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Research report

Sex differences in the development of axon number in the splenium of the rat corpus callosum from postnatal day 15 through 60

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Abstract

Axon number in the splenium was examined at 15, 25 and 60 days of age in male and female rats. The splenium (posterior fifth) of the corpus callosum was found to contain the axons from the visual cortex at all three ages and was extensively sampled with electron microscopy. Overall, there was a 15% decrease in the total number of axons between postnatal day 15 and day 60 in both sexes. The observed decrease in axon number between day 15 and 25 in both males and females is consistent with Elberger's (A.J. Elberger, Transitory corpus callosum axons projecting throughout developing rat visual cortex revealed by DiI, Cereb. Cortex 4 (1994) 279–299) data which suggest that the pattern of visual callosal projections in the rat visual cortex is not restricted to the adult form until the fourth postnatal week. There was a further decrease in axon number between day 25 and day 60 in females only such that by 60 days of age, the total number of axons was equivalent between the sexes. Thus in the rat splenium, males appear to attain the adult number of axons earlier than females. These results also indicate that there is a sex difference in the timing of axon withdrawal in the rat splenium, with axon withdrawal continuing in females after it has ceased in males. © 1997 Elsevier Science B.V.

Keywords: Visual cortex; Horseradish peroxidase; Ultrastructure; Axon withdrawal

1. Introduction

Since the issue of sex differences in the human corpus callosum continues to generate considerable controversy (for a meta-analysis, see [7]), interest in the rat corpus callosum has been high because factors influencing the structure can be manipulated and the cellular basis for changes in gross size can be investigated. Sex differences [3], environmental effects [3,20], and hormonal influences [10,12,34] have been described in the gross size of various regions of the rat corpus callosum. In addition, sex differences in the density (not number) of axons have been reported [28].

Research in our laboratory is primarily focused on the splenium, the region of the corpus callosum which carries visual fibers. The sex differences we have found in the gross size and cellular composition of the visual cortex [19,39–41,44] make an examination of the axonal composition of the splenium particularly relevant. In adult rats, we have recently reported that there are no sex differences in the total number of axons in the splenium. Males do, however, have more myelinated axons than females [23]. Preliminary data from a developmental study suggest that changes may occur in the number of axons in the rat splenium after the second postnatal week [24]. We have found, however, that a more thorough sample is necessary to accurately estimate axon number because axon density varies rostrocaudally and dorsoventrally in the splenium [23]. In addition, Elberger [8] has reported that the distribution of callosal projections in the rat visual cortex continues to be pruned from the initial exuberant distribution to the adult-like pattern through the end of the fourth postnatal week. It is unknown whether changes in the axonal composition of the splenium accompany this occurrence. In the present study, we define the proportion of the corpus callosum containing visual axons at postnatal days 15, 25 and 60 and then thoroughly sample this region with electron microscopy in order to achieve an accurate estimate of axon number. Our goal is to examine potential sex differences and the time course of axon elimination as well.

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2. Material and methods

Subjects were 15-, 25- and 60-day-old male and female rats. Postnatal day 15 was chosen since by this age there is evidence that the distribution of callosal projection neurons has reached its adult-like distribution [36]. Day 25 is weaning age, and day 60 is past puberty and is also the age at which Juraska and Kopcik [20] examined the splenium. Data from the 60-day-old animals have been reported previously [23]. All rats, born in the laboratory colony, were first generation descendants of Long–Evans hooded rats from Simonsen Laboratories, Gilroy, CA. All three ages were drawn from every set of matings, and some litters were used for more than one age. Litters were undisturbed for the first 10 postnatal days to avoid effects of early handling [3]. The rats were socially housed in same sex groups of 2–3 after weaning at 25 days of age.

