Quantitative analysis of myelinated axons of commissural fibers in the cat brain

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ABSTRACT

Objective: There are limited data related to the number of commissural axons found in various species. Although the corpus callosum has been investigated in cat, there are no data on the number of its myelinated axons. Additionally, the number of myelinated axons of anterior, posterior and habenular commissures are not documented for the cat. Therefore, we aimed to examine the topographic distribution and regional numerical differences of myelinated axons in encephalic commissures of the cat.

Methods: This study was carried out at the Faculty of Medicine, Hacettepe University, Ankara, Turkey, from March 2003 to July 2003. The myelinated axons of the encephalic commissures of one cat were counted in this study.

Results: In parts of the corpus callosum, a statistically significant difference was found between rostrum and genu, rostrum and truncus, genu and truncus, genu and splenium and truncus and splenium. However, no statistically significant difference was found between rostrum and splenium. When comparing the number of myelinated axons of anterior, posterior and habenular commissures, statistically significant differences were found between anterior and habenular commissures, and between posterior and habenular commissures. No statistically significant difference was found between anterior and posterior commissures.

Conclusion: These numerical data, evaluated by quantitative analytical statistical methods, might be useful in filling the lack of information on the mean numbers of myelinated axons of different commissures. Knowledge of the number of myelinated axons in different parts of the corpus callosum may also be very important when performing surgical procedures of the corpus callosum, such as commissurectomies.

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The corpus callosum and the anterior, posterior and habenular commissures are the commissural fibers that connect the cortical regions of the 2 cerebral hemispheres with each other.[1] Surprisingly, little is known about the number of commissural axons found in various species.[2] The corpus callosum and anterior commissure are the largest cerebral commissures, and interconnect the frontal, parietal, temporal and a small part of the occipital cortices of both cerebral hemispheres.[3,4] Although the corpus callosum has been investigated in the cat, there are no data about the number of its myelinated axons.[5-9] Additionally, in the cat there are only very few studies related to the posterior commissure,[10] and to our knowledge there are no studies about the anterior and habenular commissures. Consequently, the number of myelinated axons of these 3 commissures is not documented for the cat. This study aimed to examine the topographic distribution and regional numerical differences of myelinated axons in the encephalic commissures of the cat, by using the camera lucida and quantitative analytical statistical methods.

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Methods. This experimental study was performed on the brain of an autopsy specimen, which was a 2-year-old male cat (Felis silvestris), weighing 2840 g. The cerebral hemispheres were removed from the skull and were separated from each other by a median section. By means of a stereomicroscope, at 8X magnification, tissue samples were obtained from the rostrum, genu, truncus and splenium parts of the corpus callosum and from the anterior, posterior and habenular commissures. Additionally, we also examined the diameters of the myelinated axons and reported the differences between all these commissures. The tissue samples were put into 10% formalin and prepared according to routine paraffin embedding tissue preparation technique. Following this, 10 sections approximately 5 µm in thickness were cut from each of the tissue samples, stained with Luxol fast blue and examined by a Nikon Optiphot (Nikon Corporation, Tokyo, Japan) light microscope by the same researcher (**Table 1**). The myelinated axons of the rostrum, genu, truncus, splenium, anterior commissure, posterior commissure and habenular commissure were counted in areas of 0.01 mm^2 under 200X magnification using a camera lucida. Parts of myelinated axons located at the lower and left-hand edges were included in the counting, whereas those at the upper and right hand edges were not recorded. Statistical differences between the 10 samples of each specimen were compared by using a Kruskal-Wallis variance analysis test and Mann-Whitney U test for comparison of the different groups of commissural fibers with each other.

