

**RECOVERY OF FUNCTION AFTER CINGULATE CORTEX INJURY
IN RATS**

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Abstract

The current studies investigate the behavioral and anatomical changes after lesions at different ages of the cingulate cortex. Rats received lesions of the posterior cingulate cortex (PCing) or the anterior and posterior cingulate cortex (Total) at: postnatal day 4 (P4); day 10 (P10), or in adulthood (P120). Rats were trained in the Morris water maze, the Whishaw reaching task, conditioned taste aversion (CTA), and their activity was monitored over 48 hours. The general finding was a significant behavioral recovery on P10 animals regardless the size of the lesion. This recovery was associated with an increase in dendritic arborization in P10 animals with the PCing removed and a partial regeneration of the midline tissue in the Total P10 animals. These results suggest that damage to the cingulate cortex at P10 is associated with substantial behavioral and anatomical plasticity and that removal of the frontal midline tissue stimulates a regenerative process in more posterior cortex that does not occur otherwise.

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Table of Contents

Title page.....	I
Signature page.....	II
Abstract.....	III
Acknowledgements.....	IV
Table of contents	V
List of tables.....	VII
List of figures.....	VIII
<u>Chapter 1: General Introduction</u>	1
<u>Chapter 2: Background</u>	3
Cortical development in the rat	
Plasticity in the normal cortex	
Plastic changes in the adult-injured brain	
Plastic changes in the early-injured brain	
<u>Chapter 3: The Cingulate Cortex</u>	15
Cingulate anatomy	
Functions of the cingulate cortex in the rat and humans	
<u>Chapter 4: The Problem</u>	21
<u>Chapter 5: Rationale for the Behavioral and Anatomical Measures</u>	22
Morris water task	
Conditioned taste aversion	
Whishaw's reaching task	
Motor activity	
Anatomical measures	

<u>Experiment 1:</u>	31
Method	
Results	
<u>Experiment 2:</u>	43
Method	
Results	
<u>Experiment 3:</u>	54
Method	
Results	
<u>General Discussion</u>	87
Behavioral aspects	
Anatomical aspects	
Future directions	
<u>References</u>	99
<u>Appendices</u>	106

List of Tables

Table		Page
1.	Exp. 1: Mean of brain weight.....	34
2.	Exp. 2: Mean of brain weight.....	46
3.	Exp. 3: Mean of brain weight (PCing)	60
4.	Exp. 3: Mean of brain weight (Totals)	61
5.	Exp. 3: Summary of behavioral results.....	80

List of Figures

Figure	Page
1. Outline of Brain Development.....	4
2. Representation of the Subventricular Zone.....	5
3. Cingulate Areas.....	17
4. The Morris Water Task.....	23
5. The Conditioned Taste Aversion.....	25
6. Reaching Box.....	27
7. Cages used for monitoring general activity.....	28
8a. Exp. 1: Sholl Analysis, Apical Dendrites.....	35
8b. Exp. 1: Sholl Analysis, Basilar Dendrites.....	36
9a. Exp. 1: Swim distance by trial block on Morris water task.....	38
9b. Exp. 1: Sum latency on Morris water task.....	39
10. Exp. 1: Dwell time of probe trial on Morris water task.....	40
11. Sections through the rat brain for measuring cortical thickness	45
12. Exp. 2: Mean cortical thickness by group.....	48
13. Exp. 2: Swim distance by trial block on Morris water task.....	49
13b. Exp. 2: Sum latency on Morris water task	50
14. Exp. 2: Dwell time of probe trial on Morris water task.....	52
15. Exp. 3: Mean cortical thickness by group (PCing).....	63
16. Exp. 3: Mean cortical thickness by group (Totals).....	64
17a. Exp. 3: Swim distance by trial block on Morris water task (PCing).....	67
17b. Exp. 3: Sum latency on Morris water task (PCing).....	68
18. Exp. 3: Dwell time of probe trial on Morris water task (PCing).....	69

19a.	Exp. 3: Swim distance by trial block on Morris water task (Totals).....	70
19b.	Exp. 3: Sum latency on Morris water task (Totals).....	71
20.	Exp. 3: Dwell time of probe trial on Morris water task (Totals).....	72
21.	Exp. 3: Mean of reaching performance (PCing).....	75
22.	Exp. 3: Mean of reaching performance (Totals).....	76
23.	Exp. 3: General activity monitored during 48 hours (PCing).....	78
24.	Exp. 3: General activity monitored during 48 hours (Totals).....	81
25.	Exp. 3: Retention test of conditioned taste aversion (PCing).....	82
26.	Exp. 3: Retention test of conditioned taste aversion (Totals).....	83
27.	Exp. 3: Pictures of representative brains (PCing).....	84
28.	Exp. 3: Pictures of representative brains (Totals).....	85

1. GENERAL INTRODUCTION

The systematic study of sparing and recovery of function after brain damage began with the work of Margaret Kennard in the early 30's. In 1936 she performed a series of experiments with monkeys in which she observed that small motor cortex lesions in infancy allowed significant recovery of function relative to similar lesions in adult monkeys (Kennard, 1936). This observation of recovery after cortical lesions in infancy became known as "the Kennard principle" which states that it is better to have brain damage early in life because this allows better functional recovery than if the damage occurs later. In the early stages of her work Kennard suggested that the recovery observed in young animals was probably due to the fact that the cortical areas were not yet mature at the time of the damage. Later on she introduced the idea that some changes in the synaptic organization of the brain in early development must have taken place in order to allow the functional outcome (Kennard, 1942). Although she did not specify what the changes might be, she hypothesized that the remaining cortical areas were mediating the recovery.

A closer examination of Kennard's animal studies revealed that the behavioral functions in her monkeys were performed adequately by the young animals shortly after the injury but these same animals demonstrated motor dysfunction as they reached adulthood (Goldman, 1974; Kennard, 1942).

There is now considerable evidence to support the Kennard principle, but many studies have failed to confirm it. For example, Passingham et al., (1983) suggested that there may be virtually no difference between the effects of motor cortex lesions in infant and adult monkeys when the entire system is damaged

(Passingham et al., 1983). Moreover in his studies with children with infant frontal lobe injuries Hebb, (1949) concluded that brain damage early in life may be worse than similar damage sustained later in life because certain cognitive functions depend on the integrity of particular structures at particular developmental times. Thus, if a system is altered during a critical period of development the function performed by that system or structure may not develop normally.

It is now clear that there are a number of conditions in which early injury has greater long-term impact than later-sustained brain insult (Hart & Faust, 1988, for a review). These include variables such as type of insult, location, and extent of injury. Questions such as how plastic is the developing brain; how do the plastic changes in the brain relate to function; and what are the factors influencing this plasticity are the issues that I approached in the present studies.

In order to examine the factors influencing recovery from early brain injury I shall first review how the mammalian brain is generated during development and what factors may be influential in brain organization and development throughout life. Next, I will review some of the mechanisms mediating recovery after early and late brain damage. I will then examine the anatomical and functional aspects of the cingulate cortex, which is the specific system addressed in this thesis. Finally, I will present the rationale for the specific experiments.

2. BACKGROUND

Cortical development in the rat

The rat nervous system (like that of most mammals) receives its primary source of cells from a proliferative germinal matrix called the neuroepithelium [Fig 1]. During gastrulation the neuroepithelium differentiates on a region of the dorsal surface of the embryo called the neural plate. The neural plate forms a neural tube that extends caudally from the tip of the presumptive spinal cord in a rostral direction where programmed cell proliferation produces balloon-like expansions known as the rhombencephalon (hindbrain), mesencephalon (midbrain) and prosencephalon (forebrain). The rhombencephalon subdivides later into the myelencephalon (medulla) and the metencephalon (cerebellum and pons); the mesencephalon into the tectum (superior and inferior colliculi) and the tegmentum; and the prosencephalon into the diencephalon (thalamus and hypothalamus), and the paired telencephalic vesicles the cerebral cortex and the basal ganglia (Paxinos, 1995).

One major germinal layer, the subventricular zone (SVZ), gives rise to most neurons and glial cells in the brain [Fig 2]. The SVZ forms during embryonic development adjacent to the telencephalic ventricular zone and is more prominent in the ganglionic eminences (Altman & Bayer, 1995). At late stages of embryonic development the SVZ generates cells destined not only to the diencephalon but also to the cortex (Anderson et al., 1997). Interestingly, the SVZ's capacity to generate both neurons and glia persists into adulthood (Lois & Alvarez-Buylla, 1993). In adulthood, cells born in the SVZ continue to migrate along a restricted pathway, called the rostral migratory stream (RMS; Altman, 1969), to the olfactory bulb.

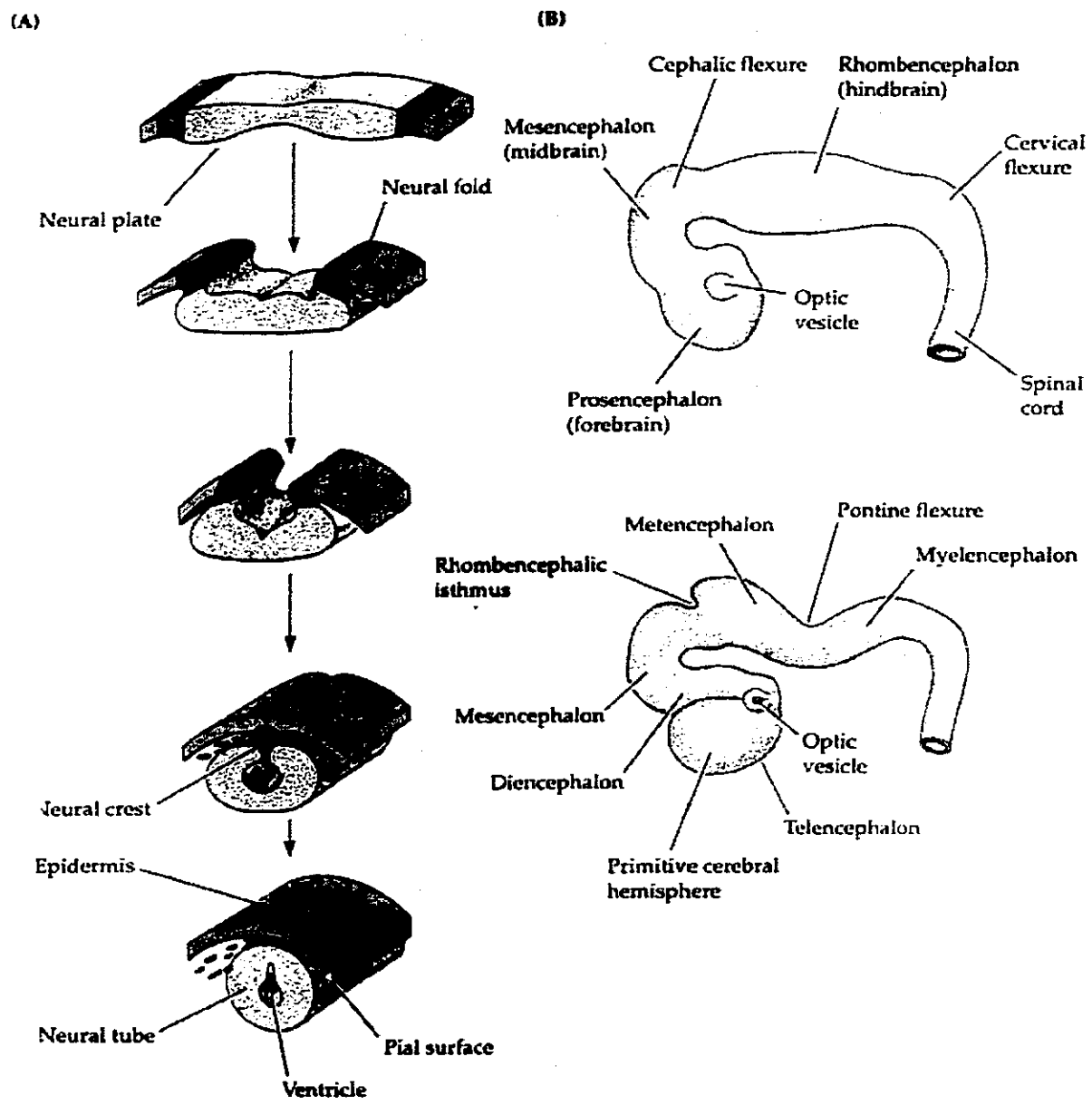


Fig. 1. A schematic outline of brain development. (a) Early in development. (b) Later in development.

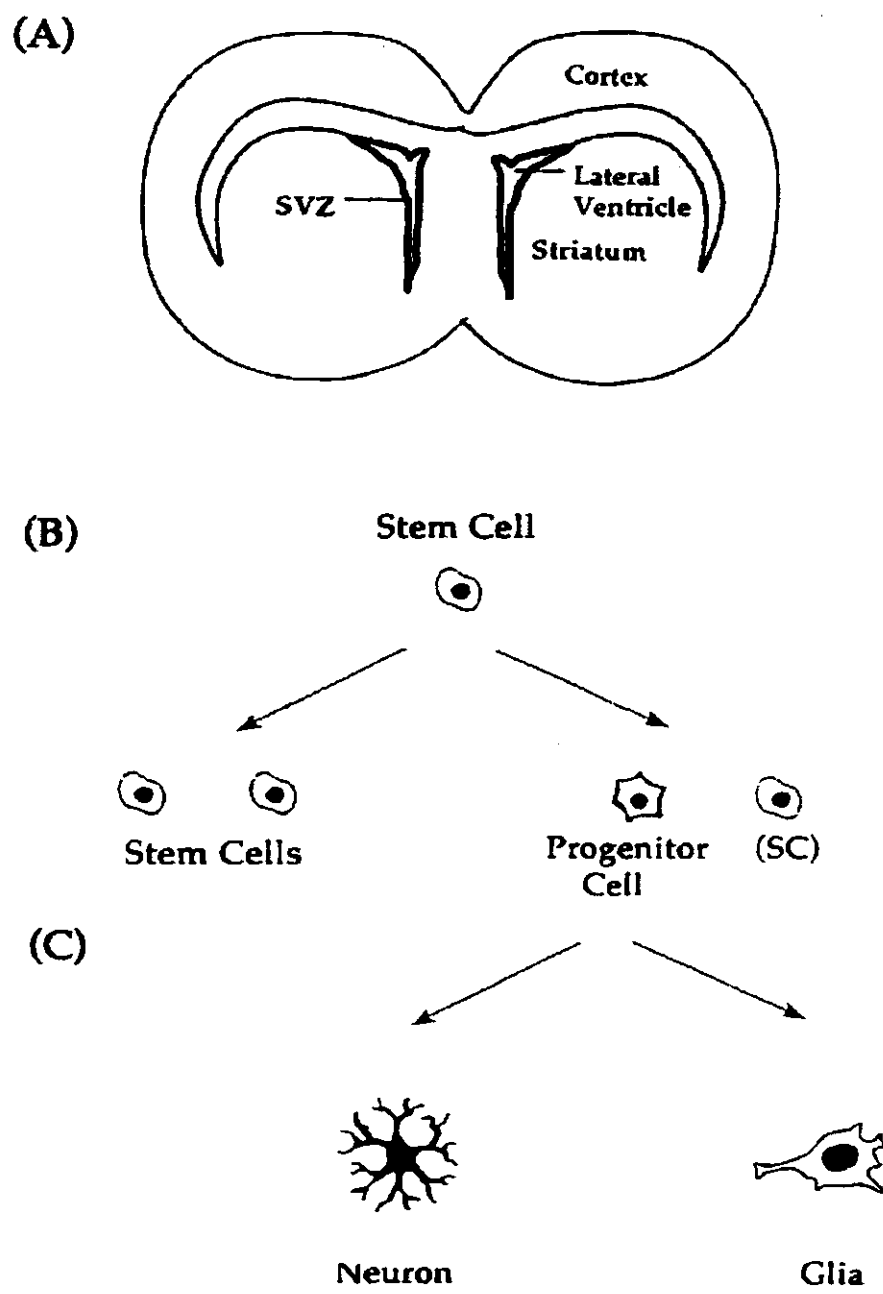


Fig. 2. (A) A schematic representation of a coronal section through the forebrain of a rat showing the subventricular zone. General schematic of cell development within the SVZ. (B) A stem cell can divide symmetrically giving rise to two stem cells, or asymmetrically to give rise to a stem cell and a progenitor cell. (C) A Progenitor cell can divide to produce neurons or glia.

These cells migrate as elongated aggregates of cells called chains (Lois et al., 1996).

Neurons arise from neural stem cells in the SVZ that can divide symmetrically to produce two stem cells, or asymmetrically to produce a stem cell and a progenitor cell. It seems that progenitor cells that divide to produce neurons and/or glia can migrate away from the SVZ and lie quiescently in the white or gray matter (Kolb et al., 1999). Most neocortical neurons are generated during embryogenesis, between embryonic day 14 and 20. Once the cells are generated they migrate throughout the brain and are largely in their appropriate cortical locations by about postnatal day 6. The period of cell differentiation continues for another 5-7 days. During the next 2-3 weeks there is an exuberant growth of dendrites, axons and synapses followed by a period of programmed cell death to remove unnecessary synapses and cells.

It was traditionally believed that the capacity of the SVZ to generate neurons was restricted to the embryonic period. However, it is now well known that new neurons continue to be added to certain regions of the adult brain especially in the olfactory bulb and in the hippocampus (for review see Alvarez-Buylla & Lois, 1995), but also in the cortex (Shankle et al., 1998; Gould et al., 1999).

How SVZ neurogenesis is maintained in the adult brain is not well understood. The discovery that the SVZ continues a process of neurogenesis throughout life has opened experimental opportunities of studying and understanding the differentiation of cells and regeneration of tissue in the adult central nervous system. The cerebral cortex is the end product of cell proliferation in the SVZ, thus it seems appropriate to look in the cortex for plastic changes that can occur after brain damage. In order to understand these changes

it is first important to review some of the changes that occur during normal development of the cerebral cortex.

Plasticity in the normal cortex

One of the most interesting phenomena in neurobiology is the correlation between certain neuronal connections and certain behaviors. A remarkable aspect of this phenomenon is the ability of the brain to be plastic so that new experiences can modify the original set of neuronal connections causing the creation of new neurons or the formation of new synapses between existing neurons.

The interaction between the organism and its environment has been shown to influence brain and behavior. In a well-known experiment by Hebb, in which he allowed some laboratory rats to explore his home for some weeks (like pets) and then returned them to the laboratory, he reported that these animals showed better problem-solving abilities than rats that had remained in the laboratory (Hebb, 1949). Many subsequent investigators have found that experience in an enriched laboratory environment improves learning and problem-solving abilities on a wide variety of tests (see Rosenzweig & Bennett, 1996, for a review). We now can identify a large number of neural changes associated with experience such as increases in brain size, cortical thickness, neuron size, dendritic branching, spine density, and synapses per neuron. I will illustrate these phenomena with a few examples.

One widespread effect of experience is a general increase in synaptic density in different structures throughout the brain (Saito et al., 1994). It has been reported that following exposure to an enriched environment an increase in the

number of synapses per neuron in the occipital cortex of rats is observed (Turner and Greenough, 1985). There are also reports that show an increase in dendritic arborization in the somatosensory and motor cortex in monkeys (Bryan and Riesen, 1989).

Similar experience-dependent changes have also been reported in other cortical areas. Animals exposed to enriched environments right after weaning, in young adulthood, or in senescence showed an increase in brain weight, increased dendritic arborization, increased dendritic length, and altered spine density. With the exception of spine density, all of the measures showed qualitatively similar changes across the life span (Kolb et al., 2000). That animals exposed to enriched environments show more proficient production of a variety of behaviors has opened another field for studies to determine whether the environmental condition can promote recovery of function following brain damage. Moreover, animals exposed to enriched environments show different neural changes: For example, recent studies have shown that challenging adult mice by housing them under the stimulating conditions of an enriched environment results in a greater number of new neurons in the hippocampus (Kemperman et al., 1998).

There are, however, other environmental factors that can have a deleterious effect on some structures in the brain. For example, studies show that exposure to highly stressful experiences during the first two weeks of life in the rat (time of maximal neurogenesis of hippocampal neurons) inhibits the production of granule neurons. Because certain stressors can elevate adrenal steroids during the first two weeks of life, it is possible that such experiences are inhibiting the

production of hippocampal granule cells via some action of the steroids. Tanapat et al., (1998) exposed rat pups to a potentially stressful experience (the odor of adult male rats which are predators to rat pups) and determined the level of circulating glucocorticoids and the number of proliferating cells in the dentate gyrus. They found that the exposure to the odor of an adult male rat was a sufficient stressor to elevate circulating levels of corticosterone during that period. Because corticosterone treatment suppresses cell proliferation in the developing dentate gyrus, it is likely that the decrease in the number of cells observed was the result of the elevated glucocorticoid levels. Their findings suggest that one of the factors that suppresses adult neurogenesis in the dentate gyrus is the early exposure to threatening experiences. By these mechanisms, environmental cues could influence granule cell formation in the developing rat and affect the behavioral outcome.

Overall, these findings suggest that exposure to specific experiences during development induce a wide variety of structural modifications in the brain. These anatomical changes produce in turn a range of different behavioral consequences.

Plastic changes in the adult-injured brain

An increasing number of studies have shown that neural plasticity can occur in the adult brain in response to injury. Recovery of behavioral function after injury is generally correlated with anatomical changes in the brain: these changes, or plastic processes, are based on specific physiological and morphological mechanisms. In some cases recovery is associated with increased dendritic arborization and spine density in cortical pyramidal cells, whereas the

absence of recovery is associated with decreased dendritic arborization and decrease spine density (Kolb & Gibb, 1993). It was originally believed that injury-induced brain plasticity was restricted to the period of early brain development, but evidence has shown that it actually occurs throughout life. I will review separately some evidence showing different plastic changes in different regions of the adult brain.

a. *Hemidecortication*. Schallert and Whishaw, (1984) showed in an extensive study of hemidecorticated rats that an initial slow functional improvement on a sensorimotor task becomes almost complete over the course of a year. Further experiments showed that this recovery seems to be mediated by changes of the pyramidal cells (an increase in branching) of the contralateral hemisphere (Kolb, 1995).

b. *Visual Cortex*. When the visual cortex is removed in adulthood Kolb et al., (1996) found no recovery of either visual discrimination learning or visually guided spatial navigation learning. Furthermore, when the brains were analyzed for specific arborization changes, they found a significant decrease in branching on the pyramidal cells of the somatosensory area.

c. *Motor Cortex*. After bilateral motor cortex lesions there is a severe impairment of different motor abilities such as reaching for food, walking on narrow beams and tongue protrusion (Kolb & Whishaw, 1989). Although these deficits decrease over time there are always residual behavioral deficits. For example, Kolb (1995) gave bilateral motor cortex lesions and found that 6 months later the animals showed a remodeling of sensorimotor cortex, which could be responsible for mediating the modest behavioral recovery.

d. *Frontal Cortex*. There is a general correlation between recovery of spatial learning and motor behavior and synaptic changes after frontal cortex lesions. This functional outcome seems to be dependant on the time of recovery post-lesion: animals trained briefly after the lesion showed no behavioral recovery and an absence of brain reorganization, whereas animals trained 4 months post-operation showed much better behavioral outcomes and changes in dendritic branching in the adjacent parietal cortex (Kolb & Gibb, 1991a).

In sum, we can conclude from the studies of hemidecortication, motor, visual and frontal cortex lesions that the adult brain is capable of remodeling its cortical circuitry after an insult and that these changes seem to be, in some cases responsible for functional restitution.

Plastic changes in the early-injured brain

When the brain is damaged during the early period of life, the specific age of injury and the location of the insult produce very different behavioral and anatomical outcomes. Over the past two decades Kolb and his colleagues have been studying the anatomical and behavioral sequelae of lesions in different cortical areas at different developmental ages. Here there is a brief summary of some important findings that will be relevant for a more complete understanding of how the brain changes after damage.

a. *Deneocortication and Hemidecortication*. Kolb & Whishaw, (1981) showed that when the entire cortex is removed there is no functional recovery regardless of the age of injury. This story becomes quite different when only the cortex of one hemisphere is removed (hemidecortication): the earlier the removal, the better

the recovery. In general, neonatal hemidecorticated animals perform much better in adulthood than adult hemidecorticates on walking, swimming and reaching (Kolb & Tomie, 1988). This behavioral outcome is presumably sustained by the intact hemisphere. Kolb et al., (1983) found that when one hemisphere is removed there is an increase in the cortical thickness in the contralateral cortex. There is also an increase in the dendritic arborization of the pyramidal cells in the motor and parietal cortices and less, but also significant, increase in the more posterior cortex (Kolb et al., 1992).

b. *Visual Cortex.* Kolb et al., (1996) gave bilateral visual cortex lesions to rats at different postnatal ages (day 4, 10, or adulthood) and then tested them in adulthood on the Morris water task and on a visual pattern discrimination task. The general finding for these two conditions was an absolute absence of visually-guided learning regardless of the age at lesion. Curiously, the P10 animals showed significantly longer whiskers, and an associated enhancement of somatosensory functions. In addition, animals with lesions at P10 showed an increase in dendritic branching in somatosensory cortex.

c. *Motor Cortex.* There seem to be different results if the lesion occurs unilaterally or bilaterally. When the injury is unilateral at P1 or P10 there seems to be a considerable amount of recovery on skilled reaching tests compared with similar lesions sustained in adulthood (Kolb et al., 2000; Whishaw & Kolb, 1988). This behavioral outcome is correlated with an increase in dendritic arborization in pyramidal cells of the parietal cortex in both the damaged and the normal hemisphere. In another study (Kolb et al., 2000) animals received bilateral lesions

and the results were quite different: while P10 animals showed better recovery on various motor and cognitive tasks, animals with similar lesions at P1 or in adulthood were totally devastated, particularly P1 animals tested in a spatial task (Morris water task).

d. *Parietal Cortex*. When the posterior parietal cortex of the rat is removed in infancy (P1 or P5 or P7 or P10) there are severe functional deficits relative to adults with similar lesions and no compensatory changes in dendritic arborization (Kolb et al., 1987; Kolb & Cioe, 1998).

e. *Frontal Cortex*. Rats given frontal cortical lesions during a window of time (P7-P12) show a significant recovery of function on various cognitive and motor tasks. Moreover, if the lesion is restricted to the medial portion of the frontal cortex, without removing adjacent motor cortex, the recovery can be complete. This recovery is correlated with marked changes in the remaining neocortex. In particular, there is an expansion of dendritic fields and an increase in spine density of cortical pyramidal cells throughout the remaining neocortex (Kolb & Gibb, 1993). Surprisingly, in addition to the changes in dendritic arborization, there appears to be regeneration of some of the lost tissue.