2.1. Definition of splenial boundaries

For more precise details on surgical procedure and histology, see Kim et al. [23]. Rats were anesthetized with ketamine hydrochloride (120 mg/kg) and xylazine (15 mg/kg). Each animal received a single intracortical injection of a 2% solution of horseradish peroxidase conjugated with wheat germ agglutinin (WGA-HRP) as used by Olavarria and colleagues [35,37]. The injection site, one per animal, was aimed at the 17/18a border region of the anterior visual cortex or the posterior parietal cortex (areas 7 and 39). After 24 h, the animals were deeply anesthetized with sodium pentobarbital and perfused with a fixative solution (1.25% glutaraldehyde and 2% paraformaldehyde) followed by 10% sucrose. Brains were stored in sucrose at 4°C until the following day. Beginning from the posterior pole of the cortex, 60 μm frozen sections were cut in the coronal plane until the anterior end of the genu of the corpus callosum was reached. All sections were processed according to the tetramethyl benzidine protocol of Mesulam [31]. Alternate sections were also counterstained with Methylene blue–Azure II.

The total number of sections containing the corpus callosum was multiplied by section thickness to determine the anterior-to-posterior length of the corpus callosum for each animal. The injection site, label in the corpus callosum (Fig. 1), and transported label in the contralateral cortex were examined under dark-field illumination. Counterstained sections were viewed under light-field illumination to confirm, through cytoarchitectonic characteristics, the cortical location of the injection site and the contralateral label. The atlas of Zilles [46] was used for identification of the primary and secondary visual areas as well as the border between the visual areas and parietal cortex. It was found that these cytoarchitectonic areas could be identified as early as day 15 by using characteristics of layer IV. The thalamus ipsilateral to the injected cortex was also examined to ascertain that the appropriate thalamic nuclei were labeled.

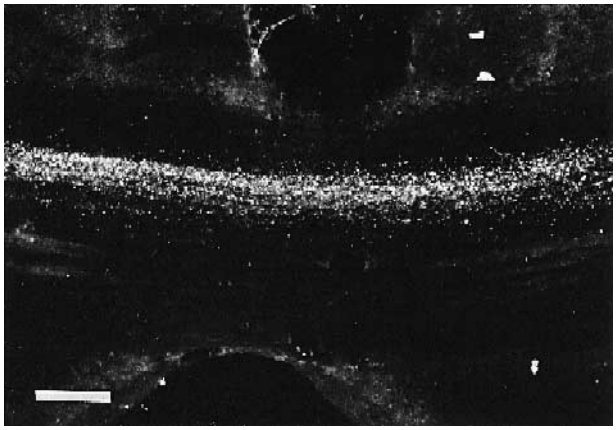

Fig. 1. A dark-field coronal section illustrating HRP within the axons of the corpus callosum. Bar = 1.0 mm.

The fraction of the corpus callosum carrying visual axons was determined only in those animals in which the injection site extended to the anterior border of the visual cortex. At least five males and five females at each age met this criterion. The most anterior section containing labeled axons in the corpus callosum was selected in these animals. The number of sections caudal to and including this section was divided by the total number of sections containing the corpus callosum. The resulting fraction represented the proportion of the corpus callosum which carries axons between the visual cortices. Animals with injection sites restricted to somatosensory cortex, including its most posterior aspect, were used to determine the amount of overlap between visual and somatosensory axons within the corpus callosum.

2.2. Axonal composition of the splenium

Another group of littermate pairs of male and female rats was used. Seven to eight littermate pairs were used at days 15, 25 and 60. Data from the 60-day-old animals have been reported previously [23]. Housing conditions were the same as described above. For precise details on materials and methods, see Kim et al. [23]. Animals were anesthetized with sodium pentobarbital and perfused intracardially with Ringer's solution followed by a solution of 1% glutaraldehyde and 4% paraformaldehyde containing 2.5 mmol of calcium chloride. The brains were cut midsagittally.

One hemisphere was used to determine the gross size of the corpus callosum. A sagittal cut about 1 mm lateral to the midsagittal surface of the hemisphere was made. The resulting 1 mm slab of tissue was stained in cold 2% osmium tetroxide for 1–2 min and photographed at a magnification of 4 $\times$. The corpus callosum was traced and the total area of the corpus callosum was computed using Sigma-Scan (Version 3.10, Jandel Scientific [18]). The anterior-to-posterior length of the corpus callosum was

measured and the posterior fifth of the corpus callosum was defined relative to this length.

