and stained with Cresyl violet for Nissl bodies. The animals were about 200 days old at the time of sacrifice.

2.4. Brain measurements

2.4.1. Brain weight

In order to estimate the loss of brain tissue, the brains were weighed immediately following removal from the skull. Before weighing, the spinal cord was cut even with the caudal edge of the cerebellum, the cerebellar paraflocculi were removed, the optic nerves were severed 1–2 mm anterior to the chiasm, the pineal gland was removed, and all remaining dura was stripped off.

2.4.2. Cortical thickness

The cortical thickness of the brains was measured by projecting the Nissl-stained sections on a Zeiss DL 2 POL petrographic projector set at a magnification of 13×. Measurements were taken with a plastic millimetre ruler. The cortical thickness was measured at an anterior, middle, and posterior plane corresponding to about 1 mm anterior to the bregma, 2 mm posterior to the bregma, and 6 mm posterior to bregma, respectively. Measurements were made at 3 different points at each of the planes in each hemisphere, as illustrated in Kolb [12].

2.4.3. Cross-sectional area

In order to estimate the area of lesion and area of remaining cortex, the cross-sectional area was measured at the last section anterior to first the appearance of grey matter in the striatum. The slides were placed on a light table and viewed with a video camera hooked to a computer with NIH IMAGE software. The total area of the cerebral hemisphere above the olfactory bulb was calculated, as was the remaining midline tissue dorsal to the tanea tecta.

2.5. Behavioural methods

2.5.1. Morris water maze

The method followed in this test is virtually identical to that used by Sutherland, Whishaw, and Kolb [34]. The maze consisted of a circular pool (diameter 1.5 m, height 45 cm), the inside of which was painted white and filled to a height of 25 cm with approximately 18°C water in which one litre of instant powdered milk was dissolved. A clear Plexiglas platform (11 × 12 cm) was present inside the pool; its top surface was 1 cm below the surface of the water, and thus the platform was invisible to a viewer inside the pool.

The trial consisted of placing a rat by hand into the water, facing the wall of the pool, at one of 4 starting locations (north, south, east or west) around the pool's perimeter. Within each block of 4 trials, each rat started at the 4 starting locations, but the sequence of locations was randomly selected.

The behavioural testing was conducted on 6 consecutive days, with each rat receiving 8 trials per day. 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 swimming path for each trial was recorded via a video camera mounted above the tank. A computer system was able to extract the black head of the rat from the white background of the milk and subsequently determine the angle relative to the platform toward which the rat was heading (12 cm, approximately one body length) after release (heading error). The latency to find the platform was timed by an experimenter standing by the pool's edge.

2.5.2. Forepaw reaching for food

This procedure was adapted from the method developed by Whishaw and colleagues [43]. 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 45 mg food pellets (chicken feed), 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 pellet. If it reached into the tray without touching a pellet, no attempt was scored. Animals were trained for a minimum of 10 days, by which time their performance had asymptoted.

2.5.3. Swimming

Swimming tests were conducted in a swimming pool made of fibre glass-coated wood with the exception of one of the long sides which was made of clear Plexiglas. The pool was 130 × 22 × 15 cm and was filled with 20°C water to the depth of 25 cm. At one end of the alley was a 14 cm^3 mesh platform onto which the animals could climb. A trial consisted of placing the rat at one end of the alley and allowing it to swim to the other end. Each rat received 5 trials with approximately a 2-s rest between trials.

We were particularly interested in forepaw movement by the rats, and so a rating scale of forepaw movement was used: 0, continuous paddling with the forepaws; 1, one forepaw held motionless under the chin for at least one stroke; 2, both forepaws held motionless for at least one stroke; 3, both forepaws held motionless for a number of strokes; and 4, both forepaws held motionless under the chin for most of the swim. Each rat was rated without the rat's surgical history being know to the rater.

2.5.4. Food hoarding

Food hoarding behaviour was studied in an apparatus consisting of 8 individual alleys each 150 × 20 × 18 cm, which were constructed in horizontal layers like bookshelves, the front wall being made of clear Plexiglas. Each hoarding alley was divided into two unequal chambers by a wooden partition (20 × 18 cm) containing an opening allowing the rat to move freely between the two compartments. The smaller compartment was 20 × 20 × 18 cm and contained a thin layer of wood chips, which served as bedding material. Twenty Purina rat chow pellets were scattered in the alley and the measure of hoarding behaviour was the number of pellets either moved into the nest box or piled outside the nest box at the end of 24 h.

2.5.5. Claw cutting

After perfusion, the claws of the hind paws were measured from the cuticle to the tip.

3. Anatomical results

Overall, there was a clear difference in the appearance of the lesion between brains with lesions at different ages. For the youngest and the oldest operates (P3, P6, P30) the lesion was obvious as there was nearly always a cavity (Fig. 1). In contrast, however, most of the lesions at P9 and P15 were very difficult to observe before the brain was sectioned as there was virtually no indication of a lesion on the surface of the brain. The lesion location was clear in the sectioned brain as there was a glial scar (Fig. 2). The cortical tissue ventral to the scar was abnormally thin but did have some laminar organization. In order to get a sense of the developmental difference in the appearance of a cavity versus only a scar we calculated the percentage of animals showing either a cavity or a scar (Fig. 3). This classification is not exclusive as some animals have a cavity on one side and a scar on the other, and many day 6, 15, and 30 animals have both a small cavity and a scar. Note, however, that no day 9 animals had a cavity. When we examined the brains sacrificed at different postoperative intervals over the first hours and days, we found an obvious cavity at birth but this shrunk rapidly over the first few days (Fig. 4). By 2 weeks postlesion the cavity is gone. This is not simply due to a shifting of the hemisphere into the region of lesion, but rather there appears to a be 'regrowth' of the missing region (Fig. 5).