In an extensive study Kolb et al., (1998) showed that the cavity following medial frontal lesions was filled with new tissue that contained neurons that made appropriate connections with other brain regions. Furthermore, there is evidence to suggest that the functional recovery that these animals show is sustained by the newly generated neurons of the regrown cortex. Kolb and colleagues, (1999a) showed that pre-treating animals with the mitotic marker

BrdU at embryonic days 12 to 17 (E12-E17) blocks the regenerative process after medial frontal lesions on P10. The absence of filling of the lesion cavity is correlated with a lack in functional recovery usually observed after this kind of lesion on P10. In another study, frontal lesions at P10 were performed, the tissue was allowed to reform, and then the new tissue was removed again in adulthood. This treatment produced severe functional deficits relative to animals with only P10 or adult lesions (Temesvary et al., 1998).

It is puzzling that this phenomenon of neurogenesis occurs only in the medial frontal cortex and not in other cortical areas. One explanation could be the proximity of the midline frontal cortex to the subventricular zone. Newly formed neurons would need to migrate only 1-2 mm to reach the midline frontal cortex, if this proved to be the mechanism of the regeneration. Another possibility could be that the midline frontal area lies adjacent to the olfactory bulb and it is known that the olfactory bulb of rodents is constantly being supplied with newly generated neurons throughout life (Altman, 1969). These cells originate in the SVZ and they migrate to the olfactory bulb on a route that passes directly by the damaged medial frontal cortex (Lois & Alvarez-Buylla, 1994). It may be the case that some of the neurons that filled in the frontal cavity were originally destined to go to the olfactory bulb. Furthermore, removal of the olfactory bulb at P10 leads to regeneration of the bulb. This age-dependent regeneration of another midline structure suggests that there must be something special about the mid-line area at this particular developmental stage. The cingulate cortex, which will be the subject of the following chapter, also lies in the midline region of the cortex.

3. THE CINGULATE CORTEX

Sutherland et al., (1988) found that lesions to the posterior cingulate cortex in adult rats produce a profound deficit in two spatial navigation tasks. In a later study by Kolb and Whishaw, (1991) the posterior cingulate cortex was removed at postnatal day 1, 3 or in adulthood and the general finding was an absence of recovery at any age. Although animals with lesions at P3 performed better on the spatial tasks than the adult operates, they did not reach the performance level of the control animals. Animals with lesions at P1 performed as poorly as animals with similar lesions in adulthood. An interesting finding in this study, which may account for the better performance of P3-lesioned animals, is the presence of normal hippocampal electroencephalographic activity (EEG). It is known that lesions of the posterior cingulate cortex disrupt hippocampal EEG because the lesion interferes with the serotonergic input to the hippocampus (Vanderwolf et al., 1985). The EEG disruption indicates abnormal hippocampal functioning, which is probably one reason why lesions of the posterior cingulate cortex impair performance on spatial learning. The presence of normal hippocampal EEG in neonatal-lesioned animals suggests however, a plastic change or reorganization of the serotonergic routes to the hippocampus, which supports its normal hippocampal functioning. Before explaining the functions of the cingulate cortex, it is worthwhile examining the development and connectivity of this cortical area in relation with some other cortical and subcortical structures.

Cingulate Anatomy

The cingulate cortex in the rat can be divided into two major areas that have very different inputs and projections: the anterior cingulate cortex and the posterior cingulate cortex including the retrosplenial area [Fig. 3].

The anterior cingulate cortex is commonly divided into areas Cg1, Cg2 and Cg3 (Zilles, 1985) or areas 24b, 24a and 25 (Vogt & Peters, 1981) respectively, and they represent the medial part of the prefrontal cortex. Zilles, (1985) divided the posterior cingulate cortex into dorsal agranular (RSA) and ventral granular (RSG), areas corresponding to Vogt's (1981) areas 29c and 29d, respectively.

Neurogenesis for areas Cg1 and Cg3 occurs simultaneously in the anterior/posterior plane, mainly on E15-E16 (layer VI), E16-E18 (layer V) and E18-E19 (layer IV-II). Neurons in Cg2 are generated slightly earlier than Cg1 and Cg3. For areas Cg1 and Cg3, 33% of cells are generated before E19 and for area Cg2, 49% are generated before E19 (Bayer & Altman, 1991).

In the retrosplenial area there is a more distinct deep (older) to superficial (younger) neurogenetic gradient between layers. Neurons in layer VI are generated mainly on E16-E17, in layer V on E17-E18, and layers IV-II on E18-E19. The superficial neurons in RSG originate slightly earlier (47% are generated before E19) than those in the same layers in RSA (39% are generated before E19), (Bayer & Altman, 1991).

Although the anterior and the posterior cingulate cortices are intimately interconnected and share numerous projections with structures disseminated throughout the entire brain, their connections with the neocortical regions, and with the thalamic nuclei and limbic structures, differ. Among the neocortical regions, the anterior cingulate cortex (ACC) has extensive reciprocal connections

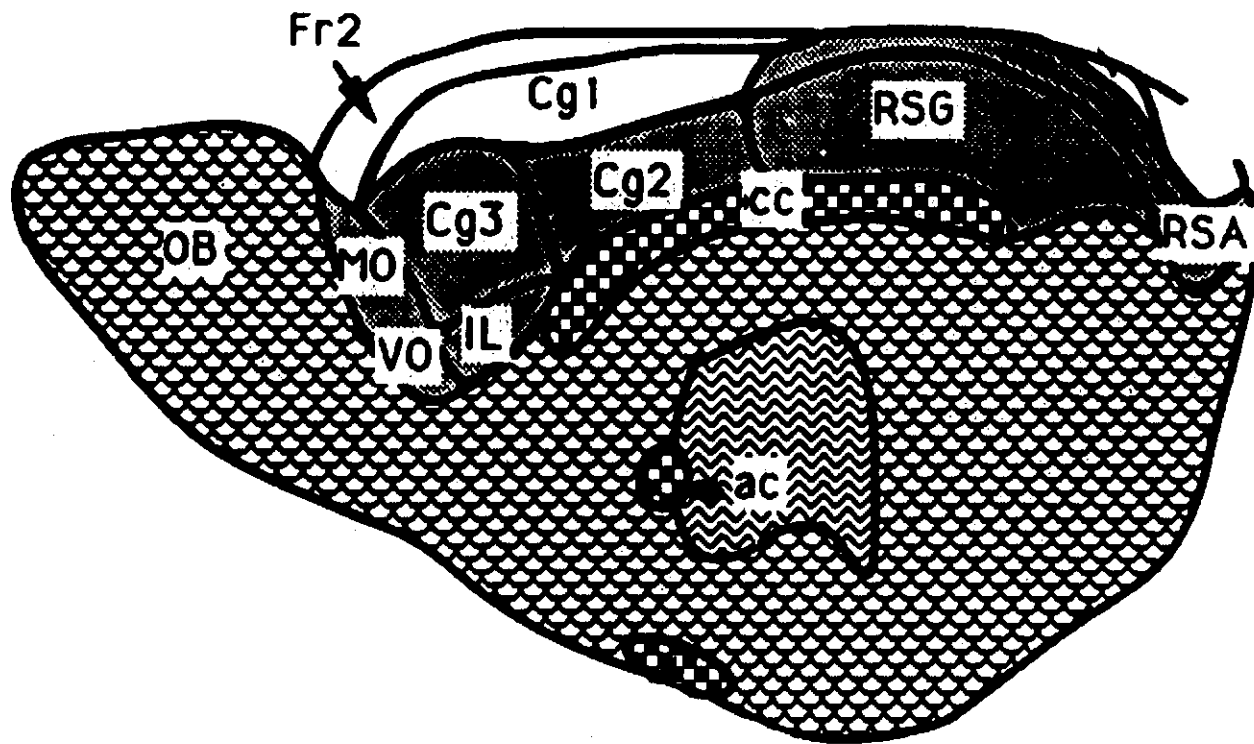


Fig. 3. Medial view of the cortical map in an adult rat showing cingulate areas. Abbreviations are: ac, anterior commissure; cc, corpus callosum; Cg1, cingulate area 1; Cg2, cingulate area 2; Cg3, cingulate area 3; Fr2 frontal area 2; IL, infralimbic area of the medial frontal cortex; MO, medial orbital area; OB, olfactory bulb; RSA, agranular retrosplenial cortex; RSG, granular retrosplenial cortex; VO, ventral orbital area.

with the rostral association areas, in particular the prefrontal cortex. By contrast, the posterior cingulate cortex (PCC) is predominantly interconnected with the caudal association areas. Within the thalamus, the anterior cingulate cortex is primarily linked with the dorsal medial and medial anterior nuclei, whereas the posterior cingulate cortex is specifically connected with the ventral and dorsal anterior nuclei. Within the limbic system, the ACC possesses important reciprocal projections with the basolateral nucleus of the amygdala but not with the subicular complex and parahippocampal cortex. Conversely, the PCC is interconnected with the subicular complex and parahippocampal cortex, but not with the amygdala (for a review, see Domesick, 1969 and Finch et al., 1984).

An anatomical distinction has been made between the anterior cingulate cortex and the medial frontal cortex. The medial frontal cortex includes Zilles's area Cg3 as well as the overlying Cg1 that lies rostral to the genu of the corpus callosum and the infralimbic cortex (IL). This area projects to the nucleus accumbens (Brog et al., 1993), the mediodorsal nucleus of the thalamus (Uylings & van Eden, 1990) and the amygdala (Ray & Price, 1992). The anterior cingulate cortex corresponds to Zilles's areas Cg1 and Cg2 caudal to the genu of the corpus callosum, and has connection with the mediodorsal caudate nucleus (Groenewegen et al., 1990), mediodorsal nucleus of the thalamus (Uylings & van Eden, 1990) and the amygdala (Divac & Diemer, 1980).

The different thalamic and cortical connections of the ACC and PCC has led several authors (Vogt et al., 1979; Baleydier & Mauguire, 1980, cited from Vogt, et al., 1992) to propose a functional dichotomy between these two regions. Because of its reciprocal connection with the amygdala, the anterior cingulate cortex has been implicated in emotional behaviors, and because of its connection

with the hippocampus, the posterior cingulate cortex has been implicated in learning and memory processes.

Functions of the cingulate cortex in the rat and humans

Anterior cingulate cortex:

The anterior cingulate cortex in humans is involved in direct control of skeletal and visceromotor systems, response selection, cognitively demanding processing, and retrieval from short-term memory (e.g. Kolb, 1992). For example, blood flow in anterior cingulate cortex increases during semantic processing of single words or letters (Petersen et al., 1988; Frith et al., 1991; Grossman et al., 1992). Other studies have shown the role of the anterior cingulate cortex in attention and depression (Bench et al., 1992). Studies on pain perception have also suggested the involvement of the anterior cingulate cortex in pain (Jones et al., 1991). Lesions of the anterior cingulate cortex reduce pain sensitivity: "the perception of the pain as such does not appear to be modified, but the patient's total reaction to pain and the threat to existence that it represents is modified markedly" (Foltz & White, 1968). Most of Foltz and White's patients stated that they continued to have pain but it was not particularly bothersome, 'it doesn't worry me....' (Foltz & White, 1968). Furthermore, there is evidence of the involvement of the cingulate cortex in mnemonic processes. Valenstein described a human case with a small infarct in the posterior cingulate cortex and a profound disruption of both anterograde and retrograde amnesia (Valenstein et al., 1987). PET studies have confirmed an increase in blood flow to the anterior cingulate cortex when learning a list of nouns or verbs, which later declines when the list has been practiced (Raichle et

al., 1994, Grasby et al., 1993). Finally, studies on epileptic patients have shown that cingulate cortex seizures can alter the level of attention or consciousness, voluntary and involuntary skeletomotor activity, and affective expression (Levin & Duchowny, 1991).

Posterior cingulate cortex:

Functional studies demonstrate that lesions of the posterior cingulate cortex impair place learning of rats in the water maze: "posterior cingulate areas are essential for the ability to move accurately to points in space using the relationships among distal cues" (Sutherland et al., 1988). Other studies demonstrate the presence of head direction cells (that is, cells that are maximally active when the rat is pointing its head in one particular direction) in the retrosplenial cortex (Chen et al., 1994), and that the integrity of this structure seems to be important for maintaining stability of a head-direction cell's preferred direction (Basset & Taube, 1999). Gabriel and colleagues (Gabriel et al., 1989, Gabriel & Sparenbog, 1986; 1987) have shown that the response of retrosplenial cortex neurons in a learning paradigm is in part dependent on intact connections from the hippocampal formation to the retrosplenial cortex.

In sum, these results demonstrate that these interconnections provide the anatomical basis for the retrosplenial cortex's role in processing and integrating information related to memory, learning and emotional functions. Taken together, it appears that the cingulate cortex contributes to a broad spectrum of cognitive functions.

4. THE PROBLEM

Three main questions were formulated at the beginning of the present investigation.

1) Are the plastic changes observed in medial frontal lesions at P10 of age also observed in animals with the posterior cingulate cortex removed at P10?

2) Are there any behavioral differences in the performance of cognitive and sensorimotor tasks in animals that have received posterior cingulate cortex lesions in infancy or in adulthood?

3) What are, in adulthood, the anatomical correlates of functional recovery after neonatal cingulate lesions?

Experiment 1 describes the adult behavioral and anatomical effects of posterior cingulate cortex removals at postnatal day 10. Questions that developed from results of the first experiment then were assessed in experiments 2 and 3. Experiment 2 describes the anatomical and behavioral effects of combined anterior and posterior cingulate cortex removals at postnatal day 10. Experiment 3 describes the anatomical and behavioral effects of anterior and/or posterior cingulate cortex removals at postnatal days 4, 10, and 120. But, before I start describing the methodology used in the present study, I would like to describe some of the behavioral procedures that were utilized in the assessment of the recovery of function after brain injury.

5. RATIONAL FOR THE BEHAVIORAL AND ANATOMICAL MEASURES

In the study of neurobiological processes, the use of different behavioral tasks that challenge the organism to execute different behaviors (e.g. mazes, motor tasks), opens the possibility to study the same behavior repeatedly over time and to characterize recovery, if any, after brain injury. In order to understand the evolution of a particular behavior, or the emergence of any deficit after brain damage, more than a single test of learning is needed. A single behavioral measure provides a restricted estimate of behavior so an assessment of a wider variety of species-typical and learned behaviors is required in the evaluation of the effects after brain injury.

Animals in experiments 1 and 2 were only tested in the Morris water task. Animals in experiment 3 were subjects in two learning tasks (Morris water task and conditioned taste aversion) and one motor task (Whishaw reaching task). In addition, one species-typical behavior was evaluated (locomotor activity). I will present a brief description of each task.

Morris Water Task

The Morris water maze (Morris, 1982) has been used as a tool for evaluating the effects of different types of brain damage or drug manipulations on spatial learning. In this task, rats are placed in a large circular pool of water from which they can escape onto a hidden platform which is located in the middle of one of the four imaginary quadrants of the pool [Fig. 4]. The platform is rendered invisible, both by ensuring that its top surface is just beneath the water surface, and by making the water opaque. Thus, the platform offers no local cues to guide

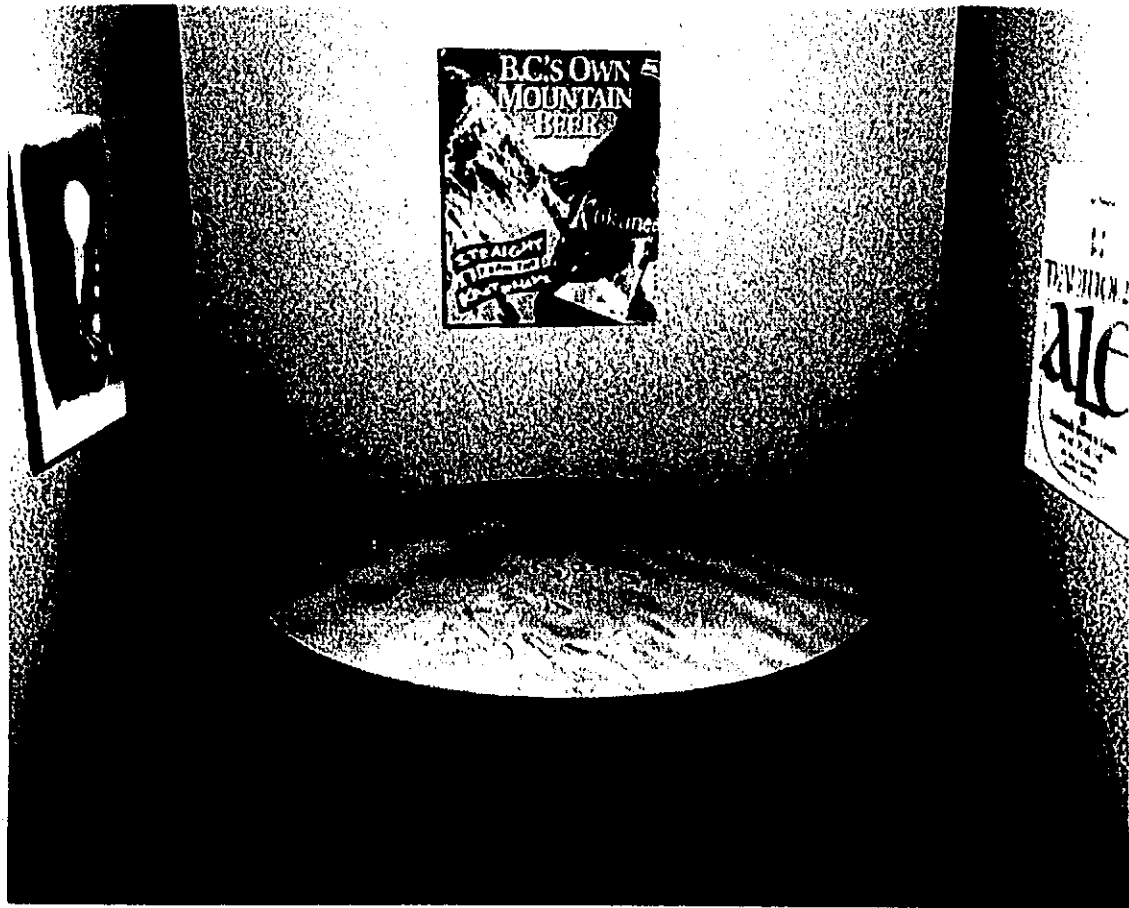


Fig 4. Schematic representation of the pool and room used in the Morris water task. The animal is required to learn the location of a submerged, hidden platform. The only cue to the position of the platform is its spatial relationship to cues about the room.

escape behavior. Normal rats very quickly learn to swim directly towards the platform from any starting position at the circumference of the pool. The accurate directionality of the escape behavior provides evidence that the rat escapes by learning the spatial position of the platform relative to distal cues. Performance on this task can be measured by recording the time taken to swim to the platform, the distance traveled, or measuring directionality of the head and body to conduct their swim to the platform. Animals with various cortical or hippocampal lesions are severely impaired at learning the location of the platform (e.g., Whishaw & Maaswinkel, 1998; Whishaw et al., 1994; Kolb & Gibb, 1991b; Sutherland et al., 1983; Eichenbaum et al., 1990), and in many cases the animals are unable to acquire the task even with extensive training.

Conditioned Taste Aversion

One model widely used in the study of learning and memory processes is conditioned taste aversion (CTA; Garcia et al., 1985). In this procedure, rats are exposed to a novel flavored solution, and a few minutes later receive an induced gastric malaise. As a result, when the flavored solution is presented again, animals reduce their consumption [Fig. 5]. The anatomical substrate of CTA has been well described (Kiefer, 1985) and includes the gustatory portion of the insular cortex (IC) as a projection site for taste-visceral information. However, IC lesions do not produce obvious deficits in gustatory or gastrointestinal sensitivity, or taste perception. These processes seem to take place in the pontine areas of the brainstem (Yamamoto, 1993). Lesions of the IC as well lesions of the nucleus basalis magnocellularis (which provides the primary cholinergic

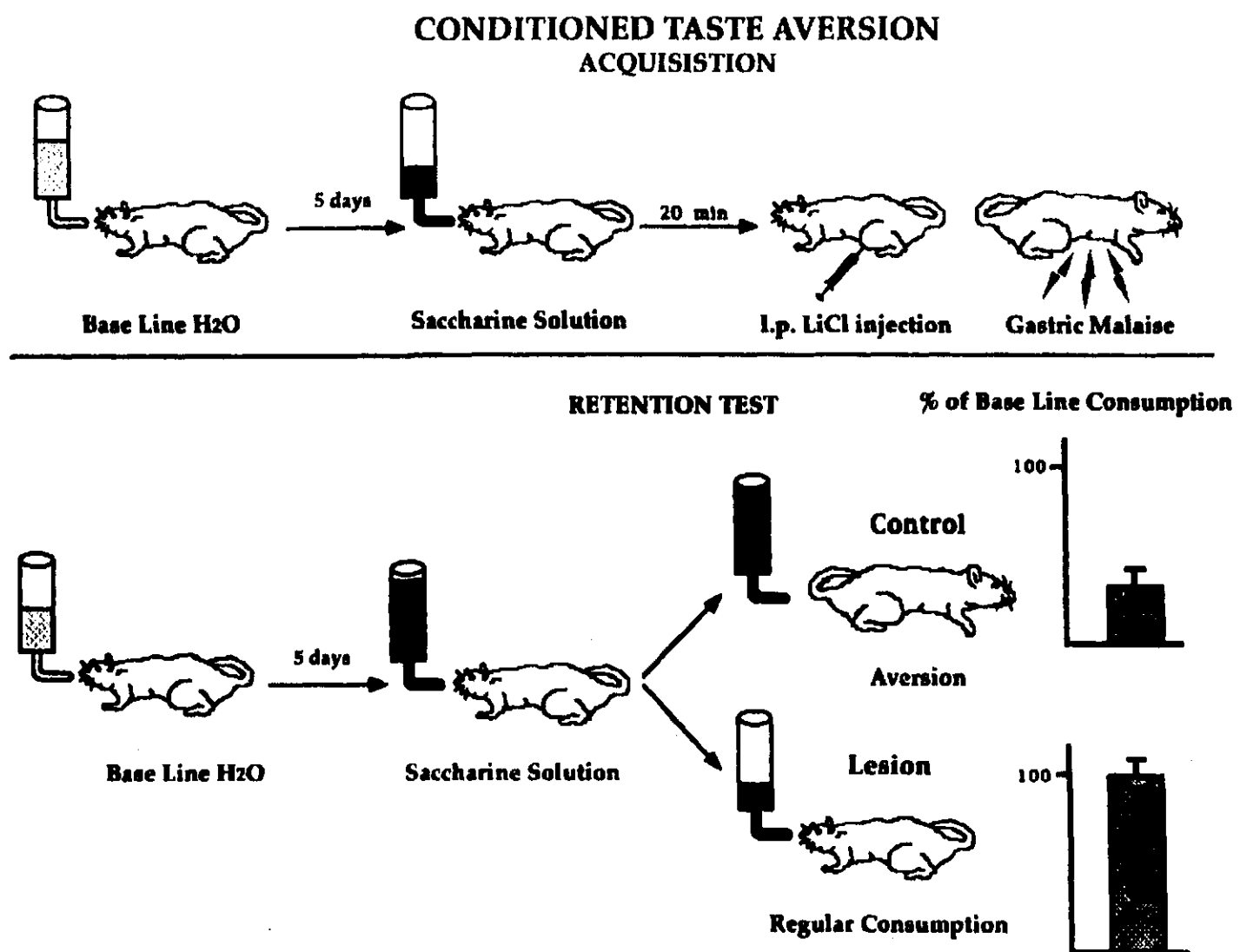


Fig. 5. Schematic representation of the conditioned taste aversion, and expected results of an imaginary control and lesion animal. The top part shows the acquisition phase and the bottom part shows the retention test.

projection to the cerebral cortex Bigl et al., 1982; Saper, 1984) disrupt acquisition of CTA (Braun et al., 1982; Lopez-Garcia et al., 1993; Gutierrez et al., 1999).

