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Increases in size and myelination of the rat corpus callosum during adulthood are maintained into old age

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Abstract

Although there are indications of growth in the size and myelination of the rat corpus callosum during adulthood, it is not known how long this growth continues. In addition, the potential for age-related changes in these measures to affect the sex differences seen in adulthood has not been examined. Here the size of callosal subregions and area occupied by myelin were examined in the genu and splenium of male and female rats in adulthood, middle-age, and old age. Our findings revealed increases both in size and in the area composed of myelin between adulthood and middle-age that were maintained into old age, with no indications of age-related loss in either the genu or splenium of the rat.

Keywords

aging; sex differences; genu; splenium; myelination

1. Introduction

There is recent evidence that the process of myelination in the rat corpus callosum continues into adulthood, and this may underlie continuing increases in callosal size. Previous work from our laboratory indicates that increases in size and myelination in the rat splenium continue at least beyond 120 days of age (Nunez et al., 2000), and others have found increases in callosal area after 12 months of age (Sullivan et al., 2006). Evidence for continued myelination in the adult corpus callosum has also been found in the cat (Looney & Elberger, 1986) and mouse (Sturrock, 1980), and continued increases in white matter volume have also been reported in humans until middle-age (Bartzokis et al., 2001; Courchesne et al., 2000). How long myelination continues in the rat corpus callosum is unknown.

Sex differences have also been found in the size of the rat corpus callosum, including the splenium (posterior 20% of the corpus callosum) (Nunez & Juraska, 1998), with males having a larger callosal area compared to females (Berrebi et al., 1988; Bimonte et al., 2000; Bimonte, Fitch, & Denenberg, 2000; Fitch et al., 1991). Differences in callosal area reflect differences in the underlying axonal composition of the corpus callosum. Males have a higher number of myelinated axons in the splenium (Kim & Juraska, 1997) and a higher myelinated to

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unmyelinated axon ratio in the genu (Mack et al, 1995) which results in a larger area of the corpus callosum in males due to the substantially larger size of myelinated axons (Kim & Juraska, 1997). Males and females differ in the timing of splenial axon development between days 15 and 60 (Kim & Juraska, 1997), but possible sex differences in the course of aging in the rat corpus callosum have not been investigated. Continued growth or age-related loss in the corpus callosum could alter the sex differences present in adulthood. For example, losses associated with aging may eliminate sex differences as in other regions of the rat brain (Markham et al., 2005; Markham & Juraska, 2002). The present study investigated possible age-related changes and the influence of sex on the size and on the area occupied by myelin in the genu and splenium of the corpus callosum in adulthood, middle-age, and old age.

2. Results

Analyses of variance were run for brain weight, callosal size, area occupied by and proportion of myelin and of other material using Systat (Version 11, 2004). The factors were age (adult, middle-age, and old), sex (male, female), and region (genu, splenium). The infrequent occurrence of glial cell bodies (mean= 38/1100 counts in the genu and 29/1320 counts in the splenium) and blood vessels (mean= 17/1100 counts in the genu and 3/1320 counts in the splenium) resulted in counts that were insufficient for analysis. Estrous phase and estropausal state showed no significant effect on any variable, so that at each age, all females were combined. Due to the large age range in the old group, comparisons of old (18–21 months) and oldest old (22–26 months) animals were made. However, no significant differences were found between the old and oldest old groups. For example, the area of the genu was 0.62 in old animals compared to 0.64 in the oldest animals and in the splenium the area for old animals was 0.77 compared to 0.78 in the oldest animals. As a result, data from these ages were combined.

Brain weights showed significant effects of sex ($p=.001$) and age ($p=.001$). Males had larger brains compared to females at all ages, and brain weights increased with age (4% from adulthood to middle age; 5% from middle to old age).

For area, there were significant effects of sex ($p=.001$; male>female), age ($p=.001$; older>younger), and region ($p=.001$; splenium>genu) (Fig. 1). Post-hoc analysis of the age effect showed significant increases in area between adult and middle-age animals ($p=.029$) and between adult and old animals ($p=.005$) but not between middle and old age ($p<.80$). Neither sex nor age interacted with region, indicating that similar patterns were occurring in both the genu and splenium. There was also a strong trend for a sex by age interaction ($p=.052$), resulting from two different patterns of change. One was an increase in size between adulthood and middle-age for females ($p=.001$) but not males. In addition, sex differences (male>female) were also found in both adult ($p=.002$) and old age animals ($p=.001$), but not in middle-age. Thus, both sex and aged-related differences in these subregions of the corpus callosum did not completely parallel the differences in overall brain weight.

The area occupied by myelin (proportion multiplied by area) showed a significant effect of sex ($p=.002$; male > female), but no effect of region or age (Fig. 2). A similar pattern was found for both regions. Post-hoc analyses showed a trend for an increase in area occupied by myelin between adulthood and middle-age ($p=.077$). In addition, the proportion of grid intersections occupied by myelinated axons showed no significant effect of age or sex. In all, this indicates that myelination was maintained into old age.

