

NEURON NUMBER DECREASES IN THE RAT VENTRAL, BUT NOT DORSAL, MEDIAL PREFRONTAL CORTEX BETWEEN ADOLESCENCE AND ADULTHOOD

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Abstract—Neuroimaging studies have established that there are losses in the volume of gray matter in certain cortical regions between adolescence and adulthood, with changes in the prefrontal cortex being particularly dramatic. Previous work from our laboratory has demonstrated that cell death can occur as late as the fourth postnatal week in the rat cerebral cortex. The present study examined the possibility that neuronal loss may occur between adolescence and adulthood in the rat prefrontal cortex. Rats of both sexes were examined during adolescence (at day 35) and young adulthood (at day 90). The volume, neuronal number, and glial number of the medial prefrontal cortex (mPFC) were quantified using unbiased stereological techniques. Neurons were lost from the ventral, but not dorsal, mPFC between adolescence and adulthood, suggesting a late wave of apoptosis that was region-specific. This was accompanied by a decrease in the volume of the female ventral mPFC. In contrast to neuron number, the number of glial cells was stable in the ventral mPFC and increased between adolescence and adulthood in the dorsal mPFC. Sex-specific developmental changes in neuron number, glial number, and volume resulted in sex differences in adults that were not seen during adolescence. The loss of neurons at this time may make the peri-adolescent prefrontal cortex particularly susceptible to the influence of environmental factors. © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: sex differences, anterior cingulate, white matter, development, cortical volume.

Adolescence is a time of increased risk for depression, anxiety, schizophrenia, and drug abuse (Spear, 2000; Steinberg, 2005). The prefrontal cortex has been implicated, directly or indirectly, in each of these conditions, but little is known regarding the relevant changes occurring in the adolescent prefrontal cortex, especially at the cellular

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Abbreviations: ACd, dorsal anterior cingulate cortex; ACv, ventral anterior cingulate cortex; CE, coefficient of error; Fr2, frontal cortex, area 2; IL, infralimbic cortex; mPFC, medial prefrontal cortex; PL, prelimbic cortex; PrCm, medial precentral cortex.

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level. Recent magnetic resonance imaging studies of the adolescent human brain have shown decreases in the volume of gray matter of some cortical regions, including frontal, between early adolescence and young adulthood. Maturation changes in the human cortex are non-linear, region-specific, and often differ between males and females (Jernigan et al., 1991; Giedd et al., 1996, 1997, 1999; De Bellis et al., 2001). Differences between the adolescent and adult brain are greatest in the frontal cortex (Sowell et al., 1999, 2001b), where gray matter volume increases until around adolescence and then decreases between adolescence and young adulthood (Jernigan et al., 1991; Pfefferbaum et al., 1994; Giedd et al., 1999; Sowell et al., 1999; Shaw et al., 2006). These volumetric changes can be correlated with intelligence tests and cognitive development, most notably on tasks that rely on the prefrontal cortex (Casey et al., 2000; Sowell et al., 2001a, 2004; Shaw et al., 2006).

The cellular bases for late decreases in cortical volume are unknown and for technical reasons are difficult to determine in humans. Some investigators have found evidence for changes in synaptic density in the peri-adolescent primate cortex which may accompany the observed volumetric changes (Huttenlocher, 1979; Bourgeois et al., 1994; Anderson et al., 1995). Another intriguing possibility is suggested by our previous work in rats: the neuronal loss in the cortex which takes place relatively late in development (Nunez et al., 2001, 2002). Loss of neurons could contribute to the observed changes in both synaptic density and cortical volume.

Prior work from our laboratory has shown that a small rise in cell death in the visual cortex occurs at day 25 which is especially notable in females (Nunez et al., 2001). This finding indicates that cortical neuron number continues to be modified relatively late in development. It also appears to contribute to the 20% lower number of neurons found in adult female rats (compared with males) in the visual cortex (Reid and Juraska, 1992), because removal of the ovaries (but not testes) as late as postnatal day 20 results in equal numbers of neurons in both sexes in adulthood (Nunez et al., 2002). It is unknown whether the late occurring loss of cells that plays a role in the formation of sex differences in neuron number may also occur in non-sensory cortical association areas, such as the medial prefrontal cortex (mPFC).

