Morphometric analysis of myelinated fibre composition in the optic nerve of adult C57BL and CBA strain mice and (C57BL x CBA) F1 hybrid

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Morphometric analysis of myelinated fibre composition in the optic nerve of adult C57BL and CBA strain mice and (C57BL × CBA) F1 hybrid: a comparison of interstrain variation

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ABSTRACT

In a study involving 50 optic nerves isolated from 3 different strains of adult male mice, C57BL, CBA and (C57BL × CBA) F1 hybrids, and from adult female CBA strain mice, we observed that the mouse had a lower mean total myelinated nerve fibre count than other mammals such as the rat, cat, rabbit, monkey and man where similar information was available from the literature. The nerve fibre spectrum, however, which mostly consisted of small diameter fibres, was similar to the distribution seen in these other species. The largest myelinated nerve fibres observed in any of the strains of mice investigated had a diameter of not more than 1.92 μm. The C57BL optic nerve had the largest population of large diameter fibres, while the F1 had the largest population of small diameter fibres. In all the strains of mice investigated, the distribution of nerve fibres was unimodal, with a modal diameter of 0.48 μm. The mean nerve fibre diameter was 0.62 ± 0.02 μm (S.E.M.), 0.57 ± 0.03 μm and 0.55 ± 0.01 μm for C57BL, F1 and CBA, respectively. The F1 had the lowest population of fibres around the modal diameter. The myelinated nerve fibres were most densely packed in the CBA strain of mice, whereas the C57BL was the least densely populated. There was a significant interstrain difference in the parameters measured between the 3 strains of mice studied, whereas there was no significant intrastrain difference.

INTRODUCTION

Few detailed studies are available on the myelinated fibre composition of the mouse optic nerve, although much work has been undertaken in a variety of other mammalian species (see Discussion). The only relevant studies in the mouse that we have so far located have been those of Gyllensten & Malmfors (1963) and Gyllensten et al. (1966) who analysed the influence of visual stimulation on myelination and fibre composition in the mouse optic nerve. These early morphometric studies, although of interest in some respects, are incomplete in that the authors failed to establish whether there was any significant difference in myelinated fibre composition between, for example, the left and the right optic nerve, or between male and female mice of the same and different strains.

The principal aim of the present study was to establish baseline information which would be useful for subsequent teratological studies. The optic nerves from adult male C57BL, CBA and (C57BL × CBA) F1 hybrid mice, and from female CBA mice were isolated in order to undertake morphometric analyses. Both the cross-sectional areas of the optic nerves and the numbers and diameters of myelinated nerve fibres present, and nerve fibre density, were established for the various groups analysed. This information has allowed a comparison to be made between our findings in the mouse and comparable findings from a wide range of other mammalian species including man.

MATERIALS AND METHODS

Three different strains of 11-wk-old male mice were used in this study. C57BL (× 5) and CBA (× 10) strain mice and (C57BL × CBA) F1 hybrid (× 5) mice were deeply anaesthetised following an intraperitoneal injection of 0.02 ml/g body weight of a 1.2% solution

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of Avertin dissolved in 0.9% saline. An intracardiac perfusion of fixative, using 2 ml/g body weight of a 2.5% solution of glutaraldehyde in 0.1 M phosphate buffer was given via a 20 G needle into the left ventricle while the heart was still beating.

The optic nerves from each mouse were carefully dissected out, avoiding traction on the nerves. Each nerve was cut just posterior to the orbit, and anterior to the optic chiasma, immediately put into a separate bottle of fixative and left in this for a total of about 12 h. The isolated nerves were washed in buffer and then transferred into a secondary fixative consisting of 1% osmium tetroxide in 0.1 M phosphate buffer for a further 2 h. They were then dehydrated through a graded alcohol series and finally embedded in Araldite.

Semithin transverse sections of ~ 1 μm thick were cut perpendicular to the long axis of the nerve using a Reichert-Jung Ultracut E microtome and stained with 1% toluidine blue in 1% borax suitable for light microscopy. Thin sections 80 nm in thickness were then cut, put onto copper grids (200) and subsequently stained with 0.2% lead citrate and a saturated solution of uranyl acetate (Reynolds, 1963). A selection of photomicrographs was then taken from the centre to the periphery of each nerve using a Philips EM301 transmission electron microscope. The micrographs were developed and printed at a final magnification of $\times 3000$, sufficient to ensure that all fibres with the thinnest myelin sheaths could be unequivocally identified in counts.

