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Morphometric analysis of prefrontal cortical development following neonatal lesioning of the dopaminergic mesocortical projection

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Summary. In this study the possibility that dopamine (DA) plays a trophic role in cortical development was studied by analysing cortical morphology and dendritic arborization of pyramidal cells after neonatal depletion of DA. The prefrontal cortex (PFC) was depleted of a DA innervation from postnatal day 1 onwards by thermal lesions of the DA cell group (A10) in the ventral tegmental area. Measurements of the cortical thickness and volume of the PFC subareas did not reveal any gross alterations. The DA-depleted animals, however, showed a 30% decrease in the total length of the basal dendrites of the pyramidal cells in layer V of the medial PFC. These cells constitute the primary target of the dopaminergic innervation in the prefrontal cortex. The decreased dendritic length was due mainly to a reduced branching frequency of the basal dendrites. The present results of the dendritic measurements support a trophic role for DA in neuronal differentiation.

Key words: Golgi-study – Dendrite – Pyramidal cell – Cortical thickness – Volumetry – Immunocytochemistry – Regression – Rats

Introduction

The major catecholaminergic cell groups are formed and undergo differentiation during early stages of development (Tennyson et al. 1973; Lauder and Bloom 1974). In rats these axons reach the subplate of the neocortex approximately 1 week before birth (Kalsbeek et al. 1988a), at a stage of development when other types of neurones – including the ones that will eventually be their target cells – are either unformed or not fully differentiated. At birth the synaptic structures of the rat neocortex are mainly monoaminergic terminals (Coyle and Molliver 1977), which suggests that monoamine neurones play an important ontogenetic or developmental role in the cerebral cortex, prior to the actual onset of neurotransmission (Lauder and Bloom 1974; Buznikow 1984; Mattson 1988).

There are, however, some marked differences between the full-grown cortical innervation patterns of the three monoamines. Noradrenaline and serotonin have a widespread, rather homogeneous innervation pattern, whereas dopamine has a more "focused" innervation pattern (Morrison and Magistretti 1983; Parnavelas and McDonald 1983). In rodents, the prefrontal cortex (PFC) is virtually the only neocortical area, besides the entorhinal cortex, with a dense and extensive dopaminergic innervation (Van Eden et al. 1987; Doucet et al. 1988).

Removal of the mesocortical dopaminergic fibres shortly after birth has previously been shown to cause both behavioural deficits (Kalsbeek et al. 1988b, 1989a) and a reduction in the cortical thickness of the medial part of the PFC (Kalsbeek et al. 1987). The exact mechanisms that underlie the reduction in cortical thickness are not clear, as the cortical thickness may reflect both the number of cells and the size of their tree structures.

The goal of the present study was to examine further the effects of perinatal depletion of the DA innervation in the PFC. In addition to a more comprehensive measurement of cortical thickness, volumetric quantifications were performed in the different PFC subareas. Dendritic arbors of pyramidal cells were examined as a possible point of impact of the mechanisms underlying the reduction in cortical size. Measurements were focused on the pyramidal cells in layer V, as this layer contains the highest density of dopaminergic fibres and the

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pyramidal cells form the primary target of the dopaminergic projection (Van Eden et al. 1987; Doucet et al. 1988).

Material and methods

Subjects

The experimental animals were rats of the Wistar strain (CPB–WU) bred at the Netherlands Institute for Brain Research. Subjects for the present study were selected from 12 litters, containing both males and females. The animals were housed in air-conditioned rooms with controlled temperature and 12/12 h light-dark schedules. Six pups from each litter received a bilateral lesion aimed at the ventral tegmental area (VTA) within 24 h after birth, while the remaining two pups were sham-operated. Electrothermal lesions were made under ether anaesthesia using a stereotaxic procedure adapted for neonatal rat pups (Hoorneman 1985). The sham-operated pups underwent the same surgical procedure, the only difference being that the electrodes were not heated. The pups were returned to their mothers after surgery and remained there until weaning when they were 21 days old. They were subsequently housed in groups of four with food and water available ad libitum. Body weights were recorded weekly.

Tissue preparations

Subsequent litters were processed for either Experiment 1 or Experiment 2 (5 and 7 litters, respectively). Both the lesioned and the sham-operated animals were perfused with buffered 5% glutaraldehyde (pH 4) on postnatal day 36 ± 2. Brains were removed from the skull, postfixed for 30 min and then weighed immediately. Next, using a simple blocking device (Fig. 1), the brains were cut immediately rostral to the optic chiasm. This "blocking" procedure allowed the different brains to be cut in the same plane and prevented the exclusion of tissue from analysis because of asymmetrical or oblique cutting (Kolb and Whishaw 1981; Uylings et al. 1984; Kalsbeek et al. 1987). The caudal part of the brain was processed for immunocytochemical staining to verify the location and extent of the lesions. Antibodies were kindly donated by Dr. R.M. Buijs (Geffard et al. 1984). Coronal Vibratome sections, 50 μm, were stained alternatively for dopamine, serotonin and Nissl. The forebrains were processed for either Golgi-Kopsch impregnation (Experiment 1) or Nissl staining (Experiment 2). The modification of the Golgi-Kopsch method used was based on both the original Kopsch procedure and the one described by Colonnier (1964). After glutaraldehyde perfusion a 1.5 mm piece of the forebrain was left in the same fixative overnight. It was then transferred to the Golgi-Kopsch fixative 2.5% $K_2Cr_2O_7$: 25% glutaraldehyde = 4 : 1 (v/v) for 24 hours, followed by 3.5% $K_2Cr_2O_7$ for 4–5 days, and impregnated with 0.75% $AgNO_3$ for 3–4 days. Subsequently the tissue blocks were transferred to 70% alcohol saturated with silverchromate and the 150 µm coronal vibratome sections were dehydrated and mounted in Histomount (Diagnostics). For Experiment 2, the rostral half of the brain was dehydrated and embedded in celloidin. The coronal serial sections (thickness 50 µm) were cut on a sliding microtome and stained with cresyl violet.

