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Regional variability in age-related loss of neurons from the primary visual cortex and medial prefrontal cortex of male and female rats

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Abstract

During aging, changes in the structure of the cerebral cortex of the rat have been seen, but potential changes in neuron number remain largely unexplored. In the present study, stereological methods were used to examine neuron number in the medial prefrontal cortex and primary visual cortex of young adult (85–90 days of age) and aged (19–22 months old) male and female rats in order to investigate any age-related losses. Possible sex differences in aging were also examined since sexually dimorphic patterns of aging have been seen in other measures. An age-related loss of neurons (18–20%), which was mirrored in volume losses, was found to occur in the primary visual cortex in both sexes in all layers except IV. Males, but not females, also lost neurons (15 %) from layer V/VI of the ventral medial prefrontal cortex and showed an overall decrease in volume of this region. In contrast, dorsal medial prefrontal cortex showed no age-related changes. The effects of aging clearly differ among regions of the rat brain and to some degree, between the sexes.

Section 2

Nervous system development; regeneration; aging

Keywords

sex differences; stereology; neuron number; volume; aging; anterior cingulate

1. Introduction

During aging, the rat shows behavioral deficits on a variety of tasks, including the spatial version of the Morris water maze (Frick et al., 1995), the Barnes circular platform task, the eight-arm radial maze (reviewed by Rosenzweig and Barnes, 2003), spatial delayed nonmatch-to-sample (Aggleton et al., 1989), recall of passive-avoidance (Winocur, 1988), and Hebb-Williams mazes (Winocur and Moscovitch, 1990). It is well established that there is no loss of neurons with age in the hippocampus, even in rats with spatial learning deficits (Rapp and

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Gallagher, 1996; Rasmussen et al., 1996), though decreases in the size of perforated synapses do occur in learning-impaired aged rats (Nicholson et al., 2004). We have also shown that male rats lose dendritic branches from hippocampal neurons during aging (Markham et al., 2005). Nevertheless, evidence of structural changes in the cerebral cortex, such as changes in the number of neurons that may be contributing to changes in task performance, remains limited. In fact, deficits in learning the spatial version of the Morris water maze have instead been correlated with degree of photoreceptor loss in the retina (O'Steen et al., 1995; Spencer et al., 1995), suggesting a possible role for the visual system in age-related performance deficits. Interestingly, decreases in dendritic spine density and dendritic extent during aging have been found in the primary visual cortex (Oc1) (Ruiz-Marcos et al., 1992; Feldman and Dowd, 1975). Changes in electrophysiological properties of neurons in the visual cortex also occur in the aging rat (Wang et al., 2006; Mendelson and Wells, 2002), further supporting the possibility of other age-related changes in the primary visual cortex.

The medial prefrontal cortex (mPFC) also undergoes age-related structural changes which may impact cognitive decline in aging. Deficits in tasks which engage the mPFC, such as delayed match-to-sample (Winocur, 1992) and attentional set-shifting (Barense et al., 2002) also occur during aging. Spine density and dendritic complexity are reduced in the rat mPFC during aging (Markham and Juraska, 2002), and increases in glial density and reductions in neuronal nucleus size in the frontal cortex have also been reported (Peinado et al., 1993). Structural changes known to occur during aging in other regions of the cerebral cortex of the rat include losses in synaptic density in parietal cortex (Adams and Jones, 1982), reduced dendritic arborization in auditory cortex (Vaughan, 1977), decreased synaptophysin content in frontal and occipital cortices (Saito et al., 1994), and decreases in neuron size (see review by Flood and Coleman, 1988). In contrast, neuron number in the barrels of somatosensory cortex (calculated using the Abercrombie correction) does not decline in aging mice (Curcio and Coleman, 1982) nor does the number of inhibitory interneurons in sensorimotor cortex decrease between middle and old age in male rats (Shi et al., 2006).

Aging may follow a different pattern in the brains of males and females, as we have observed in the mPFC and hippocampus, where males undergo more prominent age-related changes in dendritic extent and spine density compared to females (Markham and Juraska, 2002; Markham et al., 2005). Age-related changes in some cognitive abilities have been found to differ between males and females (Bowman et al., 2006; Warren and Juraska, 2000; Markowska, 1999), further suggesting that the course of aging in the cerebral cortex may differ between the sexes. Basal forebrain neurons also decrease in size in aging males, while remaining unchanged in females (Veng et al., 2003). Changes in neuron number in aging mPFC and Oc1 of the rat have not been examined however, leaving open the possibility of a differential vulnerability in males and females to a loss of neurons with age.