Whishaw's Reaching Task

The Whishaw reaching task was developed to assess the ability of rats to reach using the forepaws (Whishaw et al., 1986). In the task the animal has to learn to reach through bars to retrieve small pieces of food [Fig. 6]. Reaching ability can be assessed by measuring endpoint success such as accuracy of reaching (i.e., percent of reaches in which the animal successfully retrieves food) or by videotaping the animal's behavior and doing more refined kinematic analysis on different aspects of the actual movements. Animals with various cortical lesions show deficits at acquiring the task (e.g., Whishaw & Coles, 1996; Whishaw et al., 1992).

Motor Activity

One of the advantages of measuring behaviors other than the learning of cognitive tasks is that it is possible to evaluate other behaviors that the animal might exhibit after brain trauma that were not part of their original repertoire or that were altered after the injury. General activity would provide information about any fluctuation on the circadian rhythms or changes in motor activity. The animals are placed in a bank of 15-wire photocell cages with two parallel horizontal infrared beams 1 cm above the floor [Fig. 7]. A computer records the total number of beam breaks which can then be analyzed statistically. The cages

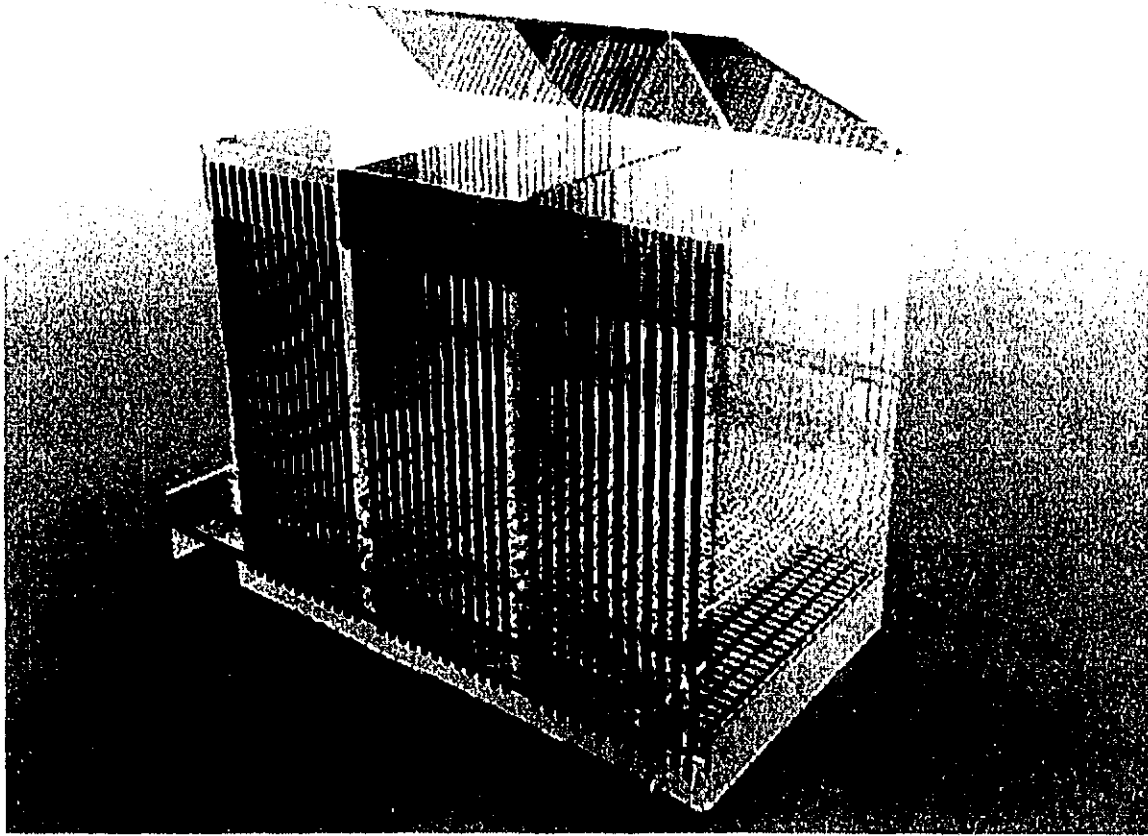


Fig. 6. Illustration of the reaching box. For the task, the animal must reach through the bars to grasp a piece of food and retrieve it.

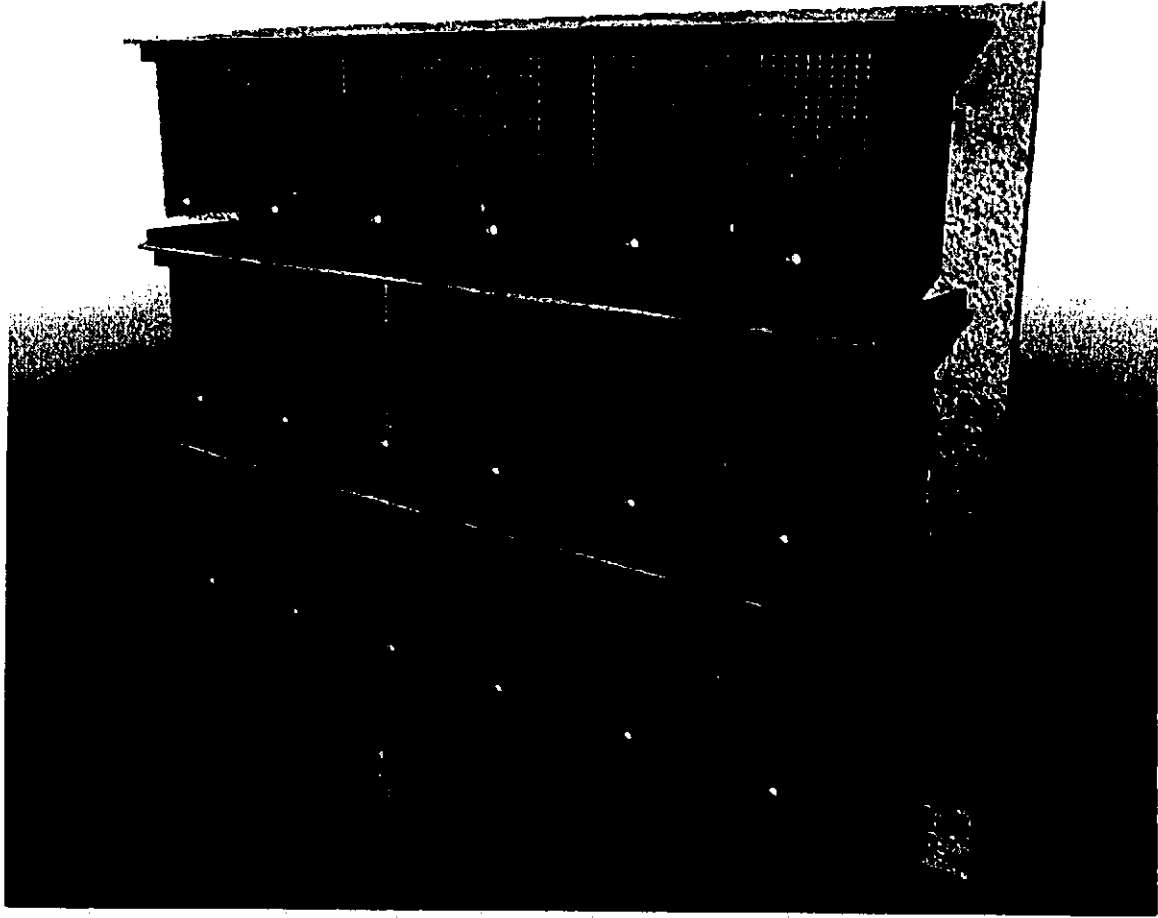


Fig. 7. Illustration of the cages used for monitoring general activity. The animal is placed in the cage that contains two parallel horizontal infrared beams that are interrupted as the animal moves about the cage.

simulate a home cage thus the animals can be placed in them for few hours or for several days.

Anatomical Measures

Kolb (e.g., Kolb 1992) has proposed that one possible compensatory mechanism in rats with good functional outcome after neonatal lesion is an increase in dendritic arborization in the remaining neocortical areas. In order to address this issue a Golgi-Cox study was carried out. In the Golgi-Cox technique (Gibb & Kolb, 1998) a small percentage (less than 5%) of all cortical cells (randomly) is stained by a precipitate of a heavy metal such as mercury. Once cells are stained they are drawn under camera lucida and different aspects such as the dendritic length, the number of dendritic branches or the number of spines can be quantified providing an indirect measure of synaptic space.

In addition to the Golgi study, some other anatomical measures were done at the end of the behavioral testing. These are brain weight, cortical thickness and thalamic organization. In doing so, the behavioral results can be correlated with morphological and/or anatomical changes in the brain.

EXPERIMENT 1.

6. Experiment 1

Recovery of function after cortical brain damage is age and locus dependent. The best functional outcome in rats takes place when the lesion occurs during a window of time that goes from P7 to P12. Functional outcome is nearly complete after medial frontal lesions, partial with parietal lesions, and nearly absent with occipital or motor lesions. The present experiment investigates the effects of lesions to the posterior cingulate cortex given at P10. The posterior cingulate cortex is particularly interesting because, like the medial frontal cortex, it is a midline structure and, in addition, it has substantial connections with medial frontal and posterior-parietal cortices.

METHOD

Subjects.

Thirteen Long-Evans rats (5 females; 8 males) were used in this study. Seven rats had the posterior cingulate cortex (areas RSA and RSG) removed bilaterally on the 10th day of life (P10), and six were used as controls. Behavioral testing began at 120 days of age. One additional rat was given a complete removal of the midline cortex. He was assessed behaviorally but he was not included in the statistical analysis.

Surgical procedures.

The animals were anesthetized at day 10 by cooling them in a Thermanon cooling chamber set at -5°C. They were cooled for 15 min, or until their rectal temperature was in the range of 18-20°C, and they were immobile. The bone was

removed from bregma to lambda by cutting it with iris scissors and then removing the cortex posterior to bregma by aspiration. The wound was then sutured with silk thread. The control rats were anesthetized in the same manner and the skin was incised and sutured. Animals were slowly warmed up to 35°C under a heat lamp before being returned to the mother.

Anatomical procedures.

At the conclusion of the behavioral testing the animals (males only) were given an overdose of sodium pentobarbital and intracardially perfused with 0.9% saline. The brains were removed and weighed before being immersed in 20 ml of Golgi-Cox solution where they remained 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 & Kolb, (1998).

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.

Golgi analysis.

In order to be included in the data analysis the dendritic processes of pyramidal cells had to fulfill the following criteria: 1) the cell had to be well impregnated and not obscured with stain precipitations, blood vessels, or heavy clusters of dendrites from other cells; 2) the cell had to lie approximately in the middle of the section thickness so that the apical and basilar dendrites were

clearly visible in the plane of section. The cells were analyzed by using the concentric ring procedure of Sholl, (1956). A mean of the total dendritic length can be estimated by multiplying the total number of intersections by 20. For each cell, the number of intersection of dendrites with a series of concentric circles at 20 μm intervals from the center of the cell body was counted. The cells were then analyzed by branch order in which each division of a branch was numbered and counted.

Behavioral Procedures

Morris water task. The maze consisted of a circular pool (diameter 1.55 m, height 46 cm), the inside of which was painted white and filled to a height of 25 cm with approximately 22°C water in which instant powdered milk was dissolved. A clear plexiglas platform (11 X 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. A trial consisted of placing a rat by hand into the water, facing the wall of the pool, at one of four starting positions (north, south, west and east) around the pool's perimeter.

Animals were tested with eight trials per day for five consecutive days. 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 60 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 swimming paths were recorded via a video camera mounted above the tank. A target scanning system was able to track the black head of the rat from the white

background of the pool. After the 8 trials, the animals were returned to their home cages and the same procedure was repeated the next day. For the probe trial the platform was removed from the tank on the 8th trial of the last day of training, and the rat was allowed to swim for 30 sec. Its swim trajectory was then recorded.

RESULTS

Anatomical Results

Gross Anatomy. The lesions removed the posterior cingulate region including most of Zilles' areas RSA and RSG. There was no direct damage to subcortical structures, including the hippocampus.

Brain weight. The lesion resulted in a loss of brain weight up to 7% (Table 1). Analysis of variance revealed a significant main effect for lesion group ($F(1,9) = 7.87, P = 0.020$).

Table 1

Group	Brain Weights
Control	2.162 ± 0.03 (n = 6 Males)
P10 Cingulate	2.018 ± 0.04* (n = 5 Males)

*Significantly different from control animals.

Dendritic Analysis. The mean of the total dendritic length derived from the Sholl analysis showed an increase in both apical and basilar dendrites of parietal

SHOLL ANALYSIS Apical Dendrites

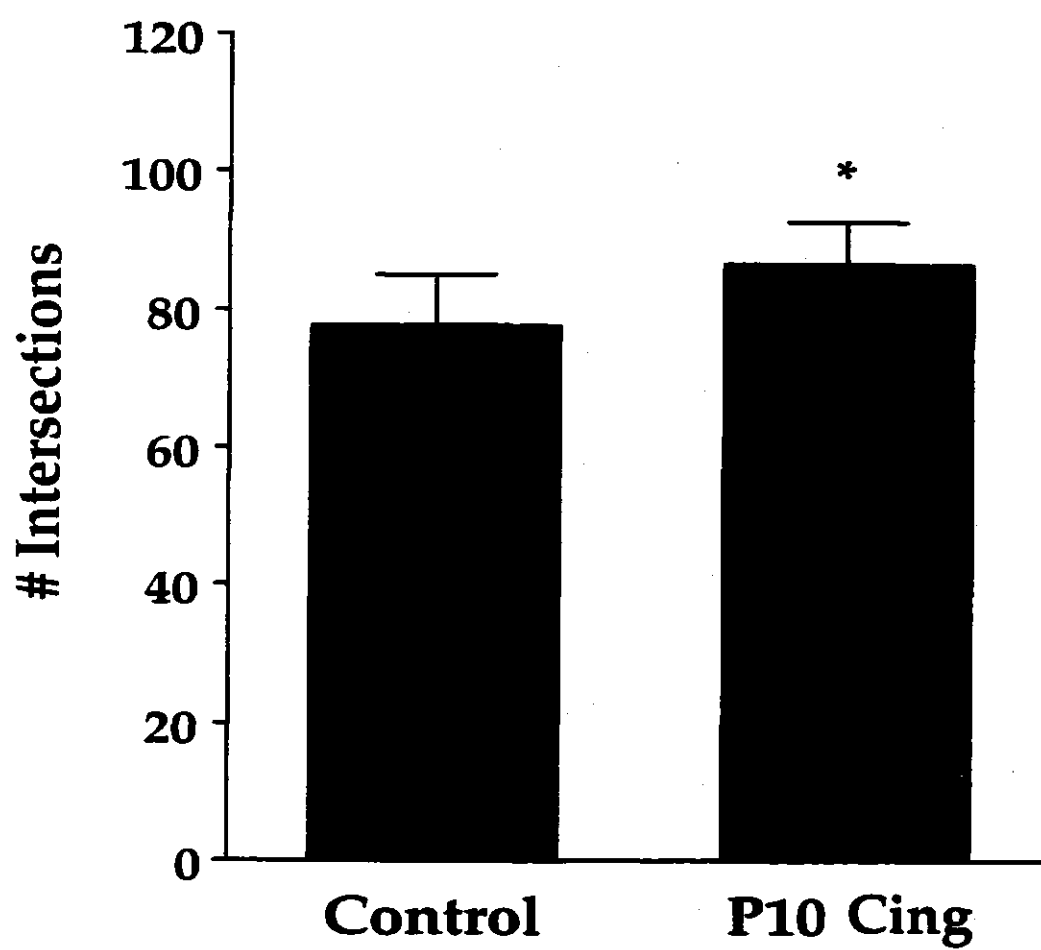


Fig. 8a. Summary of mean number of total apical dendritic branches (\pm SE) on layer III pyramidal neurons in Zilles' area Par1. Animals received P10 lesions of the posterior cingulate cortex. (* significantly different than control).

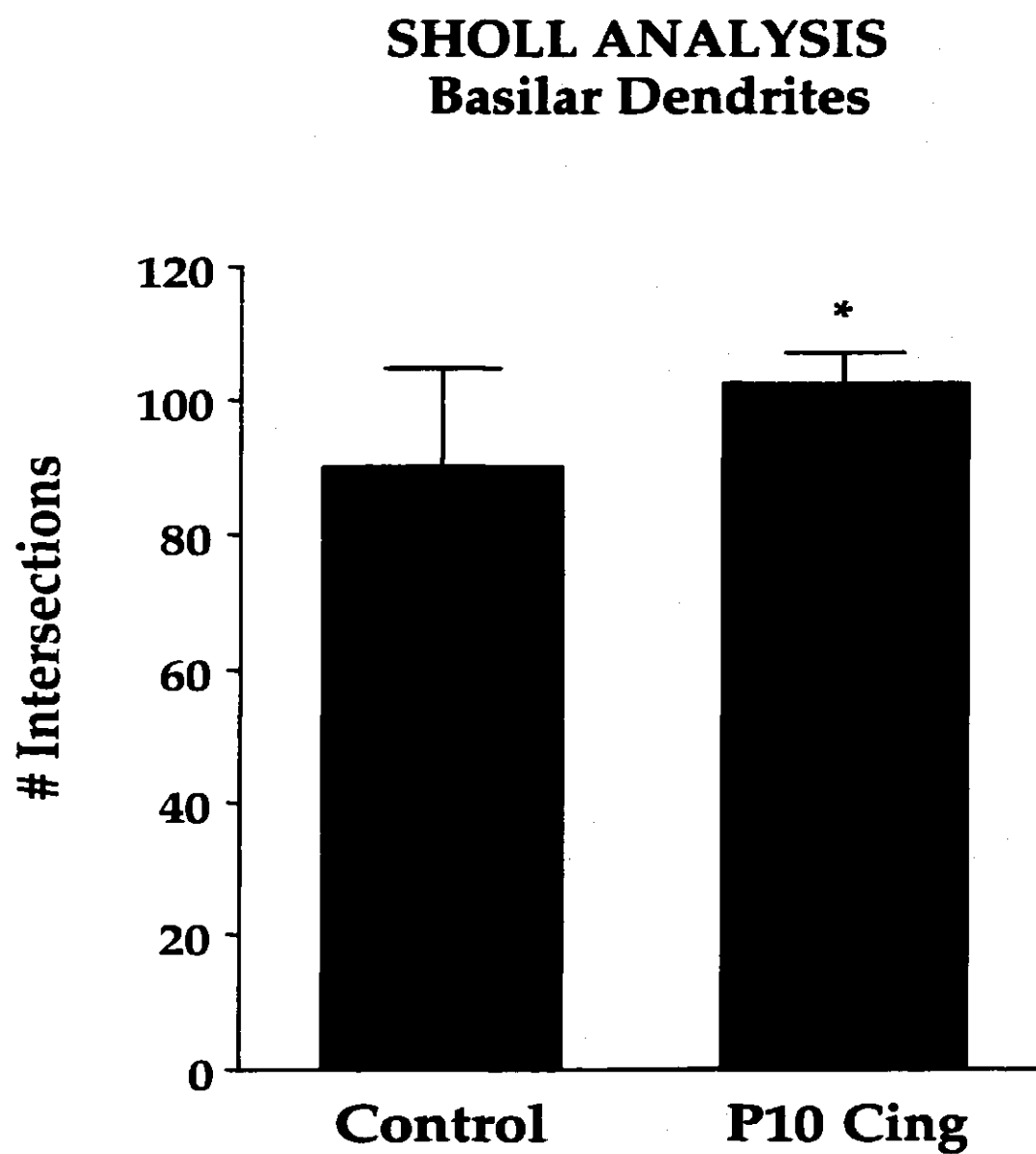


Fig. 8b. Summary of mean number of total basilar dendritic branches (\pm SE) on layer III pyramidal neurons in Zilles' area Par1. Animals received P10 lesions of the posterior cingulate cortex.
(* significantly different than control)

cortex 1 layer III in animals with cingulate lesions [Fig 8a & 8b]. Analysis of variance on the Sholl analysis for the total dendritic length for both the apical ($F(1,26) = 6.43, P < 0.02$), and basilar ($F(1,26) = 4.03, P < 0.05$) dendrites showed significant group effects.

Behavioral Results. Control animals quickly learned to find the platform and to swim directly toward it when released from any starting location. This improvement was reflected in the decline of the latencies in finding the platform. By the last day of training the escape latency for all animals reached an asymptote at around 4 sec. When the platform was removed on the MWT probe trial all animals swam around the previously correct location before heading off to swim in other directions. There was no obvious sex difference in the performance of this task. Although animals with cingulate lesions differed from control, they showed better performance than previous studies of similar lesions in adulthood (Kolb & Whishaw, 1991) reaching an asymptote at around 8 sec by the last day of training. Analysis of variance comparing the control and the P10 cingulates showed a significant main effect of group ($F(1, 11) = 4.85, P < 0.05$), and trial block ($F(9, 99) = 30.6, P < 0.0001$), but no interaction ($F(9, 99) = 1.01, P = 0.43$) [Fig. 9a & 9b].

On the probe trial P10 cingulate animals swam around the previously correct location in the same way as the controls did. Analysis of the probe trial revealed that although lesioned animals spent slightly less time in the quadrant where the platform used to be, this difference was not statistically significant ($F(1,11) = 4.67, P = 0.054$), [Fig. 10]. This result suggests that even though P10

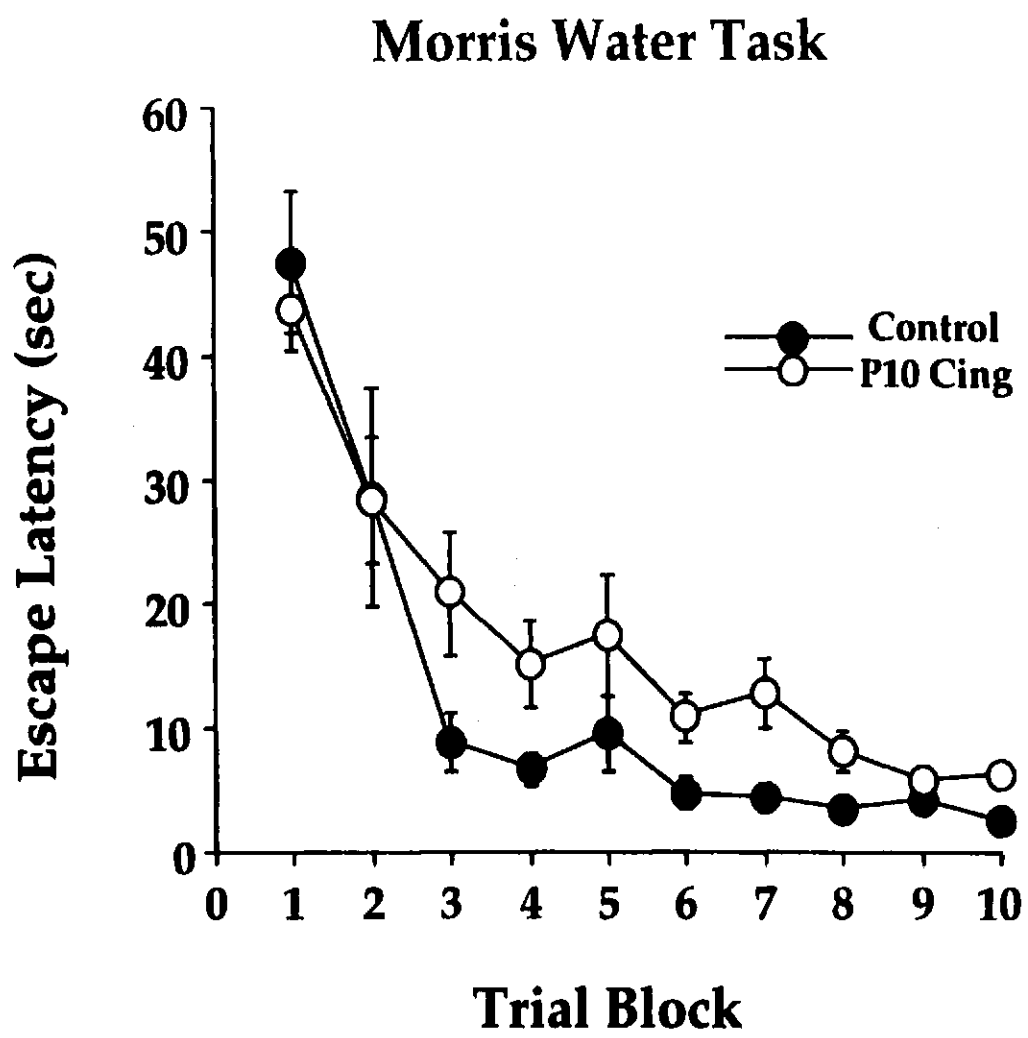


Fig. 9a. Summary of performance (\pm SE) in the Morris water task for P10 posterior cingulate lesioned animals. Mean escape latency across the 10 trial blocks.