Data acquisition

Experiment 1. To be included in the data analysis the dendritic trees of the large pyramidal cells of layer V had to fulfil the following criteria: (1) the cell body had to lie in the middle third of the section thickness, (2) only well-impregnated cells were selected, of which (3) the dendritic trees were not obscured by heavy clusters of dendrites or stain precipitations. The three-dimensional branching pattern was measured with a semi-automatic dendrite measuring system developed at the Netherlands Institute for Brain Research (Uylings et al. 1986a). On the basis of the three-dimensional measurements, the following dendritic parameters were reported: the area of the pyramidal cell soma projected onto a coronal plane, to indicate the size of the soma; the number of basal dendrites per cell; the total number of basal dendritic segments per cell, indicating the branching frequency; the total basal dendritic length per cell; and the length of individual basal dendritic segments. In order to analyse the length of individual segments, dendritic segments were classified into different subgroups based upon their different length distributions (Uylings et al. 1986a). Since the length of terminal segments may differ, depending on their distance from the dendritic origin, the terminal segments were analysed versus the centrifugal order. The root segments are called 0 order and after each bifurcation the order is raised by one. The intermediate segments were analysed according to their degree (i.e. the number of terminal endpoints to which the pertinent segment can lead). Three subdivisions were discerned (1) degree-2, i.e. intermediate segments which branch into two terminal segments; (2) degree-3, i.e. intermediate segments branching into a terminal and an intermediate segment with two endpoints in its peripheral subtree; and (3) degree $\geqq 4$, i.e. intermediate segments having a peripheral subtree with four or more terminal segments. In addition to segment length, the radial distance from the terminal tips to their dendritic origin (radial length) and their distance along the dendrites (path length) were analysed.

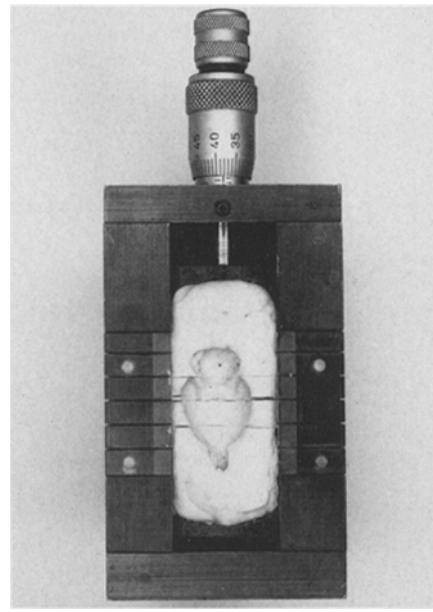

Fig. 1. Blocking device. A plaster mould of the desired brain weight is used to fix the different brains in an identical position. With the screw the coronal cut can be made at any desired anterior/posterior position

Experiment 2. Using every third section, the cytoarchitectonically defined boundaries of the PFC and its subareas were drawn, with the aid of a computerized digitizing tablet attached to a microscope equipped with plan objectives. The criteria for the discrimination of the PFC subareas and the analysis of volumes were the ones proposed by Van Eden and Uylings

(1985). The following subareas were discerned: the prelimbic area (PL), the dorsal part of the anterior cingulate (ACd) and the medial precentral area (PrCm) within the medial prefrontal cortex, and a dorsal as well as a ventral part of the agranular insular cortex (AId and AIv, respectively). The volumes of ACd and PrCm were divided in a pregenual and a supragenual part, since the dopaminergic projection to the supragenual part is partly derived from the substantia nigra, which was not lesioned in this study.

The measurement of cortical thickness was performed on the same material as was used for the volumetry, at three different levels: (1) bregma +2.7 of the atlas of Paxinos and Watson (1982), i.e. 200 µm caudal to the most rostral tip of the nucleus accumbens (the same level was used in a previous study (Kalsbeek et al. 1987); (2) bregma +2.2, i.e. the most rostral level of the caudate nucleus; and (3) bregma +1.2 approximately 500 µm caudal to the genu of the corpus callosum. The cortical thickness was measured along the curvilinear borderlines within the cortex between the cytoarchitectonic subareas of the PFC, as indicated in Fig. 6 ((1) IL1, PL1, ACd1, PrCm1, AId, AIv; (2) IL2, PL2, ACd2, PrCm2, SS; (3) ACv, ACd3, PrCm3). ACv is the ventral part of the anterior cingulate cortex. The cortical thickness was also measured in the agranular part of the somatosensory cortex (SS).

Statistics

Possible differences in the mean cortical thickness, the mean volume of prefrontal subareas, and the dendritic parameters between the experimental and control groups were tested by means of the non-parametric Mann-Whitney U-test. Since the variation of the dendritic variables between the animals was larger than the variation within the animals, each animal was treated as an individual case (Uylings et al. 1989). Therefore, comparisons between the control and experimental groups were based upon mean values of each animal.