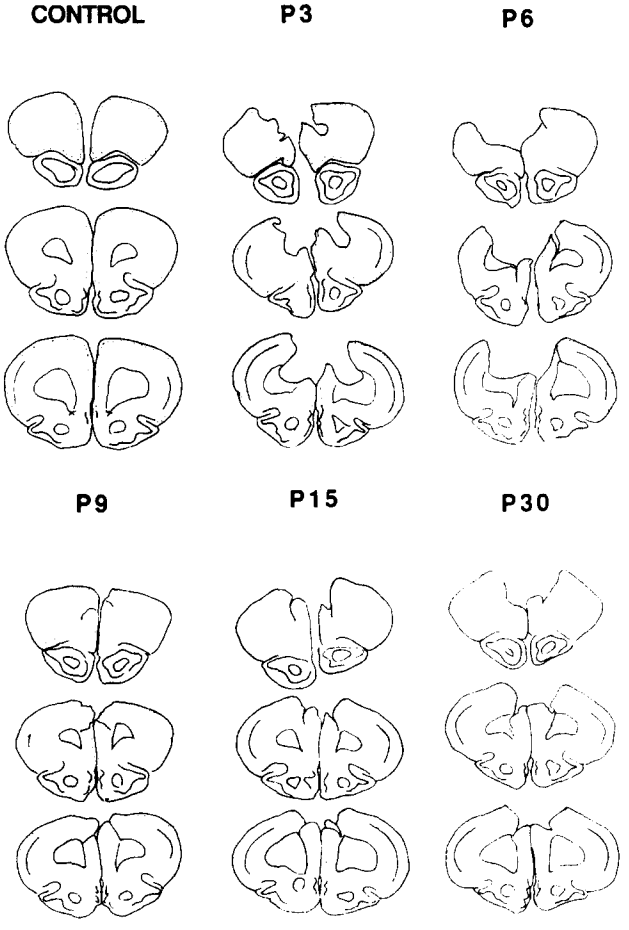

Fig. 1. Reconstructions of serial sections through the brains of rats with representative lesions at different ages. The brains of the rats with lesions on day 3, 6, and 30 show clear cavities. The brains of rats with lesions on day 9 and 15 show only a scar to indicate the boundary of the lesion. Layer IV of area Par 1 is indicated by a line in the cortex.

In view of the obvious difference in macroscopic appearance at different ages, it is difficult to be confident in the lesion comparisons at different ages. Nevertheless, it is possible to locate reliably the border of parietal cortex by the prominent layer IV (Figs. 1, 2). In all lesion cases this layer ends more dorsally than in the normal brain, and is often adjacent to the scar. Thus, even though there is no cavity, it appears that Zilles' [45] regions Fr 2, and possibly Fr 1, are damaged at all ages.

3.1. Brain and body weights

The lesions reduced brain weight in all groups, with little difference between the lesion groups (Fig. 6). Since brain weight is sexually dimorphic we did separate analyses of variance on each sex and found a significant main effect for lesion group in each analysis, (females, $F(5,29)=9.85$, $P=0.0001$; males, $F(5,32)=13.26$, $P=0.0001$). The posthoc between group comparisons (Fisher's LSD, $P<0.05$ or better) are summarized in Fig. 6, which shows that with the exception of P9 male brains, all groups differ from their respective control group. There were no differences between the lesion groups for either sex.

We have reported previously [12] that body weight is reduced in male but not female rats with large mPFC lesions at day 1 or 5. A similar result was seen here as male rats with the lesions at P3, or P6 were lighter at the time of sacrifice than the control, P9 or P15 rats.

An analysis of variance on male body weights revealed a significant main effect for lesion group ($F(5,32)=3.09$, $P=0.025$). In contrast, a similar test on the females was not significant ($F(5,29)=1.53$, $P=0.22$). Posthoc tests for the male rats are summarized in Fig. 6. The early (P3, P6) and late (P30) lesions decreased body weight compared to both the controls and the P9 lesions.

3.2. Cortical thickness

Overall, the lesions produced cortical thinning in all the groups at the anterior and middle planes but had little effect in the posterior plane (Fig. 7). The P3 brains had the thinnest cortex and the P30 had the most normal cortex.

Like brain weight, cortical thickness tends to be sexually dimorphic, so we tested for an overall sex difference in cortical thickness. It was not significant ($F(1,48)=0.40$, $P=0.53$), and so we collapsed across sex

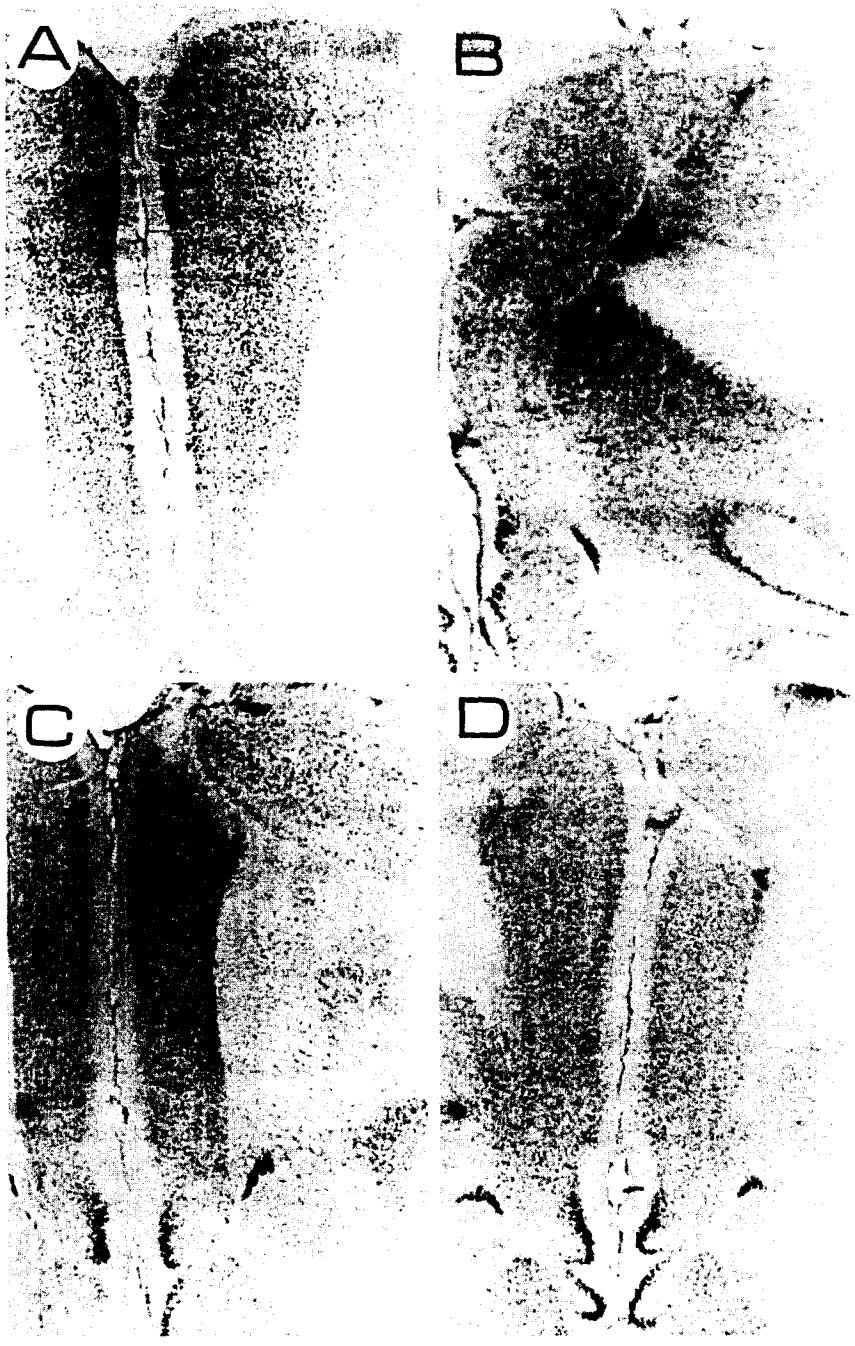

Fig. 2. Photomicrographs illustrating a 'slit' in brains that sustained a mPFC lesion. A: control, B: day 6 lesion, C and D: day 9 lesion. Note that the cortex ventral to the slit is markedly thinner than normal, especially in the more dorsal aspect. The day 6 brain is grossly deformed and appears to have two 'slits'.

for subsequent analyses. We then performed a two-factor repeated measures analysis of variance (lesion group by cortical plane), which showed a significant main effect of the lesions ($F(5,44)=8.64$, $P=0.0001$), a significant main effect of cortical plane ($F(4,176)=337.67$, $P=0.0001$), and a significant interaction between lesion and plane ($F(20,176)=2.27$, $P=0.0024$). The interaction reflected the fact that the lesion effect on cortical thickness decreased going from anterior to posterior. Posthoc tests (Fisher's LSD, $P<0.05$) at representative planes are summarized in Fig. 7.