The present study was designed to investigate potential neuron number changes between adolescence and adulthood in the rat mPFC. If late changes in cell number occur in the mPFC, this could provide a potential cellular

mechanism underlying changes in cortical volume in humans. Additionally, the potential contribution of late changes in neuron number to the development of sex differences in this region would be elucidated. Neuron number was quantified in dorsal and ventral divisions of the mPFC separately because of the regional differences in both afferent and efferent connections (e.g. Neafsey et al., 1993; Uylings et al., 2003), as well as behavioral functions (see Ragozzino et al., 2002; Kesner and Ragozzino, 2003).

EXPERIMENTAL PROCEDURES

Subjects

Subjects were Long-Evans hooded rats, descended from Simon-sen Laboratory (Gilroy, CA, USA) stock and bred in the vivarium of the Department of Psychology at the University of Illinois. Pups were weaned at day 25, same-sex and litter-mate pair-housed, and handled once weekly thereafter until the time of kill. Food and water were available *ad libitum*; animals were maintained on a 12-h light/dark cycle with the lights on at 07:00 h. Subjects were examined at day 35 (peri-adolescent; females $n=9$, males $n=9$) or day 90 (young adulthood; females $n=9$, males $n=11$). All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. The experiment conformed to U.S. National Institutes of Health guidelines on the ethical use of animals, and efforts were made to minimize the number of animals used and their suffering.

Histology

All rats were deeply anesthetized with sodium pentobarbital (2 mg/kg of a 65 mg/ml solution), and intracardially perfused with Ringer's wash (20 s) followed by a solution of 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed and stored in a solution of 4% paraformaldehyde in 0.1 M phosphate buffer for one week, followed by cryoprotection in fixative solution with 30% sucrose added. On the first day after sinking in solution, brains were coronally sectioned at 60 μm using a freezing microtome. Every fourth section was collected and placed in 0.1 M phosphate buffer. Free-floating sections were immediately mounted on gelatinized slides, allowed to dry, and then stained with Methylene Blue/Azure II.

Volume estimation

Dorsal mPFC (anterior cingulate cortex, both dorsal and ventral regions (ACd and ACv)) and ventral mPFC (prelimbic (PL) and infralimbic (IL)) regions were parcellated according to cytoarchitectonic criteria (Krettek and Price, 1977; Van Eden and Uylings, 1985a) at 31.25 \times using a camera lucida (frontal cortex, area 2 (Fr2) or medial precentral cortex (PrCm) was not included) (Figs. 1 and 2). The border between IL and PL cortical areas is made principally on the basis of the transition between layers I–III; the most superficial cells in layer II of IL extend into layer I whereas the boundary between layers I and II in PL is much more distinct. Layers II–V of IL consist of much more evenly distributed, ovoid-shaped cells, and the lamination pattern is much less defined in this region. Additionally layer II cells are more densely packed in PL as compared with IL. The border between PL and ACd is marked by a broadening of layer V and an increase in the density