Stereological investigations of overall neuron number have not been previously completed within specific regions of the rat cerebral cortex to determine if any age-related changes occur. To address this question, the present study examined the potential influences of aging and sex on neuron number in the medial prefrontal cortex (an association area) and primary visual cortex (a primary sensory region) in male and female rats in adulthood and old age. Information regarding possible changes in neuron number is critical in forming the context for understanding other structural changes during aging in the rat cerebral cortex, and for understanding the neuroanatomical basis of age-related cognitive decline.

2. Results

Medial prefrontal cortex (Figure 1 and Figure 2)

Ventral—Data from the mPFC of the adult group has been previously reported in Markham, Morris, and Juraska (2007). While neither aging nor sex significantly impacted overall neuron number in the ventral mPFC (Figure 3a), males showed a loss of neurons in ventral mPFC layer V/VI of 15% ($p < .039$) (Figure 4). In addition, there was a sex difference in adult animals such that adult females had approximately 13% fewer neurons in this region than did adult males ($p < .03$). No sex difference was present among aged animals. An adult sex difference in neuron number was also present in layer V/VI ($p < .049$). Glial number of the ventral mPFC was not influenced by age and did not differ between the sexes (Table 1).

Age and sex also interacted to influence the volume of the ventral mPFC (Figure 5a; $p < .005$). There was an age-related reduction of 15% in ventral mPFC volume among males ($p < .04$), which was not present in females. Similar to neuron number, there was a sex difference in ventral mPFC volume among adults (females < males by 18%, $p < .01$) which was not present among aged animals. A sex by age interaction was also seen for the volume of ventral mPFC layers II/III ($p < .021$) and V/VI ($p < .005$; Table 1).

Dorsal—In the dorsal mPFC, neither neuron and glial number nor volume were influenced by age and did not differ between the sexes (Fig 3b, 5b, and Table 1).

Primary visual cortex (Figure 6 and Figure 7)

Both age ($p < .001$) and sex ($p < .001$) significantly affected neuron number in Oc1 (Figure 3c). Neuron loss occurred with age in both sexes (18% loss in males, 20% in females). A sex difference was seen at day 90 (males > females by 18.3%; $p < .014$) and was preserved in old age (males > females by 20.8%; $p < .028$). Analyses by layer showed that age and sex affected neuron number in layers II/III (sex $p < .001$; age $p < .001$), V (sex $p < .001$; age $p < .024$), and VI (sex $p < .010$; age $p < .005$). Only layer IV showed no effect of either age or sex (Figure 8). Glial number was not affected by age or sex (Table 1). Glia-to-neuron ratios were within the range previously reported in the rat cortex (Gabbott and Stewart, 1987).

Sex ($p < .008$) and age ($p < .001$) both also impacted the volume of Oc1, with both males and females showing a reduction with age (21.4% loss in males, 13.0% in females; Figure 5c). Age and sex affected volume in layer II/III (sex $p < .005$; age $p < .001$), V (sex $p < .004$; age $p < .001$), and VI (sex $p < .020$; age $p < .027$). Layer IV was not affected by age or sex (Table 1).

3. Discussion

There is regional variation in the loss of neurons in the aging rat cortex. While the primary visual cortex lost neurons with age in both sexes in all layers except IV, neuron loss in the ventral mPFC was specific to males and was localized primarily in layers V/VI. In contrast, the dorsal mPFC showed no loss of neurons with age.