The cross-sectional areas (*csa*) of the optic nerves were determined by viewing and measuring complete cross-sections of the optic nerves in a Magiscan image analysis system (Applied Imaging). Detailed morphometric analysis was performed on the electron micrographs using a systematic random sampling procedure (Mayhew, 1990) to establish the numbers and diameters of the myelinated nerve fibres by means of the image analyser. In order to determine the latter, the approximate centre of each optic nerve was located, and from this point sectors of 10° were drawn 120° apart which produced 3 sectors/nerve. A grid of 1 cm × 1 cm squares (equivalent to 7.8 μm^2 of nerve cross-sectional area) was placed over each sector. Starting from the centre of each nerve, and using the systematic random sampling method referred to above, every 4th square in each direction (i.e. 1 in 16) was sampled for estimating nerve fibre counts. The aim was to sample between 150 and 200 myelinated nerve fibres, and to measure as many profiles of the nerves as possible. In this method all nerve fibres whose centres were within a sampled square were counted and their transverse diameter measured. The image analyser allowed a histogram to be plotted which displayed the distribution of diameters of all the nerve fibres studied from each optic nerve sample. When all the information from the various sample groups was available, it was then possible for histograms to be plotted which displayed the overall distribution of the nerve fibre diameter composition for each of the mouse strains studied.

An estimate of the number of myelinated nerve fibres present in the entire nerve and their density was calculated using a ratio technique (Matheson, 1970; Mayhew, 1988, 1990). A 2-tailed Student's t test was then performed on the pooled data for each strain of mouse, to establish whether there was any significant difference ($P \leqslant 0.05$) in cross-sectional area and total nerve fibre count and size distribution between the left and right optic nerve for each of the strains studied, and to establish whether interstrain variation existed. Furthermore, to establish whether evidence of sexual dimorphism was present, an additional series of 11-wk-old female CBA ($\times 5$) mice was treated as indicated above, and the findings obtained compared with those obtained previously from the analysis of the male CBA mice.

RESULTS

Cross-sectional areas (csa)

Analysis of the *csa* of the left and right optic nerves of the male mice studied showed that, except in the C57BL, the right optic nerve was consistently larger than the left (i.e. in the CBA strain, and in the (C57BL × CBA) F1 hybrids). The smallest difference between the left and right optic nerves was seen in the CBA and the largest difference observed in the F1. However the difference observed was not significant. The data from the left and right optic nerves of each strain studied were therefore pooled. This allowed the sample size to be doubled in each case, so that in the C57BL, CBA and F1 hybrid mice, the pooled sample size of the optic nerves studied was 10, 20 and 10 respectively. The C57BL had the largest mean *csa* (72968 ± 1876 μm^2 (S.E.M.)) and the CBA the smallest (57113 ± 1513 μm^2) (S.E.M.). Further analysis of the pooled data has revealed that there was a significant difference between all the strains ($0.01 < P < 0.05$) (see Tables 1, 2).

In an additional series, a total of 5 left and 5 right optic nerves were isolated from 11-wk-old female CBA mice. Analysis of the *csa* of this group revealed that there was no significant difference between the two sides. A comparison of the findings from this

Table 1. *Mean cross-sectional area, myelinated nerve fibre count and nerve fibre density of optic nerves isolated from (C57BL × CBA) F1 hybrid, C57BL and CBA strains of adult mice*

Strain	Mean cross-sectional area ± S.E.M. (μm²)			Mean nerve fibre count ± S.E.M.			Mean nerve fibre density per 1000 μm² (L+R) ± S.E.M.		
	Left (L)	Right (R)	L+R	Left (L)	Right (R)	L+R	Left (L)	Right (R)	L+R
(C57BL × CBA) F1 hybrid (male, n = 9)	62925 ± 1849	70452 ± 1384	67107 ± 1603	98694 ± 2907	90399 ± 6017	94086 ± 3691	1569 ± 18	1279 ± 48	1408 ± 61
C57BL (male, n = 10)	75264 ± 1219	70667 ± 3410	72968 ± 1876	95016 ± 8418	79781 ± 4005	87398 ± 5076	1258 ± 98	1129 ± 17	1194 ± 51
CBA (male, n = 20)	57200 ± 2040	57308 ± 1788	57254 ± 1320	80924 ± 4439	78586 ± 2530	79755 ± 2500	1426 ± 80	1378 ± 48	1402 ± 46
CBA (female, n = 10)	54654 ± 2809	59005 ± 7483	56830 ± 3847	80524 ± 6400	79934 ± 6825	80229 ± 4432	1475 ± 100	1382 ± 69	1429 ± 60
CBA (male & female, n = 30)	56351 ± 1625	57874 ± 2603	57113 ± 1513	80791 ± 3517	79035 ± 2696	79913 ± 2180	1442 ± 61	1380 ± 38	1411 ± 36