Regression lines were computed for brain weight and cortical thickness and for brain weight and volumes in both experimental and control groups. If a linear regression exists, the line has been calculated for a group of bivariate points with the standard major axis method, and the control and experimental group are statistically compared with the test of the residual bivariate deviations (RDi) of bivariate points described previously (Uylings et al. 1986b, 1987).

Results

Before the dendritic and volumetric measurements were started, the lesions were inspected and categorized. The following groups were discerned: (1) sham-lesions (Fig. 2a), (2) lesions completely destroying the VTA, at least unilaterally (Figs. 2c,d), (3) lesions just dorsal to the interpeduncular nucleus, hardly affecting the DA cells of the VTA (Fig. 2b), and (4) lesions which only partly damaged the VTA or were completely misplaced. The

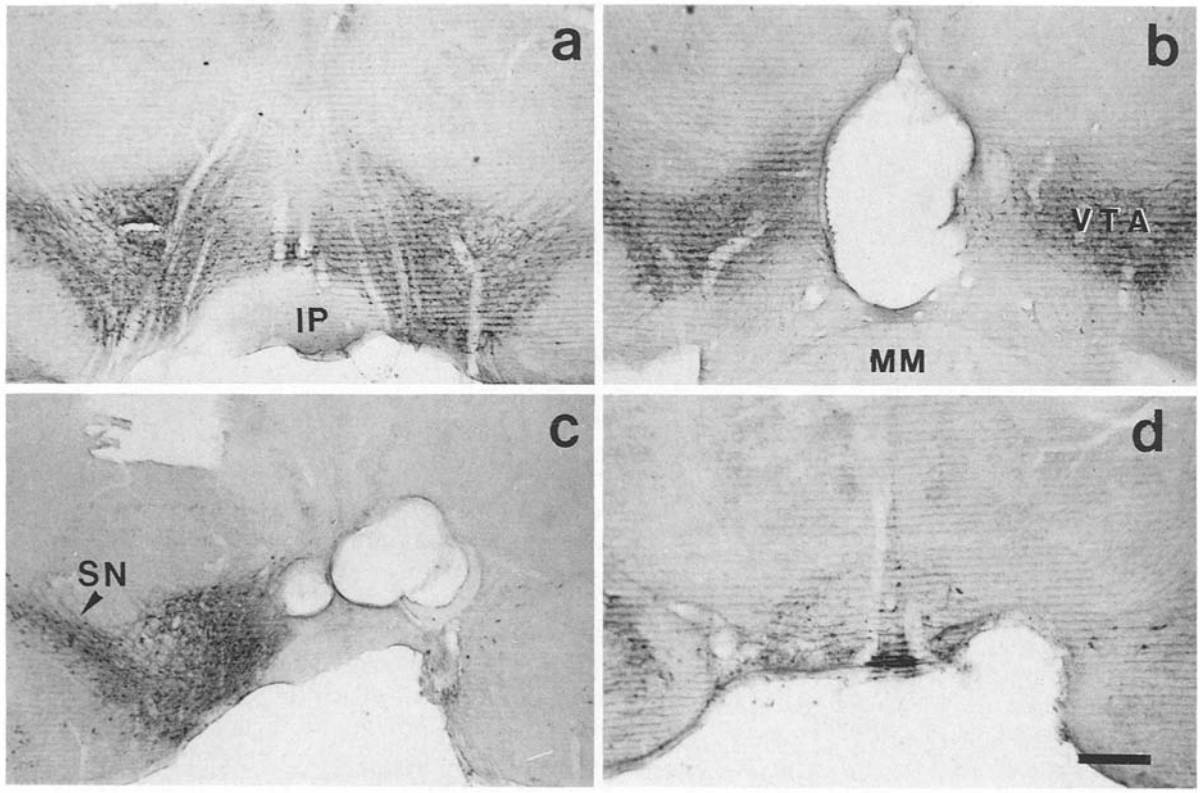

Fig. 2a–d. Different lesion types as discerned in this study: **a** dopamine staining of the ventral tegmentum, **b** type (3) lesion; mediodorsal to the VTA, hardly affecting the dopaminergic cell bodies but lesioning the serotonergic fibre system. **c** Type (2) lesion; a unilateral destruction of the major part of the dopaminergic cells in the VTA. **d** Type (2) lesion; but now a bilateral destruction. Bar is 0.5 mm

Table 1. Effect of neonatal VTA lesions on brain weight and body weight (g)

	Experiment 1					Experiment 2				
	n m/f	brainweight		bodyweight		*n* m/f	brainweight		bodyweight	
		mean	sd	mean	sd		mean	sd	mean	sd
Group 1 (control)	2/4	1.67	0.05	142.9	13.4	10/2	1.62	0.07	137.5	29.7
Group 2 (DA-depleted)	6/4	1.60*	0.06	139.2	10.1	13/8	1.54*	0.07	123.9	26.1
Group 3 (5HT-depleted)	0/2	1.61	0.08	135.6	2.6	4/3	1.49**	0.06	111.6**	8.5

*$p<0.05$, **$p<0.01$ according to the Mann–Whitney test m = male; f = female

Since animals were categorized after analyzing the lesion placement, brain and body weights could not be matched for the different groups. 40% (38 out of 96) of the animals had to be discarded because of misplaced lesions (type 4 lesions), bad impregnation or unsatisfactory embedding