The area occupied by other material (glial processes and unmyelinated axons, which are not resolvable using the light microscope) showed an effect of age ($p=.014$; older>younger) and region ($p=.001$; splenium>genu) but only a trend for an effect of sex ($p=.068$; male>female)

(Fig. 3). The effect of age is seen in post-hoc analysis as a trend towards an increase between adult and old age animals ($p=.074$). The proportion of grid intersections overlapping other material did not reveal differences due to age or sex.

3. Discussion

Increases in size and myelination occurred between adulthood and middle-age in the splenium and genu of the rat corpus callosum and were stable into old age. These changes fit with previous reports of callosal growth and continued myelination in adult rats and humans (Sullivan et al., 2006; Nunez et al., 2000; Bartzokis et al., 2001; Courchesne et al., 2000; Steen et al., 1997), as well as continued myelination in adult cats and mice (Looney & Elberger, 1986; Sturrock, 1980). Reports of increases in corpus callosum size are partially the result of continuing myelination, due to the large size of myelinated axons ($0.35\mu\text{m}$ diameter for myelinated axons, not including the myelin sheath compared to $0.16\mu\text{m}$ diameter of unmyelinated axons; Kim & Juraska, 1997). Increases in other material (glial processes and unmyelinated axons) also contribute to the growth in size but to a lesser degree because of the lower proportion of area occupied by other material. Glial processes may increase with age, resulting in a larger area occupied by other material. Such age-related changes in glia have been reported in parietal cortex of the rat, where increases in density and area of astrocytes have been found between adulthood and old age (Björklund et al., 1985; Peinado et al., 1998).

Consistent with previous work in our laboratory, we found a sex difference in size and myelination in the splenium (Kim, Ellman, & Juraska, 1996; Nunez & Juraska, 1998). We also found similar sex differences in size and myelination in the genu. Interestingly, we found that only females showed increases in callosal size between adulthood and middle-age. This is similar to a study in humans in which females continued to show an increase in callosal area into middle-age while males did not (Cowell et al., 1992). In the present study, sex differences were still present in old age, due to the absence of any age related losses in the size of the genu and splenium as well as in total brain weight. This is in contrast to the age-related elimination of sex differences in the dendritic tree in other regions of the rat brain, such as the anterior cingulate cortex and hippocampus (Markham et al., 2005; Markham & Juraska, 2002). In these regions, greater age-related deterioration in males eliminated the sex differences in the dendritic tree. Possible reasons for some areas remaining protected from deterioration during aging remain unclear.

Even though remyelination is slower in aging rats (Shields et al., 1999; Sim et al., 2002), the continuing ability of the adult rat brain to produce myelin may help to improve chances of remyelination in cases of injury or in response to demyelinating diseases. Remyelination progresses faster in aging female rats compared to males however (Li et al., 2006), possibly reflecting the greater oligodendrocyte proliferation and turnover in females (Cerghet et al., 2006).

In the current study, we report increases between adulthood and middle age in size and myelination in the genu and splenium, which is similar to work in humans showing continued increases in white matter into middle-age (Bartzokis et al., 2001; Courchesne et al., 2000). However, our data also show that these measures are maintained in old age which is in contrast to the loss of white matter volume and corpus callosum size found 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). While frontal regions sometimes show greater susceptibility to age-related deterioration in humans (Resnick et al., 2003; Raz et al., 1997), we saw no loss in either the genu or splenium. Evidence of myelin loss with age in humans is further supported by diffusion tensor imaging studies

which show decreases in fractional anisotropy and increased diffusivity in white matter (including the corpus callosum), which reflects a breakdown of the underlying axon structure (O'Sullivan et al., 2001; Ota et al., 2006; Sullivan, Adalsteinsson, & Pfefferbaum 2006). Similar to the volumetric data, diffusion tensor imaging studies show evidence of greater age-related deterioration in anterior regions of white matter and the corpus callosum (O'Sullivan et al., 2001; Ota et al., 2006; Sullivan, Adalsteinsson, & Pfefferbaum 2006; Abe et al., 2002; Sullivan et al., 2001; Salat et al., 2005).

In aging humans, smaller corpus callosum size (Ryberg et al., 2006) and reductions in fractional anisotropy (Sullivan, Adalsteinsson, & Pfefferbaum 2006) have been related to poorer cognitive performance. While the aging rat does experience cognitive decline on hippocampal based tasks such as the spatial version of the Morris water maze, the eight-arm radial maze, and the Barnes circular platform task (reviewed by Rosenzweig & Barnes, 2003), and an attentional set-shifting task that requires the medial prefrontal cortex (Barense et al., 2002), the possible implications of corpus callosum size on cognitive performance in rats have not been examined. Sex differences in aged rats are largely unexplored. These data imply that any task that did depend on the integrity of the corpus callosum would probably continue to show sex differences into old age. However at this point, it is clear that the known age-related cognitive declines in rats are not the result of obvious callosal deterioration.