The other hemisphere was processed for electron microscopy. The splenium was blocked, fixed in cold 2% osmium tetroxide and embedded in Medcast epoxy resin. The blocks were sectioned in the sagittal plane with a Sorvall ultramicrotome. A few 1 μm semi-thin sections were cut from each block and stained with Toluidine blue, and the splenium was traced at a magnification of 100 $\times$ using a camera lucida. The anterior border of the posterior fifth of the corpus callosum (relative to the overall length) was demarcated. The posterior fifth was divided into an anterior and a posterior half (each comprising one-tenth of the anterior-to-posterior length of the corpus callosum). The cross-sectional areas of the two halves of the splenium were calculated from these drawings using Sigma-Scan (Version 3.10, Jandel Scientific [18]). These areas were summed to get the area of the posterior fifth of the corpus callosum. Thin sections (600–900 Å range) were cut and mounted on formvar coated grids. The grids were stained with uranyl acetate and lead citrate and examined with a JEOL 100C electron microscope.

The sampling method used was described in Kim et al. [23]. Sampling was restricted to the posterior fifth of the corpus callosum. The posterior splenium (posterior tenth of the corpus callosum) was divided into three equally spaced

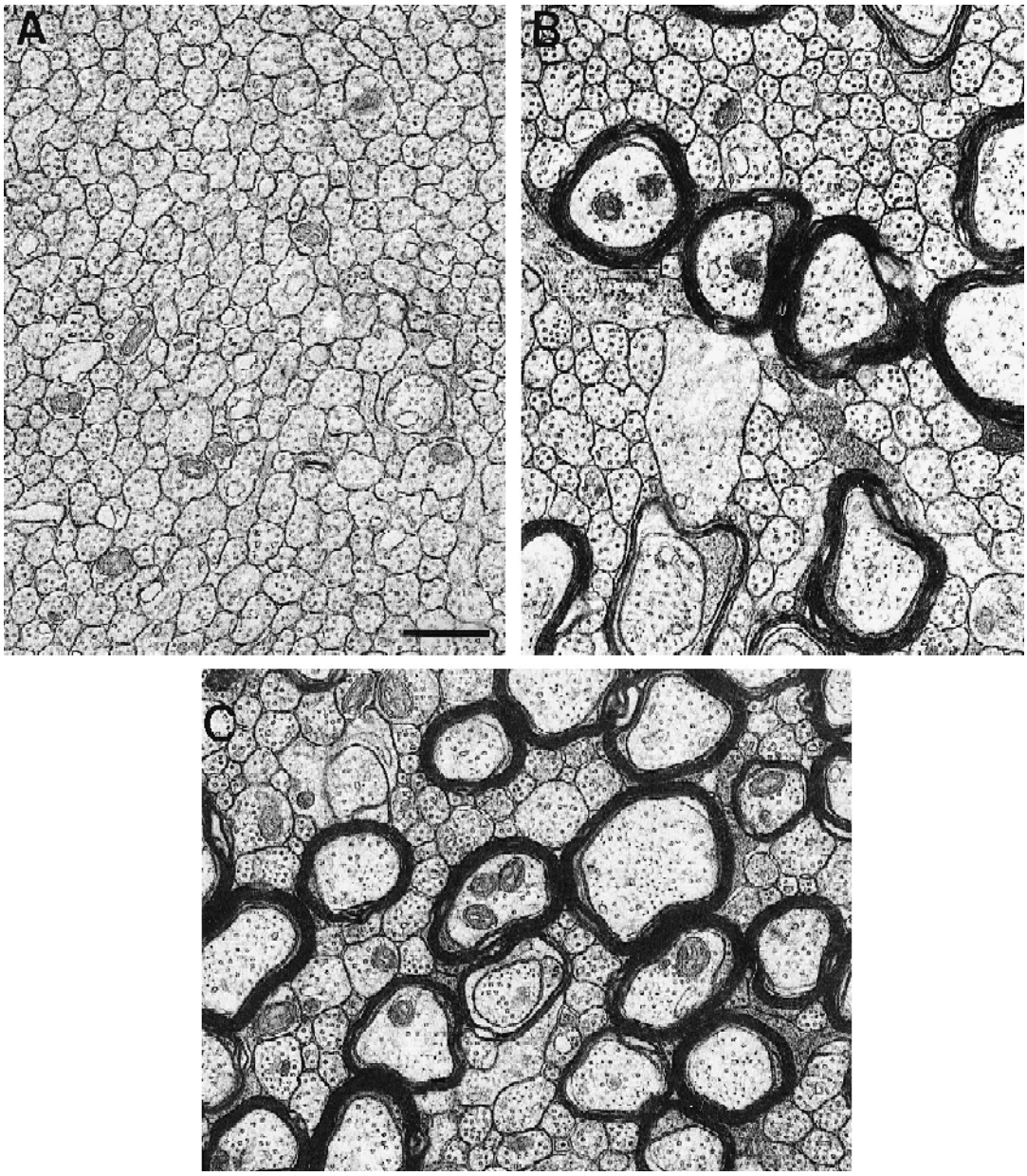

Fig. 2. Representative electron micrographs from the rat splenium at postnatal days (A) 15, (B) 25, and (C) 60, showing unmyelinated and myelinated axons. Bar = 0.6 μm.

columns. Four to nine photographs (depending on the width of the corpus callosum) were taken per column, at a magnification of 10 000 ×, such that the entire dorsal-to-ventral extent of the corpus callosum was sampled. To investigate possible dorsoventral variation in axon density, micrographs were divided into three groups (dorsal, middle and ventral) depending on their dorsoventral location.

The anterior half of the splenium was also divided into three equally spaced columns. Since the corpus callosum is considerably thinner in this region, only two micrographs (corresponding in location to the dorsal and middle regions in the posterior splenium) were taken per column.