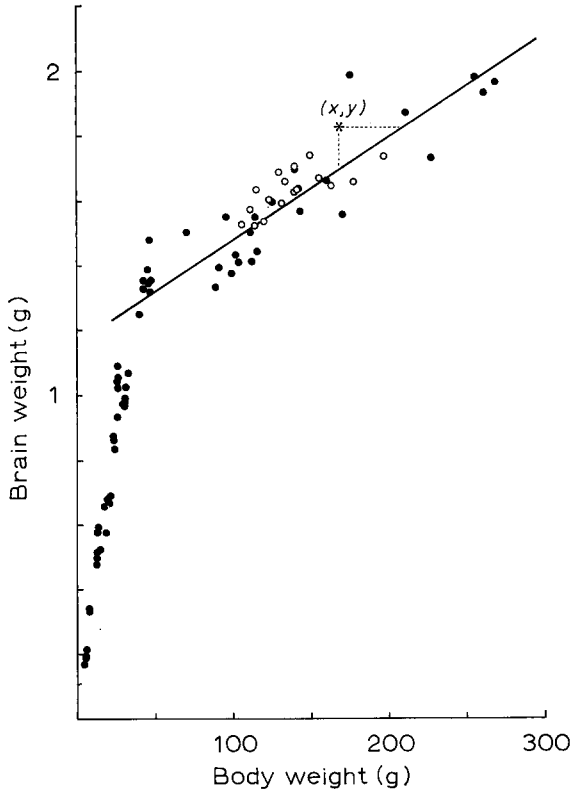

Fig. 3. Brain weight vs body weight for a developmental serie of "normal" Wistar rats (●), and the sham-operated animals (○) of the present study. For the developmental series, the data of a previous experiment were used (Kalsbeek et al. 1988a), in which the same perfusion procedure was employed. For each bivariate point (x, y) from experimental and control groups the product of deviation in the x and y direction from the reference line is determined (i.e. RDi). These RDi values are used in testing the existence of differences between groups

type (2) lesions depleted the dopaminergic innervation of the prefrontal cortex, whereas, depending on the extent of this lesion, the 5HT innervation of the cortex was also affected. Previous studies have shown that lesions as discerned in group 3 predominantly deplete cortical serotonin (Kalsbeek et al. 1989a,b).

Using observers unaware of an animals classification, we only performed measurements with the forebrains of the animals in groups 1, 2 and 3. Table 1 shows brain and body weight data from animals in the control and two experimental groups. DA-depletion significantly lowered brain weight in both experiments, whereas 5HT-depletion had no effects in Exp. 1, but lowered both brain and body weight in Exp. 2.

The body/brain weight relation of a developmental series of "normal" Wistar rats that were not operated on is shown in Fig. 3. According to the RDi test the bivariate data of the sham-operated animals in the present study did not differ significantly from the regression line of the reference group. In other words, the sham-operation does not appear to perturb normal development.

Dendritic analysis (Experiment 1)

In Uylings et al. (1989) procedures are described for designing the optimum sample size for the different sampling levels, in order to examine the presence of significant differences between groups of neurones. These procedures indicate a minimum of 6 animals per group and 8 cells per animal for detecting significant differences in somatic surface, number of segments in basal dendrites, and total basal dendritic length. Control and bilaterally lesioned animals with more than eight Golgi-impregnated Layer V pyramidal cells in each hemisphere revealed no left-right hemisphere differences for the different dendritic parameters. This supports the recent findings of Bruch et al. (1988), who could not detect