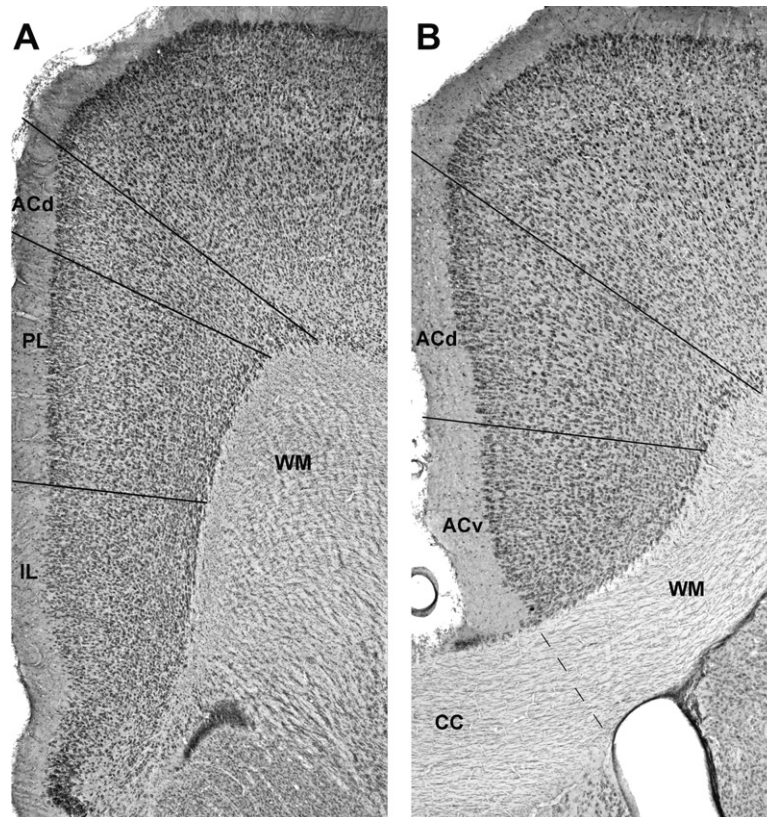


Fig. 1. Coronal sections through the mPFC showing parcellated borders (A, B). Frontal cortical white matter (WM) is also shown. In B, the portion of WM that is medial to the dashed line was considered corpus callosum (CC).

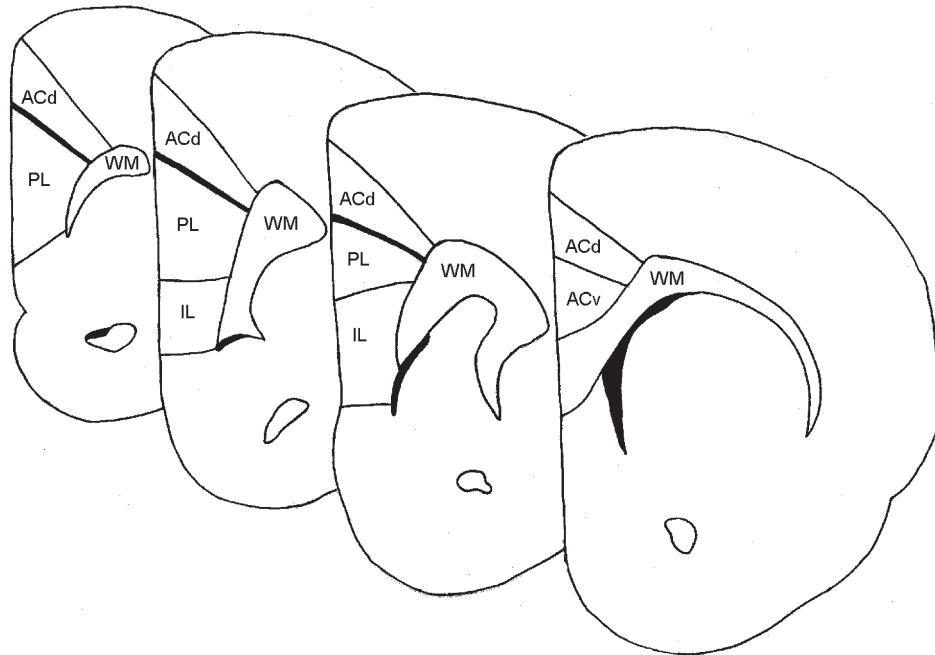


Fig. 2. Volume, neuron number, and glial cell number were estimated in the dorsal mPFC, consisting of areas ACd and ACv, and the ventral mPFC, consisting of areas PL and IL. Division between dorsal and ventral regions is emphasized with a bolded line. Frontal white matter volume (WM) was also estimated from the same sections.

of layer III cells in the ACd as compared with the PL. A thin, cell-poor lamina between PL layers III and V may be observed in sections from some animals, and near the border between PL and ACd this disappears. The border between ACd and PrCm (Fr2) is marked by a thin, cell poor band between VIa and VIb in PrCm; layers V and VI of the ACd do not contain sublayers. This characteristic was often more noticeable on anterior sections, whereas on posterior sections the thickening of layer II and the thinning of layer I in PrCm tended to be the more noticeable cytoarchitectonic differences between the two regions. ACv is distinguished from ACd by the blending of (lack of a clear border between) layers II/III and V/VI. The border is drawn where the first distinct lamination pattern is observed. The posterior boundary of the dorsal mPFC is marked by a much denser layer II and a layer III which is clearly distinguishable from both layers II and V.