Examples of electron micrographs from the rat splenium at postnatal days 15, 25 and 60 are illustrated in Fig. 2. Micrographs were printed at a final magnification of 39 000 ×. The number of unmyelinated and myelinated axons was counted for each micrograph and used to determine axon density. Axon number for the anterior and posterior halves of the splenium was calculated by multiplying axon density by their respective areas. The total number of axons in the posterior fifth of the corpus callosum was calculated as the sum of the number of axons in the anterior and posterior splenial regions.

The average diameters of the unmyelinated and myelinated axons were calculated by measuring the minimum diameter of each axon. Twenty unmyelinated and up to 20 myelinated axons were measured from each micrograph. Between 350 and 600 unmyelinated and myelinated axons were measured for each animal.

For gross size and axon number, an analysis of variance was run with sex, age, and litter as factors. For axon density and diameter, an analysis of variance was run with sex, age, litter, rostrocaudal column and dorsoventral location as factors. Each micrograph measure was nested within animal. Post-hoc investigations of interactions were performed with an analysis of variance in which sex or age were run separately. The SAS program package (Version 6, SAS Institute [42]) was used.

3. Results

3.1. Definition of splenial boundaries

The density of label in the cortex opposite the injection site did not obviously differ between the sexes or across ages. Similarly, for comparable injections, there were no detectable differences in the density of labeled fibers in the corpus callosum.

As reported earlier for 60-day-old animals [23], visual fibers were contained within the posterior 20% of the corpus callosum at days 15 and 25 in both males and females. Posterior parietal fibers project in the posterior midbody of the corpus callosum, just rostral to visual fibers. There was some overlap, however, with the posterior 17–22% of the corpus callosum containing both visual and parietal axons. Thus, in spite of a progressive increase with age in the anterior-to-posterior length of the corpus callosum, the relative location of visual axons in the corpus callosum does not vary.

3.2. Axonal composition of the splenium

The gross size measurements are shown in Table 1. The anterior-to-posterior length ($P < 0.0001$), the total area ($P < 0.0001$), and the area of the splenium (posterior fifth) of the corpus callosum ($P < 0.0001$) increased significantly with age. The area increase was evident in the measurements of the posterior portion as well as the anterior portion of the splenium. There were no significant sex differences in the callosal area or length or splenial area.