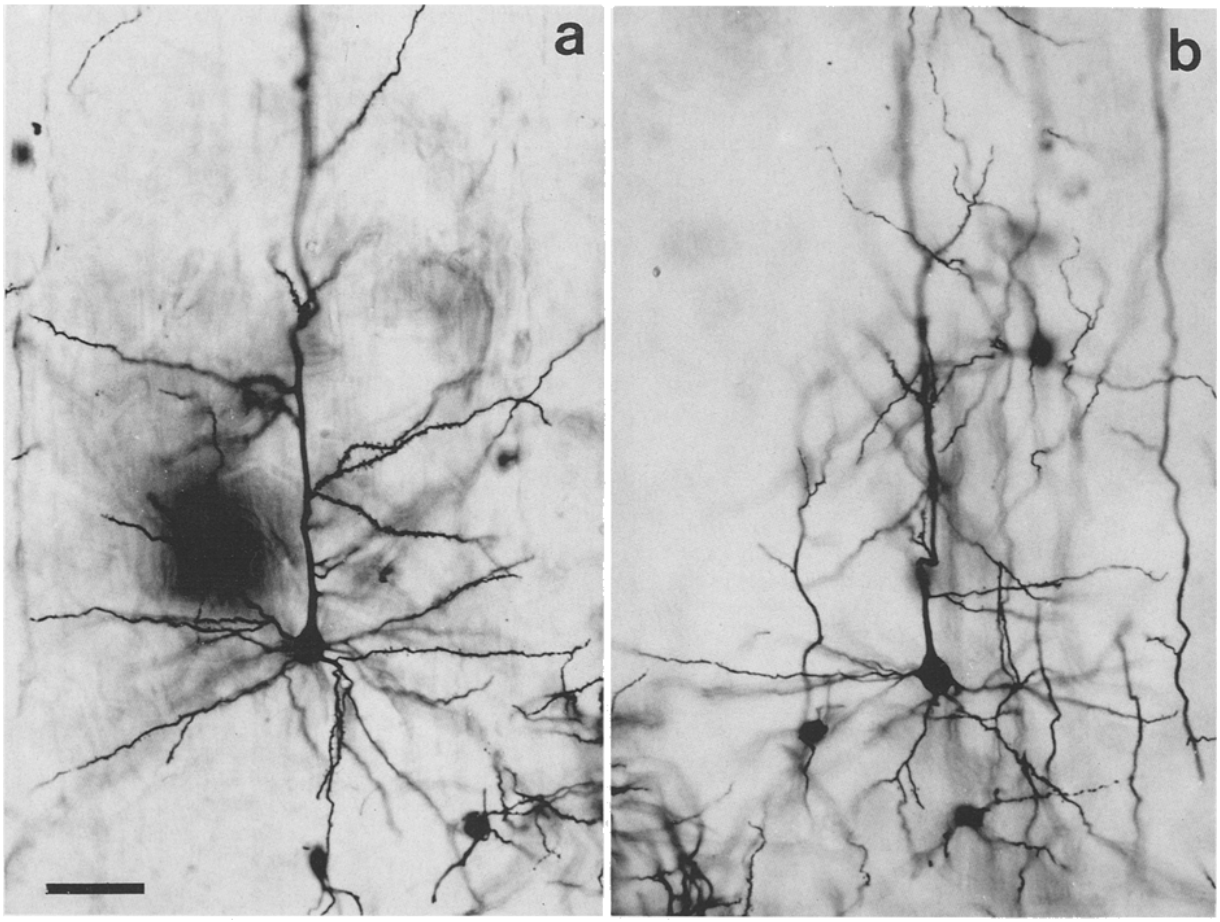

Fig. 4a, b. Examples of Golgi-impregnated pyramidal cells in cortical layer V of a control **a** and a DA-depleted **b** animal, as analysed in the present study. Bar is 0.1 mm

asymmetry in the dendritic parameters of layer–III and Layer–V pyramidal cells in the motor cortex of adult rats. Therefore, the cells of both hemispheres were combined and all animals with eight or more Golgi-impregnated cells fulfilling the analysis criteria were used for further analysis (Fig. 4). The somatic area and the number of basal dendrites in DA depleted animals showed a slight, but significant decrease (-7%; $p<0.05$) (Tables 2 and 3). Neonatal DA depletion reduced the total number of dendritic segments by 26% and the total dendritic length per cell by 30% ($p<0.01$), as compared with control values (Tables 4 and 5). Further analysis showed that the segment length of the separate dendritic segments was not affected significantly. Only the length of the terminal segments of the first order of DA-depleted animals was reduced significantly (-14%, $p<0.05$; Fig. 5). The radial distance from the terminal tips of the basal dendrites to their dendritic origin showed no significant differences (mean values: Control – 108 µm, DA-depleted – 102 µm, 5HT-depleted – 114 µm). In addition, there was no significant difference between the groups in the percentage of cut segments per cell (mean values: Control – 16%, DA-depleted – 20%, 5HT-depleted – 17%). The lack of a significant correlation between brain weight and dendritic length suggests that differences between the

Table 2. Somatic area (µm²) of pyramidal cells in layer V used for dendritic analysis

Control				DA-depleted			
Case	Mean	SEM	*n*	Case	Mean	SEM	*n*
C1	170.4	9.1	20	E1	121.3	6.7	17
C2	166.2	10.0	21	E2	149.8	8.6	18
C3	164.3	10.9	21	E3	165.1	9.7	22
C4	147.6	9.5	10	E4	163.6	10.0	18
C5	169.7	13.1	9	E5	163.8	10.6	12
C6	180.1	17.3	8	E6	151.8	15.3	8
				E7	145.6	8.4	14
S1	194.8	17.3	16	E8	148.0	8.9	18
S2	150.8	8.1	25	E9	154.2	21.9	8
				E10	178.1	13.1	10
$\bar{X}_C$	166.4	4.4	6	$\bar{X}_E$	154.1	4.8	10

Table 3. Number of basal dendrites per cell

Control				DA-depleted			
Case	Mean	SEM	*n*	Case	Mean	SEM	*n*
C1	6.2	0.4	20	E1	4.8	0.4	18
C2	5.6	0.3	21	E2	5.6	0.4	19
C3	5.4	0.3	21	E3	5.3	0.3	22
C4	5.2	0.2	10	E4	5.2	0.3	18
C5	5.8	0.4	9	E5	5.5	0.4	12
C6	5.4	0.5	8	E6	4.8	0.3	8
				E7	5.1	0.2	14
S1	5.8	0.3	16	E8	5.0	0.3	18
S2	5.0	0.3	25	E9	5.5	0.6	8
				E10	5.2	0.3	10
$\bar{X}_C$	5.6	0.1	6	$\bar{X}_E$	5.2	0.1	10

Table 4. Total number of segments of basal dendrites per cell

Control				DA-depleted			
Case	Mean	SEM	*n*	Case	Mean	SEM	*n*
C1	38.2	2.8	20	E1	19.2	1.5	18
C2	37.2	2.6	21	E2	24.9	2.8	19
C3	40.2	2.6	21	E3	27.1	2.2	22
C4	36.2	4.2	10	E4	33.6	3.1	18
C5	49.6	3.1	9	E5	31.2	2.5	12
C6	32.6	4.2	8	E6	24.5	2.7	8
				E7	30.5	2.5	14
S1	44.3	3.7	16	E8	32.8	2.5	18
S2	34.6	2.4	25	E9	31.5	3.5	8
				E10	32.2	3.2	10
$\bar{X}_C$	39.1	2.3	6	$\bar{X}_E$	28.8	1.5	10

Table 5. Total length of basal dendrites per cell (μm)