Parcellation for the entire study was conducted for groups of litter-matched animals by a single experimenter who was blind to the animal's condition. At regular intervals throughout the study, animals were selected at random for re-parcellation (without the aid of previous drawings), and areas of the parcellated regions were recalculated to confirm the consistency of the experimenter's judgment of cytoarchitectonic borders. Consistency of area measurements within 5% between parcellation drawings for a given animal (which were conducted weeks or months apart) was routinely demonstrated.

Cortical layer I was excluded from analysis due to its variability in thickness and paucity of neuronal soma. Layers II/III and V/VI were measured separately (all rat mPFC areas are agranular; i.e. without layer IV). Prefrontal cortical areas and frontal white matter volume were parcellated on each section on the slide containing them both (every 4th section had been mounted on the slide) until the first section in which the genu of the corpus callosum appeared. Beginning with the appearance of the genu, parcellation occurred on every 3rd section appearing on the slide (because only two mPFC areas appear on these sections, ACd and ACv). To conform to the stereological rule of systematic random sampling, a skipping interval was randomly assigned (0, 1, or 2 sections on the slide were skipped) immediately after the first section

containing the genu, and thereafter every third section was parcellated. This resulted in parcellation of both hemispheres in eight to 11 sections per animal. The volume of each mPFC region was estimated by scanning the camera lucida tracings of the parcellated areas into a computer and quantifying the area of each with NIH image (version 1.62, 1999), then applying the Cavalieri method by which volume is estimated to be the product of the area measurements and the tissue thickness between section planes. Volume of the entire frontal white matter was estimated as above, using each section from which parcellations of the mPFC gray matter regions were drawn, beginning with the first section containing white matter and ending with the last section containing (parcellated) mPFC. This resulted in the volume estimation being made from eight to 11 drawings of all underlying cortical white matter in both hemispheres. The small portion of the white matter crossing between hemispheres (i.e. the corpus callosum) was excluded (see Fig. 1B).

Mounted section thickness was measured by determining the difference between the focal depth of the top and bottom of the tissue, using the focus position meter feature of the StereoInvestigator software program (MicroBrightField), which receives information about movement in the z axis from the motorized stage controller (Prior Scientific, Cambridge, Cambridgeshire, UK). Forty-eight measurements of thickness were taken per animal, evenly distributed among sample sites and sections (six measurements \times two layers (II/III, V/VI) \times four regions (ACd, ACv, PL, IL)). An average section thickness was calculated per animal and used in the calculations for that animal. Tissue shrinkage in section thickness was equivalent among all groups (average thickness: 20.2 μ m) and was not influenced by age or by sex.

Neuron number estimation

Quantification of neuron number was carried out as we have previously described (Nunez et al., 2002), following stereological principles (Howard and Reed, 1998) and using the StereoInvestigator software program (MicroBrightField). Neuronal density was quantified within each mPFC subregion using the optical disector,

an unbiased stereological technique (Gundersen et al., 1988). The software program automatically and randomly moves a counting frame containing appropriate 'acceptance' and 'forbidden' lines (area or Aframe of $45 \times 45 \mu\text{m}$ and height (h) of the section thickness excluding the $3 \mu\text{m}$ thick guard zones) within each region of interest in order to obtain correct stereological estimates of cell density that were unbiased for cell size and shape. In this method, only cells falling fully inside the counting frame or those that contact the 'acceptance' line without also contacting the 'forbidden' line are counted. Any cell contacting the 'forbidden' line is excluded from the count. Sampling frames that did not contain any neurons were included in the analysis in order to obtain an accurate estimate of cell density for a region. The optical disector, like the physical disector, uses a 'reference' section and a 'look-up' section, although in this case the two sections are optical (meaning they are two planes of focus within the thickness of a single tissue section). A cell was only counted if the bottom of the cell was observed within the volume of the counting frame—i.e. appeared in focus at the 'reference' section or between the two sections but not at the 'look-up' section. Any cell that was transected by the 'look-up' section was excluded from the count. The StereoInvestigator program allows individual cells to be tracked with the use of markers and will perform a tally of multiple counts if separate markers are used for different cell types as was done here for neurons and glia. In the cortex, neurons and glia can be unambiguously distinguished based on morphological characteristics; neurons are identified by their larger size and the presence of a clearly defined nucleus and nucleolus, whereas glia are identified by their smaller size, amorphous shape, and cytoplasmic and nuclear textural differences. Through systematic random sampling, at least 400 neurons and 140 glial cells within each layer (II/III, V/VI) of each region (dorsal, ventral) were counted in each subject. To obtain cell density, the area of the counting frame (Aframe) was multiplied by the height (h) to obtain the volume for the counting frame [v(dis)]. The cell counts made within this volume (in a particular lamina of a particular region) were divided by the volume of the counting frame [v(dis)] to obtain cell density measures (Nv). This cell density measure was then multiplied by the volume of the appropriate reference space (a particular layer of a particular region) to obtain cell number (glial density multiplied by reference space volume to obtain glial cell number; neuronal density multiplied by reference space volume to obtain neuron number).