3.2.1. Axon density

The density of unmyelinated axons decreased significantly across ages, while myelinated axon density increased (Table 2). Total axon density decreased signifi-

Table 1
Gross size measures of the rat corpus callosum

	Total length (mm)		Area of posterior 1/5 (mm^2)		Total area (mm^2)	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
15 days						
Males ($n = 7$)	5.52	0.09	0.28	0.01	1.09	0.02
Females ($n = 7$)	5.60	0.12	0.27	0.02	1.07	0.04
25 days						
Males ($n = 7$)	6.48	0.11	0.37	0.01	1.45	0.06
Females ($n = 7$)	6.53	0.12	0.37	0.01	1.40	0.04
60 days						
Males ($n = 8$)	7.30	0.13	0.46	0.02	1.95	0.07
Females ($n = 8$)	6.93	0.10	0.45	0.01	1.79	0.06

Table 2
Axon density in the rat splenium

	Unmyelinated axons ($\times 10^6$/mm^2)		Myelinated axons ($\times 10^6$/mm^2)	
	Mean	S.E.M.	Mean	S.E.M.
15 days				
Males ($n = 7$)	17.2	0.29	0.03	0.06
Females ($n = 7$)	18.5	0.34	0.04	0.08
25 days				
Males ($n = 7$)	10.7	0.32	0.42	0.02
Females ($n = 7$)	12.2	0.29	0.46	0.02
60 days				
Males ($n = 8$)	7.42	0.21	1.42	0.03
Females ($n = 8$)	7.85	0.22	1.35	0.03

cantly across ages because of myelination and an overall increase in mean axon diameter across ages.

Sex differences are shown in Table 2. At postnatal days 15, 25, and 60, there were significant sex differences in unmyelinated axon density (female > male, $P < 0.001$). A similar sex difference (female > male) was found at days 15 and 25 in total axon density (day 15: $P < 0.002$; day 25: $P < 0.0001$) because there are few myelinated axons at these ages. At 25 days of age, females had a higher myelinated axon density than males ($P < 0.04$). Males, however, had a higher myelinated axon density than females at day 60 ($P < 0.04$).

There was significant variation in axon density in the rostrocaudal and dorsoventral dimensions in the rat splenium. This points to the importance of an extensive sampling strategy when examining the axonal composition of the rat splenium and of any other tracts with a non-uniform axon density.

In 60-day-old animals, the variation in axon density has been reported in detail elsewhere [23]. In summary, myelinated axon density was highest dorsally and lowest ventrally; unmyelinated axon density displayed the opposite pattern. There was also a significant rostrocaudal variation in axon density: myelinated axon density was higher and unmyelinated axon density was lower in the anterior splenium than in the posterior splenium. In 25-day-old animals, rostrocaudal and dorsoventral variations ($P < 0.0001$) in axon density similar to that seen at postnatal day 60 are present. Thus, even at a relatively early stage, when less than 4% of the axons are myelinated, regional variation in unmyelinated and myelinated axon density is evident. At day 15, while rostrocaudal variation in axon density similar to that found in the older animals is significant ($P < 0.0001$), there was no evidence of any dorsoventral variation in axon density.

The regional differences in axon density were associated with the extent of myelination, but not with axon size. Thus, sectors that were heavily myelinated tended to have lower axon densities, and sectors that were sparsely myelinated had higher overall axon densities.

3.2.2. Axon number

These results are illustrated in Fig. 3. There were very few myelinated axons at postnatal day 15 (less than 0.5% of the total axons). This is consistent with studies indicating that myelination in the rat corpus callosum does not begin until postnatal day 12 [45]. At postnatal day 25, 3.8% of the axons were myelinated, and 15% were myelinated at day 60.

In contrast to the progressive increase in the number of myelinated axons across the 3 ages, there was a significant decrease in unmyelinated axon number ($P < 0.0001$). Total axon number (unmyelinated plus myelinated) decreased significantly between day 15 and day 25 in both males and females ($P < 0.0001$). Overall, in both sexes, there was