Table 2. *Intrastrain and interstrain comparison for variables measured for the optic nerve of adult F1, C57BL and CBA strains of mice*

Strain comparison	Variable/level of significance*		
	Mean cross sectional area (μm²)	Mean myelinated nerve fibre count	Mean myelinated nerve fibre density (per 1000 μm²)
F1 (L vs R, n = 9)	n.s.	n.s.	n.s.
C57BL (L vs R, n = 10)	n.s.	n.s.	n.s.
CBA male (L vs R, n = 20)	n.s.	n.s.	n.s.
CBA female (L vs R, n = 10)	n.s.	n.s.	n.s.
CBA male vs CBA female (n = 30)	n.s.	n.s.	n.s.
CBA male and female (L vs R, n = 30)	n.s.	n.s.	n.s.
F1 male vs CBA male (n = 29)	$0.01 < P < 0.05$	$0.01 < P < 0.05$	n.s.
F1 male vs CBA male and female (n = 29)	$0.01 < P < 0.05$	$0.01 < P < 0.05$	n.s.
F1 male vs C57BL male (n = 19)	$0.01 < P < 0.05$	n.s.	$0.01 < P < 0.05$
C57BL male vs CBA male (n = 30)	$0.01 < P < 0.05$	n.s.	$0.01 < P < 0.05$
C57BL male vs CBA male and female (n = 40)	$0.01 < P < 0.05$	n.s.	$0.01 < P < 0.05$

* Student's t test; n.s., not significant.

series with their CBA male littermates revealed no significant difference between the two sexes. For this reason the pooled data for this strain have been given in Table 1.

Total myelinated nerve fibre counts

Although the *csa* of the optic nerve of the right eye, except for the C57BL, was consistently greater than that of the left, the opposite finding was observed with respect to the mean myelinated nerve fibre counts, where the left optic nerve consistently showed a higher myelinated nerve fibre count than the right in all groups of mice studied. This difference between the left and right optic nerve, however, was not significant. As in the *csa* analysis indicated above, this has allowed the sample size in each strain to be doubled. The total myelinated nerve fibre count varied between 79913 ± 2180 (S.E.M.) (CBA) and 94086 ± 3691 (S.E.M.) (F1). Further analysis of the pooled data has revealed that the F1 differ significantly from the CBA ($0.01 < P < 0.05$). There was no significant difference, however, between the C57BL and either of the other strains. As for *csa* the CBA findings provided no evidence of sexual dimorphism, so that the individual male and female findings, as well as the pooled data have been provided in Tables 1 and 2.

Myelinated nerve fibre density per 1000 μm²

No significant difference was observed in the mean myelinated nerve fibre density between the left and