Control				DA-depleted			
Case	Mean	SEM	*n*	Case	Mean	SEM	*n*
C1	1845	198	20	E1	789	70	18
C2	1968	139	21	E2	1094	126	19
C3	2023	152	21	E3	1064	101	22
C4	1739	227	9	E4	1591	122	18
C5	2870	224	8	E5	1452	145	12
C6	1684	230	8	E6	970	115	8
				E7	1933	166	14
S1	2472	180	16	E8	1818	136	18
S2	1679	122	25	E9	1753	177	8
				E10	1679	205	10
$\bar{X}_C$	2016	180	6	$\bar{X}_E$	1414	128	10

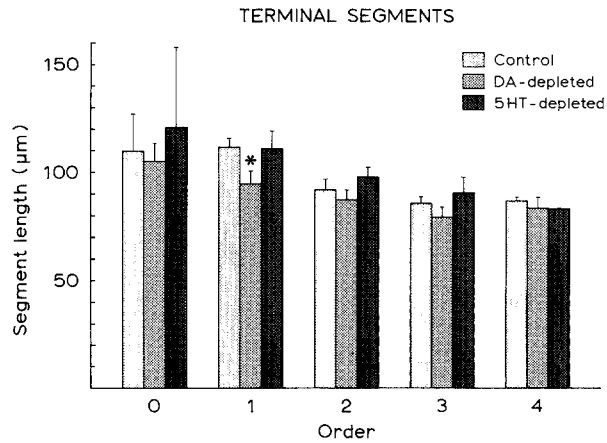

Fig. 5. Effect of experimental treatment on the length (µm) of terminal segments of basal dendrites versus their centrifugal order. $*p<0.05$

layer–V pyramidal neurones of the DA-depleted and control animals are attributable to factors other than simply brain size or weight (Table 1).

Volumetric and cortical thickness measurements (Experiment 2)

The brain weight was slightly decreased in both the DA- (−4%, $p<0.01$) and the 5HT-depleted (−8%F, $p<0.01$) animals as compared with the control group (Table 1). Body weight was significantly decreased only in the 5HT-depleted group. The mean cortical thickness in depleted hemispheres (left and right combined) is visualized in Fig. 6. DA-depleted animals show no significant differences compared with the control group (Fig. 6b). Compared with the controls, the cortical thickness of the 5HT-depleted animals was reduced in several areas, especially in the rostro-medial part of the cortex (Fig. 6c). The results of the volumetric measurements are presented in Table 6. The 5HT-depleted animals show a significant decrease in the volume of PL and the pregenual part of ACd.

Since brain and/or body weight were reduced in experimental animals (Table 1), regression analysis was applied to control for the variability in these weight parameters (Fig. 7; see also legend Fig. 3). In more than half of the cases this method revealed a significant positive regression effect between brain weight and cortical thickness or volume of the subareas. Using the RDi values, 5HT-depleted animals no longer showed significant decreases in cortical thickness and volumes (Fig. 7b). In fact, a relative sparing of some parts of the cortex may occur (Fig. 7a).

Discussion

To detect a possible trophic role of dopamine (DA) in the development of the prefrontal cortex (PFC),