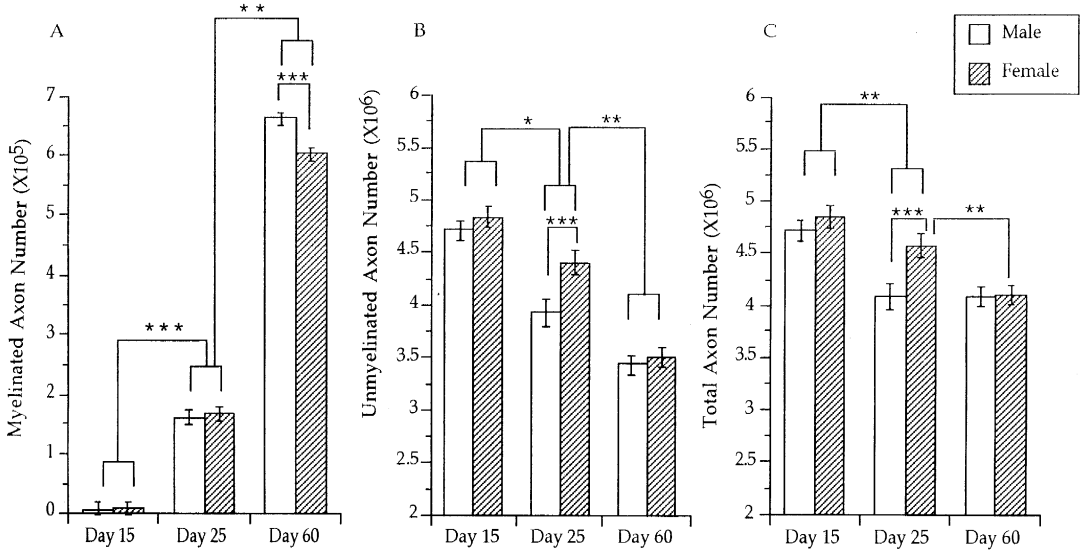

Fig. 3. (A) Myelinated, (B) unmyelinated, and (C) total axon number in the rat splenium. $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$.

approximately a 15% decrease in the total number of axons between postnatal days 15 and 60.

There was a sex difference in the number of unmyelinated axons (females > males) at day 25, but not at day 15 or day 60. There were no sex differences in myelinated axon number at day 15 or day 25. At day 60, there was a sex difference in myelinated axon number, with males having a significantly greater number than females; however, since unmyelinated axons outnumber myelinated axons by approximately a factor 7:1, there was no sex difference in total axon number.

There was a sex by age interaction for total axon number. Post-hoc tests in which each age was run separately revealed that there were no sex differences in total axon number at either postnatal day 15 or day 60. There was, however, a sex difference in total axon number at day 25, with females having significantly more axons than males. This sex difference in axon number occurred in spite of the lack of a sex difference in gross size.

The sex by age interaction also occurred when axon number was compared across ages for each sex. Total axon number in females decreased significantly between 25 and 60 days, whereas it remained the same in males. Thus, although the overall percentage of axons which are eliminated between day 15 and day 60 is similar in males (14.5%) and females (16%), the time course of elimination varied. In males, virtually all of the elimination occurred between day 15 and day 25. In females, on the other hand, 11% of the axons underwent elimination between day 25 and day 60.

3.2.3. Axon diameter

These data are summarized in Table 3. Average unmyelinated axon diameter increased significantly between postnatal day 15 and day 25 ($P < 0.005$). There was no change in average myelinated axon diameter across ages. However, due to the increasing number of myelinated axons, there was a progressive increase in overall axon diameter across the ages (15 days, 0.152 μm; 25 days, 0.172 μm; 60 days, 0.189 μm). This is similar to that reported in cats [2] and monkeys [26].

Table 3
Axon diameter in the rat splenium

	Unmyelinated axons (μm)		Myelinated axons (μm)	
	Mean	S.E.M.	Mean	S.E.M.
15 days				
Males ($n = 7$)	0.15	0.002	0.34	0.010
Females ($n = 7$)	0.15	0.002	0.35	0.010
25 days				
Males ($n = 7$)	0.17	0.002	0.38	0.010
Females ($n = 7$)	0.16	0.002	0.38	0.010
60 days				
Males ($n = 8$)	0.16	0.003	0.35	0.004
Females ($n = 8$)	0.16	0.002	0.35	0.010

There were no sex differences in the diameter of unmyelinated or myelinated axons at postnatal day 15 or 60. At postnatal day 25, there was no sex difference in myelinated axon diameter. There was, however, a sex difference in unmyelinated axon diameter at this age, with males having significantly larger axons than females ($P < 0.0005$). There was a sex by age interaction for unmyelinated axon diameter at days 25 and 60. There was a significant decrease in unmyelinated axon diameter between these ages in males but not in females ($P < 0.001$), possibly due to more of the larger axons becoming myelinated in males.