Coefficient of error (CE) for the neuronal and glial density of each animal within each area was calculated using equation 10.32 in Howard and Reed (1998) to verify that our sampling was sufficient. Average CE values for day 35 and day 90 animals are presented in Table 1. Neuronal density for a given region was multiplied by the volume of the corresponding reference space

Table 1. CE values for neuronal and glial density in ventral mPFC layers II/III and V/VI and in dorsal mPFC

Layer	Day 35	Day 90
Ventral mPFC		
II/III Neurons	0.044	0.045
II/III Glia	0.046	0.046
V/VI Neurons	0.036	0.036
V/VI Glia	0.047	0.046
Dorsal mPFC		
II/III Neurons	0.040	0.042
II/III Glia	0.043	0.042
V/VI Neurons	0.038	0.039
V/VI Glia	0.048	0.046

There were no differences in CE for any measure between animals of different ages (day 35, day 90; group means shown).

Table 2. Neuron number, glial cell number, and volume of the ventral and dorsal mPFC, and volume of the frontal white matter

	Day 35	Day 90
Ventral mPFC		
Neuron number [†]		
♀	364,342±26,660	294,257±15,300
♂	350,305±19,139	331,567±11,684*
Glial number		
♀	158,387±15,622	144,377±8,076
♂	155,501±10,247	170,108±9,102*
Volume (mm ³) [‡]		
♀	1.56±0.13	1.28±0.07
♂	1.48±0.10	1.51±0.07*
Dorsal mPFC		
Neuron number		
♀	576,649±24,601	587,551±15,991
♂	583,678±24,699	599,715±23,674
Glial number [†]		
♀	253,592±14,857	292,850±9,024
♂	274,343±14,789	317,579±22,241
Volume (mm ³)		
♀	2.55±0.17	2.58±0.13
♂	2.57±0.12	2.88±0.16
Frontal white matter		
Volume (mm ³) ^{†, +, ‡}		
♀	0.491±0.029	0.611±0.025
♂	0.506±0.010	0.706±0.008*

N=9 For all groups except 90 day old males where N=11.

[†] Denotes a significant main effect of age, + denotes a significant main effect of sex, [‡] denotes a trend towards an age by sex interaction ($P < .06$ in both cases) and * indicates males > females at day 90. See text for details. Group means ± standard error of the mean (SEM) are shown.

(see volume estimation, above) in order to obtain neuron number for each region for each animal.

Statistical methods

A two-way analysis of variance for the factors of sex and age was conducted for each dependent variable, with litter included in the analysis as a blocking variable (SPSS statistical package, version 13.01). Post hoc comparisons were specified using lmatrix contrast statements in SPSS. The lmatrix subcommand is used to request the test of specific pairwise contrasts (rather than testing all possible pairwise comparisons). Significance was considered to be $P < 0.05$.

RESULTS

Ventral mPFC

Total neuron number in the ventral mPFC decreased between days 35 and 90 (age: $F = 4.1$, $P < 0.021$). Although the interaction between sex and age was not significant, this effect was more pronounced in females (19% loss in females compared with just over a 5% loss in males), resulting in a sex difference in neuron number of this region in adulthood (females < males by approximately 13%, $P < 0.03$) (Table 2). When the superficial and deep cortical layers of the ventral mPFC region were examined