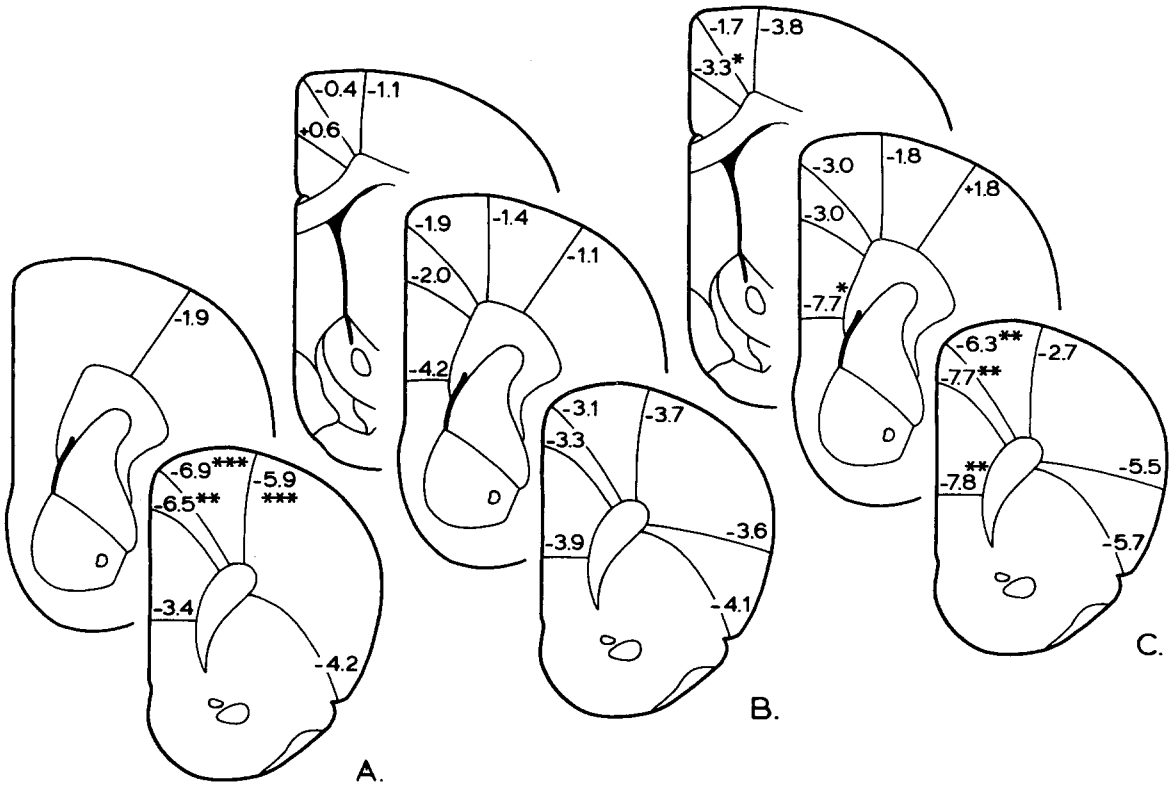

Fig. 6A–C. Cortical thickness along the borderlines of PFC subareas, at three different levels: **A** data from previous experiment (Kalsbeek et al. 1987), **B** DA-depleted, **C** 5HT-depleted. Percentages indicate changes compared to control values (left and right hemispheres combined)

Table 6. Influences of neonatal VTA lesions on volumina (mm) of PFC subareas

	Control		DA-depleted			5HT-depleted		
	Mean	SD	Mean	SD	%change	Mean	SD	%change
PrCm (p)	0.99	0.17	1.00	0.13	+1.0%	0.92	0.13	− 7.1%
PrCm (sg)	0.60	0.14	0.59	0.18	−1.7%	0.54	0.20	−10.0%
ACd (p)	0.86	0.10	0.83	0.09	−3.5%	0.73	0.12	−15.1%**
ACd (sg)	0.49	0.08	0.49	0.11	0.0%	0.46	0.17	− 6.1%
PL	1.16	0.16	1.16	0.12	0.0%	1.02	0.13	−12.1%*
AId	0.24	0.09	0.27	0.08	+12.5%	0.20	0.08	−16.7%
AIv	0.22	0.07	0.25	0.08	+13.6%	0.19	0.06	−13.6%

*$p < 0.05$, **$p < 0.01$, according to the Mann–Whitney test

a number of morphometric techniques have been applied in the present study to assess changes that may occur in the cortical cytoarchitecture. Cortical-thickness measurements and volumetric analysis can be used to gain insights into the more general alterations in brain morphology. Alterations of these brain-size variables have been found in a variety of experimental designs (Kolb and Whishaw 1981; Lidov and Molliver 1982; Bedi 1984; Friedman and Price 1986; Zagon and McLaughlin 1986; Johnson et al. 1987; Stewart and Kolb 1988). In the present study no differences were found between the volume of PFC subareas in DA-depleted hemispheres and those in the homologous non-depleted hemispheres. Similarly, the cortical thickness measurements revealed no effect of the DA depletion (Fig. 6). To control for the concomittant depletion of 5HT after VTA lesions, animals which showed only a depletion of cortical 5HT were also analyzed. In these 5HT-depleted animals both the cortical thickness and volume estimates seemed reduced (Fig. 6c, Table 6). Taking into account the numerical variation due to differences in brain weight by regression analysis,

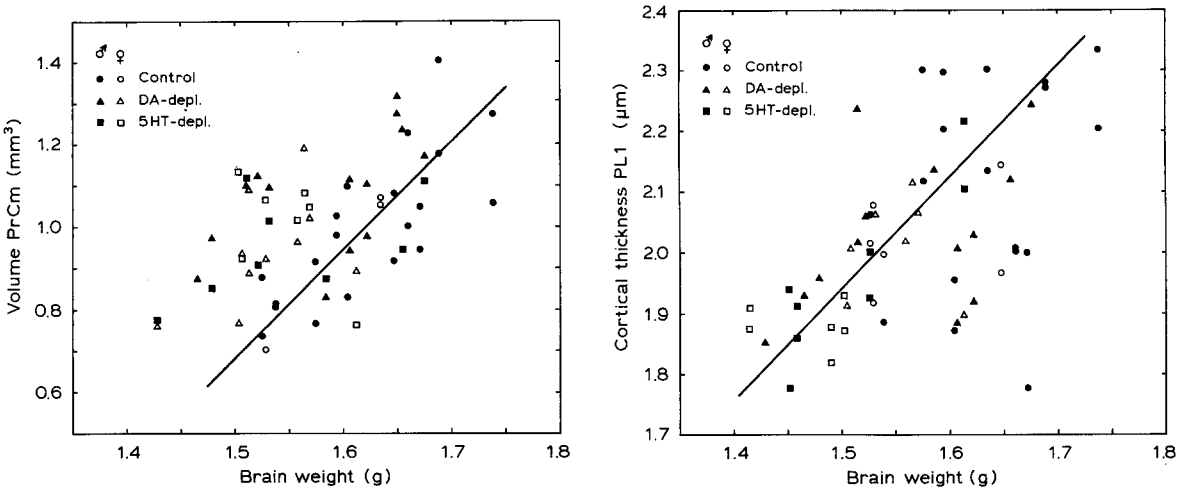

Fig. 7. Relation between brain weight and the volume of the the PrCm subarea (left figure) and brain weight and cortical thickness at PL1 (right figure), for the different treatment groups. The left figure shows a relative sparing of the volume of PrCm in DA- and 5HT-depleted animals compared with the control group ($p<0.05$), whereas the right figure indicates that there are no differences in cortical thickness in spite of the decreases in absolute means in this area as shown in Fig. 6

however, shows that these reductions can be attributed to the reduced brain weights of this experimental group (Fig. 7b).