MORRIS WATER TASK

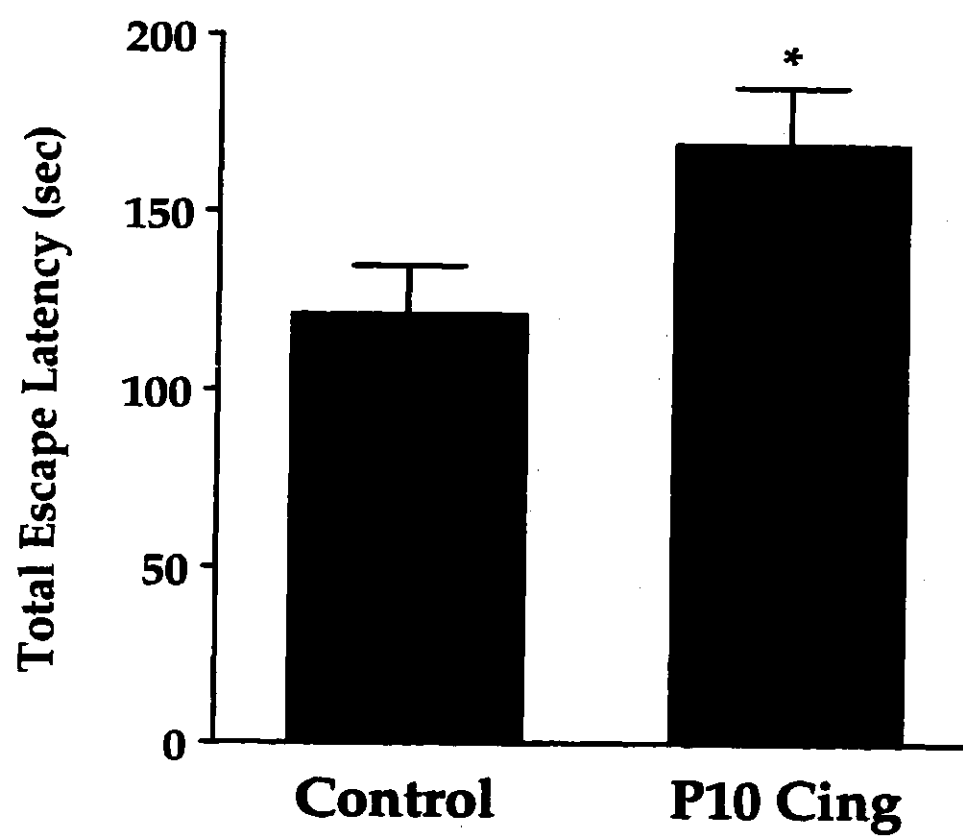


Fig. 9b. Summary of performance in the Morris water task. Total escape latency (\pm SE) summed across the 10 trial blocks. Animals received P10 lesions of the posterior cingulate cortex. (* significantly different than control)

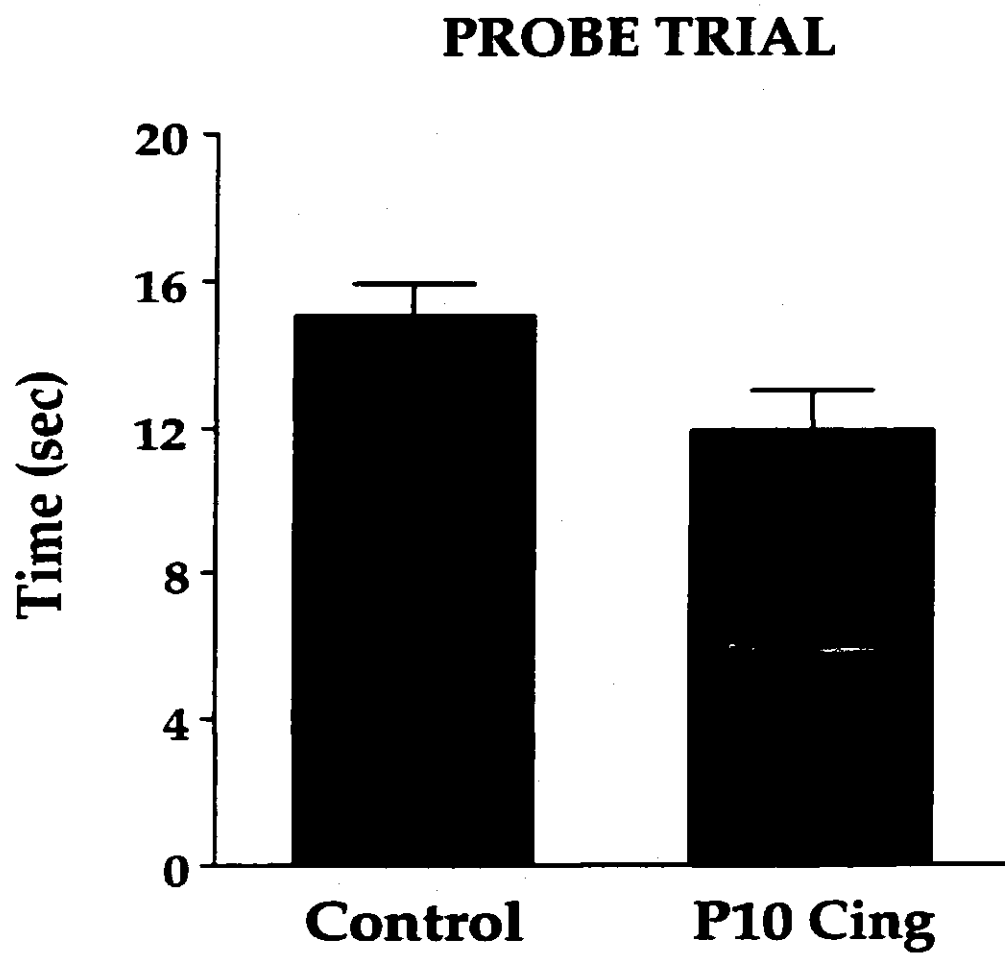


Fig. 10. Summary of time spent (\pm SE) by the animals swimming in the previously correct quadrant during the probe trial. Animals received P10 lesions of the posterior cingulate cortex. Total swim time = 30 s.

cingulate animals showed longer latencies in finding the platform, they eventually learned the correct location of the platform in the pool.

One curious finding was a spontaneous-partial regeneration of tissue on the single animal that had both the anterior and posterior cortices removed. This phenomenon was correlated with a surprisingly good behavioral outcome, the animal's performance was indistinguishable from controls in the MWT.

The results of the present experiment showed that although animals with posterior cingulate cortex removed showed a mild deficit in acquiring the Morris water task, they performed surprisingly well compared to animals with similar lesions in other cortical areas. The removal of the PCC did not lead to regrowth, but there was an increased dendritic arborization in pyramidal neurons in the parietal cortex. This dendritic growth presumably reflects increased synaptic formation that could be responsible of the functional recovery.

EXPERIMENT 2.

7. Experiment 2

One unexpected finding was observed in Experiment 1: Removal of the anterior cingulate in addition to the posterior cingulate cortex ($n = 1$) resulted in a partial regrowth of the lost frontal and cingulate. This animal showed a complete recovery of function on the water task and its performance was indistinguishable from controls. The purpose of the second study was to explore the possibility that the additional removal of the anterior cortex was stimulating neural generation such that cells appeared to migrate beyond the damaged area to partially restore the posterior cortex, and to determine if this regeneration was correlated with functional recovery.

METHOD

Subjects.

Seventeen Long-Evans rats (8 females ; 9 males) were used in this study. Eight rats (5 males; 3 females) had the anterior and posterior cingulate cortex (areas CG1, CG2, CG3, RSA and RSG) removed bilaterally on the 10th day of life (P10Total), and nine (4 males; 5 females) were used as controls. Behavioral testing began at 120 days of age.

Surgical procedures.

The animals were anesthetized at day 10 by cooling them in a Thermanon cooling chamber set at -5°C . They were cooled for 15 min or until their rectal temperature was in the range of $18-20^{\circ}\text{C}$ and they were immobile. The midline bone (from the frontal suture to lambda) was removed by cutting it with iris scissors and then removing the cortex by aspiration. The wound was then

sutured with silk thread. The control rats were anesthetized in the same manner and the skin was incised and sutured. Animals were slowly warmed up to 35°C under a heat lamp before being returned to the mother.

Anatomical procedures.

At the conclusion of the behavioral testing all the animals were given an overdose of sodium pentobarbital and intracardially perfused with 0.9% saline followed by 4% paraformaldehyde solution. The brains were removed, weighed and placed in a 30% sucrose formalin solution for 3 days before being cut frozen at 40 µm. Every seventh section was saved, mounted on slides, and stained with Cresyl violet.

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.

Cortical thickness was measured by projecting the Nissl-stained sections on a Zeiss DL 2 POL petrographic projector set at a magnification of 20 X. Measurements were made with a clear plastic millimeter ruler and taken at three points at each of the following 5 planes: plane I, first section with caudate putamen visible, measures in Zilles', (1985) areas Gu, Par 1, Fr 2; plane II, centre of anterior commissure, measures in Par 2, Par 1, Fr 1; plane III, first hippocampal section, measures in areas Gu, Par 1, Fr 1; plane IV, posterior commissure, measures in areas Te 1, Oc 2L, RSA; plane V, most posterior hippocampal section, measures in Te 1, Oc 1B, Oc 2ML [Fig. 11].

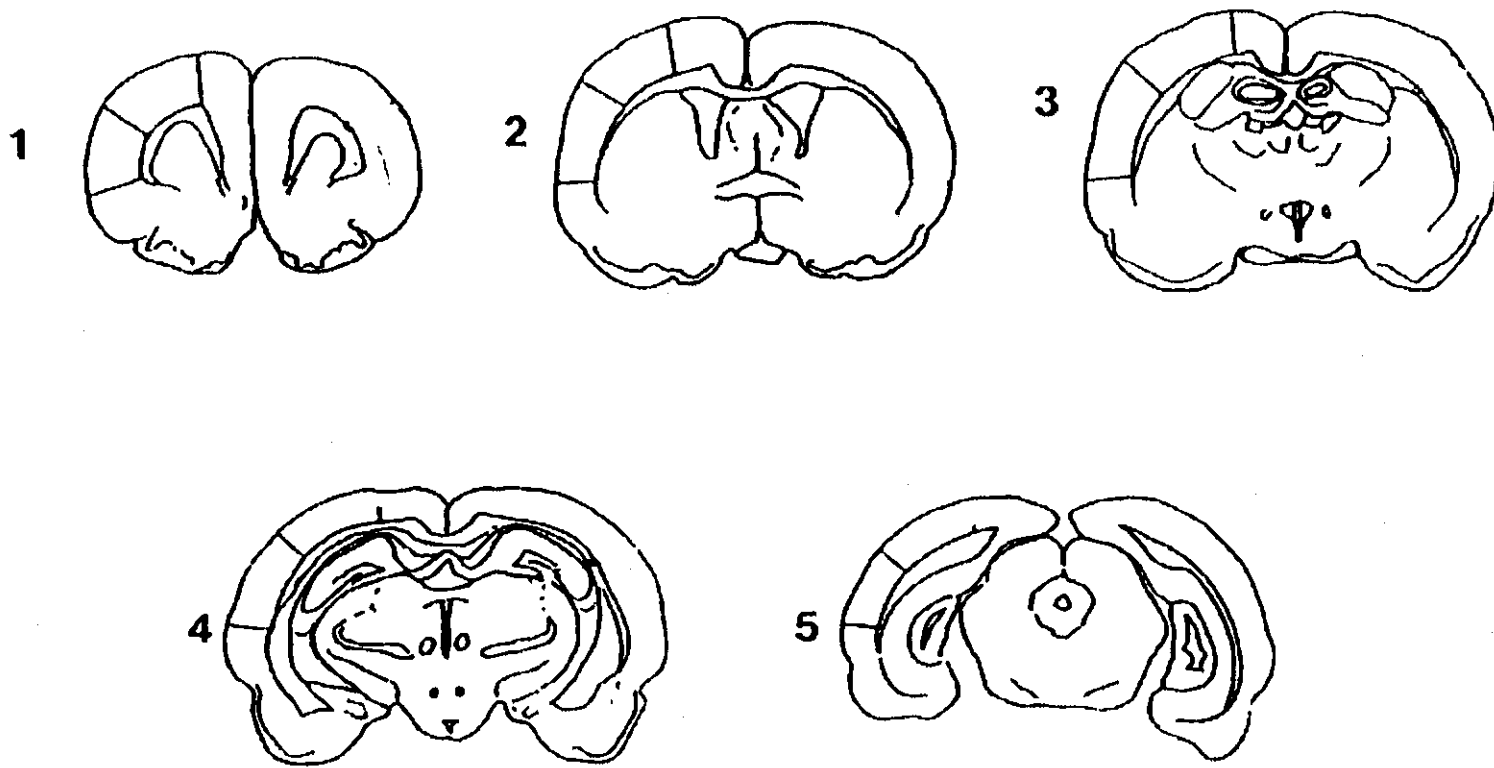


Fig. 11. Sections through the rat brain showing the planes at which cortical thickness was measured. The landmarks for the planes are as follows: Plane 1, external capsule; Plane 2, anterior commissure; Plane 3, first hippocampal section; Plane 4, posterior commissure; Plane 5, most posterior hippocampal section.

Behavioral Procedures.

Morris water task. The maze and procedures were the same than those used in Experiment 1.

RESULTS

Anatomical Results

Gross Anatomy. The lesions removed the anterior and posterior cingulate region including most of Zilles' areas CG1, CG2, CG3, RSA and RSG. All animals showed a partial regrowth primarily in the anterior cingulate cortex but in some cases it extended to the posterior region. There was no direct damage to subcortical structures, including the hippocampus.

Brain weight. Overall, the lesion reduced brain weight up to 9%, (Table 2). Because brain weight is sexually dimorphic, a two-way analysis of variance (Sex by Lesion) was conducted and this revealed a significant main effect of the lesion group ($F(1,13) = 40.13, P < 0.0001$), sex ($F(1,13) = 35.01, P < 0.0001$), but not the interaction ($F(1,13) = 0.01, P = 0.922$). Follow-up tests (Fisher's LSD) showed that the P10 total animals and control groups differed for each sex ($P < 0.01$).

Table 2

GROUP	SEX	
	Male	Female
Control	2.095 ± 0.03 (n = 4)	1.928 ± 0.01 (n = 5)
P10 Total	1.916 ± 0.02* (n = 5)	1.743 ± 0.02* (n = 3)

*Significantly different from control animals.

Cortical Thickness. The overall result was that the lesion reduced cortical thickness in the five different planes, and that there was a sex difference (females had thinner cortices). Sex therefore was included as a factor in the statistical analyses [Fig. 12]. Analysis of variance showed a significant main effect of group ($F(1, 22) = 79.06, P < 0.0001$), sex ($F(1, 22) = 4.92, P < 0.05$) and the interaction ($F(1, 22) = 4.59, P < 0.05$). There was also a significant effect plane ($F(4, 88) = 153.66, P < 0.0001$), but not the interaction by group ($F(4, 88) = 1.94, P = 0.110$), or by sex ($F(4, 88) = 0.27, P < 0.89$). Follow-up tests (Fisher's LSD) showed that the P10 total animals and control groups differed for each sex ($P < 0.01$).

Behavioral Results. Control animals quickly learned to find the platform and to swim directly toward it when released from any starting location. This improvement was reflected in the decline of the latencies in finding the platform. By the last day of training the escape latency for all animals reached an asymptote of around 4 sec. When the platform was removed on the MWT probe trial all animals swam around the previously correct location before heading off to swim in other directions. There was no obvious sex difference in the performance of this task. Although animals with the complete cingulate cortex removed differed from controls, they performed surprisingly well, reaching an asymptote of around 7 sec (mean) by the last day of training. Analysis of variance comparing the control and the P10 total cingulates showed a significant main effect of group ($F(1, 15) = 6.65, P < 0.05$), and trial block ($F(9, 135) = 28.29, P < 0.0001$), but no interaction ($F(9, 135) = 1.39, P = 0.19$) [Fig. 13a & 13b]. On the probe trial P10 total animals swam around the previously correct location in a very similar way that the controls did. Analysis of variance of the probe trial

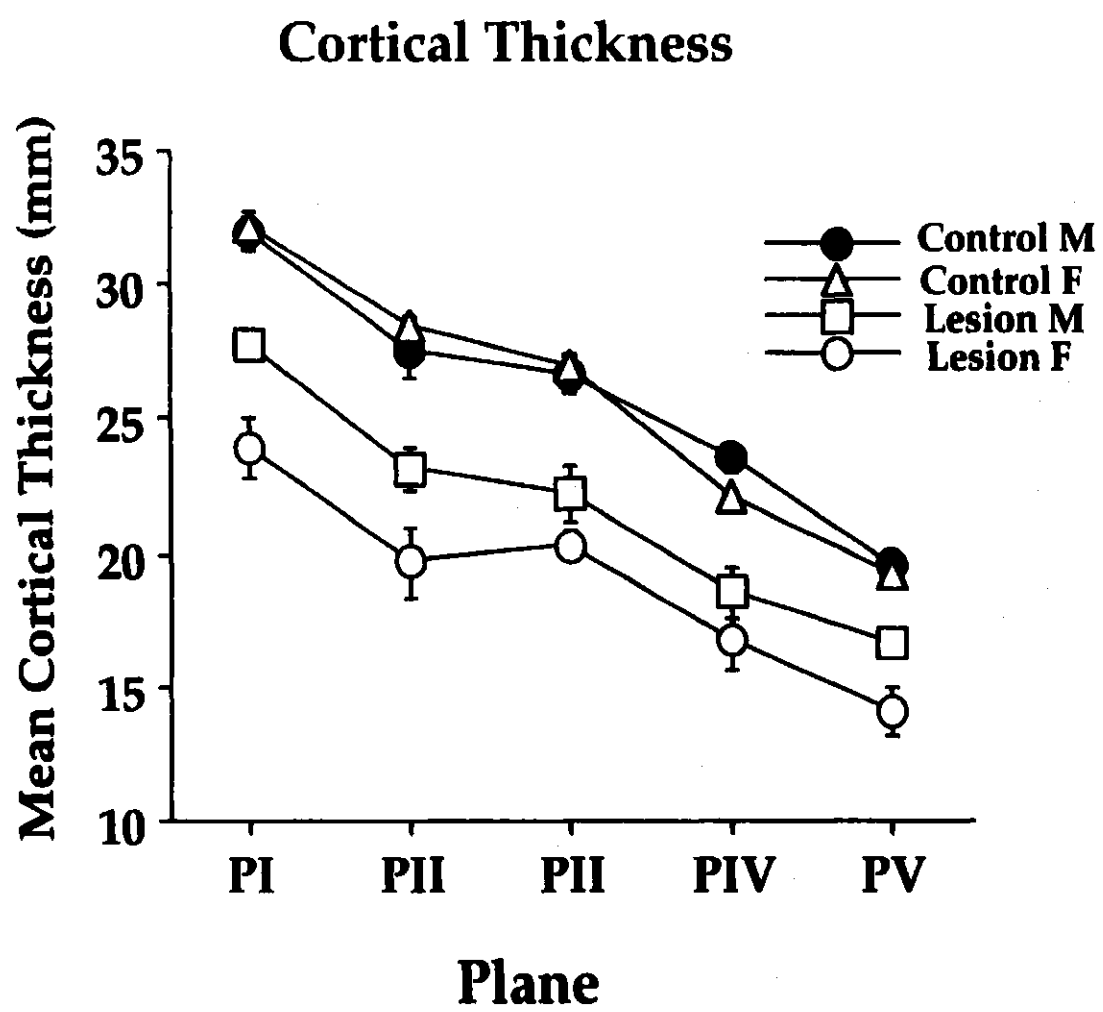


Fig. 12 Cortical thickness summary showing effects of complete cingulate cortex lesions at different ages. Mean cortical thickness (\pm SE) at each of 5 planes. M or F represent masculine or feminine respectively.

Morris Water Task

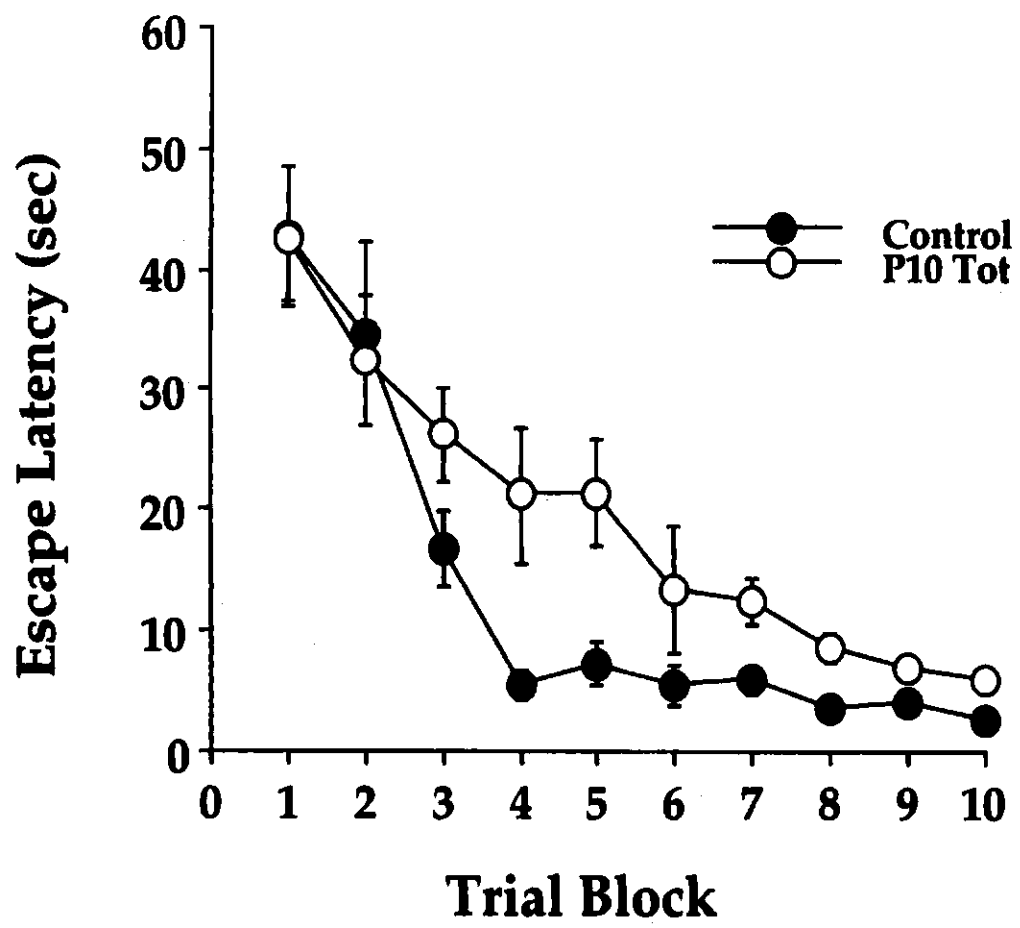


Fig. 13a. Summary of performance (\pm SE) in the Morris water task for P10 complete cingulate-lesion animals. Mean escape latency across the 10 trial blocks.

MORRIS WATER TASK

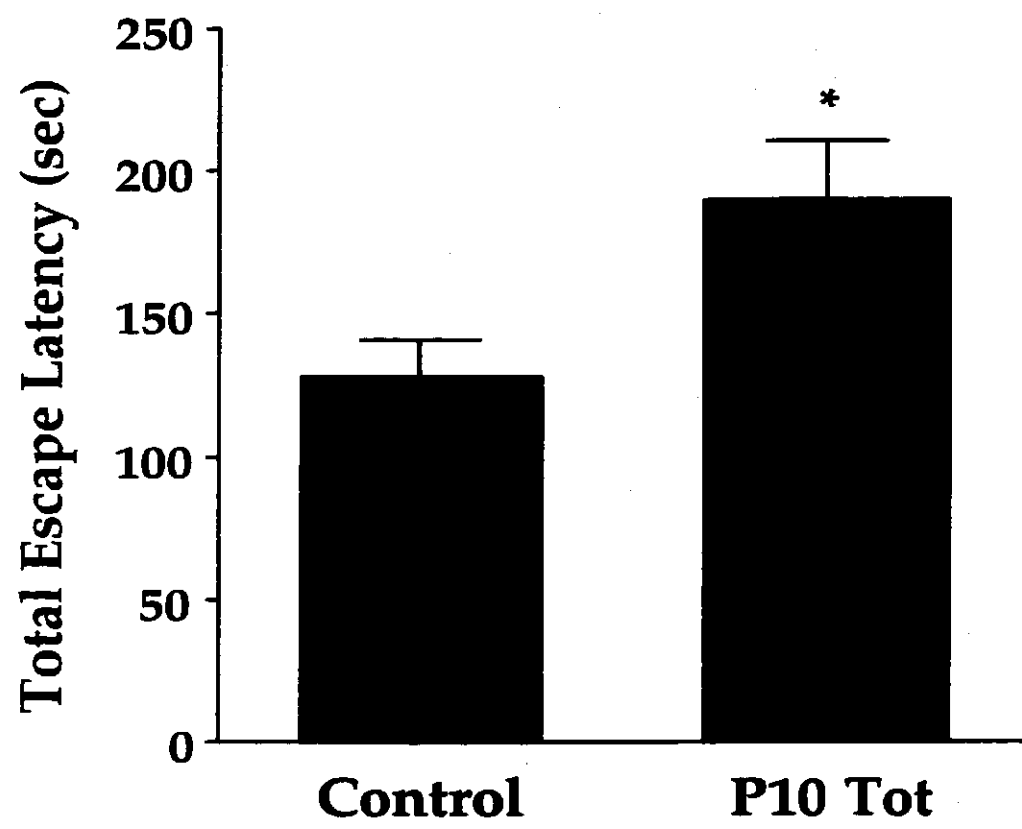


Fig. 13b. Summary of performance in the Morris water task. Total escape latency (\pm SE) summed across the 10 trial blocks. Animals received P10 lesions of the complete cingulate cortex. (* significantly different than control)

revealed that the lesion animals spent equivalent time in the quadrant where the platform was previously located. Analysis of variance showed no differences among groups, ($F(1,15) = 0.504, P = 0.488$), [Fig. 14]. This result suggests that even though P10 total animals showed longer latencies in getting to the platform they knew the correct location of the platform in the pool.