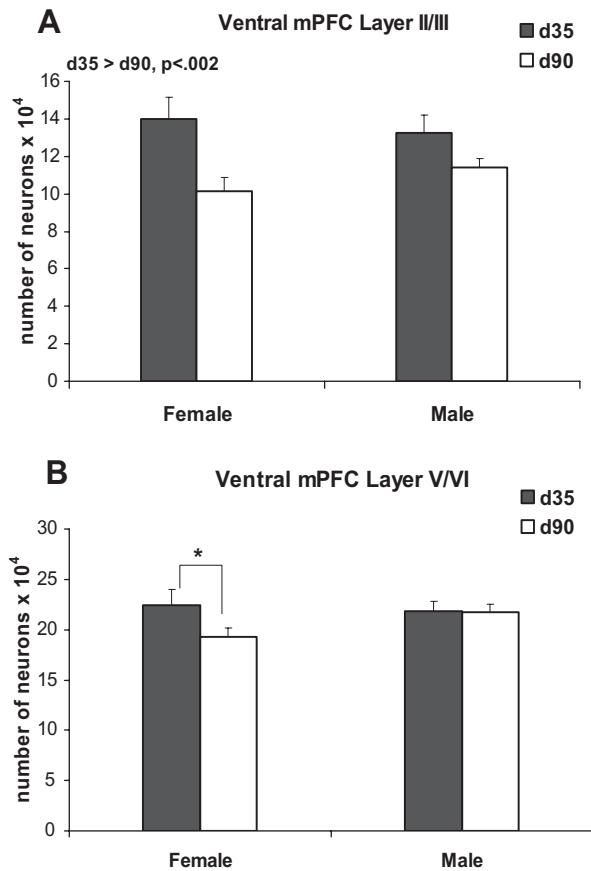


Fig. 3. Neuron number in the ventral mPFC. (A) Reduction of neuron number in layer II/III of the ventral mPFC between days 35 (periadolescence) and 90 (young adulthood). (B) Neuronal loss between days 35 and 90 in ventral mPFC layer V/VI occurs only in females. Group means \pm standard error of the mean (S.E.M.) are shown. * $P < 0.05$.

separately, it was found that neuron number was significantly reduced between days 35 and 90 in both sexes in layers II/III ($F = 11.5$, $P < 0.002$; Fig. 3A) but not in layers V/VI, although planned comparisons revealed that females did undergo a loss of neurons in layers V/VI ($P < 0.04$) but males did not (Fig. 3B).

Glial cells accounted for between 30 and 35% of total cells in the ventral mPFC in all four groups, similar to what has previously been found in the adult rat primary visual cortex (Gabbott and Stewart, 1987; Nunez et al., 2002). In contrast to neuron number, glial cell number in the ventral mPFC remained stable between these ages (Table 2). There was, however, a sex difference among young adults with males having nearly 18% more glial cells in the ventral mPFC than females ($P < 0.01$) (Table 2).

Finally, there was a trend toward an interaction of sex and age on the volume of the ventral mPFC gray matter ($F = 4.1$, $P < 0.052$), due to an age-related reduction in volume in females of approximately 18% ($P < 0.02$) but no change in volume among males. The amount of the volumetric reduction among females closely corresponds to the adult sex difference in volume of this region (females < males by approximately 18%, $P < 0.01$) (Fig. 4).

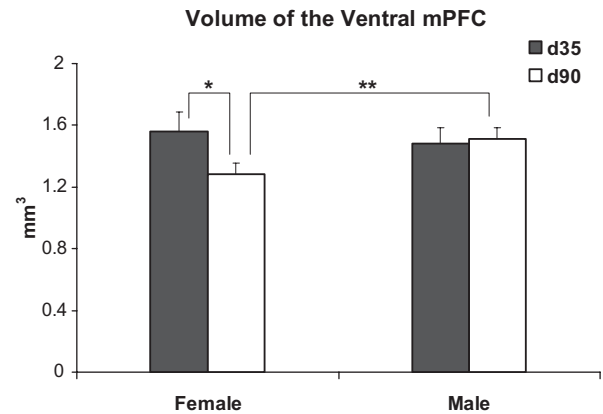


Fig. 4. Ventral mPFC volume. Volume of the ventral mPFC is reduced in females but not males between day 35 (periadolescence) and day 90 (young adulthood) (age \times sex $P < 0.052$), resulting in adult males having a larger volume than adult females. Group means \pm standard error of the mean (S.E.M.) are shown. * $P < 0.05$, ** $P < 0.01$.