3.3. Cross-sectional area

The total area of remaining tissue was reduced in all lesion rats relative to controls (Fig. 8). The reduction ranged from 30% in the day 6 and 9 groups to about 20% in the older groups. Analysis of variance showed a significant effect of group ($F(5,41)=28.1$, $P<0.0001$). Posthoc tests, which are summarized in Fig. 8, showed that the day 6 and 9 groups had smaller areas than the day 9, 15 and 30 groups.

Analysis of the extent of midline tissue at the time of

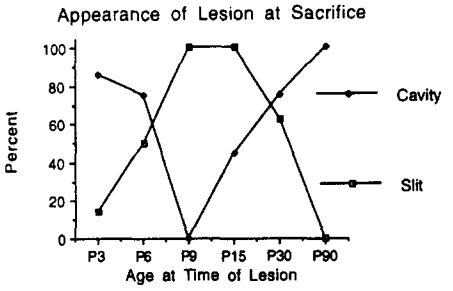

Fig. 3. Summary of the probability of the occurrence of a slit or a cavity in either hemisphere of brains at different ages. Note that lesions at day 9 or 15 virtually never leave a cavity whereas lesions at other ages normally leave a cavity. Data from adult rats are taken from previously our published studies [13].

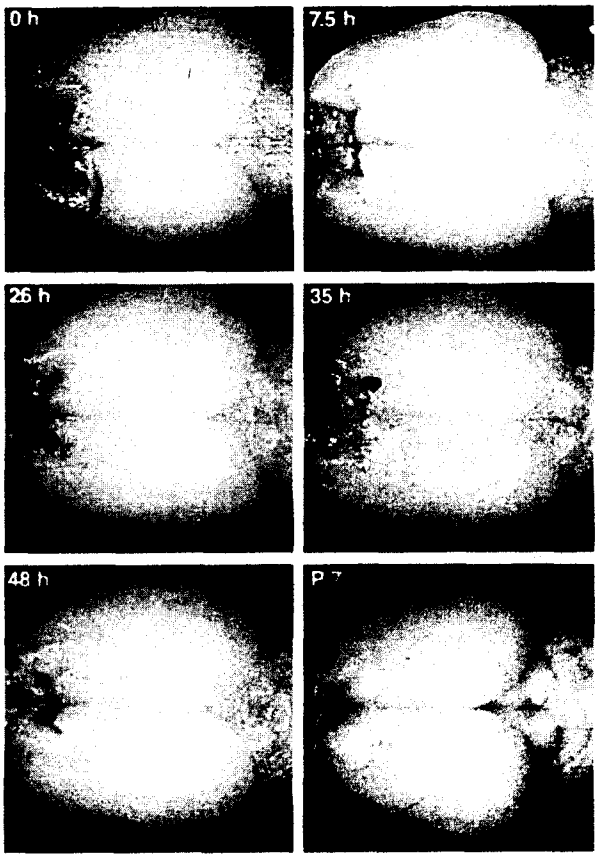

Fig. 4. Photographs of brains of rats given mPFC lesions on postnatal day 9. Note that there is a lesion cavity visible on the day of lesion but that the cavity becomes progressively smaller in the brains of rats harvested over the ensuring hours and days.

sacrifice showed that the amount of tissue varied from about 30% of control in the youngest groups to about 60% in the older groups. Thus, in spite of the apparent regrowth of the lesion region, the tissue did not completely replace the original tissue: there was still about 40% less tissue in the animals with no cavity. Analysis of variance found a significant main effect of group, ($F(5,41)=47.9$, $P<0.0001$). Posthoc tests once again showed that the two youngest groups had significantly less tissue than the older lesion groups, who had significantly less tissue than controls.

In order to determine if the shrinkage in the overall cross-sectional areas in the lesion groups was due just to the loss of midline tissue, we estimated the area of the remaining non midline tissue by subtracting the midline from the total area (Fig. 8). The results showed that the area of the remaining hemisphere was reduced in all groups relative to control. Analysis of variance showed a significant group effect, ($F(5,41)=11.1$, $P<0.0001$). Posthoc tests again showed that the youngest groups had smaller areas than the older groups, who all differed from the controls.

In sum, the cross-sectional area measurements revealed that all groups had missing midline tissue and that the lesions produced an over shrinkage of the remaining hemispheric area. Furthermore, there was an age-related effect as the day 3 and 6 groups has less midline tissue and less frontal tissue overall than the day 9, 15 and 30 groups.

4. Behavioural results

4.1. Morris water task

The control rats performed like those described in detail elsewhere [34]. When initially placed in the tank, the normal control rats traversed a wide area until they bumped into the hidden platform. Performance improved rapidly on successive trials until it reached asymptote around 5 s. By the last trial block the animals appeared to swim fairly directly to the platform. Rats with mPFC did not appear to perform much differently from control animals as they learned to find the platform as quickly as the normal controls (Fig. 9), although they were somewhat more variable in their performance and they did not appear to be quite as accurate in their orientation to the platform. We quantified two aspects of the performance on this task: (1) latency to find the platform, and (2) the angle at which the rat swam relative to the platform after it had swum about one body length (heading error).

4.1.1. Latency

Since we had previously found water maze performance to be sexually dimorphic in frontal rats [13], we included sex as a factor in our statistical analysis. Analysis of variance confirmed that the lesions did not adversely affect the latency to find the hidden platform ($F(5,46)=0.56$, $P<0.7$). There was also no sex difference ($F(1,46)=0.84$, $P<0.36$) nor interaction ($F(5,46)=0.6$, $P=0.70$).