There was no rostrocaudal variation in average unmyelinated or myelinated axon diameter at any of the three ages examined. There was, however, some variation according to dorsoventral location at day 25 and day 60. At day 60, average unmyelinated axon diameter was significantly higher in the ventral and middle sampling regions than in the dorsal sampling region ($P < 0.0035$). At day 25, unmyelinated axon diameter was highest in the middle sampling region of the splenium ($P < 0.0005$). Within each age, there was no association between axon size and axon density. This is in contrast to the situation in the monkey corpus callosum [25] where smaller axon diameters were generally found in regions of high axon density, and larger axon diameters were found in regions of low axon density.

4. Discussion

This study is the first to examine developmental changes in the number of callosal axons originating from a specific portion of the cortex and to document that axon elimination can continue after 25 days of age in the rat splenium. Prior investigations of axon number in the corpus callosum during development have focused on the total number of axons [2,14,26]. However, given evidence of regional variation in the time course of axon elimination [2,5,22,26], total axon number may not reflect changes that are occurring in subpopulations of callosal axons. We showed that axons originating from visual cortical areas are located in the posterior fifth of the corpus callosum at postnatal days 15, 25 and 60, so that the loss of axons across these ages does not change the overall topography of visual axons in the splenium. Our results indicate that axon elimination in the rat splenium occurs between postnatal days 15 and 25 in both sexes, and beyond day 25 in females. This supports recent data suggesting that the distribution and also the number of visual callosal projections are subject to change through the end of the fourth postnatal week [8].

The present results indicate that there are significant sex differences in the time course of axon withdrawal in the

splenium of the rat corpus callosum. Although axon number decreased between postnatal days 15 and 25 in both sexes, the absolute decrease was greater in males than in females. As a result, females had significantly more axons than males by 25 days of age. There was no further decrease in axon number between day 25 and day 60 in males; however, axon number continued to decrease in females such that by day 60, total axon number in the two sexes was equivalent.

There are indications from the work of Fitch et al. [12] on the gross size of the rat corpus callosum that estrogen may play a role in the late withdrawal of axons in females. They found that ovariectomy on postnatal days 8, 12, or 16 resulted in a larger callosal area in adulthood than controls [12]. The effects of ovariectomy on callosal size were not detectable by days 30 or 55 but were present by day 90 [11]. Ovariectomy at 78 days was without effect at 115 days [30]. Thus, between days 16 and 78, the presence of ovaries results in a smaller corpus callosum. A role specifically for estrogen is further implicated: an estradiol implant on day 25 stopped the size increase that follows ovariectomy on day 12 [29]. Estrogen, perhaps associated with puberty, may be promoting the withdrawal of axons which results in a somewhat smaller corpus callosum. While we have not found the corpus callosum to be smaller in females than males in the present study, the direction of the means (male > female) is congruous, and we have found a significant sex difference (male > female) in callosal size in a related experiment [34]. Thus our work is compatible with the possibility of estrogen promoting the withdrawal of axons between postnatal days 25 and 60, but direct tests of the hypothesis need to be performed.

The phenomenon of late elimination of axons in females and the temporary appearance of sex differences before the elimination occurs may be concordant with similar developmental patterns in the dendritic tree in the visual cortex. For example, Muñoz-Cueto and colleagues [32,33] demonstrated that the dendritic spine density on pyramidal neurons in the rat visual cortex peaks at day 20 in females resulting in a temporary sex difference. Spine density in females subsequently fell to male levels, which had stayed constant, by day 60. Ovariectomy at day 30 prevented the decrease. Somewhat more indirect evidence also comes from studies from our lab: female rats have more total length in the apical tree of pyramidal neurons in the visual cortex at day 25 [44], and this difference was not found at day 55 in a separate study [19]. Thus dendrites and spines appear to peak and regress in the female visual cortex in the same time frame as axons in the splenium.