Dorsal mPFC

Neuron number and regional volume of the dorsal mPFC did not change between days 35 and 90 in either males or females, and there were no differences between the sexes (Fig. 5 and Table 2). In contrast to neuron number, glial cell number in the dorsal mPFC increased with age by over 15% in both males and females ($F = 5.5$, $P < 0.03$) (Table 2). As in the ventral mPFC, glial cells accounted for between 30 and 35% of total cells in the dorsal mPFC in all four groups.

Frontal white matter

In contrast to mPFC gray matter, volume of the frontal cortical white matter increased dramatically with age ($F = 63$, $P < 0.0001$; Fig. 6 and Table 2). The increase in males (nearly 40%) was greater than in females (nearly 25%) (age by sex interaction, $F = 4.0$, $P < 0.055$; sex $F = 7.9$, $P < 0.01$), resulting in an adult sex difference in frontal white matter volume of approximately 15% ($P < 0.04$).

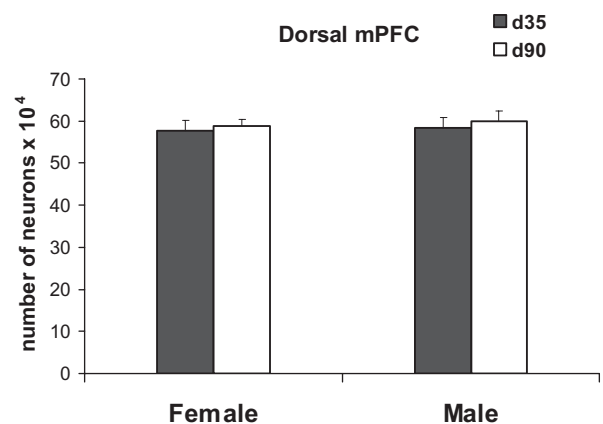


Fig. 5. Neuron number in the dorsal mPFC. Neuron number is equivalent between males and females and remains stable between days 35 (periadolescence) and 90 (young adulthood). Group means \pm standard error of the mean (S.E.M.) are shown.

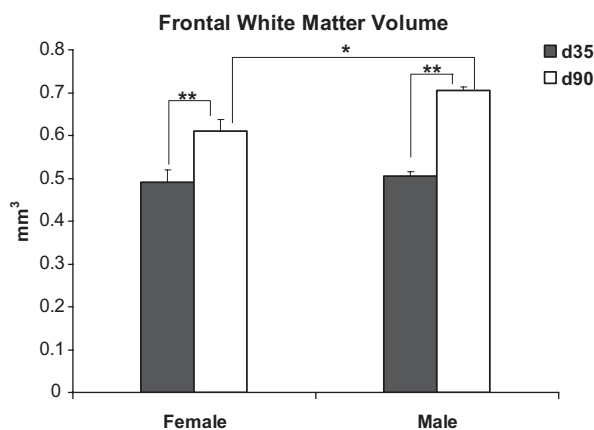


Fig. 6. White matter volume. Frontal white matter volume increases between day 35 and day 90 in both sexes ($P < 0.0001$), but more so in males (age \times sex, $P < 0.055$), resulting in an adult sex difference. Group means \pm standard error of the mean (S.E.M.) are shown. * $P < 0.05$, ** $P < 0.01$.

DISCUSSION

The present study investigated whether the number of neurons in the rat mPFC is altered between adolescence and young adulthood, and if so, whether these changes contribute to the sexual differentiation of this cortical region. We report that neuronal number is reduced in the ventral mPFC between postnatal days 35 and 90 in the rat, whereas glial cell number in this region remains stable during this time. Although we found that both sexes underwent a reduction in neurons, the effect was more pronounced among females, resulting in an adult sex difference in neuron number with females having approximately 13% fewer neurons in the ventral mPFC than males. The findings support our previous work indicating that neuron number continues to be shaped relatively late in postnatal development and that these late changes contribute to adult sex differences in cortical neuron number (Reid and Juraska, 1992; Nunez et al., 2001, 2002).