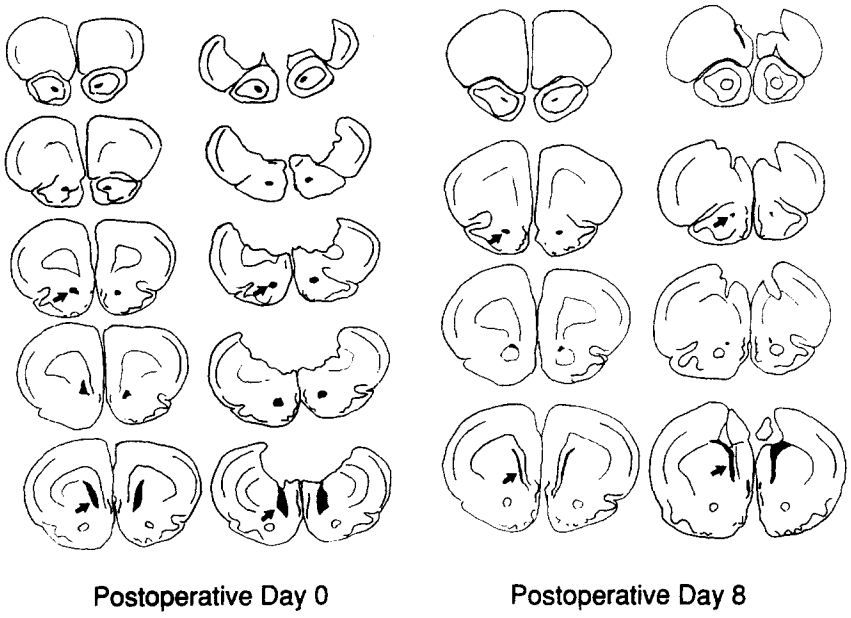

Fig. 5. Coronal sections through the lesion area of rats sacrificed immediately after surgery at PN9 (Postoperative Day 0) or 8 days after surgery (Postoperative Day 8). Unoperated control animals are on the left and the lesioned animals are on the right. Arrows indicate cell dense subventricular zone which is characteristic of young brains.

4.1.2. Heading error

When normal animals first learn the water task they swim randomly from the start point, which gives a heading error that is not different from chance (39°). Heading error improves more slowly than latency, and it is not usually until about the 4th or 5th day of training that controls differ from chance [34]. In contrast, rats with frontal lesions in adulthood usually begin with heading angles close to 90°, which reflects their tendency to hug the pool wall, rather than swimming into the open water. They never learn to orient very accurately and after 5 days of testing they typically have dropped to chance levels, reflecting their acquisition of the strategy of swimming out from the wall but after 12 cm they have not yet oriented to platform. Accordingly, we analyzed heading error statistically on the first and last day of testing.

A two-way analysis of variance (sex and group) on day 1 found a significant main effect of lesion group ($F(5,45)=5.29$, $P=0.0007$) but not of sex ($F(1,45)=0.06$, $P=0.81$), or the interaction ($F(5,45)=0.87$, $P=0.51$). The significant group effect reflected the fact that only the control animals had heading errors approaching chance ($M=48°$) while the other groups were much higher ($M=64°$ or higher), which reflects their strategy of hugging the pool wall. A two-way analysis on day 5 showed a significant main effect of group ($F(5,45)=2.7$, $P=0.03$) and, unexpectedly, also revealed a trend toward a sex effect ($F(1,45)=2.82$, $P=0.099$) and an interaction ($F(5,45)=2.17$, $P=0.07$). Subsequent closer inspection of the data by sex suggested that normal male rats, and possibly P9 male rats, differed from all others in their performance: they were the only rats that had heading errors different from chance (Fig. 9). A one-way ANOVA on the mean heading error for males showed a significant effect ($F(5,25)=10.64$, $P=0.0001$). Fig. 9B shows that the P3 and P30 males made significantly larger heading errors as compared to the male controls. Their heading angles were very high, which indicates that they were still hugging the wall when first released into the tank. The P30 males were also worse on this measure than all of the other lesioned males; P3 males did worse than all of the other lesioned groups except the P15 males (Fisher's LSD, $P<0.05$). There were no differences among the groups, however, for the females (Fig. 9C; ANOVA, $F(5,25)=0.25$, $P=0.93$). Curiously, even female control rats had poor heading angles and were worse than control males, even though they are smaller and might be expected to orient to the correct heading direction more quickly. Direct comparison of male and female rats showed that the male controls did significantly better on this measure than the female controls (Fig. 9B and 9C; $F(1,15)=5.09$, $P=0.04$).

In sum, the data from the analysis of heading error suggested that: (1) there was a sex difference with males orienting more accurately than females; and, (2) male rats with frontal lesions at P6, P9, and P15 performed differently than male rats with P3 or P30 lesions. Indeed, since the P9 and control males did not differ, it appears that the P9 males might have been the only operated group to perform the task like the male control rats.

4.2. Forepaw reaching for food

Normal rats learn to reach quickly and typically asymptote at about 50–60% accuracy. Failed reaching

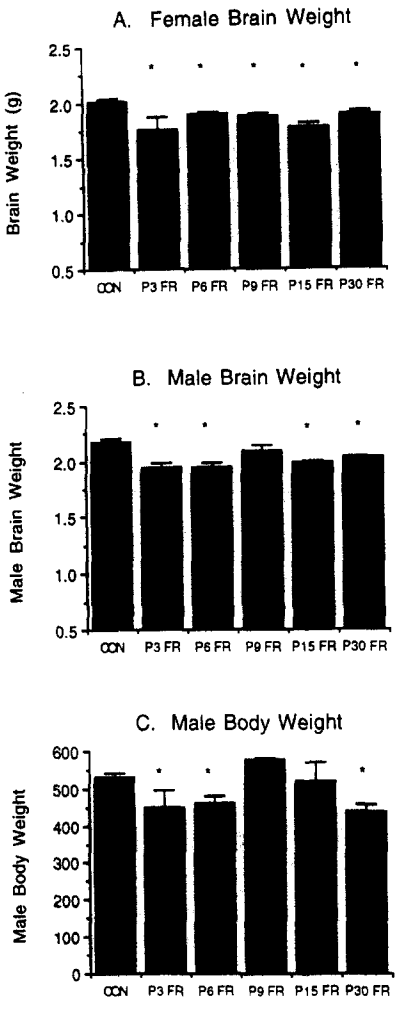

Fig. 6. Summary of the brain weight in female (A) and male (B) rats and of body weight (C) in male rats. Lesions at all ages reduce brain weight. Body weight is reduced only in male rats with lesions at day 3, 6, or 30. Asterisks indicate a statistical difference from controls $Ps < 0.05$ or better. Values are means and standard errors.