The results of the present experiment confirmed the serendipitous finding of Experiment 1. When the anterior cingulate cortex is removed along with the posterior cingulate cortex, there is partial regrowth of the anterior tissue that in some cases extended to the more posterior region. Although animals with the complete cingulate cortex removed differed from controls on their performance in the MWM, their execution was better than animals with only the ACC or the PCC removed in adulthood or at P3 (Sutherland et al., 1988; Kolb & Wishaw, 1991).

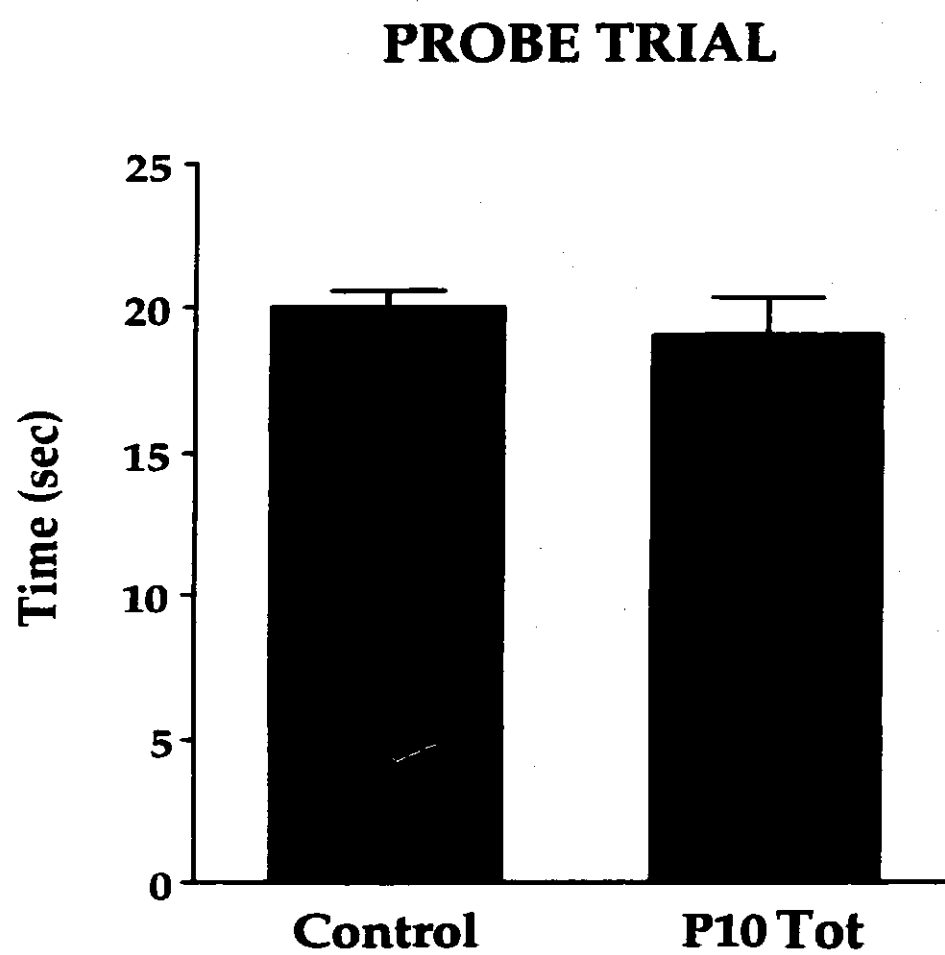


Fig. 14. Summary of time spent (\pm SE) by the animals swimming in the previously correct quadrant during the probe trial. Animals received P10 lesions of the complete cingulate cortex. Total swim time = 30 s.

EXPERIMENT 3.

8. Experiment 3

The extent of recovery following focal cortical injury in the rat varies with the age of the insult. The worst behavioral outcome occurs if the cortex is injured in the first few days after birth, which is a time of neural migration and cell differentiation. In contrast, if the lesion occurs during the early stages of cortical neurogenesis the ability of the brain to compensate for injury is remarkable (for a review see Kolb 1995). For example, if the medial frontal cortex of the rat is removed at day 10 there is a spontaneous regeneration of the damaged tissue that supports behavioral outcome. Similar injury on day 3 or in adulthood does not lead to regeneration or functional recovery. In the present experiment I assessed the findings of experiments one and two in a broader manner. Animals received lesions of either the posterior cingulate cortex, or the anterior and posterior cingulate cortices at three times of development: Postnatal day four, postnatal day ten, or in adulthood (120 days). All animals were tested in two cognitive tasks: the Morris water maze and conditioned taste aversion; in one motor task: the Whishaw reaching task, and one species typical measurement: general activity over 48 hours.

METHOD

Subjects

Fifty Long-Evans rats were used in this study. Nineteen (10 males; 9 females) received bilateral lesions of the posterior cingulate cortex and nineteen rats (10 males; 9 females) received lesions of the entire midline cortex. Posterior cingulate cortex removals which included areas RSA and RSG bilaterally were performed at the following ages: postnatal day 4 (P4; 3 males, 2 females),

postnatal day 10 (P10; 3 males 3 females) or postnatal day 120 (P120; 4 males, 4 females). Animals with the anterior and posterior cingulate cortex lesions had areas CG1, CG2, CG3, RSA and RSG removed bilaterally at the same ages described above with the same distribution of subjects per group respectively. Twelve rats (6 males; 6 females) served as sham-operated controls. To simplify the analysis, the posterior cingulate and complete midline lesion group will be compared to the control group separately. Behavioral testing began at 150 days of age.

Surgical procedures

The P4 and P10 animals were anesthetized by cooling them in a Thermanon cooling chamber set at -5°C. They were cooled for 15 min or until their rectal temperature was in the range of 18-20°C and they were immobile. Animals with posterior cingulate lesions had the bone removed from bregma to lambda. Animals with the anterior and posterior cingulate lesions (totals) had the entire midline bone (from the frontal suture to lambda) removed. In both cases, this procedure was done by cutting the bone with iris scissors and then removing the cortex by aspiration. The wound was then sutured with silk thread. The control rats were anesthetized in the same manner and the skin was incised and sutured. Animals were slowly warmed up to 35°C under a heat lamp before being returned to the mother.

The adult rats were anesthetized with sodium pentobarbital (65 mg/kg for males and 45 mg/kg for females), the skull was opened and the posterior cingulate cortex or the anterior and posterior cingulate cortex were removed by suction under an operating microscope. The wound was then closed with wound

clips and the rats were under observation for an hour before being returned to their home cages.

Anatomical procedures

At the conclusion of the behavioral testing animals were divided into two groups: All males were processed for Golgi analysis, and the females were processed for Cresyl Violet staining. 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.

Golgi-Cox staining:

Male animals were given an overdose of sodium pentobarbital and intracardially perfused with 0.9% saline. The brains were removed and weighed before being immersed in a 20 ml of Golgi-Cox solution where they remained 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 & Kolb (1998).

Cresyl Violet staining:

Female animals were given an overdose of sodium pentobarbital and intracardially perfused with 0.9% saline followed by 4% paraformaldehyde solution. The brains were removed, weighed and placed in a 30% sucrose

formalin solution for 3 days before being cut frozen at 40 μm . Every seventh section was saved and stained with Cresyl violet.

Cortical thickness was measured by projecting the Golgi or the Nissl-stained sections on a Zeiss DL 2 POL petrographic projector set at a magnification of 20 X. Measurements were made with a clear plastic millimeter ruler and taken at three points at each of the following 5 planes: plane I, first section with caudate putamen visible, measures in Zilles', (1985) areas Gu, Par 1, Fr 2; plane II, centre of anterior commissure, measures in Par 2, Par 1, Fr 1; plane III, first hippocampal section, measures in areas Gu, Par 1, Fr 1; plane IV, posterior commissure, measures in areas Te 1, Oc 2L, RSA; plane V, most posterior hippocampal section, measures in Te 1, Oc 1B, Oc 2ML.

Behavioral Procedures

Morris water task. The swimming pool and test procedures were the same than those used in Experiments 1 and 2.

Forepaw reaching for food. Measures were done using a procedure adapted from the method devised by Whishaw et al., (1986). Each animal was food-deprived to 90% body weight for the training and testing. Animals were placed in the test cages (20 X 28 X 25 cm high) with floors and fronts constructed of 2 mm bars, 9 mm apart edge to edge. A 5 cm wide and 1 cm deep tray, containing chicken feed pellets was mounted in the front of each box. 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 25 min every day for a total of 12 days, by which time their performance had reached asymptote. On day thirteen, five minutes of continuous reaching activity for each rat was videotaped and scored. If the rat made a reaching movement (forepaw inserted through the bars, but no food was grasped or the food was dropped), the movement was scored as a "reach", whereas if the rat obtained the food and consumed it, the movement was scored as a "reach" and a "hit". Scoring was achieved by calculating the percentage of hits to total reaches.

Activity. Locomotor activity was tested in a bank of 15-wire photocell cages. 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, 34 cm apart, and perpendicular to the long axis of the cage. The total number of beam breaks, registered incrementally by an Apple II + computer, was used as an index of activity. Activity was monitored for 48 hours.

Conditioned Taste Aversion (CTA). Acquisition: Animals were water deprived for 24 hours and habituated for seven days to obtain their water intake twice a day (baseline). Twenty minutes before each morning drinking session, food was removed from the cages to make sure that all animals were drinking without periods of food intake, and returned after drinking. Distilled water was given to each rat in graded test tubes and the consumption was recorded for 10 min. On the acquisition day a novel 0.1% saccharin solution was given instead of water. Twenty minutes after drinking, animals were injected i.p. with a LiCl solution (LiCl 0.15 M; 7.5 ml/kg) to induce gastric malaise. After this, water and

food were available again *ad libitum*. **Retention Test:** Two days after acquisition animals were again water deprived for 24 hr and a baseline of water consumption was taken daily for the next 5 days. In the following morning session, saccharin solution was substituted for the water and consumption was measured. To correct for individual differences in baseline drinking, the total intake for the taste stimulus is expressed as a percentage of the baseline of water (Dugas-du-Villard et al., 1981), taking the arithmetical mean of the previous two morning sessions as 100%.

RESULTS

Anatomical Results

Gross Anatomy. The posterior cingulate lesions were extensive in all groups and included all of the midline posterior cingulate cortex (Zilles' RSA and RSG, posterior Cg 1 and Cg 2) as well as the medial edge of the posterior parietal cortex and part of Zilles' area Oc2M. No animal had damage to the hippocampus or other subcortical areas, although the hippocampus was distorted in appearance after the neonatal lesions. The complete cingulate lesions included the same region of posterior cingulate injury and, in addition, included all of the anterior cingulate regions (Zilles' Cg1, Cg2, Cg3) and the infralimbic cortex.

There was no direct damage to the caudate-putamen in any animals. There was an obvious loss of the descending cortical fibers in the medial part of the caudate-putamen. There was also damage to the medial part of Zilles' area Fr2.

Posterior cingulates: The lesions removed the posterior cingulate region including most of Zilles' areas RSA and RSG. There was no direct damage to subcortical structures, including the hippocampus.

Brain Weight. Removal of the posterior cingulate cortex at P4, P10 or P120 of age led to a lighter brain in both males and females. Because males and females were processed for two different histological analysis (Golgi and Cresyl Violet respectively), the statistical analysis was done separately. For the male brains ($n = 16$), the lesion reduced its weight in all groups with little difference between the lesion groups (Table 3). Analysis of variance revealed a significant main effect for lesion group ($F(3,12) = 24.091$, $P = 0.0001$) and post hoc test (Fisher's LSD, $P < 0.05$) showed that the three lesioned groups differed from the control group, but did not differ significantly between themselves.

Table 3

Group	Brain Weights	
	Males	Females
Control	2.320 ± 0.037 (n = 6)	2.047 ± 0.021 (n = 6)
P4	2.077 ± 0.023* (n = 3)	1.940 ± 0.010* (n = 2)
P10	1.980 ± 0.033* (n = 3)	1.887 ± 0.039* (n = 3)
P120	2.055 ± 0.006* (n = 4)	2.0 ± 0.031 (n = 4)

*Significantly different from control animals.

For the female brains ($n = 15$), the lesion reduced its weight in P4 and P10, but not in P120 group (Table 3). Analysis of variance revealed a significant main

effect for lesion group ($F(3,11) = 6.073$, $P = 0.0108$) and post hoc test (Fisher's LSD, $P < 0.05$) showed that the P10 lesioned group differed from both the control and P120 groups, the P4 differed from the control and no differences were found between the control and P120 groups.

Total cingulates: The lesions removed the anterior and posterior cingulate region including most of Zilles' areas CG1, CG2, CG3, RSA and RSG, although some of the P10 animals showed a partial regrowth primarily in the anterior cingulate cortex but in some cases it extended to the posterior region. There was no direct damage to subcortical structures, including the hippocampus.

Brain weight. For the male brains ($n = 16$), the lesion reduced brain weight in all groups with little difference between the lesion groups (Table 4). Analysis of variance revealed a significant main effect for lesion group ($F(3,12) = 27.77$, $P = 0.0001$) and post hoc test (Fisher's LSD, $P < 0.05$) showed that the three lesioned groups differed from the control group, but did not differ significantly among themselves.

Table 4

Group	Brain Weights	
	Males	Females
Control	2.320 ± 0.037 (n = 6)	2.040 ± 0.021 (n = 6)
P4	1.960 ± 0.036* (n = 3)	1.850 ± 0.09* (n = 2)
P10	1.950 ± 0.031* (n = 3)	1.750 ± 0.031* (n = 3)
P120	1.925 ± 0.043* (n = 4)	1.840 ± 0.05* (n = 4)

*Significantly different from control animals.

For the female brains ($n = 15$), the lesion reduced its weight in the three lesioned groups (Table 2). Analysis of variance revealed a significant main effect for lesion group ($F(3,11) = 11.54$, $P = 0.0010$) and post hoc test (Fisher's LSD, $P < 0.05$) showed that the three lesioned groups differed from the control group, but did not differ significantly between themselves.

Cortical Thickness. The overall result was that the lesion reduced cortical thickness in the five planes for all lesioned groups. Because there were no left/right differences, the values of each side were grouped for further analysis.

Posterior cingulates. Analysis of variance showed a significant main effect of group ($F(3, 28) = 8.98$, $P < 0.001$), planes ($F(4,112) = 59.37$, $P < 0.0001$) and the interaction ($F(12, 112) = 2.59$, $P < 0.005$). Follow-up tests (Fisher's LSD) showed that P120 animals differed from the other three groups on planes I, II and III ($P < 0.05$); P10 animals differed from controls in planes II and III ($P < 0.05$) and P4 animals differed from controls on the five different planes ($P < 0.05$) [Fig. 15].

Total cingulates. One animal (P4) had only the anterior cingulate cortex removed, thus was excluded from the analysis. Analysis of variance showed a significant main effect of group ($F(3, 26) = 40.35$, $P < 0.0001$), planes ($F(4,104) = 30.91$, $P < 0.0001$) and the interaction ($F(12, 104) = 9.78$, $P < 0.0001$). Follow-up tests (Fisher's LSD) showed that the three lesioned groups differed from the control group in the five different planes ($P < 0.01$) [Fig. 16].

Thalamic Degeneration. All adult posterior cingulate rats had thalamic degeneration, which was characterized by gliosis and some calcification, in the

CORTICAL THICKNESS Posterior Cingulates

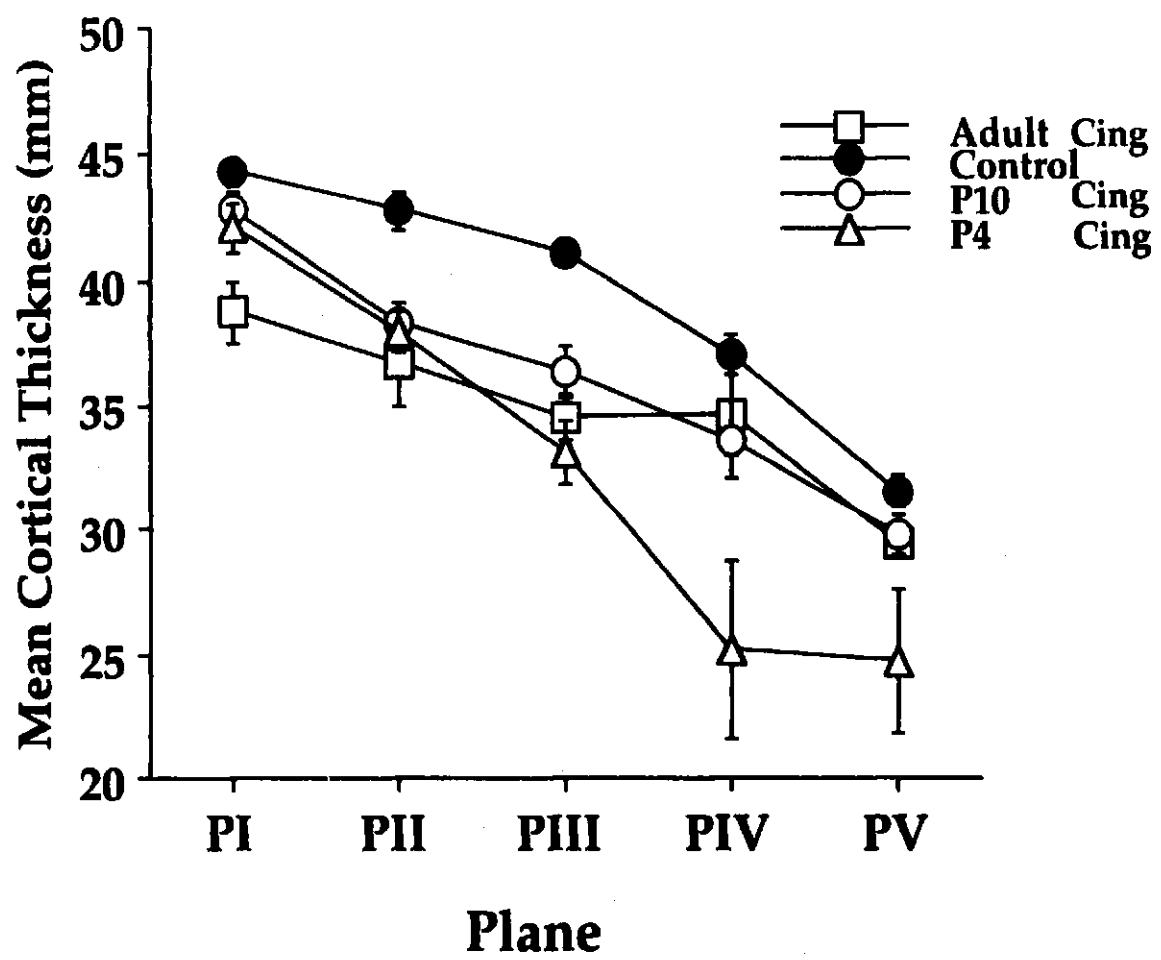


Fig. 15. Cortical thickness summary showing effects of posterior cingulate cortex lesions at different ages. Mean cortical thickness (\pm SE) at each of 5 planes.

CORTICAL THICKNESS Total Cingulates

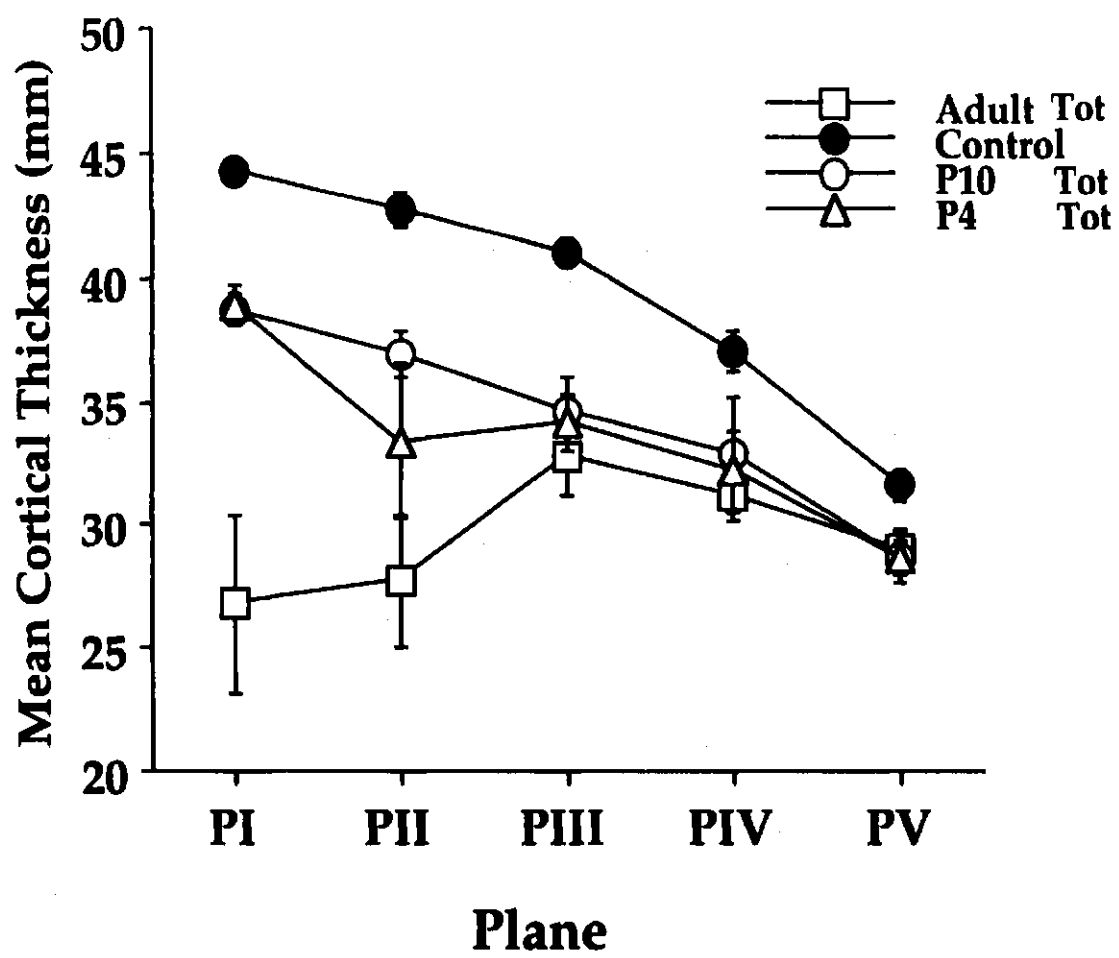


Fig. 16. Cortical thickness summary showing effects of complete cingulate cortex lesions at different ages. Mean cortical thickness (\pm SE) at each of 5 planes.

dorsal and posterior lateral nuclei, largely along the border with the posterior nucleus, as well as in the ventral anterior nucleus. There was no obvious degeneration in the lateral geniculate nucleus. The thalamus of the young animals was more severely affected, however. In contrast to the adult operates, which had gliosis in the thalamus, the infant operates had little gliosis. Rather the affected nuclei were shrunken and misshapen. This shrinkage was more severe in the P4 operates, much as has been reported elsewhere for posterior parietal lesions (Kolb et al., 1987).

The thalami of the animals with total cingulate lesions were quite similar to those of the animals with posterior cingulate lesions. In addition, there was subtle thalamic degeneration in the lateral region of the dorsal-medial thalamic nucleus in the adult operates but not in the infants, as reported previously (Kolb & Nonneman, 1978) and degeneration in the medial anterior nucleus.

Behavioral Results.