The present study demonstrates the importance of distinguishing sex differences in the rate of development and sex differences that are present at maturity. A sex difference could represent a difference that will persist throughout the life of the animal. On the other hand, if a sex difference merely reflects a difference in the rate of development, whether or not one detects a sex difference will depend on the age of the subjects examined. If, for example, myelination continues in the splenium beyond 60 days of age, the sex difference in the number of myelinated axons found at 60 days in the present study might not persist in later adulthood. Also, the sex difference in axon number (female > male) found at weaning age in the present study was due to earlier axon withdrawal in males relative to females. Thus, males appear to attain the adult number of axons earlier than females. However, the extended period of axon withdrawal in females may leave them more plastic for a longer period of time to events which may influence axon withdrawal [1,6,13].

We found that axon number in the rat splenium decreases by 15% between postnatal days 15 and 60. We could not, however, identify axons undergoing withdrawal or other degenerative processes such as death. This is consistent with another ultrastructural study of the developing rat corpus callosum [45], and also with reports in the cat [2] and monkey [26] corpus callosum. In cats, a 70% reduction in total callosal axon number during development [2] is temporally correlated with the restriction of various callosal projections across the cortex [9,16]. In monkeys, a similar 70% reduction in total axon number occurs [26]; however, the relationship between axon elimination and the reshaping of callosal projections across the cortex is less clear [5,22,43]. In rats, studies using HRP have indicated that the distribution of visual callosal projections is pruned down from an earlier, exuberant pattern to the discrete, adult pattern by postnatal day 12 [27,36]. Furthermore, a recent DiI study has suggested that the pattern does not attain its adult form until the fourth postnatal week [8]. In this case, the present results suggest that the observed reduction in the number of visual callosal axons may contribute to the formation of the restricted callosal zone in the visual cortex. The observed decline in axon number (at least in females) *after* the restriction of the callosal zone is complete would imply that this late axon withdrawal does not influence the distribution of callosal projection neurons or their terminal fields in the cortex. This suggests that there may be a decrease in the density of callosal projection neurons. This could occur when neurons that projected callosally retract their callosal collateral while maintaining projections to an ipsilateral area [4,15,17,38]. Another possibility is that axons of callosal projection neurons branch in the white matter, and only one of the branches is retracted; this process would also result in a decrease in overall axon number without influencing the density or the topography of the projection neurons. This seems unlikely, however, because there is no evidence of axon branching in the corpus callosum after the first postnatal week in rodents [21].

It is unclear how long axon withdrawal continues in the rat splenium, and it is possible that axon withdrawal continues after postnatal day 60. Gravel et al. [14] found no difference in the total number of axons in the corpus callosum at postnatal days 10 and 60, but separate calcula-

tions were not made in the splenium so their findings may not be representative of events in the splenium. Another possibility for the discrepancy between the two studies is the differences in the sampling strategy used. The sampling strategy used in the present study was very thorough, extending throughout the rostrocaudal and dorsoventral extent of the splenium. This was necessary because of the regional variation in axon density throughout the splenium [23]. The limited sampling strategy employed by Gravel et al. [14] may not have been sufficient to obtain an accurate estimate of axon number. In fact, in their examination of myelinated axon number in the rat corpus callosum, Berbel et al. [1] noted a major discrepancy between their own findings and those of Gravel et al. [14] in the number of myelinated axons in the hypothyroid rats which they attributed to differences in the sampling strategies employed in the two studies. The number of myelinated axons we found in the splenium is comparable to that found in the posterior sector of Berbel et al. [1], while the axons densities of both myelinated and unmyelinated in the present study are higher than those reported by Gravel et al. [14] in the posterior sector.

In summary, the present study has demonstrated that there is a 15% decrease in the number of axons in the rat splenium between postnatal days 15 and 60. Furthermore, there is a sex difference in the time course of axon withdrawal with axon withdrawal occurring between postnatal days 15 and 25 in both sexes and beyond 25 days of age in females. This study points to the importance of examining the number of axons in a defined portion of the corpus callosum. It also shows that sex differences need to be studied across ages.

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