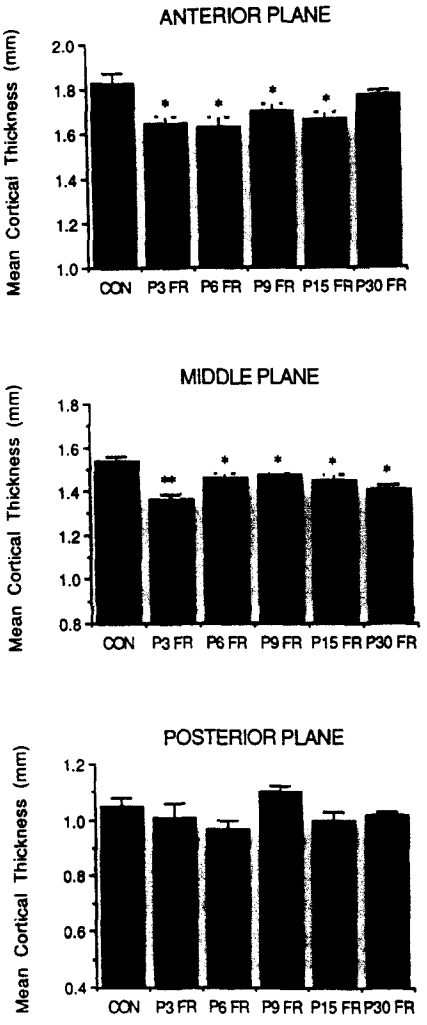

Fig. 7. Summary of cortical thickness measurements at three planes posterior to the lesions. Cortical thickness is reduced by all lesions at the anterior and middle planes. Asterisks indicate a statistical difference from controls $Ps < 0.05$ or better. Values are means and standard errors.

attempts most commonly include the inadvertent grasping of several pieces of food, which are usually dropped as the forepaw is withdrawn back to the body, as well as the occasional drop when food is grasped with both paws for eating. Rats with adult frontal lesions are less accurate and seldom score better than 20% accuracy. Their low scores reflect a larger number of errors similar to those made by normal animals, which may result from deficits in digit use or posture. With the exception of the P9 animals, all of the lesioned rats performed poorly.

After eliminating rats that did not learn to reach by the end of the training period (2 control, 1 P6, 4 P9, 3 P15), a one-way analysis of variance showed a significant effect among the groups, ($F(5,34) = 2.97$, $P = 0.02$). The posthoc tests (Fisher's LSD, $P < 0.05$) showed that the control animals did significantly better on this task than all of the lesioned animals except for the P9 rats (Fig. 10).

4.3. Swimming

All rats swam with their heads held out of the water and the forepaws held relatively motionless under the chin (forepaw inhibition). The mPFC lesions in the current study did not interfere with forepaw inhibition as all animals had inhibition ratings ranging from 3 to 4 ($F(5,44) = 1.08$, $P = 0.38$).

4.4. Food hoarding

All groups hoarded by carrying pellets from the end of the alley into the home cage and there was no hint of a group difference as all rat groups hoarded about 60% of the available pellets ($F(5,49) = 1.8$, $P = 0.13$).

4.5. Claw cutting

Normal rats trim their claws by rapid nibbles with the incisors. Rats with motor or prefrontal damage in

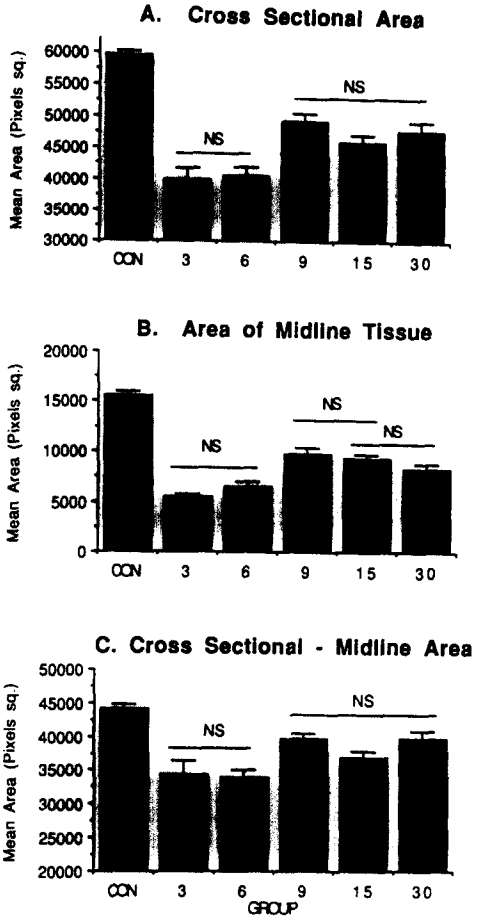

Fig. 8. Summary of areal measurements in the cortex. A: mean area of the cortex in a coronal section just anterior to the beginning of the striatum. B: mean area of the midline tissue from the top of the tanea tecta to the lesion scar. C: the mean area of the cortex exclusive of the midline area. This measure was obtained by subtracting the values in panel B from those in A.

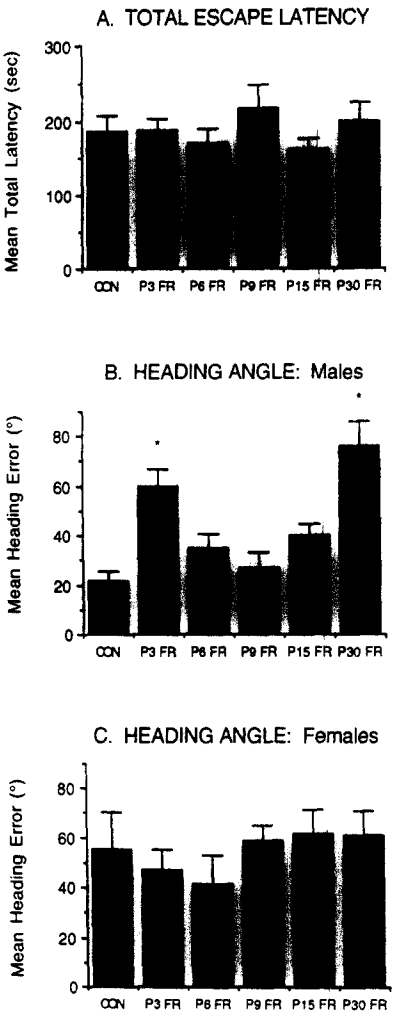

Fig. 9. Summary of performance in the Morris water task. A: total time to swim to the platform over the 10 trial blocks. There were no group differences. B and C: error in heading to the platform 12 cm after release in the pool. Male rats with lesions at day 3 or 30 were at chance. All groups of female rats performed at chance. Asterisks indicate a statistical difference from controls $Ps < 0.05$ or better. Values are means and standard errors.

adulthood are impaired at this behaviour and have claws that are nearly twice as long as normal, which presumably reflects a deficit in fine control of movement [42]. The rats in both the P3 and P15 lesioned group had longer claws than the controls (Fig. 10), but these were shorter than we have usually observed in adult operates, who usually average longer than 3 mm. Analysis of variance indicated a significant effect among groups ($F(5,43)=5.43$, $P=0.0006$) with posthoc tests revealing significant differences between the control animals and two of the lesioned groups (i.e., P3 and P15). The toenail length for P9 animals was significantly shorter than those for P3, P15 or P30 animals; the length for P6 rats was significantly shorter than for P15 which, in turn, was significantly shorter than P30 rats (Fisher's LSD, $P<0.05$).

5. Discussion

There were three principal findings of this study. First, although there were an age-related differences in details, lesions at all ages affected cerebral morphogenesis. Second, restricted mPFC lesions in even the youngest operates allowed sparing (or recovery) of function relative to adults with equivalent lesions. Third, there was an age-related gradient in extent of recovery as the best behavioural outcome was seen in the rats with lesions at day 9. We shall consider the implications of these results separately.