Results. ***Corpus callosum.*** In the light microscopic examination, small sized myelinated axons were observed in the rostrum, genu, truncus and splenium parts of the corpus callosum. No difference was found in the sizes of these axons, when the different parts of the corpus callosum were examined (**Figures 1-4**). Morphometric data on the myelinated axons of the rostrum, genu, truncus and splenium of the corpus callosum are listed in **Table 1**. When comparing these 4 groups with each other, statistically significant differences were found between the rostrum and genu (p = 0.023), rostrum and truncus (p = 0.00), genu and truncus (p = 0.004), genu and splenium (p = 0.023) and truncus and splenium (p = 0.00); however, no statistically significant difference was found between the rostrum and splenium (p = 0.912).

Anterior commissure, posterior commissure and habenular commissure. In the light microscopic examination, small sized myelinated axons were observed in the anterior (**Figure 5**) and posterior commissures (**Figure 6**). However, a heterogeneous distribution was present in the myelinated axons of the habenular commissure. The majority of the myelinated axons in the habenular commissure (88%) were small in diameter and the rest were medium-sized (**Figure 7**). Numerical data on the anterior, posterior and habenular commissures are listed in **Table 1**. The comparison of these 3 groups with each other showed statistically significant differences between the anterior and habenular commissures (p = 0.00), and between the posterior and habenular commissures (p = 0.00), whereas no statistically significant difference was found between the anterior and posterior commissures (p = 0.481).

Discussion. The axons in the brain commissures have been examined in several species

Table 1 - The number of the myelinated axons of rostrum, genu, truncus, splenium, anterior commissure, posterior commissure and habenular commissure, counted under 200X magnification, in a constant area of 0.01 mm^2 using camera lucida.

Rostrum of corpus callosum	Genu of corpus callosum	Truncus of corpus callosum	Splenium of corpus callosum	Anterior commissure	Posterior commissure	Habenular commissure
203	196	226	184	185	170	123
187	208	207	191	191	201	140
206	192	202	200	196	179	142
189	214	236	206	178	221	139
196	202	223	202	169	216	134
188	199	234	186	198	230	128
201	209	228	197	192	184	141
185	194	210	185	188	172	121
194	207	232	193	181	188	136
196	210	219	197	193	196	141
Mean 194.5	Mean 203.1	Mean 221.7	Mean 194.1	Mean 187.1	Mean 195.7	Mean 134.5
Std. Dev. 7.2303	Std. Dev. 7.5638	Std. Dev. 11.8795	Std. Dev. 7.5785	Std. Dev. 8.9747	Std. Dev. 20.9605	Std. Dev. 7.8209