The present results are in contrast with those of an earlier study, which revealed a small reduction in cortical thickness after neonatal VTA lesions (Kalsbeek et al. 1987; see also Fig. 6). A difference between both studies concerns histological procedures, which may have contributed to the disparity in results. In the present study, celloidin-embedded sections were used to measure the cortical thickness, and not vibratome sections as in the previous study. At the moment there are no clear indications for a definitive decision as to which results reflect the true effect of a neonatal DA depletion. Taking into account the smaller number of animals used for cortical thickness measurements in the previous study, it seems justified to consider the present result as the best reflection of the effect of a neonatal VTA lesion. However, both studies show that neonatal depletion of the cortical DA and/or 5HT innervation has only minor effects on the cortical thickness and volume of specific cortical regions. In addition, volume estimation, which is to be preferred over cortical thickness (Uylings et al. 1984), gave no indication of an effect of the neonatal DA depletion either. The more profound dendritic changes in one specific layer (see Experiment 1), are apparently not reflected in total cortical thickness or volume. In addition, alterations affecting the cortical thickness and/or the volume could be partly compensated for by sprouting of other systems. Westrum and Bakay (1986) showed that the shrinkage of layer I in the olfactory cortex, after adult lesions of the afferent fibres from the olfactory bulb, was prevented by the spread of cortical afferents if the lesion was applied in neonatal animals.

Our morphometric dendritic analysis shows that neonatal depletion of the dopaminergic innervation causes a substantial decrease in the branching frequency of the basal dendrites of the large pyramidal cells in layer V of the medial PFC. This reduced branching frequency, as reflected in a decreased number of basal dendritic segments (Table 4), caused a 30% reduction in the total basal dendritic length. In the present study the analysis of the dendritic tree patterns has been concentrated on the pyramidal cells of layer V in the medial part of the prefrontal cortex, since this appears to be the primary target for the DA fibres. Immunocytochemical and other neuroanatomical techniques have shown that the densest cortical DA innervation occurs in layers V and VI of the anteromedial cortex (Berger et al. 1976; Van Eden et al. 1987; Doucet et al. 1988). Electron microscopical studies have indicated that dopaminergic terminals in the prefrontal cortex predominantly form synaptic contacts with dendritic processes (Van Eden et al. 1987; Séguéla et al. 1988). The pyramidal cells in this area can also be labelled with DARP–32, a dopamine- and adenosine 3′ : 5′-, monophosphate-regulated protein, identifying dopaminoreceptive neurones (Ouimet et al. 1984). Electrophysiological experiments also support the idea that dopamine exerts its primary action on pyramidal cells in the lower cortical layers (Bunney and Aghajanian 1977; Bernardi et al. 1982; Ferron et al. 1984;

Thierry et al. 1986; Penit–Soria et al. 1987; Peterson et al. 1987).

The results of a neonatal depletion of cortical noradrenaline on the dendritic outgrowth are equivocal (Maeda et al. 1974; Wendlandt et al. 1977; Felten et al. 1982; Lidov and Molliver 1982; Lüth and Werner 1987). Felten et al (1982) reported a reduction in the dendritic branching of the cortical pyramidal cells after neonatal NA depletion. In that study, too, a systematic and extensive analysis of the dendritic morphology was performed. On the basis of qualitative inspection of our preparations, the gross morphology of pyramidal cells and their apical and basal dendrites in experimental animals was not noticeably different from their sham-operated littermates (Fig. 4). A reduction in the length of individual dendritic segments contributes only little to the observed reduction in total dendritic length (Fig. 5).

Cortical deafferentiation in neonatal animals has been shown to produce quantitatively detectable changes in cortical and neural morphology. The majority of these experiments involved some kind of sensory deprivation, i.e. visual (Valverde 1968; Ryugo et al. 1975b), vibrissal (Ryugo et al. 1975a), auditory (McMullen et al. 1988) or olfactory (Friedman and Price 1986). Other afferent systems, however, may also be important in organizing the cortical morphology. In this respect the monoaminergic systems have received a lot of attention because of their early appearance in cortical development. The monoaminergic fibres are thought to initiate differentiation and the early growth of dendrites (Lauder and Bloom 1974; Berry et al. 1978; Pinto-Lord and Caviness 1979). The reduced branching frequency, as observed in the present study, is compatible with the general idea that axon-dendritic interactions in early development provide an important stimulus to the growth and differentiation of the dendritic arbors of pyramidal cells (e.g Berry et al. 1978; Pinto-Lord and Caviness 1979; Ramirez and Kalil 1985; Purves 1986; Linden and Pinon 1987). Evidence suggests that the particular transmitter that is depleted may be at least as important as the actual reduction in the number of afferent fibres that reach the developing cortex. It has been shown previously that blocking the DA activity without lesioning the fibres, i.e. with haloperidol, reserpine or a-methyl-para-tyrosine, also retards the development of its target neurones (Tennyson et al. 1983; Iniguez et al. 1987). In the present study, lesions predominantly depleting the serotonergic innervation of the PFC seem to have no effect on basal dendritic parameters of the layer V pyramidal cells of the medial PFC. Therefore, the specific transmitter depleted and the period in development during which the depletion takes place may determine the exact nature of morphological (and functional) deficits. In an elegant in-vitro study McCobb et al. (1988) recently showed the neuron specific effects of DA and 5HT on neurite elongation.

In conclusion, neonatal depletion of prefrontal cortical DA does not drastically alter the gross features (i.e. lamination, cortical thickness and volumina) of the prefrontal cortex. However, a quantitative analysis of the cortical organization reveals clear changes at the level of layer V pyramidal neurones that support a trophic developmental role for DA in the cortex.

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