Morris Water Task. Control animals quickly learned to find the platform and to swim directly toward it when released from any starting location. This improvement was reflected in the decline of the latencies to find the platform. By the last day of training, the escape latency for all animals reached an asymptote at around 3 sec. When the platform was removed on the probe trial all animals swam around the previously correct location before heading off to swim in other directions. There was no obvious sex difference in the performance of this task.

Posterior cingulates. When the posterior cingulate cortex was removed on day 4, or in adulthood, animals were impaired in the performance of the task. In

contrast, animals with lesions at day 10 did not differ from controls as they performed surprisingly well, reaching an asymptote at around 5 sec by the last day of training [Fig. 17a & 17b]. A repeated measures ANOVA comparing all groups showed a significant main effect of group ($F(3,26) = 32.87, P < 0.0001$), trial block ($F(9, 234) = 56.08, P < 0.0001$), and the interaction ($F(27, 234) = 2.84, P < 0.001$). Follow-up tests (Fisher's LSD) showed that P4 group differed significantly from the control group ($P < 0.05$), and P120 from the control and P10 groups. Analysis of variance on the probe trial [Fig. 18], revealed a significant main effect of group ($F(3,26) = 5.602, P < 0.05$). The posthoc tests (Fisher's LSD, $P < 0.05$) showed that the P120 animals differed from the other three groups, which did not differ among themselves.

Total cingulates. A very similar pattern on performance when the anterior and posterior cingulate cortex were removed was observed in this experiment. Adult operates animals were severely impaired in comparison with the other two lesion groups and the control group. Although animals with P4 lesions were less impaired than adult operates, they were impaired, reaching an asymptote at around 17 sec by the last day of training. Day 10 lesioned animals did not differ from controls, reaching an asymptote at around 5 sec by the last day of training [Fig. 19a & 19b]. A repeated measures ANOVA comparing all groups showed a significant main effect of group ($F(3,26) = 38.09, P < 0.0001$), trial block ($F(9, 234) = 37.52, P < 0.0001$), and the interaction ($F(27, 234) = 3.72, P < 0.0001$). Follow-up tests (Fisher's LSD) showed that P120 differed from the other three groups ($P < 0.0001$), and P4 group differed significantly from the control, P4 and adult group ($P < 0.0001$). Analysis of variance on the probe trial [Fig. 20], revealed a significant main effect of group ($F(3,26) = 9.41, P < 0.001$). The posthoc tests

MORRIS WATER TASK Posterior Cingulates

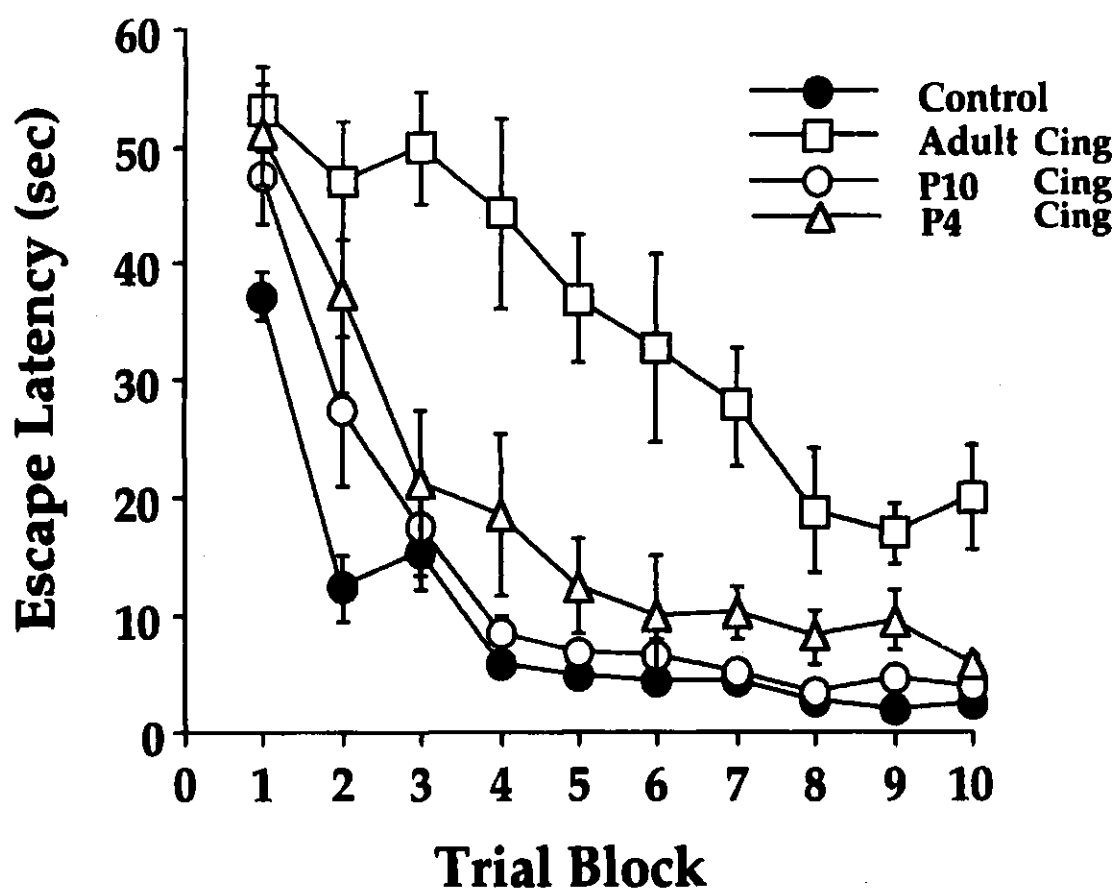


Fig. 17a. Summary of performance (\pm SE) in the Morris water task for animals with posterior cingulate cortex removals. Mean escape latency across the 10 trial blocks.

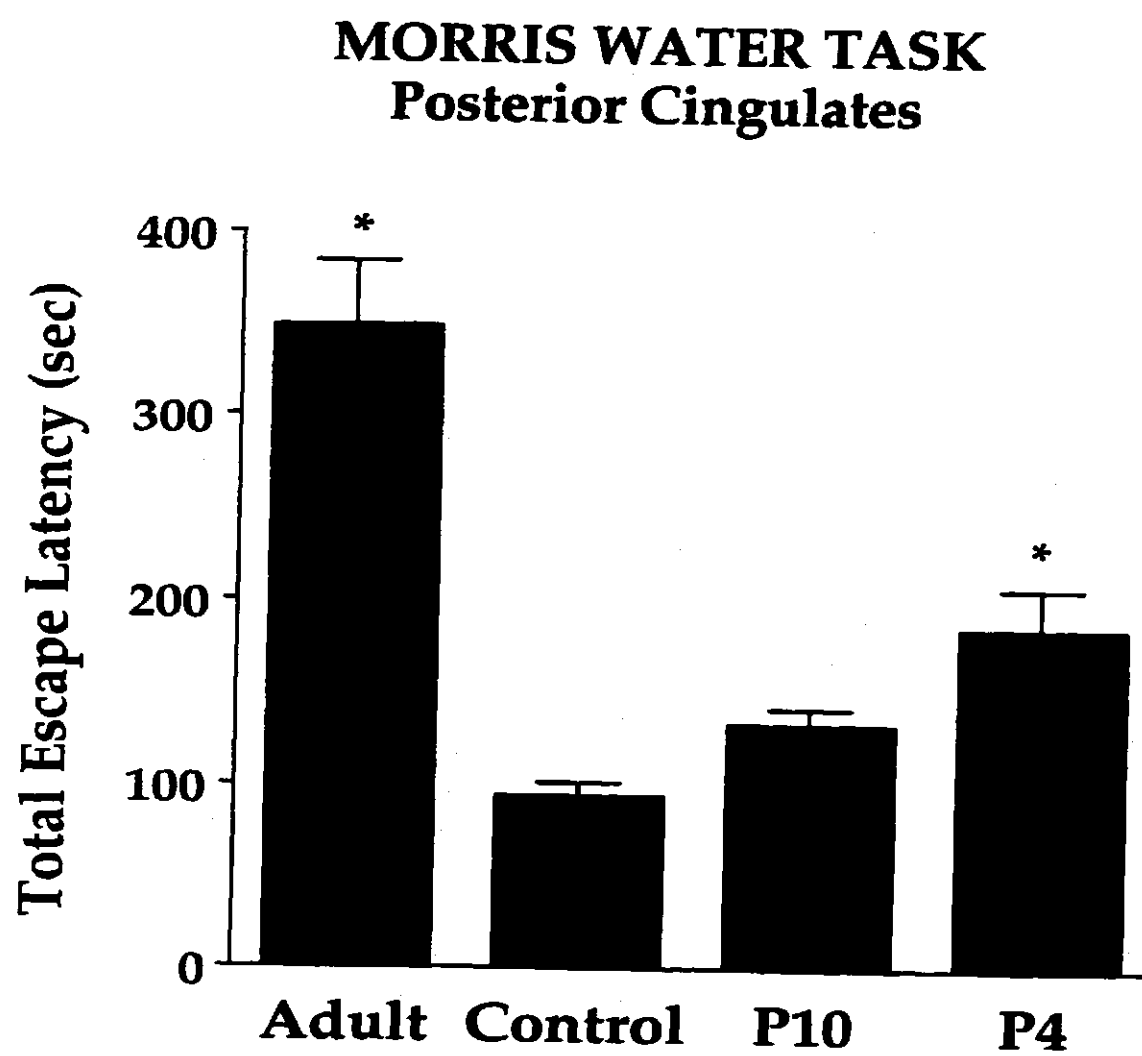


Fig. 17b. Summary of performance in the Morris water task. Total escape latency (\pm SE) summed across the 10 trial blocks for animals with posterior cingulate cortex removals at different developmental ages. (* significantly different than control)

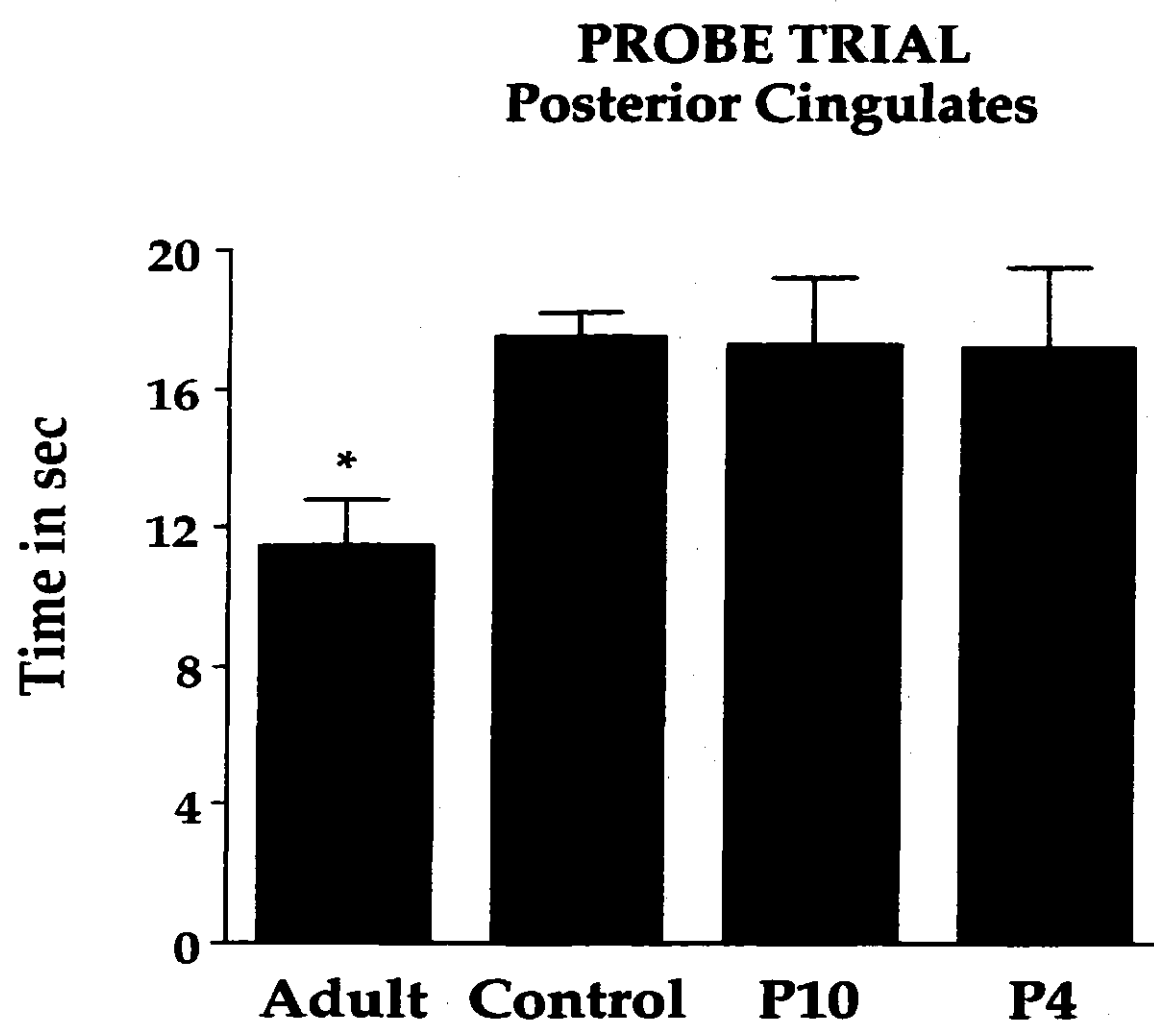


Fig. 18. Summary of time spent (\pm SE) by the animals swimming in the previously correct quadrant during the probe trial. Animals received lesions of the posterior cingulate cortex at different developmental ages. Total swim time = 30 s.
(* significantly different than control)

MORRIS WATER TASK Total Cingulates

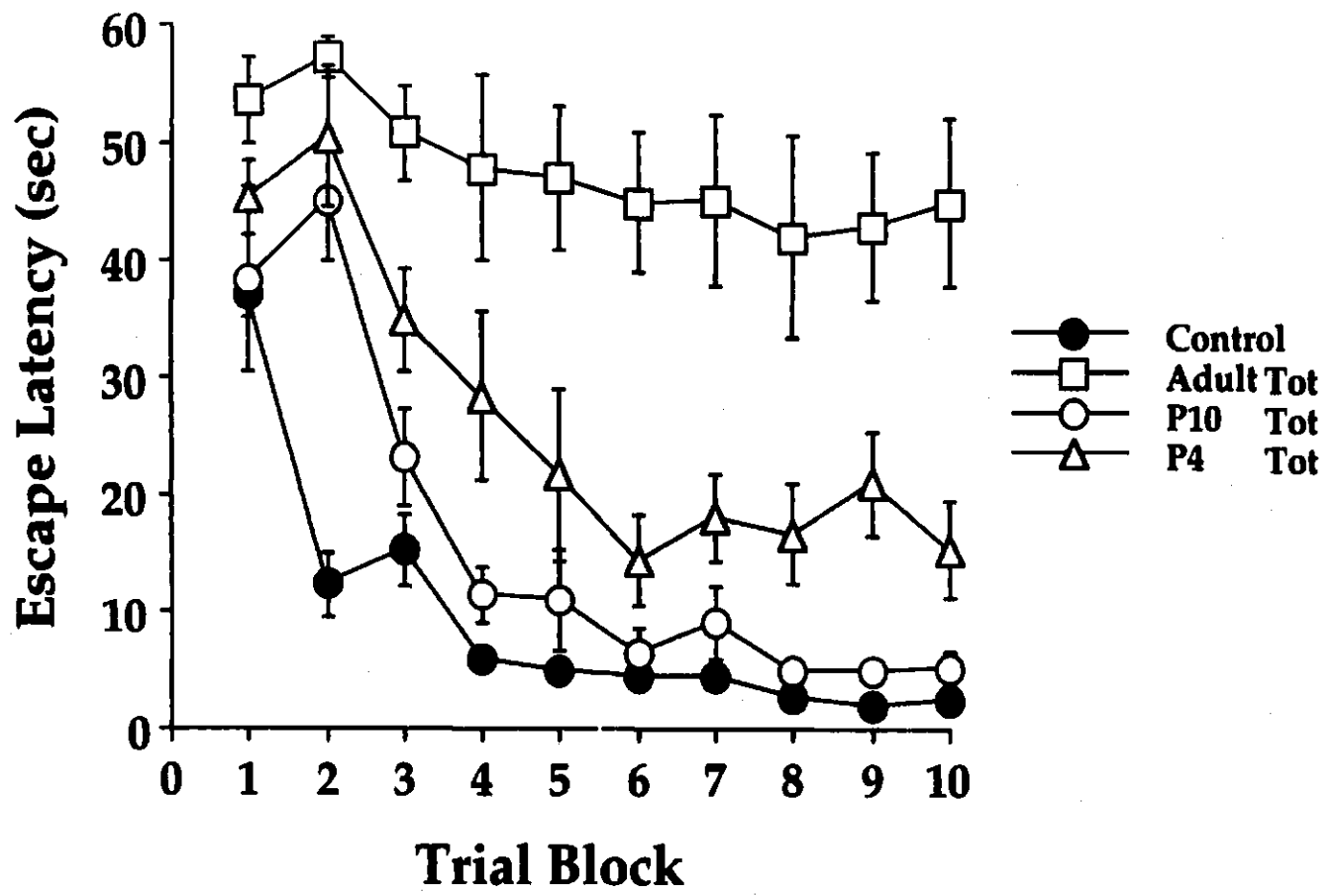


Fig. 19a. Summary of performance (\pm SE) in the Morris water task for animals with complete cingulate cortex removals. Mean escape latency across the 10 trial blocks.

MORRIS WATER TASK Total Cingulates

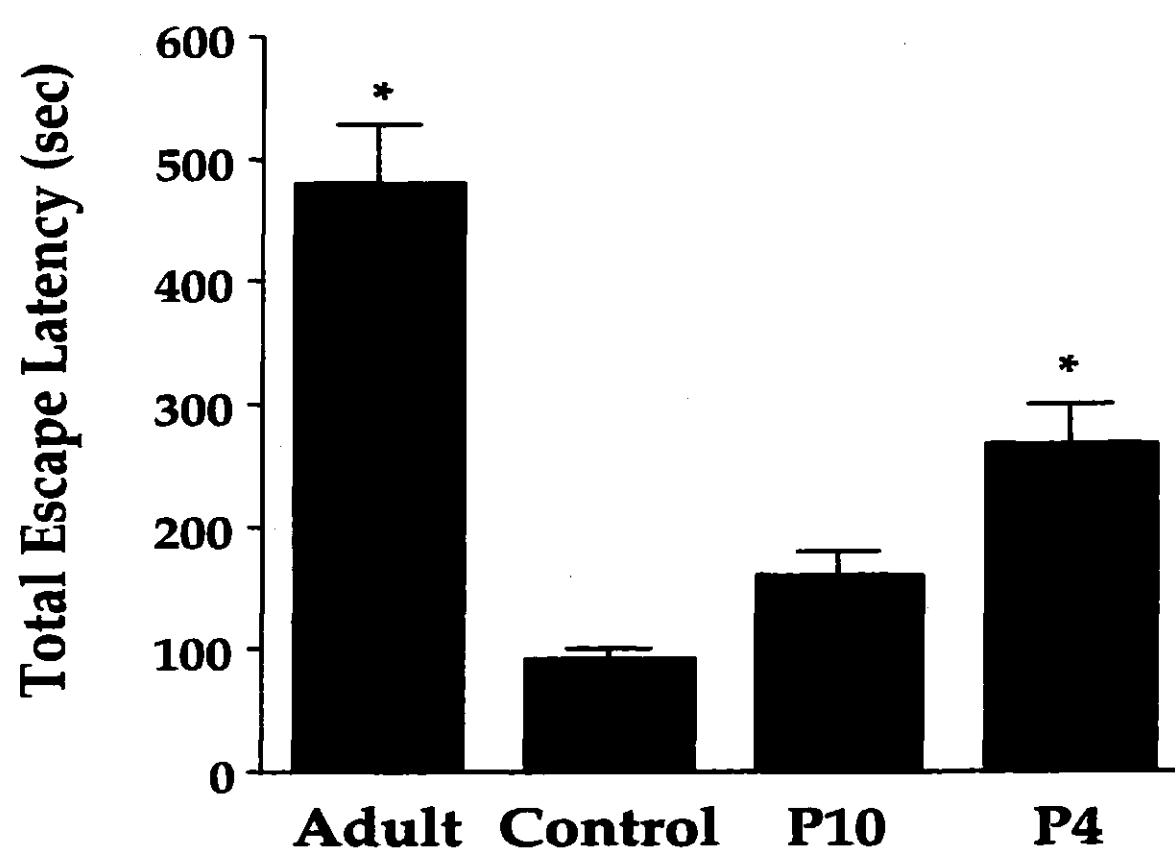


Fig. 19b. Summary of performance in the Morris water task. Total escape latency (\pm SE) summed across the 10 trial blocks for animals with complete cingulate cortex removals at different developmental ages. (* significantly different than control)

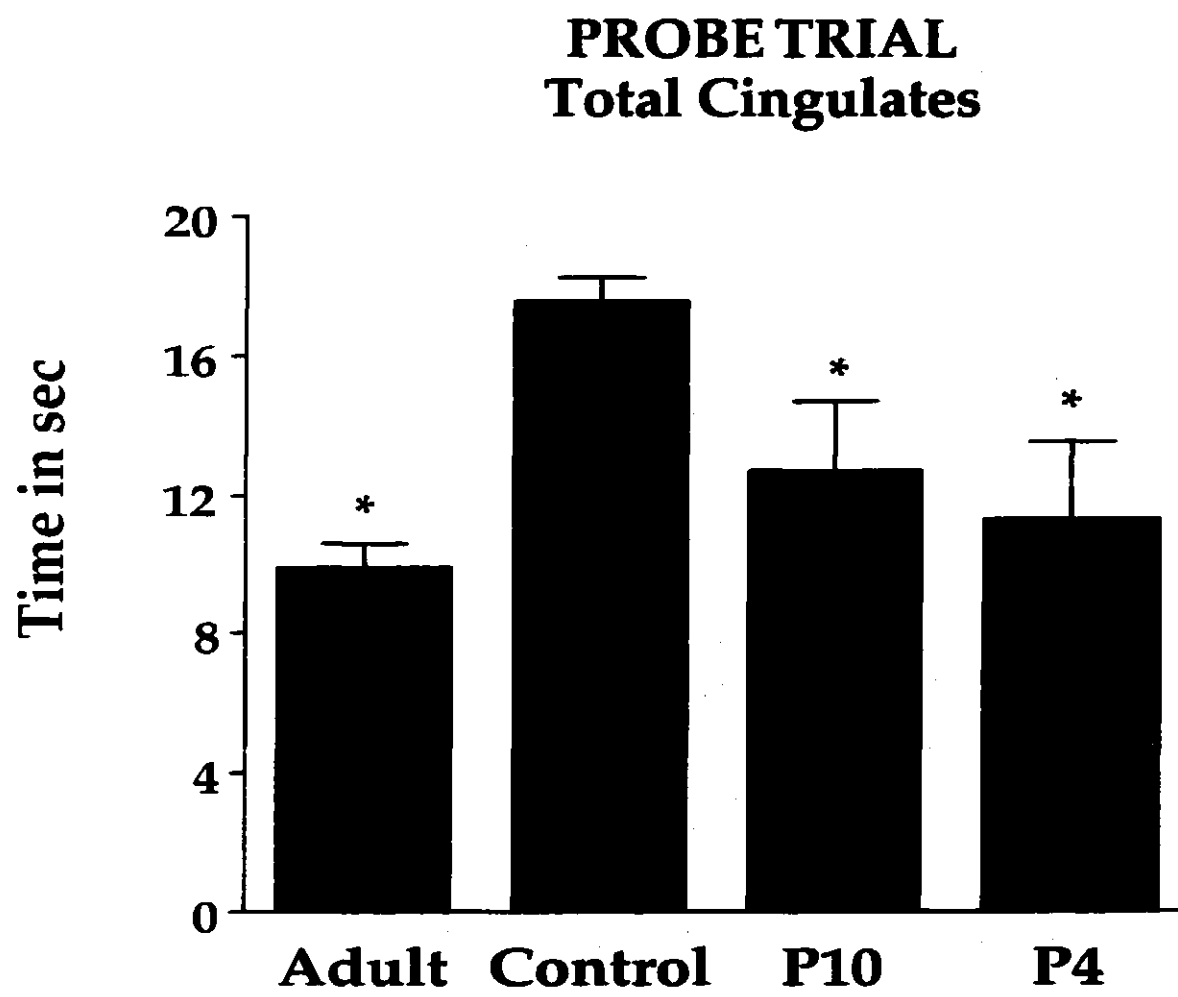


Fig. 20. Summary of time spent (\pm SE) by the animals swimming in the previously correct quadrant during the probe trial. Animals received lesions of the complete cingulate cortex at different developmental ages. Total swim time = 30 s.
(* significantly different than control)

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73

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(Fisher's LSD, $P < 0.05$) showed that the control animals differed from the other three groups, which did not differ among themselves. Thus, all of the lesion groups spent less time in the correct quadrant than the control group. This result is surprising for the P10 animals and suggests that even though P10 animals were not different from controls in the acquisition of the task, they must have used an alternate strategy to find the platform.