The absence of any age-related loss in size or myelination of the genu and splenium suggests that the rat corpus callosum is preserved from substantial age-related loss. It does not however eliminate the possibility of more subtle changes occurring at an underlying level, such as alterations in the paranodal junction of myelinated fibers that occur with age in the rat (Sugiyama et al., 2002). Nevertheless, the rat does not show the gross changes in white matter that humans undergo with age. On a speculative note, it is possible that the controlled experiences of laboratory rats have eliminated two critical factors that have been shown to impact aging: stress and immune challenges across the lifespan (Lupien et al., 1998; Brunson et al., 2005; Sandi & Touyarot, 2006; Montaron et al., 2006; Godbout & Johnson, 2006). Why the corpus callosum of the laboratory rat does not age as readily as the human may be worth exploring to better understand the course of human aging.

4. Experimental procedure

Subjects were Long-Evans hooded rats in three age groups. Adult animals were 4 months old, middle-age animals were 12–13 months old (mean=12.8 months), and old age animals were 18–26 months of age (mean=21.4). Age groups were chosen based on changes in sexual maturity and consideration of mortality rates. Puberty begins around 35 days of age, with sexual maturity reached by 2 months, so that 4 months is considered young adulthood. Twelve months is considered middle-age because females begin to show irregular estrous cycles and reduced fertility by this time. Rats are generally considered aged by 18 months of age (discussed by Coleman, 2004). At this time females no longer show estrous cycles and are in estropause, there is also an increase in tumor incidence, and deficits in cognitive behavior have been noted (reviewed by Rosenzweig & Barnes, 2003).

Tissue was collected from both the splenium and genu in a largely overlapping group of subjects. All procedures were approved by the IACUC at the University of Illinois. In the genu, tissue was used from adult animals (7 females, 6 males), middle-age animals (11 females, 7 males), and old animals (9 females, 7 males). In the splenium, tissue was available from animals in adulthood (7 females, 7 males), middle-age (8 females, 8 males) and old age (10 females, 8 males). Middle and old age animals had served as breeders until being retired at 10–12 months of age. Animals were double or triple housed with same sex cagemates with the exception of

breeding and lactation periods. Both adult animals and retired breeders were handled weekly. Prior to sacrifice, females underwent daily vaginal lavage in order to determine their estrous or estropausal status (as described in Markham & Juraska, 2002). Unlike menopausal women, estropausal rats continue to secrete moderate levels of ovarian hormones in one of two estropausal states (Dudley, 1982; Markham & Juraska, 2002). The middle and old age females had to remain in their estropausal state for 10 days to determine their classification. Males received daily handling during this time to control for the difference in treatment.

Animals were deeply anesthetized with sodium pentobarbital and perfused intracardially with Ringer's solution followed by 1% glutaraldehyde/4% paraformaldehyde. Brains were removed and placed in the 1% glutaraldehyde/4% paraformaldehyde fixative overnight. The day after perfusions, brains were weighed and blocks containing the anterior and posterior 1/3 of the corpus callosum were taken from the right hemisphere and stained with 2% osmium tetroxide. The tissue was then dehydrated in increasing concentrations of acetone before being embedded in Eponate 12 resin (Ted Pella, Redding, CA). One week after perfusions, a photo was taken of the midsagittal surface of the left hemisphere along with a calibration ruler to be used for determining the length and boundaries of the genu and splenium. The splenium was defined as the posterior 20% of the total corpus callosum length, which was established as the boundary between axons from the visual and parietal cortices in rats (Kim et al, 1996; Kim and Juraska, 1997). The genu was defined as the anterior 15%, as others have done (Mack et al., 1995). Fiber tracking indicates that axons from frontal regions cross through the genu (Xue et al., 1999).

Embedded tissue blocks were trimmed and 1–2 micron thick sections were taken using an ultramicrotome. Sections were mounted and stained with Toluidine blue before being coverslipped. An outline of the subregion (genu or splenium) contained in each section was traced under a camera lucida at a magnification of 40X. The drawings were scanned into the computer and ImageJ software (Version 1.28, 2002) was used to quantify area.

Axonal composition was quantified using a stereological point counting technique (Weibel, 1979). This involved categorizing the composition of tissue under oil immersion at 1000X while simultaneously viewing a grid with a fixed number of intersections through a camera lucida. The composition at each intersection was classified into one of four categories: myelinated axons, glial cell bodies, blood vessels and other material (glial processes and unmyelinated axons, which are not resolvable using the light microscope). Using a counting grid with 110 intersections, samples were taken throughout the entire extent of each subregion. For the genu, 10 sampling sites were used for a total of 1100 points while for the splenium 12 sites were used for 1320 total points due to its larger size. The proportion of points for each category, the area fraction, was calculated and then multiplied by the area of the subregion to get the area occupied by each type of material.

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Figure 1.

Overall area (mm^2) of the genu (A) and splenium (B). Significant effects of age ($p=.001$), sex ($p=.001$), region ($p=.001$), and a trend for a sex by age interaction ($p=.052$) were found.

Figure 2. Area occupied by myelin (mm^2) in the genu (A) and splenium (B). A significant effect of sex ($p=.002$) but not age was found.

Figure 3.

Area occupied by other material (mm^2) in the genu (A) and splenium (B). Effects of age ($p=.014$) and region ($p=.001$) were found, as well as a trend for an effect of sex ($p=.068$).