5.1. *Anatomical consequences of small mPFC lesions in young rats*

One of the most consistent anatomical effects of extensive neonatal cortical lesions in rats is a reduction in adult brain weight and cortical thickness relative to rats with similar lesions in adulthood [17,25–27]. The current study shows that removal of even relatively small

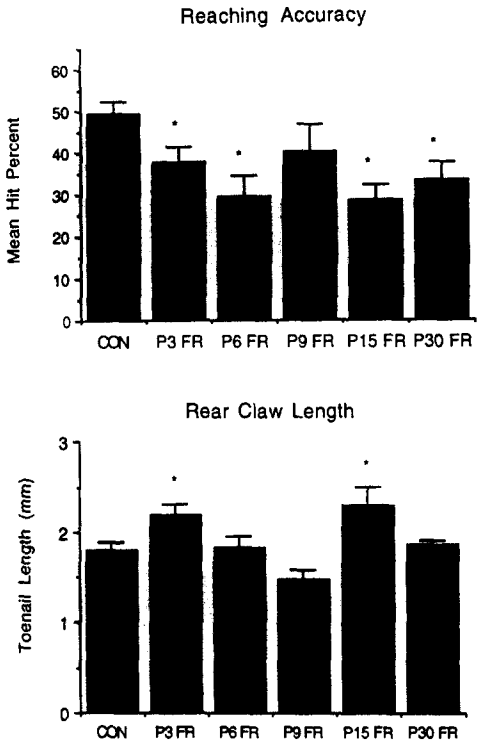

Fig. 10. Top: summary of accuracy of rats reaching through the bars for food. Bottom: summary of rear claw length. Asterisks indicate a statistical difference from controls $Ps < 0.05$ or better. Values are means and standard errors. Rats with day 9 lesions performed as well as control animals on both measures.

amounts of anterior cortex can result in the development of abnormally thin cortex throughout much of the remaining neocortical mantle. This effect seems to be largest in youngest operates but even removals at 30 days of age have an effect on cortical thickness that is not found after similar removals in adulthood. It does appear, however, that lesion size affects the extent of shrinkage as the current lesions reduced thickness by about 10% whereas larger lesions in our previous studies have been associated with reductions of up to 20%, even in the most posterior cortex [12,17].

It is difficult to determine what the cerebral shrinkage implies. We have found in other studies that the shrinkage is correlated with a loss of cortical neurons in a 0.5 mm column, which might reflect an effect of the lesion on normal developmental cortical cell loss [18]. On the other hand, the thinning could reflect a redistribution of cortical neurons as the remaining cortex is stretched after the early lesion. We have previously considered the possibility that thinning is due to loss of nonspecific projections that course through the injured region, or is due to a loss of subcortical projections, but our anatomical studies lead us to conclude that this is unlikely [15,17–20]. We have also shown that cortical thinning is not due to a reduction in neuronal processes. In fact, animals with day 10 lesions have a reduction in cortical thickness in conjunction with an *increase* in dendritic arborization in the remaining neurons [17–20]. Our best guess is that the thinning reflects a loss of cortical neurons.

One of the most puzzling, and for us frustrating, effects of early cortical lesions in rodents is the difficulty in equating lesion size at different ages [2,4,5,12,26,41]. Thus, we consistently find that lesions at 7–10 days of age appear to shrink in the 2 weeks following surgery. One or 10 day old animals sacrificed immediately after surgical excision of the frontal cortex appear to have comparable damage, but 2 weeks later it is difficult to find the lesions in those animals with injuries at 10 days. There are several possible explanations. First, the 7–15 day lesions may alter cell migration so that cells migrate into the damaged region and the lesion actually gets smaller. In fact we have preliminary evidence that this could be occurring after P9, and perhaps P15 lesions, but we do not know where the cells come from or where they normally ought to be going [3]. In view of the reduced cross sectional area of the hemisphere beyond the lesion we can speculate that the neurons may have been destined for other cortical regions, but this remains to be shown. Second, a more far-fetched explanation is that 7–15 day lesions actually stimulate mitosis of the stem cells that have been shown to line the ventricular wall of rats, even in adulthood [30,33]. One feature of this hypothesis is that it might be expected that damage to the ventricular wall, which is more likely in a very small brain, would preclude a mitotic reaction. This could account for the difference between very early lesions and those around 10 days. It does not explain, however, why mitosis does not occur in older animals. In order to test the mitosis hypothesis we have made multiple injections of bromo-d-oxy-uridine (BrdU) after lesions on postnatal day 10 and sacrificed animals in adulthood. We have subsequently double-labelled cells with an antibody to BrdU and a second one that is specific to neurons (NeuN). Thus, it appears that at least after lesions at P10 there is some mitotic activity in the midline area [14]. We do not know, however, how extensive this mitosis might be or whether the newly grown cells even contribute to normal function.

One question that arises is why the filled-in region in P9 rats is correlated with better functional outcome than we observe in the P15 and P30 rats. One possibility is that there are differences in connectivity or structure at the different ages. There are few hints about this possibility but it has been shown that P9 lesions are associated with higher than normal dopamine levels [5,6], and this may be related to increased dendritic growth in these neurons [3].

The difficulty in equating lesion sizes at different ages remains a problem for interpretation of differences in behavioural outcome after lesions at different ages. We have tried repeatedly over the last 15 years to make the lesions appear the same in chronic animals but there

does not seem to be a simple solution to this problem. As a result, for the purposes of the following discussion, we will assume that the lesions were the same size at the time of surgery, and that differences observed in adulthood reflect some difference in the reaction of the brain to the injury.

5.2. *Behavioural outcome after early frontal lesions*

Although it is generally believed that early brain damage allows better functional outcome than damage later in life (the so-called Kennard Principle), evidence is accumulating to show that the Kennard Principle is too simplistic. Thus, while there is now little doubt that although early cortical injury sometimes leads to surprisingly normal function [7,38–41], early injury also can have devastating consequences upon the developing brain [23]. We suggest that two variables may be important: lesion size and precise embryological age.

5.2.1. *Lesion size*

Medial prefrontal lesions in adulthood produce a consistent pattern of behavioural deficits, even if there is sparing of a significant part of dorsomedial thalamic projection to the ventral midline (infralimbic and orbital areas) and the insular region [14]. However, similar injuries sustained in infancy produce a much milder behavioural syndrome, especially on standard neuropsychological measures [2,4,27,31,32,37] (see Tables 1 and 2). In contrast, frontal lesions that are more extensive and include most of the prefrontal region produce a more extensive behavioural syndrome, which is especially severe if the tissue is removed in the first few days of life [12]. Lesion size therefore appears to be crucial in predicting the extent of recovery from early brain injuries. Lesions that include most of a functional zone, such as the prefrontal cortex, do not allow much functional recovery whereas lesions that are restricted to a part of a functional zone may allow the developing brain to compensate, presumably by reorganizing the spared region in some way. The importance of lesion size in predicting functional outcome from early lesions is not restricted to frontal lesions. For example, Whishaw and Kolb [42] showed that rats with small unilateral motor cortex lesions could show surprisingly normal forepaw use whereas rats with larger lesions were more impaired. Similarly, there is evidence that language can be prevented from shifting hemispheres after extensive early left hemisphere injury in humans if there is even a small injury in the potential language zones in the right hemisphere [36]. In sum, although the developing brain appears better able than the adult brain to compensate for the loss of small amounts of cerebral tissue, it seems likely that the immature brain does not compensate well for the loss of extensive amounts of tissue.