Std. Dev. - standard deviation

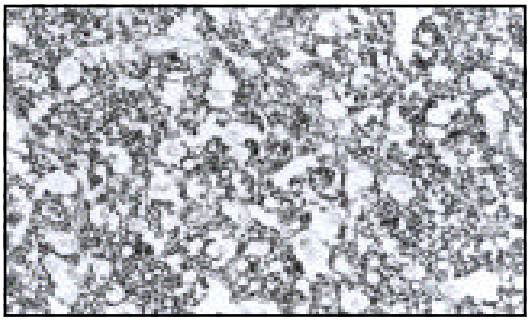

Figure 1

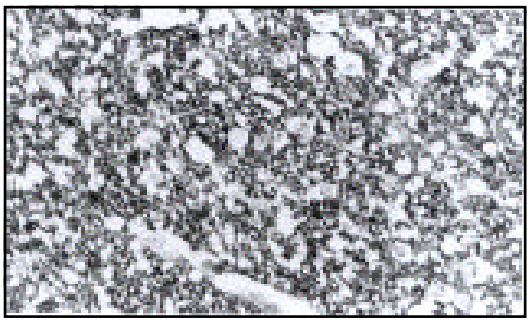

Figure 2

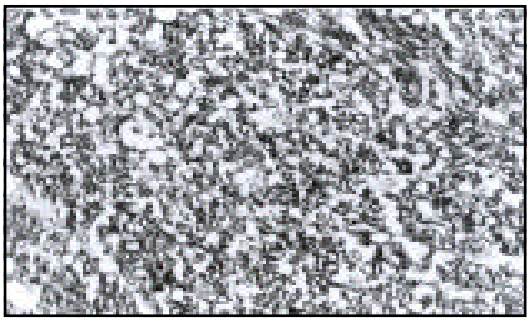

Figure 3

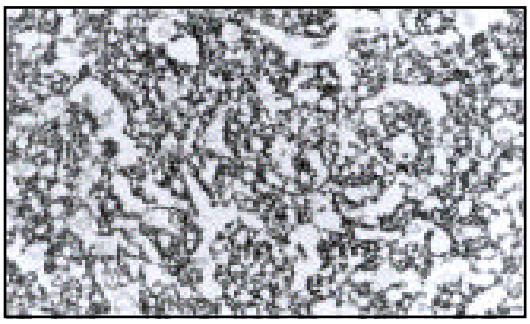

Figure 4

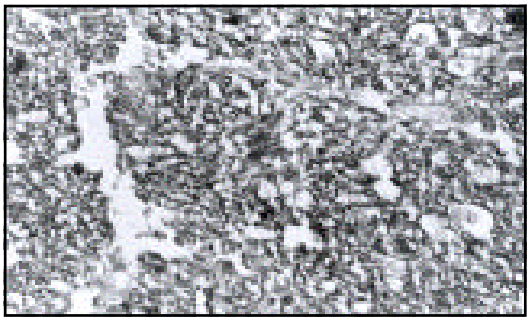

Figure 5

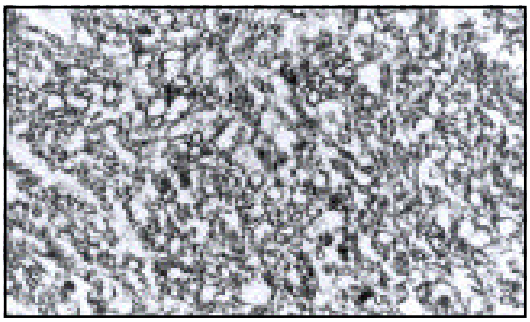

Figure 6

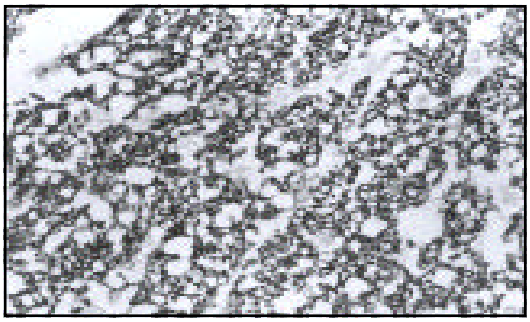

Figure 7

Photomicrographs showing the myelinated axons 200 X in all figures (1-7).

Figure 1 - Rostrum of the corpus callosum.

Figure 2 - Genu of the corpus callosum.

Figure 3 - Truncus of the corpus callosum.

Figure 4 - Splenium of the corpus callosum.

Figure 5 - Anterior commissure.

Figure 6 - Posterior commissure.

Figure 7 - Habenular commissure.