There were also regional differences in the effects of aging on the sex differences seen in adulthood, present in both Oc1 (replicating previous work from our laboratory, Reid and Juraska, 1992; Nunez et al., 2002) and the ventral mPFC. Even though the mPFC was not dramatically affected by age, sex differences present in adulthood (neuron number and volume) were eliminated during aging in the ventral mPFC. Previous work has also shown a fading of sex differences in dendritic branching and spine density in layer V of the mPFC with age (Markham and Juraska, 2002), due to greater losses in males. The loss of dendritic branches and spine density, in combination with the localized loss of neurons in the ventral mPFC that we report here in aged males, contributes to the reduction in volume for males that is not seen

in females. It is possible that the continued secretion of estrogen by aged rats during estropause may have helped to protect females from the age-related losses which occurred in males. By contrast, sex differences in Oc1 neuron number were maintained in old age due to similar losses in both males and females, suggesting that any such neuroprotective effects of estrogen, which have been previously documented in young and aging rats (e.g. Wise, 2006; also reviews by Prokai and Simpkins, 2007; Wise et al., 2005; Pozzi et al., 2006), may be regionally specific. Expression of ER- β does decrease in aging (Sharma and Thakur, 2006), but variation by region has not been explored. In young adult rats, the cellular distribution of ER- β differs between association areas such as the mPFC where it is almost exclusively restricted to parvalbumin immunoreactive cells and sensorimotor areas where many non-parvalbumin cells contain ER- β (Kritzer, 2002), suggesting that subpopulations of neurons may be differentially affected. More work is needed to examine possible regional interactions of estrogen during aging.

Performance declines on several cognitive tasks in the aging rat (Aggleton et al., 1989; Winocur, 1988; Winocur and Moscovitch, 1990; Bowman et al., 2006; reviewed in Rosenzweig and Barnes, 2003), including tasks which utilize the mPFC such as delayed match-to-sample (Winocur, 1992) and attentional set-shifting tasks (Barense et al., 2002). Since all of these studies used male rats, it is possible that a loss of neurons in the ventral mPFC contributed to these deficits. Age-related changes in visual abilities have not been explicitly examined in rats, though aging hooded rats are still able to solve tasks that involve visual cues (e.g. Markham et al., 2002; Warren and Juraska, 2000). However, among aging albino rats, the degree of photoreceptor loss is correlated with maze performance (O'Steen et al., 1995; Spencer et al., 1995). Peripheral changes such as deterioration of the retina, including a loss of photoreceptors and thinning of the retina (Weisse, 1995; Spencer et al., 1995) which also occur in pigmented rats (Weisse, 1995), may result in visual changes during aging and could be a confound in studying age-related behavioral deficits. In the current study, however, layer IV of OC1 was the only layer spared from age-related neuron losses, suggesting that thalamic input may be relatively unchanged during aging in pigmented rats. Interestingly, there is also a lack of age-related neuronal loss in layer IVB in rhesus monkeys (Hof et al., 2000). Neuron number (determined using the Abercrombie correction) and volume of the lateral geniculate nucleus does remain stable in albino rats in old age (Satorre et al., 1985), further suggesting that input from the lateral geniculate nucleus may be maintained during aging. Alterations in the visual cortex are speculated to be responsible for some of the age-related changes in visual abilities that occur in primates, as alterations in the optics themselves do not account for all of the changes seen (Spear et al., 1994; Kim et al., 1996a). Similar to functional changes seen in aging primates (Schmolesky et al., 2000), evidence for age-related alterations in neurons of the rat primary visual cortex include increased spontaneous activity and stimulus response latency, decreased signal-to-noise ratio (Wang et al., 2006), and reduced numbers of cells that respond to fast moving bars of light (Mendelson and Wells, 2002), lending further evidence for the susceptibility of Oc1 to the aging process.

Although neuronal density in the rat (Peters et al., 1983) and primate (Vincent et al., 1989; Kim et al., 1997) visual cortex, as well as neuron number in layer IVB of the primate visual cortex (Hof et al., 2000), appears to be stable during aging, changes in neuron number have not previously been examined in the aging rat. Similar to work showing no decrease in overall brain size or thickness of primary visual cortex during aging (Peters et al., 1983; however, see Ruiz-Marcos et al., 1992), we found no changes in brain weight, overall brain size, thickness of Oc1, or neuronal density (unpublished data). The reduction in Oc1 volume with age was due to an average decrease in the width (13%) and length (9%) of this region. Our results highlight the difficulty in drawing conclusions about neuron number without using volume information (i.e., on the basis of cell density estimates alone); we found density to remain unchanged in the aging visual cortex, but neuron number was decreased.