There was specificity to the changes observed here between adolescence and young adulthood. The pattern observed in the ventral mPFC (reduced neuron number but stable glial number) was not found in the dorsal mPFC; instead, neuron number in this region was stable and glial cell number actually increased by over 15% between adolescence and adulthood. Thus the nature of the periadolescent refinement of prefrontal cortex is region-specific. In addition, the white matter volume was greatly increased between adolescence and young adulthood in contrast to mPFC gray matter volumes (stable in the dorsal region, reduced in the ventral region of females). The pattern of developmental changes in frontal white matter volume found here is similar to that found in humans—linear increases in both sexes, but a steeper increase in males, resulting in an adult sex difference (Giedd et al., 1999; Casey et al., 2000; Paus et al., 2001). White matter volume is strongly correlated with myelination, which is known to continue well into adulthood, in both humans and rodents

(Yakovlev and Lecours, 1967; Benes et al., 1994; Paus et al., 1999; Nunez et al., 2000; Bartzokis et al., 2001).

A growing body of work has elucidated peri-pubertal anatomical refinement of circuitry in the primate prefrontal cortex (Lewis, 1997; Woo et al., 1997). For instance, synaptic density in this area increases early in postnatal development, plateaus for some time, and then declines during adolescence (Huttenlocher, 1979; Bourgeois et al., 1994; Anderson et al., 1995). A reduction in synaptic density could reflect a true loss of synapses (rather than dendritic elaboration which would spread a stable number of synapses over a greater area), since volume of this region is also reduced following adolescence (Giedd et al., 1999). It is not known whether peri-pubertal synaptic pruning similarly occurs in the rodent mPFC, as very little research on cortical development has included this late time frame. One study, however, did find that the volume of three rat cortical areas (the medial precentral, ACd, and PL areas) was higher prior to puberty than in adulthood (Van Eden and Uylings, 1985b), similar to what we report here for the female rat ventral mPFC (PL and IL areas). The present work indicates that, in addition to possible changes in synapse number, the reduction in volume following adolescence is at least in part due to a loss of neurons, suggesting that synaptic refinement and apoptosis are overlapping processes in late cortical development.

Our work points to a sex-specific developmental pattern which results in sex differences in adult neuron number. We found that males had an 18% larger volume of the adult ventral mPFC than females, and females had 13% fewer neurons and 18% fewer glial cells in this region in adulthood than males. These findings are comparable to the rat primary visual cortex, where males have approximately 20% more gray matter volume and 19% more neurons than females (Reid and Juraska, 1992; Nunez et al., 2002). In the visual cortex, females had the same volume and number of neurons as males if their ovaries were removed at 20 days of age which implicates the ovarian steroids in the emergence of the sex difference (Nunez et al., 2001). It remains to be demonstrated whether comparable effects would occur in the mPFC.

Human cortical development also follows a sex-specific trajectory, which results in adult sex differences in gray and white matter volumes and cortical neuron number (Pakkenberg and Gundersen, 1997; Giedd et al., 1999). Our findings in the rat cerebral cortex are consistent with this scenario, and at least in the visual cortex, gonadal hormones are implicated in this process (Nunez et al., 2002). The present findings are also consistent with the periadolescent refinement of prefrontal circuitry which has previously been observed in monkeys and humans, and they further indicate that a reduction in neurons can occur between adolescence and adulthood. The question of what the functional consequences of peri-adolescent refinement of prefrontal circuitry are is an interesting one. It has been found, for instance, that adult patterns of cerebral blood flow (Chugani et al., 1987; Chiron et al., 1992) and patterns of frontal lobe activation as measured by electroencephalography mature during late adolescence (Hud-

speth and Pribram, 1992). The structural changes are also likely to play a role in the development of competence on cognitive tasks that rely on this area. Adult levels of performance on PFC-dependent tasks, such as delayed response tasks and tests of selective attention, are not reached until after puberty in both humans and monkeys (Goldman, 1971; Alexander and Goldman, 1978; Levin et al., 1991; Casey et al., 2000). In rats also, adult skill levels on some cognitive tasks are not achieved until after puberty (e.g. Schenk, 1985; Altemus and Almlí, 1997) and, interestingly, there is evidence that sex differences in cognitive abilities also do not emerge until after puberty (Kanit et al., 2000). Our findings of region-specific neuronal loss and increases in white matter volume add to the evidence of dynamic changes occurring during adolescence in the prefrontal cortex.

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