Table 1
Summary of chronic behaviours of rats with large frontal decortications at different ages

Behaviour	Age at surgery			
	P1–5	P7–10	P25	Adult
Body weight	X	X	OK	OK
Fore paw use	X	X	X	X
Hind paw use	X	OK		OK
Tongue use	X	OK		X
Swimming	X	OK	X	X
Hoarding		X	X	X
Learning:				
Spat rev		OK	OK	X
Morris task	X	OK		X

Data from references [12,16,18,19,37].

Table 2
Summary of the chronic behaviour of rats with selective mPFC lesions at different ages

Behaviour	Age at surgery				
	P1–3	P5–6	P7–9	P25–40	Adult
Body weight	X	X	OK	X	OK
Forepaw use	X	X	OK	X	X
Swimming	OK	OK	OK	OK	OK
Hoarding	OK	OK	OK	OK	X
Learning:					
Spat rev		OK	OK	X	X
Morris task	X [a]	OK	OK	X [a]	X
Del resp/alt	OK	OK		X	X
Act avoid			OK	OK	X

Data from references [2,4,24,31,32,37].
[a] Indicates that the deficit was seen only on a measure of accuracy.

The idea that earlier might be worse if the lesion is extensive was first proposed by Hebb who suggested that such early cortical injuries may prevent the development of some behavioural capacities that an equally extensive injury, at maturity, would not have destroyed [9]. Hebb based his conclusion on his studies of humans patients who had sustained fairly extensive unilateral injuries outside the speech areas in infancy. In contrast to adults with similar lesions, Hebb found surprisingly large behavioural deficits and therefore suspected that the early lesions had interfered with the development of behavioural or physiological processes necessary for the development of normal adult functioning. What Hebb did not know, and this still needs to be determined, is whether it is the absolute size of the lesion that is important or the extent to which it destroys a functional zone. Our hunch is that it is the latter explanation.

5.2.2. *Age-related effects of cortical injury*

Various authors have proposed that there is a 'critical period' during development when the brain is especially able to compensate for injury [10,35,39]. This 'critical

period' is presumably related to the developmental status of the brain, which varies considerably at birth in different mammalian species. For example, marsupials are especially immature at birth whereas the rhesus monkey is particularly mature, at least in comparison to human brain development. When viewed in this context it appears there is one developmental time when different species of mammals are able to compensate for significant cerebral perturbations and another time, which is earlier in development, during which injury is especially disruptive. In the rat, which is nearly as immature as the hamster, this 'critical' time for recovery appears to be about 7–10 days postnatal, even if the injury is extensive (see Tables 1 and 2). Earlier injuries allow for very poor recovery, especially if the lesions are large [14]. In the cat, which is born older than the rat, the optimal time for recovery from injury appears to be postnatal, whereas prenatal lesions allow little recovery [38,39].

The optimal time for cortical injury in the monkey appears to be prenatal as Goldman and Galkin [8] made frontal lesions in prenatal monkeys and found significant sparing of function. In contrast, postnatal lesions in monkeys allow little sparing. It has been argued that Goldman and Galkin's animals had lesions at a developmental age equivalent to human newborns so one would predict that even earlier lesions might be disruptive [38,39]. Studies of human infants are more difficult to interpret, in part because the injuries result from accident or disease and thus are highly variable. Furthermore, disease or accident often leave epileptogenic foci in humans. Seizure activity might be expected to interfere not only with function but with the development of function. Nonetheless, there is little doubt that the effects of early left hemisphere injury on language functioning vary with age: postnatal damage to the language zones seldom leads to chronic dysphasia if it is sustained prior to age 5. This has led to the general belief that newborn humans may be at the optimal developmental age for recovery, but there is reason to believe that it may be somewhat older. For example, when other cognitive skills are examined it appears that cerebral injuries or disease during the first year of infancy can be more damaging to general intelligence than are later injuries [1,16,29]. It is difficult to equate this result with the animal literature since there is no easy way to index measure differences in 'intelligence' in laboratory animals. Furthermore, one must be careful of using the absence of aphasia as evidence of normal brain function.

Acknowledgement

This research was supported by an Natural Science Engineering and Research Council of Canada grant to BK and a grant-in-aid from Okanagan University College to JC. We thank Robbin Gibb and Brigitte Byers for their assistance with the histological material and J. de Brabander for her comments on an earlier version.

References

[1] Chadwick, O., Rutter, M., Thompson, J. and Shaffer, D., Intellectual performance and reading skills after localized head injury in childhood, *J. Child Psychol. Psychiat.*, 22 (1981) 117–139.

[2] De Brabander, J.M., De Bruin, J.P.C. and Van Eden, C.G., Comparison of the effects of neonatal and adult medial prefrontal cortex lesions on food hoarding and spatial delayed alternation, *Behav. Brain Res.*, 42 (1991) 67–75.

[3] De Brabander, J.M. and Kolb, B., Changes in dendritic trees of pyramidal cells in the remaining medial prefrontal cortex following partial neonatal lesions of the prefrontal cortex, submitted.

[4] De Brabander, J.M., Van Eden, C.G. and De Bruin, J.P.C., Neuroanatomical correlates of sparing of function after neonatal medial prefrontal cortex. lesions, *Brain Res.*, 568 (1991) 24–34.

[5] De Brabander, J.M., Van Eden, C.G., De Bruin, J.P.C. and Feenstra, M.G.P., Activation of mesocortical dopaminergic system in the rat in response to neonatal medial prefrontal cortex lesions. Concurrence with functional sparing, *Brain Res.*, 581 (1992) 1–9.

[6] De Brabander, J.M., Van Eden, C.G., De Bruin, J.P.C. and Feenstra, M.G.P., Monoamine concentrations in rat prefrontal cortex and other mesolimbocortical structures in response to partial neonatal lesions of the medial prefrontal cortex, *Brain Res.*, 601 (1993) 20–27.

[7] Goldman, P.S., An alternative to development plasticity: Heterology of CNS structures in infants and adults. In D.G. Stein, J.J. Rosen and N. Butters (Eds.), *Plasticity and Recovery of Function in the Central Nervous System*, Academic Press, New York, 1974, pp. 149–174.

[8] Goldman, P.S. and Galkin, T.W., Prenatal removal of frontal association cortex in the fetal rhesus monkey: anatomical and functional consequences in postnatal life, *Brain Res.*, 152 (1978) 451–458.

[9] Hebb, D.O. *The organization of behavior.* McGraw-Hill, New York, 1949.