As the visual cortex undergoes age-related neuronal loss, the splenium (which is the posterior 20% of the corpus callosum that carries axons projecting from the visual cortex; Kim et al., 1996b; Kim and Juraska, 1997) does not show any decrease in size or in area occupied by myelination during aging (Yates and Juraska, 2007). Of course, the neurons lost from Oc1 could be either interneurons or pyramidal neurons that do not project transcallosal fibers. Alternatively, due to the large difference in size of myelinated and unmyelinated axons (0.35 μ m diameter for myelinated axons, not including the myelin sheath compared to 0.16 μ m diameter of unmyelinated axons; Kim and Juraska, 1997), if the majority of neurons lost in Oc1 projected unmyelinated callosal fibers, this could remain undetectable when examining size of the splenium.

Interestingly, the regional variability in age-related changes seen in the rat brain is distinctly different from that seen in humans. In the aging human brain, decreases in volume are often found in frontal regions, while some sensory areas, such as visual cortex, are less affected (Resnick et al., 2003; Raz et al., 2005; Allen et al., 2005; Raz et al., 1997; Sowell et al., 2003). In the present study, our findings indicate the opposite in rats: the primary visual cortex shows a dramatic decrease in volume during aging in both males and females, while aging does not have a large impact on the volume of the mPFC, where only males showed a decrease in volume of the ventral mPFC. Like the rat, rhesus monkeys also do not have a decrease in the volume of the prefrontal cortex (area 46) during aging (O'Donnell et al., 1999). The only stereological examination of neuron number in the aging human cortex found that a 10% loss of neurons takes place across the entire cortex during aging (Pakkenberg and Gundersen, 1997). However it is not known if some regions are differentially affected by this loss, making it unclear how our neuron number changes compare to those that occur in humans. Other structural changes that occur in the aging human brain, such as reductions in dendritic length and spine density, do show regional variability however (see review by Uylings and de Brabander, 2002), making it likely that changes in neuron number may not affect each region of the human brain in the same way. Previous work from our laboratory has shown stability of white matter in the corpus callosum of the aging rat (Yates and Juraska, 2007), which contrasts with the loss of white matter seen in aging humans (Sullivan et al., 2002; Resnick et al., 2003; Fotenos et al., 2005; Walhovd et al., 2005; Bartzokis et al., 2001; Allen et al., 2005; Jernigan et al., 2001; Sowell et al., 2003; Courchesne et al., 2000), giving additional evidence of the disparate pattern of aging in the rat. It is possible that by strictly controlling the experiences of the laboratory rat (as well as other laboratory animals), we have eliminated basic aspects of daily living such as stress and immune challenges which have been shown to impact the cerebral cortex and the course of aging in the brain (Lupien et al., 1998; Brunson et al., 2005; Cerqueira et al., 2005; Montaron et al., 2006; Sandi and Touyarot, 2006; Izquierdo et al., 2006; Liston et al., 2006; also see reviews by Finch and Crimmins, 2004; Radley and Morrison, 2005; Godbout and Johnson, 2006). Gaining a better understanding of the factors which may contribute to the distinct pattern of aging in the rat could help us to better understand the process of neural aging in general.

4. Experimental Procedure

Subjects

Subjects were 52 Long-Evans hooded rats, descended from Simonsen Laboratory stock and bred in the Department of Psychology's breeding colony at the University of Illinois. All procedures were approved by the IACUC at the University of Illinois. All animals were double or triple housed in Plexiglas tubs with same-sex cagemates and handled weekly. As is typical for the aging animal literature (Barnes et al., 1997; Hof et al., 2000; Schoenbaum et al., 2006), aged animals had been used as breeders until 10 – 12 months of age. Animals were housed in a 12 hour light-dark cycle, with food and water provided *ad libitum*.

Histological examination of vaginal cells collected by lavage was used to characterize aged females into one of two reproductively senescent hormonal states, persistent estrus or persistent diestrus (as described in Markham and Juraska, 2002). To be categorized into one of these estropausal states, animals had to show consistent vaginal cytology for at least 10 consecutive days before sacrifice. In persistent diestrus, levels of estrogen are slightly lower than in persistent estrus but progesterone levels are elevated (Markham and Juraska, 2002; reviewed by Dudley, 1982). During this time aged males were also handled daily.