Forepaw reaching for food. Control rats learn to reach quickly and typically asymptote at about 50-60% accuracy. Failed reaching attempts usually result from careless grasping of several pieces of food, that are usually dropped when the forepaw is withdrawn back to the body, as well as the occasional drop when food is grasped with both paws for eating. Neither posterior cingulate nor total cingulate animals differed from controls, although the variance in performance was large.

Posterior cingulates. For the statistical analysis animals that failed to learn were assigned with a score of zero. A simple ANOVA showed no differences among groups ($F(3,27) = 0.835$, $P = .486$) [Fig. 21].

Total cingulates. A simple ANOVA for all animals showed no differences among groups ($F(3,27) = 1.32$, $P = 0.285$) [Fig. 22].

Activity. There was a clear circadian rhythm in cage activity, as all of the animals were more active in the dark period than in the light. Furthermore, there was a significant sex difference, as the female rats were more active than the male rats at night. For both groups, the posterior cingulates and the total cingulates, there was a significant lesion effect as P4 and P10 groups were more active than P120

REACHING Posterior Cingulates

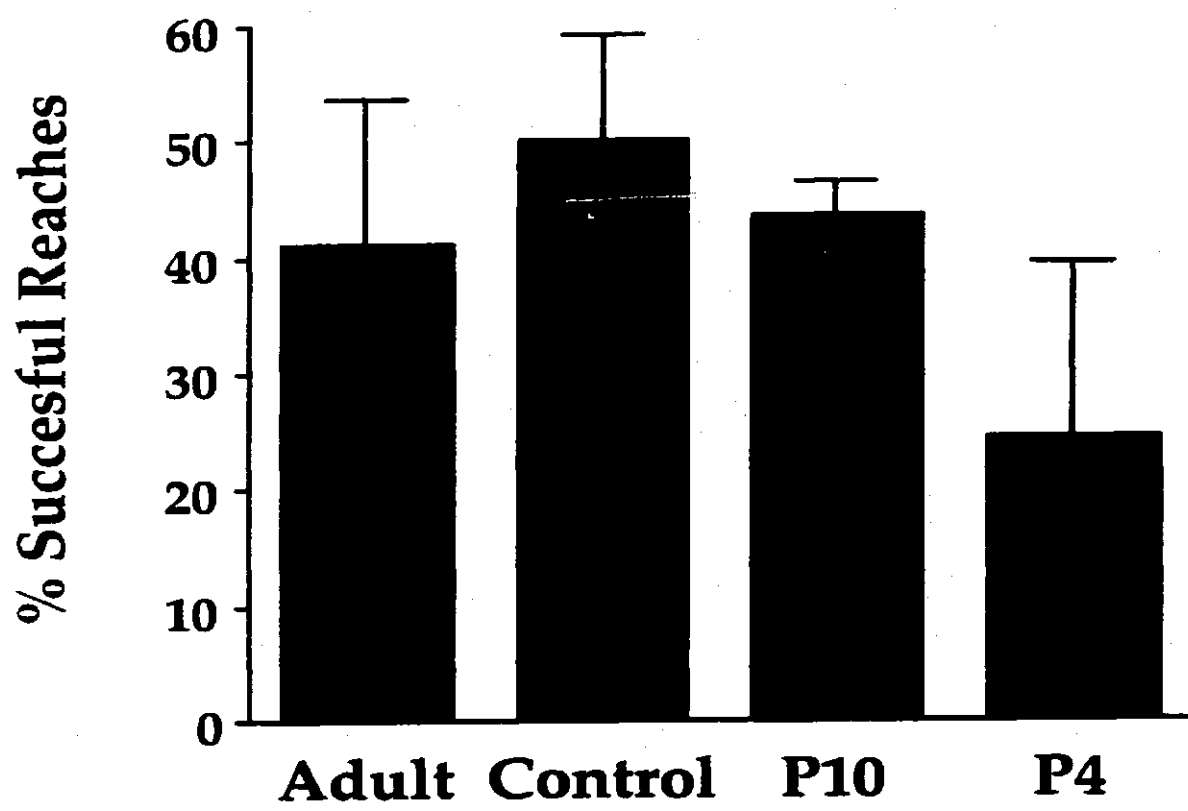


Fig. 21. Summary of mean number of successful reaches (\pm SE) in a 5 minute period. Animals received lesions of the posterior cingulate cortex at different developmental ages.

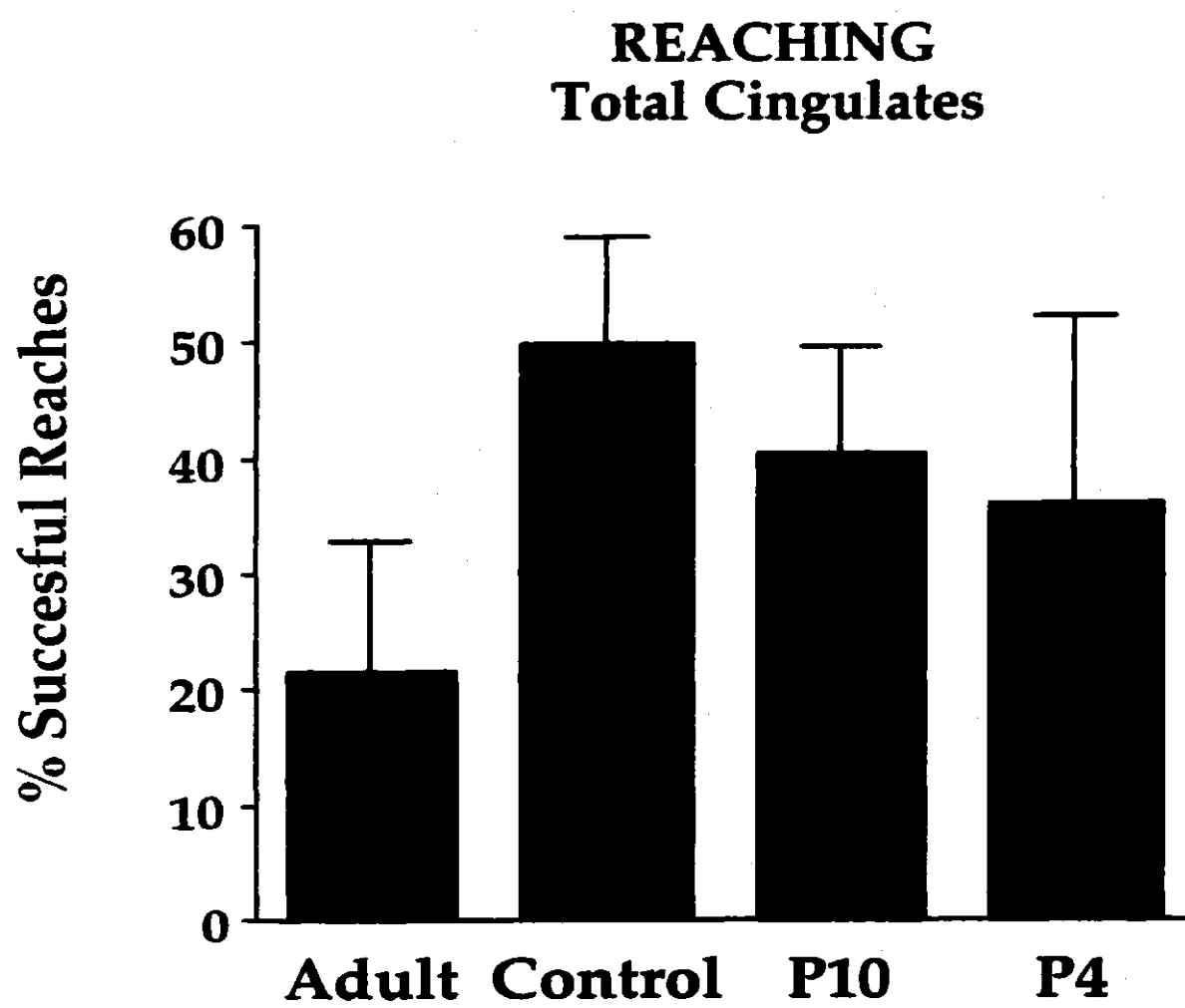


Fig. 22. Summary of mean number of successful reaches (\pm SE) in a 5 minute period. Animals received lesions of the complete cingulate cortex at different developmental ages.

or control groups, and this difference was greater in animals with the anterior and posterior cingulate cortex removed.

Posterior cingulates. Two cages were not recording accurately so two animals (P120=1, P10=1) were excluded from the analysis. On day one a repeated measures ANOVA showed a significant main effect of group ($F(3,20) = 12.96, P < 0.0001$), sex ($F(1,20) = 61.82, P < 0.0001$), and the interaction ($F(3,20) = 4.45, P < 0.02$). There was also a significant main effect of time of day ($F(23,460) = 33.52, P < 0.0001$), and the interaction with sex and group ($F(69,460) = 2.05, P < 0.0001$). Follow-up tests (Fisher's LSD) showed the following significant differences: control < adult < P4 < P10, (P 's = 0.05). On day two there was a significant sex effect ($F(1,20) = 11.04, P < 0.005$) but not of group ($F(3,20) = 2.32, P = 0.10$) nor the interaction ($F(3,20) = 1.38, P = 0.27$). There was however, a significant main effect of time of day ($F(23,460) = 2.98, P < 0.0001$) and the interaction with sex and group was also significant ($F(69,460) = 1.72, P < 0.001$). Follow-up tests (Fisher's LSD) showed the P10 differed from the other three groups ($P = 0.05$), which did not differ among themselves [Fig. 23].

Total cingulates. On day one a repeated measures ANOVA showed a significant main effect of group ($F(3,22) = 7.40, P < 0.005$), sex ($F(1,22) = 5.48, P < 0.05$), but not the interaction ($F(3,22) = 1.43, P = 0.258$). There was also a significant main effect on time of day ($F(23,506) = 27.94, P < 0.0001$), but not the interaction with sex and group ($F(69,506) = 1.01, P = 0.28$). Follow-up tests (Fisher's LSD) showed the following significant differences: control < P4 = adult < P10, (P 's = 0.05). On day two there was a significant sex effect ($F(1,22) = 5.07, P < 0.05$), but not of group, nor the interaction. There was however, a significant main effect on time of day ($F(23,506) = 1.71, P < 0.05$) and the interaction with sex

ACTIVITY Posterior Cingulates

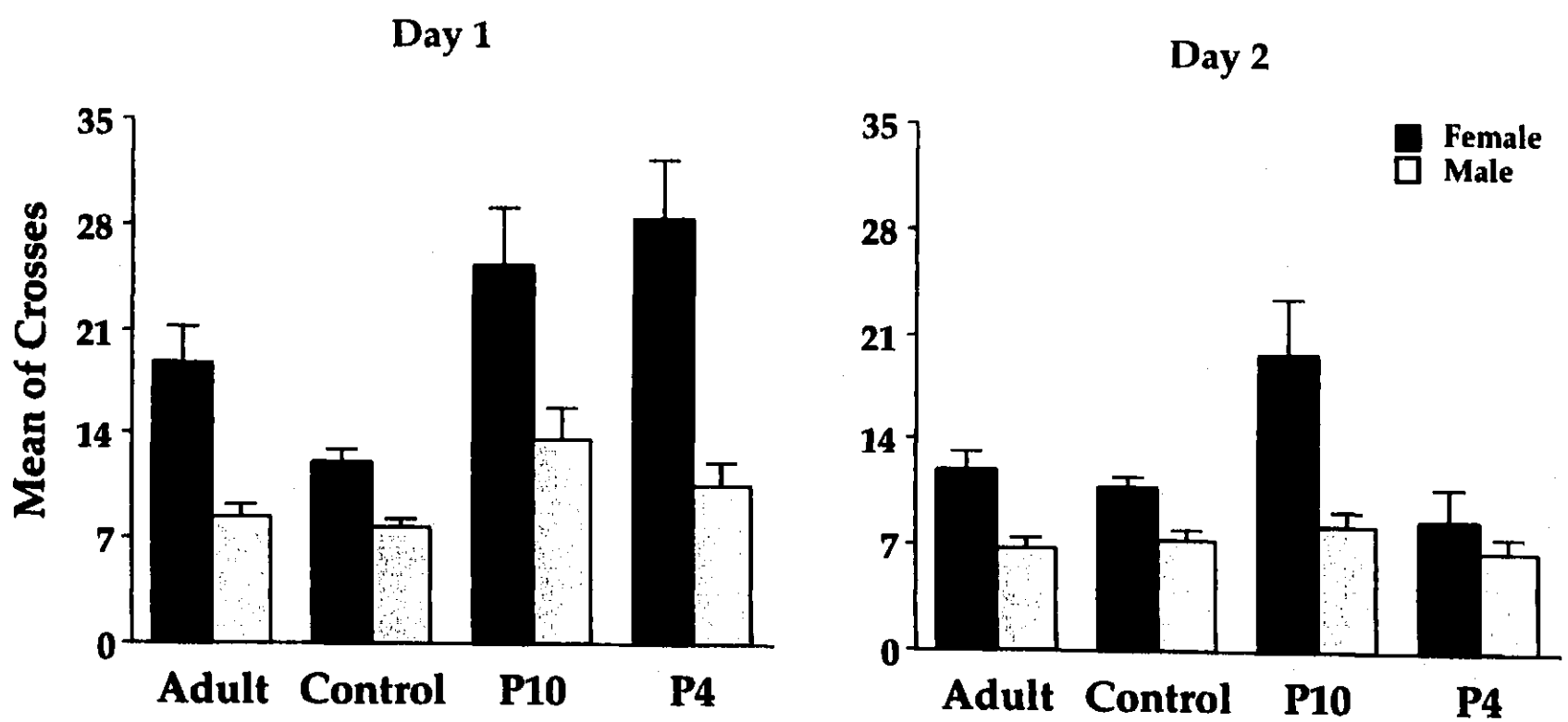


Fig. 23. Summary of mean number of crosses (\pm SE) in box recording general activity monitored during 48 hours. Animals received posterior cingulate cortex lesions at different developmental ages.

and group was also significant ($F(69,506) = 1.39, P < 0.05$). Follow-up tests (Fisher's LSD) showed the following significant differences: control < adult = P4 < P10, ($P = 0.05$) [Fig 24].

Conditioned Taste Aversion. To correct for individual baseline drinking the total intake for the taste stimulus was expressed as a percentage of the baseline of water (Dugas-du-Villard & MacLeod, 1981), taking the arithmetical mean of the previous two morning sessions as 100%. Figure 25 shows the results for the retention test for the CTA for animals with posterior cingulate cortex removals, and figure 26 shows the results for animals with the complete cingulate cortex removed. The baseline of water consumption and saccharine consumption during the acquisition of the CTA was similar for all groups (mean baseline = 10.32 ml, mean acquisition = 9.27 ml for all animals), with no differences among them. As expected, the control group showed a marked aversion to saccharine on the test as this drinking dropped to less than 30% of their baseline. Although all groups showed reduced consumption, the P120 groups show significantly less aversion than the other groups. Simple ANOVAs showed no differences in the baseline water intake among groups. A significant main effect of group was found the day of the test ($F(3,11) = 13.205, P < 0.001$) for the posterior cingulates, and ($F(3,11) = 5.206, P < 0.02$) for the complete cingulates. Follow-up tests (Fisher's LSD) showed that P120 groups differed significantly from the other three groups ($P < 0.05$), which did not differ among themselves.

In summary, the overall findings of the present experiment are summarized in table 5.

Figure 27 shows photographs of representative brains of animals with the posterior cingulate cortex removed at different ages + control, and figure 28 shows photographs of representative brains of animals with the complete cingulate cortex removed at different ages + control.

Table 5

GROUP		MWT(Lat)	MWT(PT)	CTA	Reaching	Activity
PCC	P4	X				X
	P10					X
	P120	X	X	X		
ACC+PCC	P4	X	X			X
	P10		X			X
	P120	X	X	X		

X means animals are impaired relative to sham control.

ACTIVITY Total Cingulates

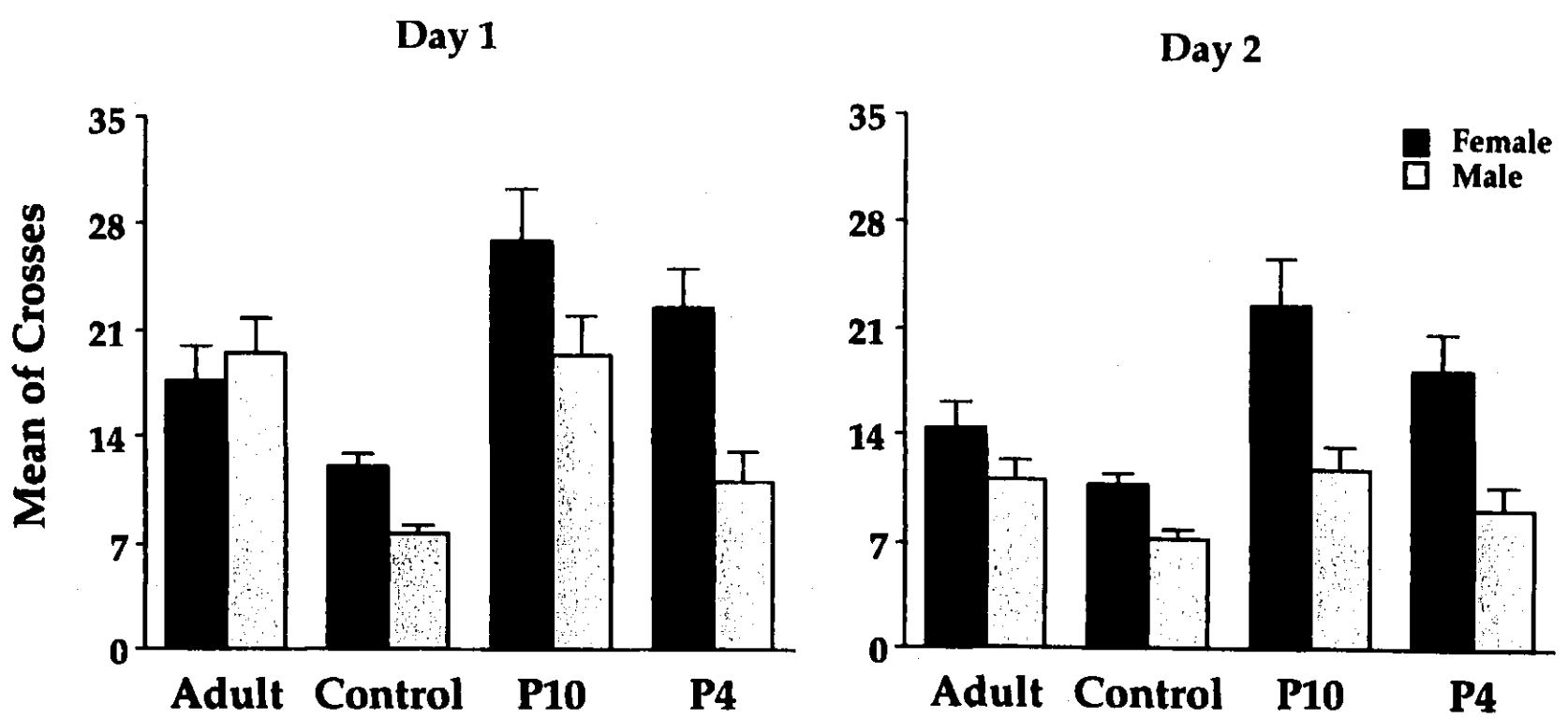


Fig. 24. Summary of mean number of crosses (\pm SE) in box recording general activity monitored during 48 hours. Animals received complete cingulate cortex lesions at different developmental ages.

CONDITIONED TASTE AVERSION Posterior Cingulates

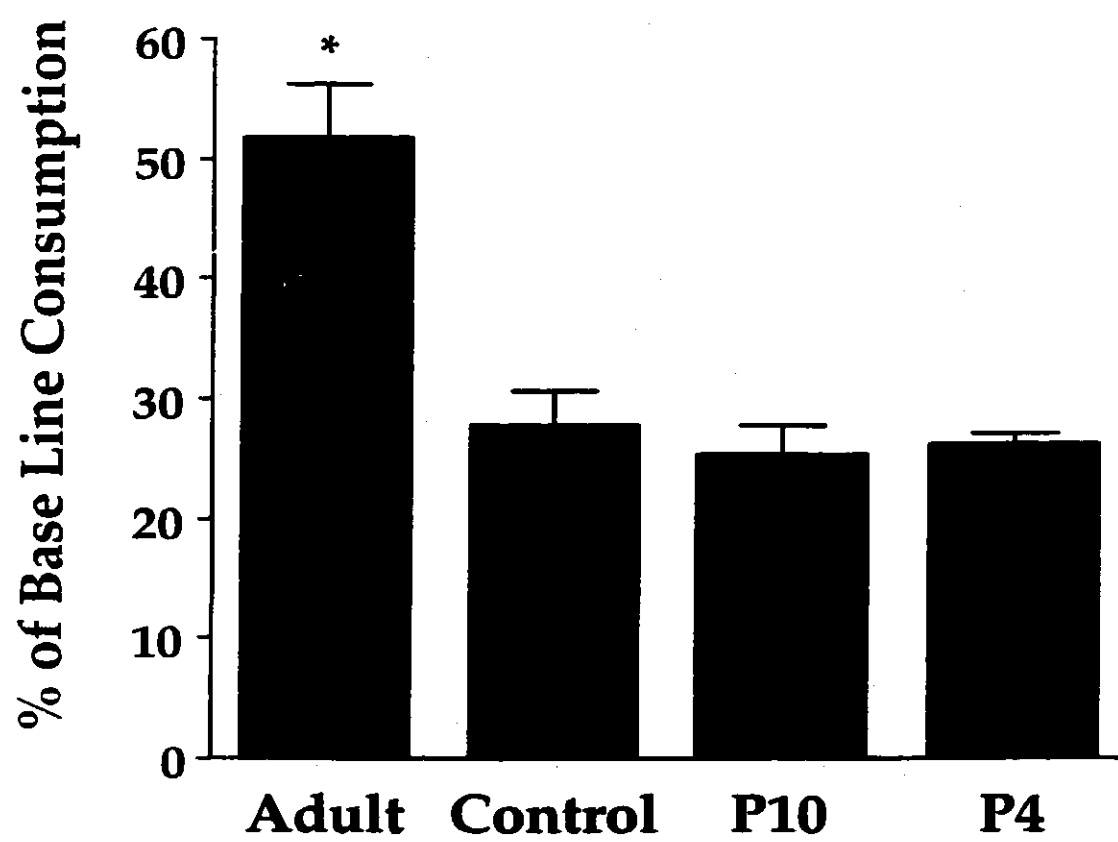


Fig. 25. Summary of the aversion index (\pm SE) expressed as a mean percentage of baseline consumption during the test trial. Animals received lesions of the posterior cingulate cortex at different developmental ages. (* significantly different than control)

CONDITIONED TASTE AVERSION Total Cingulates

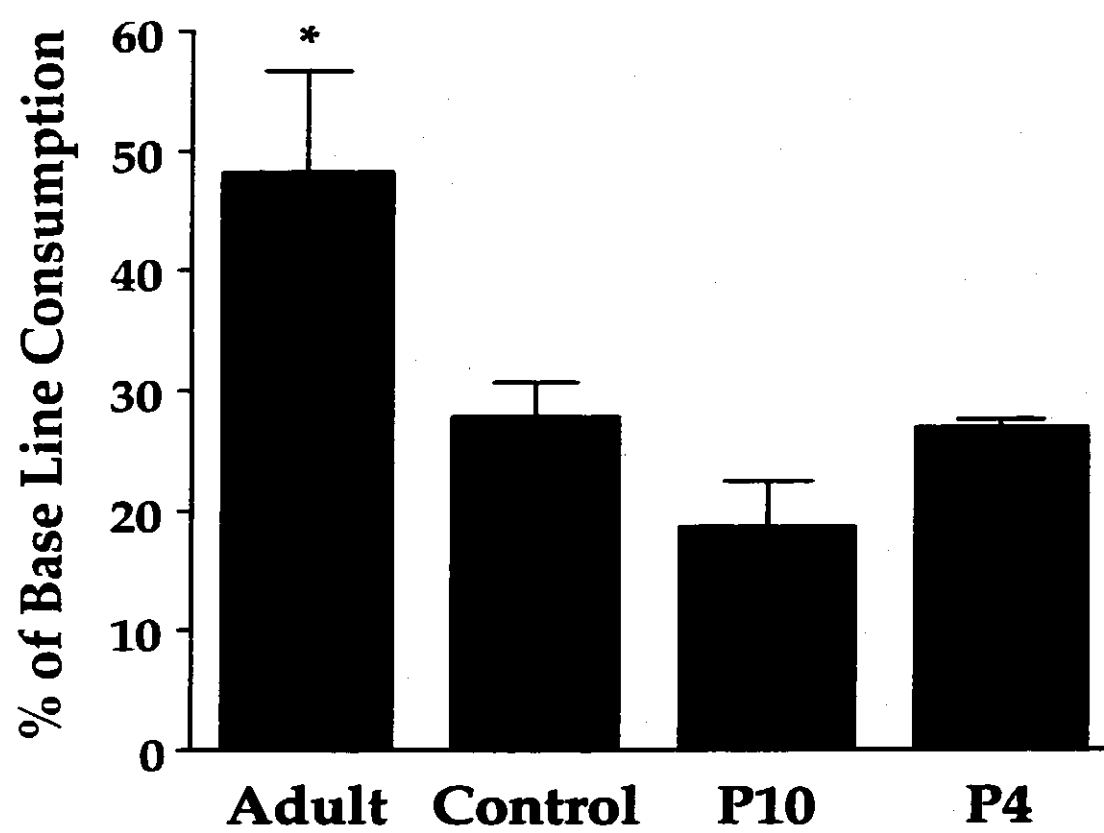
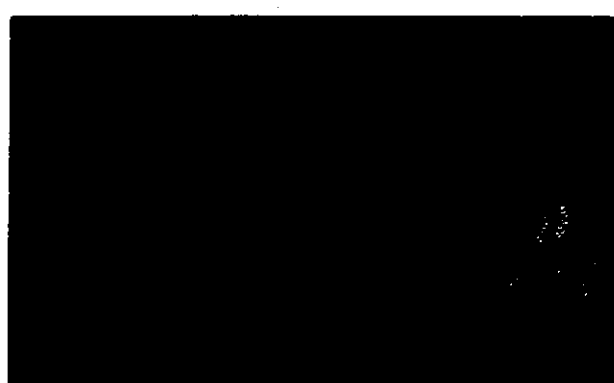


Fig. 26. Summary of the aversion index (\pm SE) expressed as a mean percentage of baseline consumption during the test trial. Animals received lesions of the complete cingulate cortex at different developmental ages. (* significantly different than control)

POSTERIOR CINGULATES



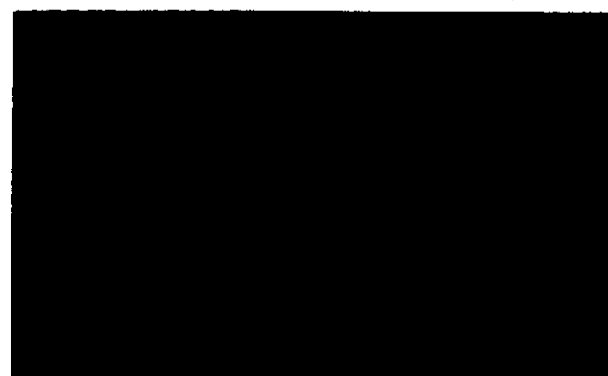
CONTROL



P4



P10



ADULT

Fig. 27. Photographs of representative brains of animals that received posterior cingulate cortex lesions at different ages. P4, postnatal day 4; P10, postnatal day 10; adult; and control.