by many authors. Lamantia and Rakic found 60 million axons connecting the 2 cerebral hemispheres in the rhesus monkey. The vast majority (56 ± 3.8 million) of these axons were present in the corpus callosum and much less (3.15 ± 0.24 million) were located in the anterior commissure.[2] Olivares et al performed a cross-species ultrastructural study of the corpus callosum in 6 domestic species: The rat, the rabbit, the cat, the dog, the horse and the cow. Their results indicated cross-species conservatism in callosal fiber composition with a good interspecies relation between fiber number and brain size.[9] Looney and Elberger examined the myelination of the cat corpus callosum in the posterior-most portion of the splenium and the anterior-most portion of the genu. Their results indicated that myelination begins and ends earlier in the anterior region of the corpus callosum.[5] Changes in the size and shape of the corpus callosum and in number, size and structure of callosal axons between embryonic day 38 and postnatal day 150 were studied by light and electron microscope in 25 kittens. In this study, the development of corpus callosum was divided into 3 phases: Embryonic development, early postnatal development and late postnatal development.[6] Houzel and Milleret analyzed the morphology of individual callosal axons linking primary visual cortices using 3 dimensional light microscopic techniques in the cat.[8] Kim et al examined the sex differences in axon density and number in the splenium of the rat corpus callosum and found no sex differences in total axon number, although male rats had significantly more myelinated axons than females.[11] Sex differences in the distribution of axon types within the genu of the rat corpus callosum were studied by Mack et al. Their results showed a significant sex difference in the ratio of unmyelinated to myelinated axons, with females having a larger proportion of unmyelinated fibers.[12] Livy et al found increased axon numbers in the anterior commissure of mice lacking a corpus callosum.[13] The anterior commissure of the Wallaby (Macropus eugenii) was studied by Ashwell et al and they found 21.7 million axons in the adult anterior commissure, of which 55-62% were myelinated. The dorsal two-thirds of the commissure containing neocortical commissural axons showed a higher percentage of larger myelinated axons than the ventral one-third which contains paleocortical commissural axons.[14] Myelogenesis and estimation of the number of axons in the anterior commissure of the chick (*Gallus gallus*) was studied by Ehrlich and Saleh. In midline sagittal sections they found about 290,000 axons, of which 32% were myelinated in the posterior commissure. The majority of myelinated fibers in the rostral zone had a diameter of less than 1 µm.[15] The existence of periaqueductal neuronal subdivision, and its close relationship with the posterior commissure were studied with silver reduced stains and the electron microscope in the hedgehog, rat and cat by Lara and this is the only study on the posterior commissure of the cat, found in the literature.[10]

The myelinated axons of the rostrum, genu, truncus and splenium parts of the corpus callosum and in the anterior, posterior and habenular commissures were previously counted in the rat brain.[16] In the parts of the rat corpus callosum; a statistically significant difference was found between the rostrum and genu, rostrum and truncus, rostrum and splenium, genu and truncus, and the genu and splenium. No statistically significant difference was found between the truncus and splenium. However in this study; a statistically significant difference was found between the rostrum and genu, rostrum and truncus, genu and truncus, genu and splenium and truncus and splenium. No statistically significant difference was found between the rostrum and splenium of the cat. When comparing the number of myelinated axons of the anterior, posterior and habenular commissures of the rat, statistically significant differences were found between the anterior and posterior commissures, and between the anterior and habenular commissures. No statistically significant difference was found between the posterior and habenular commissures.[16] However in this study; statistically significant differences were found between the anterior and habenular commissures, and between the posterior and habenular commissures. No statistically significant difference was found between the anterior and posterior commissures of the cat.

The largest bundle of axonal fibers in the entire mammalian brain, namely, the corpus callosum is the pathway through which almost half a billion neurons are scattered over all neocortical areas[8] and numerical data about corpus callosum axons are well documented for the human species.[17] Therefore, it is suggested to discuss here only data about nonhuman species as little is known about the number of commissural axons in various nonhuman species.[2] Additionally, there are only a few studies related with the posterior commissure[10] and no studies examining the anterior and habenular commissures of the cat. In the present study, we aimed to obtain information about the number of myelinated axons in different commissures and in the different parts of the major commissure, namely, the corpus callosum. These numerical data, evaluated by quantitative analytical statistical methods, might be useful in filling the lack of information about the mean numbers of myelinated axons of different commissures. Knowledge of the number of myelinated axons in the different parts of

the corpus callosum may also be very important when performing surgical procedures of the corpus callosum, such as commissurectomies. Furthermore, in contrast to the uniform pattern of small sized myelinated axons observed in the corpus callosum, and the anterior and posterior commissures, a heterogeneous distribution of myelinated axons was present in the habenular commissure of the cat. This finding has not been reported nor discussed in previous literature.

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