On the day of sacrifice, animals were deeply anesthetized with sodium pentobarbital and intracardially perfused with Ringer's solution followed by 4% paraformaldehyde in .1M phosphate buffer solution. Brains were removed, placed in the fixative, and stored at 4 degrees Celsius. Brains were then coded so that the experimenters were blind to the age and sex of the animal until final statistical analysis. One week after perfusions, tissue was weighed and transferred into a solution of 30% sucrose in fixative. One to two days after sinking in the sucrose solution, brains were coronally sectioned on a sliding microtome at 60 microns. Free-floating sections were collected in .1M phosphate buffer solution and mounted on gelatin coated slides. After drying, slides were stained with methylene blue/azure II before being dehydrated in increasing concentrations of ethanol, cleared with xylene, and coverslipped.

Tissue from the mPFC was collected from adult (day 85–90; males= 11, females= 9), and aged animals (19–22 months; males= 7, females= 8). In an overlapping sample, tissue was collected from the visual cortex in adult (males= 9, females= 9) and aged animals (males= 9, females= 7). Not all of the brains used for the mPFC were optimally stained for parcellation of the visual cortex, so that a separate group of animals containing all of the groups was added to the visual cortex data. This resulted in 17 animals being used in both areas (adult males= 5, adult females= 5, old males= 4, old females= 3) out of the 35 animals used in the mPFC and 34 used in Oc1. No differences were found between tissue from the two batches. Medial prefrontal cortex data from the adult group have been previously reported in Markham, Morris, and Juraska (2007)

Volume calculations

The mPFC was divided into the dorsal (anterior cingulate cortex, both dorsal and ventral regions: ACd and ACv) and ventral (prelimbic (PL) and infralimbic (IL)) regions based on differential afferent/efferent connections (Zeng and Stuesse, 1991; Verwer et al., 1996; Fisk and Wyss, 1999) and behavioral functions (Ragozzino and Rozman, 2007; Ragozzino et al., 2003). These two major regions were parcellated according to cytoarchitectonic criteria (Krettek and Price, 1977; Van Eden and Uylings, 1985) at 31.25X using a camera lucida (Figure 1 and Figure 2). Details of the parcellation criteria used in the mPFC have been described in Markham et al. (2007). Briefly, 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. Layer II cells are also more densely packed in PL compared to IL. The border between PL and ACd is marked by a broadening of layer V and an increase in the density of layer III cells in the ACd as compared to the PL. 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. ACv is distinguished from ACd by the 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. Layers II/III and V/VI were measured separately (rat mPFC lacks layer IV). Beginning with the first section containing white matter, prefrontal cortical areas were parcellated on each section collected until the first section in which the genu of the corpus callosum appeared. At this point, a skipping interval was randomly assigned to determine the first section of the mPFC to be parcellated following

the first section containing the genu. Thereafter, every third section was parcellated (only two mPFC areas appear on these sections, ACd and ACv) because the cortical boundaries were relatively stable across this region.

Boundaries for Oc1 were established using the parcellation guidelines of Zilles (1985) and Reid and Juraska (1991). Determination of the boundaries for Oc1 is aided by its striped appearance. Prominent cytoarchitectonic characteristics of Oc1 include the cell dense and granular layer IV, a less dense layer V which contains larger cells, and the moderate density of medium sized neurons in layer VI. The distinct boundary between layer V and VI in Oc1 due to differences in cell density fades at the boundary to Oc2 (Reid and Juraska, 1991). The first anterior section containing the visual cortex was determined in each brain and a random start location was chosen; thereafter, Oc1 was parcellated in both hemispheres on every third section until the disappearance of cortical white matter. Camera lucida drawings were completed at 125X for Oc1 layers II/III, IV, V, and VI (Figure 6 and Figure 7). For both the mPFC and Oc1, cortical layer I was excluded due to variability in thickness and relative lack of neuronal somata.

After considerable training using published criteria (see above) and examples from prior studies in the laboratory, each cortical area (mPFC and Oc1) was parcellated by a single investigator (JAM did the mPFC and SEA did Oc1) to maximize consistency. Throughout the study, brains were selected at random for re-parcellation by both the investigator doing the parcellations and a separate investigator, in order to ensure the reliability of cortical boundaries. Comparison of the secondary parcellations consistently demonstrated measurements that were within 5% of the initial areas.