[10] Kennard, M.A., Age and other factors in motor recovery from precentral lesions in monkeys, *Am. J. Physiol.*, 115 (1936) 138–146.

[11] Kolb, B., Prefrontal lesions alter eating and hoarding behavior in rats, *Physiol. Behav.*, 12 (1974) 507–511.

[12] Kolb, B., 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.

[13] Kolb, B., Prefrontal cortex. In B. Kolb and R.C. Tees (Eds.), *Cerebral Cortex of the Rat*, MIT Press, Cambridge, MA, 1990, pp. 437–458.

[14] Kolb, B, *Brain Plasticity and Behavior.* Lawrence Erlbaum and Assoc., Hillsdale, NJ, 1995.

[15] Kolb, B., Day, J., Gibb, R. and Whishaw, I.Q., Recovery from early cortical lesions in rats. 6. Cortical noradrenaline, cortical thickness, and development of spatial learning after frontal lesions or hemidecortications, *Psychobiology*, 17 (1989) 370–376.

[16] Kolb, B. and Fantie, B., Development of the child's brain and behavior. In C.R. Reynolds and E. Fletcher-Janzen (Eds.), *Handbook of Clinical Child Neuropsychology*, Plenum, New York, 1989, pp. 17–39.

[17] Kolb, B. and Gibb, R., Anatomical correlates of behavioural

change after neonatal prefrontal lesions in rats, *Prog. Brain Res.*, 85 (1990) 241–256.

[18] Kolb, B. and Gibb, R., 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.

[19] Kolb, B. and Gibb, R., Possible anatomical basis of recovery of function after neonatal frontal lesions in rats, *Behav. Neurosci.*, 107 (1993) 1–13.

[20] Kolb, B., Gibb, R. and van der Kooy, D., Cortical structure and connectivity after neonatal frontal cortex lesions in rats, *Brain Res.*, 645 (1994) 85–97.

[21] Kolb, B., Hewson, J. and Whishaw, I.Q., Neonatal motor cortex lesions alter corticospinal projections and dendritic organization in the absence of behavioural sparing, *Soc. Neurosci. Abstr.*, 19 (1993).

[22] Kolb, B. and Holmes, C., 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.

[23] Kolb, B., Holmes, C. and Whishaw, I.Q., Recovery from cortical lesions in rats. III. Neonatal removal of posterior parietal cortex has greater behavioral and anatomical effects than similar removals in adulthood, *Behav. Brain Res.*, 26 (1987) 119–137.

[24] Kolb, B. and Nonneman, A.J., Sparing of function in rats with early prefrontal cortex lesions, *Brain Res.*, 157 (1978) 135–148.

[25] Kolb, B., Sutherland, R.J. and Whishaw, I.Q., Abnormalities in cortical and subcortical morphology after neonatal neocortical lesions in rats, *Exp. Neurol.*, 79 (1983) 223–244.

[26] Kolb, B. and Whishaw, I.Q., Neonatal frontal lesions in the rat: sparing of learned but not species-typical behavior in the absence of reduced brain weight and cortical thickness, *J. Comp. Physiol. Psychol.*, 95 (1981) 863–879.

[27] Kolb, B. and Whishaw, I.Q., Earlier is not always better: behavioral dysfunction and abnormal cerebral morphogenesis following neonatal cortical lesions in the rat, *Behav. Brain Res.*, 17 (1985) 25–43.

[28] Kolb, B. and Whishaw, I.Q., Plasticity in the neocortex: Mechanisms underlying recovery from early brain damage, *Prog. Neurobiol.*, 32 (1989) 235–276.

[29] Kornhuber, H.H., Bechinger, D., Junchg, H. and Sauer, E., A quantitative relationship between the extent of localized cerebral lesions and the intellectual and behavioural deficiency in children, *Eur. Arch. Psychiat. Neurol. Sci.*, 235 (1985) 129–133.

[30] Morsehead, C.M., Reynolds, B.A., Craig, C.G., McBurney, M.W., Staines, W.A., Morassutti, D., Weiss, S. and van der Kooy, D., Neural stem cells in the adult mammalian forebrain: A relatively quiescent subpopulation of subependymal cells, *Neuron*, 13 (1994) 1071–1082.

[31] Nonneman, A.J. and Corwin, J.V., Differential effects of prefrontal ablation in neonatal, juvenile, and young adult rats, *J. Comp. Physiol. Psychol.*, 95 (1981) 588–602.

[32] Nonneman, A.J., Corwin, J.V., Sahley, C.L. and Vicedomini, J.P., Functional development of the prefrontal system. In S. Finger and C.R. Almli (Eds.), *Early Brain Damage, Vol.* 2, Academic Press, New York, 1984, pp. 139–153.

[33] Reynolds, B.A. and Weiss, S., Generation of neurons and astrocyte from isolated cells of the adult mammalian central nervous system, *Science*, 255 (1992) 1707–1710.

[34] Sutherland, R.J., Whishaw, I.Q. and Kolb, B., 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.

[35] Teuber, H.-L., Recovery of function after brain injury in man. In: *Outcome of severe damage to the nervous system, Ciba Foundation Symposium* 34, Elsevier North-Holland, Amsterdam, 1975.

[36] Vargha-Khadem, F., Watters, G. and O'Gorman, A.M., Development of speech and language following bifrontal lesions, *Brain Lang.*, 37 (1985) 167–183.

[37] Vicedomini, J.P., Corwin, J.V. and Nonneman, A.J., Role of residual anterior neocortex in recovery from neonatal prefrontal lesions in the rat, *Physiol. Behav.*, 28 (1982) 797–806.

[38] Villablanca, J.R., Harrison, J.B., Jackson, G.F., Hovda, D.A. and Infante, C., Greater neurological impairments in prenatal versus neonatal cortically-lesioned kittens, *Soc. Neurosci. Abstr.*, 17 (1991) 894.

[39] Villablanca, J.R., Hovda, D.A., Jackson, G.F. and Infante, C., 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.

[40] Villablanca, J.R., Olmstead, C.E. and Levine, M.S., Effects of caudate nuclei or frontal cortical ablations in kittens: neurology and gross behavior, *Exp. Neurol.*, 61 (1978) 615–634.

[41] 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.

[42] Whishaw, I.Q., Kolb, B., Sutherland, R.J. and Becker, J., Cortical control of claw cutting in the rat, *Behav. Neurosci.*, 97 (1983) 370–380.

[43] Whishaw, I.Q., O'Connor, W.T. and Dunnett, S.B., Contributions of motor cortex, caudate-putamen and nigrostriatal systems to skilled forepaw use in the rat, *Brain*, 109 (1986) 805–843.

[44] Whishaw, I.Q., Pellis, S.M. and Gorny, B.P., Medial frontal cortex lesions impair the aiming component of rat reaching, *Behav. Brain Res.*, 50 (1992) 93–104.

[45] Zilles, K., *The Cortex of the Rat*, Springer-Verlag, Berlin, 1985.