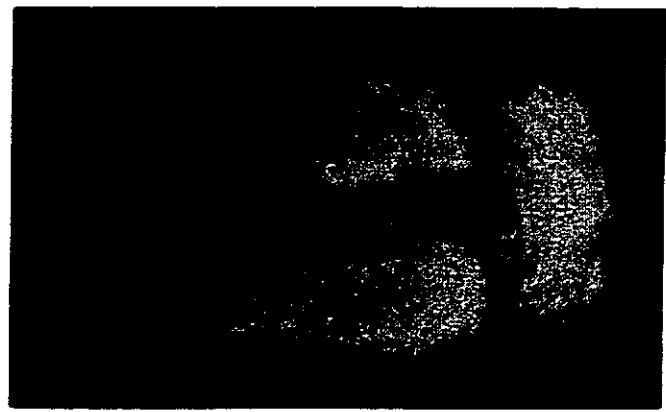
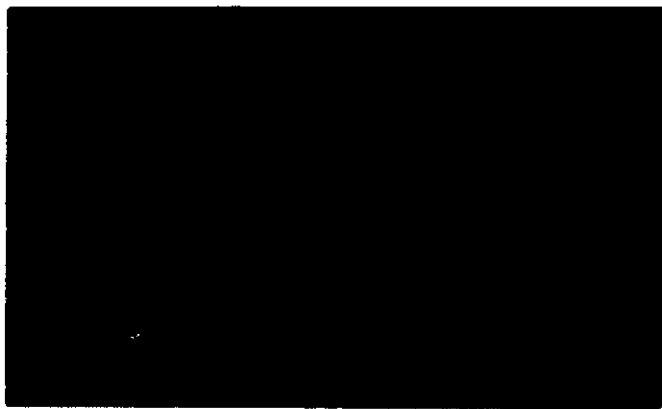
TOTAL CINGULATES**CONTROL****P4****P10****ADULT**

Fig. 28. Photographs of representative brains of animals that received complete cingulate cortex lesions at different ages. P4, postnatal day 4; P10, postnatal day 10; adult; and control. Note the regrown tissue on the P10 brain.

DISCUSSION

9. GENERAL DISCUSSION

There are five principal results that emerged from the present work. 1) animals with the posterior cingulate cortex removed at postnatal day 10 showed substantial functional recovery. 2) Animals with the complete cingulate cortex removed at any age, differed anatomically and behaviorally from animals with only the posterior cingulate cortex removed at the same age. 3) There is a behavioral difference between P4 and P10 operates, although less dramatic than that reported in previous studies. 4) Differences in the behavioral outcome varied with the precise age and site of the lesion, and this behavioral effect seemed to be dependent on specific anatomical correlates. 5) Two major neural changes were observed: an increase in dendritic branching of pyramidal cells in P10 animals with posterior cingulate cortex ablations, and an apparent spontaneous regeneration of new tissue in the lesion cavity on P10 animals with the complete cingulate cortex removed.

I will discuss the behavioral and anatomical findings separately.

9.1 Behavioral aspects

Adult lesions

Animals with complete cingulate cortex removed in adulthood showed a markedly stronger impairment in the Morris water task than animals with only the PCC removed, although they were also severely impaired. Sutherland et al., (1988) showed that animals with either posterior or complete lesions of the cingulate cortex could not learn to swim to a place in space using distal cues whereas no deficit was observed when they had to swim to a visual landmark. These results led the authors to suggest that the impairment in place navigation

produced by cingulate damage probably resulted from a difficulty in making appropriate use of the topographical relationships among distal cues rather than in swimming to a platform.

Studies from other authors (e.g., Markowska et al., 1989) have shown deficits after cingulate cortex lesions in other spatial tasks such as the T-maze and delayed non-matching-to sample. Moreover, similar deficits have also been reported in other non-spatial tasks like passive avoidance in which selective inactivation of the cingulate cortex produced a severe impairment on the acquisition of this task (Riekkinen et al., 1995).

The finding that animals with either PCC or Total cingulate removals in adulthood were impaired in the acquisition of a conditioned taste aversion has not been reported in the literature before. It is possible to attribute this result to an alteration in the cortico-cortical connectivity between the cingulate cortex and the insular cortex, which is a structure that has been known to support acquisition and retention of this aversive-learned task. The neural substrate of conditioned taste aversion involves the gustatory portion of the insular cortex, the parabrachial nucleus, the nucleus of the solitary tract, the medial thalamus, and the basolateral nucleus of the amygdala (for reviews see Kiefer, 1985 and Yamamoto, 1994). The insular cortex may serve as visceral cortex involved in the integration of gustatory and limbic information because it has extensive connections with other gustatory areas such as the parabrachial nucleus, the ventromedial thalamic nucleus and the mediodorsal thalamic nucleus; structures that are also interconnected with cingulate areas.

P10 Lesions

Animals with posterior cingulate cortex lesions performed unexpectedly well in the Morris water task. An even greater surprise was the behavioral performance of animals with complete cingulate cortex removals. Kolb and colleagues have explored the effects of P10 lesions in other cortical areas (e.g., parietal, occipital, motor, and frontal) and comparable effects in behavioral recovery are seen only in animals with medial frontal cortex lesions. Some recovery is seen when posterior parietal, occipital, or motor cortices are removed at P10 but it is incomplete at best. For example, when bilateral lesions of the occipital cortex are performed at P10, animals show poor performance in the Morris water task, but an enhancement of somatosensory functions (Kolb et al., 1996). In another study, rats with bilateral motor cortex lesions either at day 1, day 10 or in adulthood were tested in a series of motor tasks (skilled forelimb reaching, beam traversing, and tongue extension). Animals with day 10 lesions performed better than P1 or adult operates on the reaching and beam-traversing tasks, but were still markedly impaired relative to sibling controls (Kolb et al., 2000). In contrast, (for a review see Kolb, 1995) medial frontal cortex damage at day 10 leaves animals with nearly complete behavioral recovery. In the present study, the behavioral recovery seen after cingulate cortex lesions was also more complete than that observed after more lateral lesions. This suggests that there may be something special about recovery after midline telencephalic injury during development.

P4 Lesions

An unexpected result obtained from experiment 3 is the surprising behavioral recovery of P4 animals. Kolb, (1995) has shown that recovery usually

varies with precise developmental age. If the cortex is injured bilaterally during cell differentiation (P7-P12), there is virtually complete functional recovery. In contrast, if the cortex is injured during the time of neural migration (P0-P6), the consequences are behaviorally devastating. In the current studies animals with the posterior cingulate cortex removed on P4 learned to search for the platform in the Morris water task and they showed a typical learning curve very close to that shown by control animals. Like control animals, they also searched longer in the correct quadrant during the probe trial. One explanation of why P4 animals performed as well as they did in this study could be due to the restitution of hippocampal-theta activity in neonates with PCC removed.

Kolb and Whishaw, (1991) removed the retrosplenial cortex either in adulthood or at day 3 postnatally. As adults, the animals were implanted with hippocampal recording electrodes. Adult operates showed virtually no atropine-resistant hippocampal activity yet that activity was present in the hippocampus of rats with P3 cingulate removal. These electroencephalographic results suggest that damage in early infancy is associated with greater plasticity because either the serotonergic fibers that project to the hippocampus have to take an alternate route, or other serotonergic pathways to the hippocampus are modified to support atropine-resistant theta activity. It is likely that the same plastic phenomenon observed by Kolb and Whishaw, (1991) occurred in the P4 animals in the present study and that the hippocampal EEG was normal in these animals. Thus, in contrast to the adult operates, who likely had severely disrupted hippocampal EEG activity, the P4 operates would have had a more normally functioning hippocampus. There are two questions that arise from this hypothesis, however. First we can wonder how the hippocampus could

function in the absence of the rest of the cingulate inputs. We have no anatomical evidence to help us on this issue. Second, we can ask whether or not the P10 animals had normal EEG. If not, then we are left wondering if the mechanisms of recovery are different in the two groups.

It is important to note that although the functional recovery after neonatal cingulate removals, and especially that observed on P10 animals, was quite remarkable, the neonatal operates were not behaviorally normal. This is reflected in the distinctly abnormal circadian rhythm showed by the neonates when their activity was monitored over 48 hours. In general, there was a clear circadian rhythm in cage activity, as animals were more active in the dark period than in the light. There was also a significant sex difference, as the female rats were more active than the male rats at night. Furthermore, there was an effect of lesion size, as animals with complete cingulate removals were more active than those with only the posterior cingulate removed. There was also an age effect as P10 animals were more active than P4, which in turn were more active than adult operates, who did not differ from controls. These differences in general activity likely reflect the cerebral reorganization that occurs when the brain is injured during the first days of life. It is not clear from the current study whether this abnormal circadian rhythm reflects a general disturbance of other species-typical behaviors after early injury. Kolb & Whishaw, (1981; 1989) have found similar abnormalities in species-typical behaviors after early frontal lesions so this may, in fact, be one cost of recovery of cognitive functions.

9.2 Anatomical aspects

The gross anatomical measurements obtained from the animals in these experiments showed that changes occur following early cingulate lesions that lead to the development of a lighter brain, gross changes in thalamic structure, and a thinner neocortical mantle. This decrease in cortical thickness seemed to be generally diffuse over the entire cerebrum, and it was observable in regions with or without direct connections to the damaged area. That animals displayed good behavioral outcome in the presence of gross changes in cerebral development after neonatal cerebral injury could be explained in two ways.

First, the increased branching of dendrites of parietal cortex of P10 animals (experiment one) strongly suggests a plastic modification of the remaining cerebral cortex, or some portion of the remaining cortex. Kolb et al., (1994) showed that day 10 lesions of the medial frontal cortex led to increased dendritic arbor in pyramidal neurons throughout the cerebral cortex. It has been proposed that changes in dendrites (dendritic branching or spine density) may reflect a general mechanism of recovery of function after cortical injury (Kolb & Gibb, 1991a; Kolb & Sutherland, 1992). It is likely that the increased branching reflects increased available synaptic space, which could be mediating the behavioral outcome. This altered synaptic organization probably reflects changes in intrinsic organization of the cortex, rather than a whole-scale rewiring of cortical connectivity. Kolb et al., (1994) found that whereas rats with P10 frontal lesions had no obvious changes in cortical connectivity, rats with day 1 lesions showed abnormal thalamo-cortical, amygdalo-cortical, cortico-cortical, and dopaminergic connections. The rats with P1 lesions had no dendritic hypertrophy and no recovery of function. In sum, it is likely that it is the

alteration in the intrinsic organization of the cortex after day 10 cingulate lesions that is responsible for at least some of the functional recovery.

Perhaps the most important finding of the present studies is the serendipitous result of partial regrowth and complete functional recovery in P10 animals in which the medial frontal tissue was removed in addition to the posterior cingulate cortex. The animals with the complete midline removed showed better behavioral recovery than animals just with the posterior tissue removed. That animals with filling-in of cerebral tissue showed better functional recovery than animals with no regrown tissue, does not demonstrate that the filled-in tissue supports the recovery but it is suggestive. There are four pieces of evidence that support this possibility. First, Kolb and Gibb, (1991a) correlated anatomical change and behavioral recovery of the P1 and P10 frontal lesions. One prediction that can be made is that functional recovery should correlate with the actual process of regrowth. That is, there should be a behavioral deficit that is present and then recedes as the tissue regrows. A series of studies have shown that animals with regrown tissue do not show functional recovery at 22 days of age at which time the newly formed tissue is still very immature, but they do show recovery at 56 days of age, by which time the regrown tissue appears morphologically mature (de Brabander and Kolb, 1993; Kolb & Gibb, 1991; 1993).

Second, the disappearance of the cavity following medial frontal cortex lesions sustained at P7-P12 has been shown to be filled in by newly generated neurons. Kolb et al (1998) were able to identify cells in the regrown tissue with the mitotic marker BrdU. Counter labeling cells with the neuron-specific antibody NeuN allowed a positive identification of cells as newly generated neurons. These BrdU-NeuN positive cells were visible in the midline frontal

region as early as 4 days post-lesion. In addition, the authors were able to visualize neurons in the region of the lesion. There were also many dividing cells in the subventricular zone an area proximal to the lesion site. In this same work, the authors determined whether the new neurons formed normal connections. In order to address this question the retrograde tracer True Blue was injected into the posterior parietal cortex and the striatum; two regions known to be connected to the medial frontal cortex. In both cases the authors found neurons in the midline area that were positive for BrdU and retrogradely labelled with True Blue. All this evidence led the authors to conclude that the mammalian brain is capable of generating new neurons after cortical injury and that these neurons migrate to replace lost neurons making connections similar to those seen in this region of the control brain.

Third, Kolb et al., (2000) reasoned that if the new tissue is responsible for functional recovery, then by blocking the filling-in after the lesion the behavioral outcome should be diminished. The mitogenic marker BrdU is incorporated into the DNA during the S-phase of the cell cycle of any cell, and if it is administered during embryonic days 11-17 (E11-E17) of embryogenesis, it produces abnormalities in cerebral and body morphology and it prevents the lesion cavity from filling in (Kolb et al., 1999a). Kolb et al., (2000) showed that pre-treating animals with BrdU at any time from E12 to E17 blocks the functional recovery after medial frontal lesions at P10. That behavioral effect was correlated with an absence of filling-in of the lesion cavity perhaps by interfering with the mitotic activity in the subventricular zone. Indeed, a later study showed that embryonic pretreatment with BrdU severely compromised later mitotic activity of stem cells in vitro (Kolb et al., 1999b).

Finally, the question of whether removal of the regrown tissue in adulthood would block recovery was addressed in another experiment (Temesvary et al., 1998). Animals received medial frontal lesions on P10. The tissue was allowed to reform and then the new tissue was removed in adulthood. This manipulation resulted in severe functional deficits relative to animals with P10 (only) or adult lesions.

Taken together, this series of experiments provide compelling evidence that the regrown medial frontal area is supporting functional recovery. It thus seem plausible that regrown tissue may have contributed to the functional recovery in the rats with complete cingulate lesions in the current study.

In summary, the main results of the present studies suggest: 1) that damage to the posterior cingulate cortex at P10 is associated with greater neuroplasticity than similar damage at P4 or in adulthood, as it can be seen in both behavioral and anatomical data; 2) that there are specific morphological changes in remaining neocortical areas that might be supporting the behavioral recovery in the absence of regrowth; and, 3) that removing the anterior cingulate cortex along with the posterior cingulate cortex at P10, results in an almost complete regrowth of the anterior tissue and a partial regrowth of the posterior one. In other words, it seems that the neural generation that occurs when the anterior region is removed stimulates the cells to migrate and restore some posterior area as well.

9.3 *Future directions*

Neave et al., (1994) and Warburton et al., (1998) have shown that impairments in the Morris water task are not present after excitotoxic lesions of the posterior cingulate cortex, but are present after lesions of the cingulum bundle (fibers of passage between the cingulate cortex and the anterior thalamic nuclei). An additional experiment (currently underway) is examining the effects of transections of the cingulum bundle on spatial navigation. The present studies are not definitive with respect to the precise structures within the cingulate region that contribute to the deficits observed in spatial behavior after the cingulate lesions at different ages.

When the cortex is injured during the time period from postnatal day 7 to postnatal day 12, several changes occur within the brain associated with recovery of cortical function. These changes include: 1) changes in the organization of the remaining, intact, circuits in the brain; 2) the generation of new circuitry; and, 3) the generation of neurons and glia to replace at least some of the lost cells. In the present studies, we observed significant recovery of function after lesions of the cingulate cortex sustained at day 10. This recovery of function is correlated with two anatomical changes: an increase in dendritic arborization in the neocortex, and a spontaneous regeneration of some of the lost tissue. We do not, however, have any direct measure that proves that the regrown tissue observed in animals with complete cingulate cortex removals, did in fact, contain newborn neurons. In order to address this issue, an experiment wherein animals receive a mitotic marker, such as BrdU following the P10 lesions is necessary. These animals would undergo immunohistochemical processing to determine if the BrdU co-localizes with known neuronal markers such as NeuN.

It is also very likely that other morphological changes in the remaining cerebral cortex are taking place in animals with lesions at P4 and at P10. These changes are likely, at least to some extent, to be contributing to the functional recovery observed in these animals. Further analysis of the brains processed for Golgi-Cox in experiment 3 would help to answer this question, as would retrograde labeling of the hippocampal connections.

Finally, one other study (currently underway) would potentially answer the question of whether or not removal of the anterior midline portion of the cerebral cortex at P10, would stimulate cell migration to more posterior areas. In this experiment, P10 animals received unilateral lesions of the anterior cingulate cortex and bilateral lesions of the posterior cingulate cortex; or lesions of the posterior cingulate cortex on the side ipsilateral to the anterior lesion. We expect to see at least some regrowth on the posterior area, and along with this change some functional recovery.

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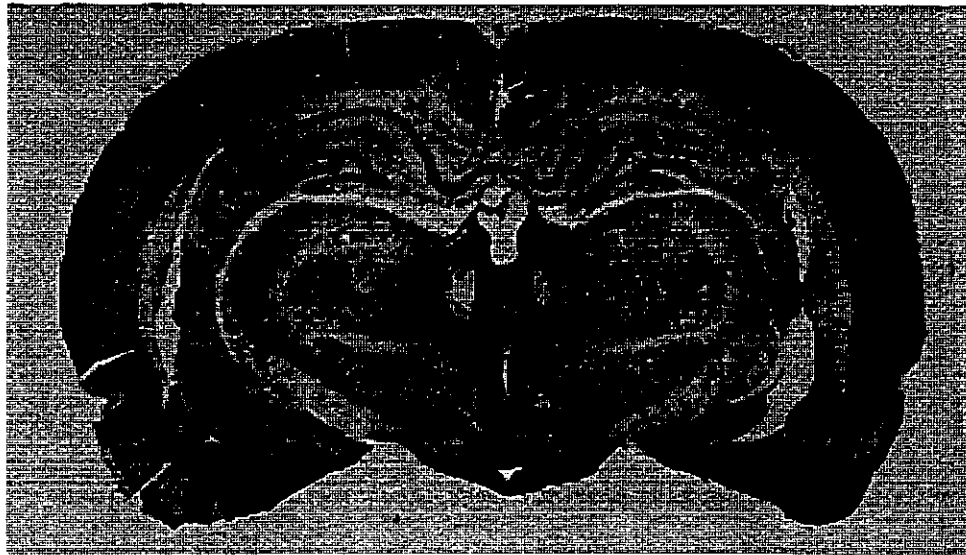
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APENDICES

APENDIX I

Photo Micrographs of representative lesions

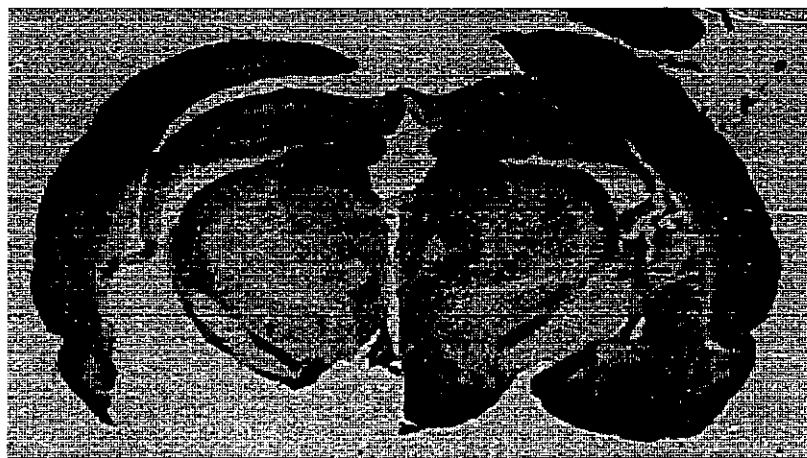
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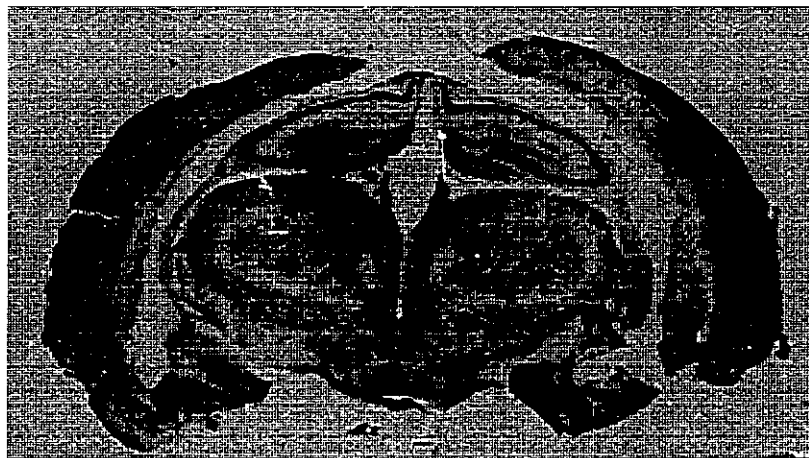
APENDIX II

Photo Micrographs of representative lesions

P4 PCING



P4 PTOTAL



APENDIX III

Photo Micrographs of representative lesions

P10 PCING



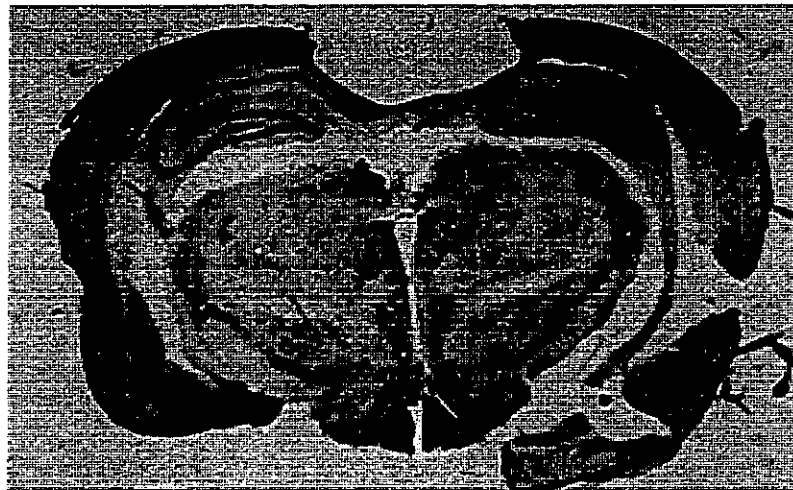
P10 TOTAL



APENDIX IV

Photo Micrographs of representative lesions

ADULT PCING



ADULT TOTAL