To determine volume, area for each parcellated section was determined using ImageJ software (Version 1.28, 2002) and the Cavalieri method (Howard and Reed, 1998) was used to calculate volume of each region (the product of area measurements and the post-processing tissue thickness between parcellated section planes). It should be noted that tissue shrinkage in section thickness did not vary by age or sex. Volumes were calculated separately for each cortical layer.

Cell density

Stereologically unbiased counts using the optical disector were used to obtain cell density in each layer of the mPFC and Oc1. Separate investigators counted the mPFC (JRM) and Oc1 (MAY) to maximize consistency. Counts were randomly checked by the same and separate investigators recounting brains throughout the study. Duplicate counts were within 5% of the initial numbers. Both hemispheres of two randomly chosen sections were used for counts in each subarea (dorsal mPFC, ventral mPFC, Oc1). In the dorsal and ventral mPFC, at least 200 neurons within each layer (II/III, V/VI) of each subarea (dorsal- ACd, ACv; ventral- PL, IL) were counted for every subject. A minimum of 200 cells per layer (II/III, IV, V, and VI) were counted in Oc1. Separate counts for neurons and glia (combined astrocyte and oligodendrocyte) were collected, using morphological characteristics. Neurons are typically larger, have a more regular shape and a clearly defined nucleus and nucleolus, while glia have a more irregular shape, are usually smaller in size, and stain a slightly different shade of blue with the methylene blue/azure II stain. Using StereoInvestigator (Version 7, MBF Bioscience), the area of interest was demarcated and counts were made at random locations throughout each layer. The area of the counting frame used for the mPFC was $45\mu\text{m} \times 45\mu\text{m}$ and it was $30\mu\text{m} \times 30\mu\text{m}$ for Oc1. Section thickness was used for the height of the disector, excluding the $3\mu\text{m}$ thick guard zones. Section thickness was measured on counted sections and was stable (20μ) across age, sex and cortical region. Cells were counted when the bottom of the cell could be seen within the thickness of the section. To calculate density, average counts for each layer were divided by

the volume of the counting frame before being multiplied by volume of the subregion to obtain cell number.

Coefficients of error

Coefficients of error (CE) were calculated for neuron and glia density in each layer of dorsal mPFC, ventral mPFC, and Oc1 according to formula 10.32 from Howard and Reed (1998) and are shown in Table 2. The higher minimum cell counts used to sample both ACd and ACv within the dorsal mPFC and PL and IL within the ventral mPFC resulted in lower CEs in these regions than in Oc1, but all CEs for neuron counts were below .08. CEs for glial counts tended to be higher because fewer glial were counted due to their lower density compared to neurons in the rat cortex (Gabbott and Stewart, 1987).

Statistical analyses

A two-way analysis of variance for the factors of sex and age (adult, aged) was conducted for each measure using Systat (Version 11, 2004). Estropausal status was without effect on any of the measures, so aged females were combined into a single group.

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List of Abbreviations

mPFC, medial prefrontal cortex; Oc1, primary visual cortex; PL, prelimbic; IL, infralimbic; ACd, dorsal anterior cingulate; ACv, ventral anterior cingulate; CE, coefficient of error; ER, estrogen receptor.

Figure 1. Coronal sections through the mPFC, showing parcellated subregions of dorsal mPFC (ACd, ACv) and ventral mPFC (PL, IL). The white matter (WM) and the corpus callosum (CC) are also shown. Reprinted with permission from Markham, Morris, and Juraska (2007).

Figure 2. Diagram depicting parcellated subareas of dorsal mPFC (ACd, ACv) and ventral mPFC (PL, IL).

Figure 3.

Neuron number in the ventral mPFC (a), dorsal mPFC (b), and primary visual cortex (c). (a) In the ventral mPFC, there was a trend for a sex by age interaction ($p = .066$) on neuron number. (b) There was no effect of age or sex in the dorsal mPFC. (c) In the primary visual cortex, neuron number was affected by both age ($p = .001$) and sex ($p = .001$). Significant post-hoc comparisons are indicated by brackets. Group means $\pm$ standard error of the mean (SEM) are shown.

Figure 4.

Neuron number in the layers of the ventral mPFC. (a) There were no significant differences in neuron number in layers II/III. (b) There was a near significant trend for an age by sex interaction in neuron number in layer V/VI ($p=.055$), due to a loss of neurons in males ($p=.039$) but not females. Significant post-hoc comparisons are indicated by brackets. Group means $\pm$ standard error of the mean (SEM) are shown.

Figure 5.

Volume of the ventral mPFC (a) dorsal mPFC (b), and primary visual cortex (c). (a) There was a sex by age interaction ($p = .005$) in the ventral mPFC. (b) Dorsal mPFC was not affected by age or sex. (c) In the primary visual cortex, volume was affected by both sex ($p = .008$) and age ($p = .001$). Significant post-hoc comparisons are indicated by brackets. Group means $\pm$ standard error of the mean (SEM) are shown.

Figure 6.
Coronal section through the primary visual cortex of the rat.

Figure 7. Diagram depicting the area containing the primary visual cortex in the rat. The subcortex is shown in only the most anterior section.

Figure 8.

Neuron number in layers II/III (a), IV (b), V (c) and VI (d) of the primary visual cortex. Age and sex significantly affected neuron number in layers II/III (sex $p < .001$; age $p < .001$), V (sex $p < .001$; age $p < .024$), and VI (sex $p < .010$; age $p < .005$). Only layer IV showed no effect of either age or sex. Significant post-hoc comparisons are indicated by brackets. Group means $\pm$ standard error of the mean (SEM) are shown.

Table 1

Neuron number, glial cell number and volume by layer of the primary visual cortex, ventral mPFC and dorsal mPFC. Group means $\pm$ standard error of the mean (SEM) are shown.

Ventral mPFC		Adult	Aged
Neuron Number			
Layer II/III	♂	114,200 $\pm$ 4,226	111,298 $\pm$ 6,661
	♀	101,583 $\pm$ 6,911	111,876 $\pm$ 3,153
Layer V/VI	♂	217,367 $\pm$ 7,884	187,030 $\pm$ 11,605 ^{<i>β</i>}
	♀	192,674 $\pm$ 8,576 ^{<i>α</i>}	197,497 $\pm$ 7,062
Glial Number			
Layer II/III	♂	49,022 $\pm$ 3,388	48,368 $\pm$ 2,371
	♀	51,276 $\pm$ 3,232	46,639 $\pm$ 2,087
Layer V/VI	♂	104,451 $\pm$ 6,200	104,573 $\pm$ 9,304
	♀	113,432 $\pm$ 6,698	107,629 $\pm$ 6,395
Volume (mm ³)			
Layer II/III †	♂	0.528 $\pm$ 0.02	0.473 $\pm$ 0.02
	♀	0.452 $\pm$ 0.03	0.523 $\pm$ 0.02
Layer V/VI ††	♂	0.987 $\pm$ 0.04	0.850 $\pm$ 0.04
	♀	0.831 $\pm$ 0.04	0.942 $\pm$ 0.04
Dorsal mPFC		Adult	Aged
Neuron number			
Layer II/III	♂	222,212 $\pm$ 10,018	209,411 $\pm$ 8,322
	♀	209,696 $\pm$ 10,507	212,956 $\pm$ 12,224
Layer V/VI	♂	377,503 $\pm$ 14,559	363,637 $\pm$ 26,593
	♀	377,855 $\pm$ 8,862	340,055 $\pm$ 13,896
Glial Number			
Layer II/III	♂	94,455 $\pm$ 6,837	87,637 $\pm$ 5,990
	♀	96,515 $\pm$ 7,199	90,032 $\pm$ 3,940
Layer V/VI	♂	212,334 $\pm$ 12,597	214,698 $\pm$ 16,664
	♀	209,522 $\pm$ 11,738	203,292 $\pm$ 13,367
Volume (mm ³)			
Layer II/III	♂	0.961 $\pm$ 0.05	0.896 $\pm$ 0.05
	♀	0.870 $\pm$ 0.05	0.944 $\pm$ 0.07
Layer V/VI	♂	1.923 $\pm$ 0.01	1.776 $\pm$ 0.09
	♀	1.714 $\pm$ 0.08	1.823 $\pm$ 0.01
Primary visual cortex		Adult	Aged
Neuron Number			
Layer II/III ††, ++	♂	230,015 $\pm$ 8,984	190,330 $\pm$ 13,670 ^{<i>β</i>}
	♀	199,796 $\pm$ 10,343 ^{<i>α</i>}	145,328 $\pm$ 5,926 ^{<i>α, ββ</i>}
Layer IV	♂	93,434 $\pm$ 5,656	94,523 $\pm$ 11,936
	♀	89,741 $\pm$ 6,342	78,940 $\pm$ 8,563
Layer V †, ++	♂	131,448 $\pm$ 7,675	104,410 $\pm$ 7,769 ^{<i>β</i>}
	♀	93,561 $\pm$ 5,258 ^{<i>αα</i>}	87,271 $\pm$ 6,230
Layer VI ††, ++	♂	190,788 $\pm$ 11,077	151,684 $\pm$ 9,740 ^{<i>β</i>}
	♀	154,414 $\pm$ 12,553 ^{<i>α</i>}	127,294 $\pm$ 7,673
Glial number			
Layer II/III	♂	86,449 $\pm$ 6,246	82,539 $\pm$ 11,833
	♀	69,345 $\pm$ 9,251	60,598 $\pm$ 5,998
Layer IV	♂	47,342 $\pm$ 3,795	49,237 $\pm$ 4,050
	♀	46,248 $\pm$ 6,010	41,672 $\pm$ 5,664
Layer V	♂	120,209 $\pm$ 8,624	94,311 $\pm$ 7,754
	♀	100,495 $\pm$ 13,968	86,677 $\pm$ 6,211
Layer VI	♂	123,764 $\pm$ 11,403	115,192 $\pm$ 19,280
	♀	122,392 $\pm$ 19,552	98,260 $\pm$ 12,070
Volume (mm ³)			
Layer II/III ††, ++	♂	0.786 $\pm$ 0.03	0.592 $\pm$ 0.03 ^{<i>ββ</i>}
	♀	0.664 $\pm$ 0.03 ^{<i>α</i>}	0.517 $\pm$ 0.04 ^{<i>β</i>}
Layer IV	♂	0.293 $\pm$ 0.01	0.279 $\pm$ 0.03
	♀	0.272 $\pm$ 0.01	0.258 $\pm$ 0.03
Layer V ††, ++	♂	0.722 $\pm$ 0.03	0.556 $\pm$ 0.03 ^{<i>ββ</i>}
	♀	0.571 $\pm$ 0.03 ^{<i>αα</i>}	0.524 $\pm$ 0.03
Layer VI †, +	♂	0.760 $\pm$ 0.03	0.638 $\pm$ 0.03 ^{<i>β</i>}
	♀	0.633 $\pm$ 0.04 ^{<i>α</i>}	0.579 $\pm$ 0.05

The following symbols are used to denote significance: main effect of age at $p < .05$: †, at $p < .01$ by ††; main effect of sex at $p < .05$: +, at $p < .01$ by ++.

For post-hoc comparisons, a sex difference at the indicated age is denoted as ^{*α*} ($p < .05$) or ^{*αα*} ($p < .01$).

Age-related differences within the indicated sex are marked by β ($p < .05$) or $\beta\beta$ ($p < .01$).

Table 2

Coefficients of error (CE) for neuron and glia densities in ventral mPFC, dorsal mPFC, and primary visual cortex.

Neuronal CE	Adult	Aged
Ventral mPFC		
II/III Neurons	0.045	0.041
V/VI Neurons	0.036	0.036
Dorsal mPFC		
II/III Neurons	0.042	0.039
V/VI Neurons	0.039	0.036
Primary visual cortex		
II/III Neurons	0.060	0.058
IV Neurons	0.061	0.064
V Neurons	0.078	0.079
VI Neurons	0.069	0.072
Glial CE		
Ventral mPFC		
II/III Glia	0.046	0.048
V/VI Glia	0.046	0.053
Dorsal mPFC		
II/III Glia	0.042	0.054
V/VI Glia	0.046	0.049
Primary visual cortex		
II/III Glia	0.109	0.106
IV Glia	0.098	0.097
V Glia	0.089	0.094
VI Glia	0.095	0.104