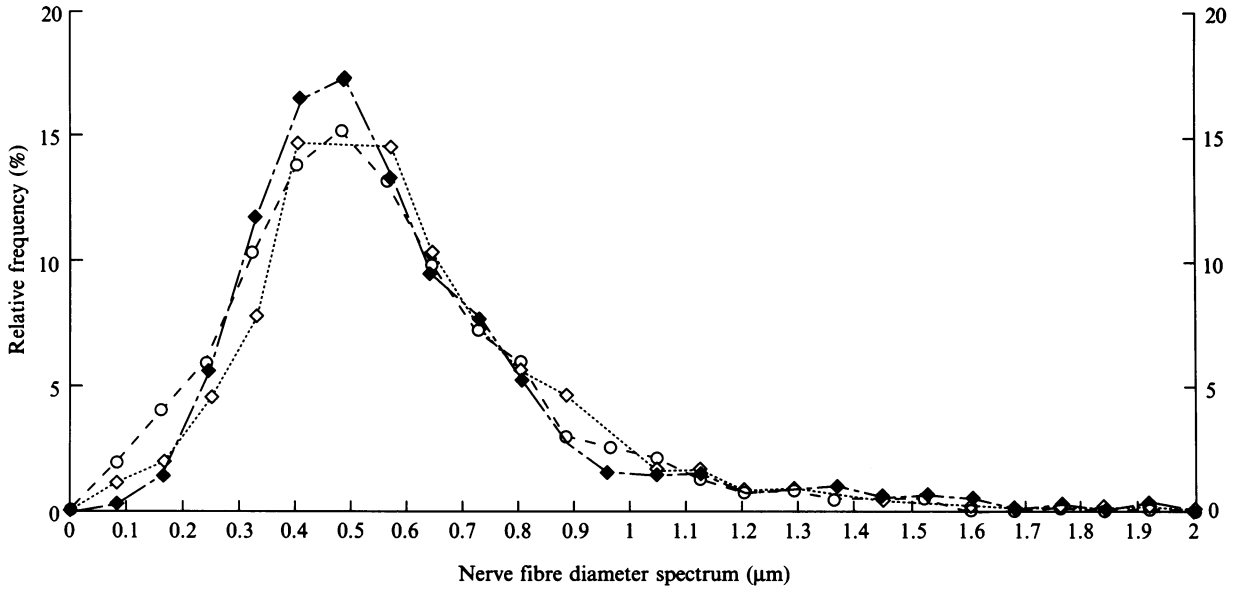

Fig. Nerve fibre size spectrum in the optic nerve of adult C57BL, CBA and (C57BL × CBA) F1 mice: –·–· ◆; ···· ◇; ––– ○.

right optic nerves in each of the strains studied. The pooled values showed a significant difference between the C57BL and both of the other strains ($0.01 < P < 0.05$) (see Table 2). There was no significant difference between the F1 and CBA. The highest mean myelinated nerve fibre density was observed in the CBA (1411 ± 36 (S.E.M.)) and the smallest in the C57BL (1194 ± 51 (S.E.M.)). As for *csa* and total myelinated fibre counts, no evidence of sexual dimorphism was seen in the CBA, so that the individual male and female findings as well as the pooled data have been included in Tables 1 and 2.

Size (diameter) distribution of myelinated nerve fibres

Over 90% of the nerve fibres in each strain of mice were not more than 1.04 μm in diameter. The largest fibres analysed were seen in the C57BL and were 1.92 μm in diameter. These constituted only 0.28% of the total fibre population of that strain. The largest fibres seen in the F1 and CBA were 1.84 μm in diameter. They constituted 0.09% and 0.04% of the total fibre population of the F1 and CBA, respectively (see Figure). The mean nerve fibre diameter was 0.62 ± 0.02 μm (S.E.M.), 0.57 ± 0.03 μm (S.E.M.) and 0.55 ± 0.01 μm (S.E.M.) for the C57BL, F1 and the CBA respectively. In all the strains examined, the distribution of nerve fibre diameter was unimodal, with a modal value of 0.48 μm. Each strain of mice exhibited a slight skewing of the fibres in favour of those with a smaller diameter. The C57BL and the CBA had similar populations of fibres (17.25% and 17.37%, respectively) with this modal diameter. The F1 had 15.2% of its fibres with the modal diameter (see Figure).

DISCUSSION

The findings reported in this study indicate that of the variables analysed, no significant differences were observed in the cross-sectional areas, total number of myelinated nerve fibres present, and nerve fibre density between the left and right optic nerves of male C57BL, CBA and (C57BL × CBA) F1 hybrid mice. In an additional analysis of the optic nerves isolated from a group of CBA females, no significant difference was observed in any of the variables studied between the left and right optic nerves, and between male and female mice. Interstrain differences were, however, seen between the different strains studied. Of the 3 strains of mice involved in this study, the C57BL strain had the largest cross-sectional area, but their optic nerves were the least densely populated with myelinated nerve fibres. Because the CBA strain has a high mean nerve fibre count and a relatively small *csa*, it has the highest nerve fibre density. The relatively small difference in the variables measured between individuals of each of the strains studied, both male and female for the CBA strain mice, indicates a considerable degree of intrastrain uniformity as might be expected in inbred strains of animals. The fact that there was a significant difference in the variables measured between the various strains studied should also not be surprising, and would seem to be reasonable evidence of genetic variation induced as a result of many generations of brother × sister matings

Table 3. *Comparison of variables measured in this study with other studies on the optic nerve*

Species	Mean nerve fibre count	Mean nerve fibre density per 1000 μm²	Fibre size range (μm)	Mean fibre diameter (μm)	Modal diameter (μm)	Distribution	Reference
Man	1100000–1300000	N.P.	N.P.	N.P.	0.5	Unimodal	Potts et al. 1972*a*, *b*
Man	815000–1000000	N.P.	0.70–8.00	N.P.	1.00	Bimodal	Chacko, 1948
Monkey (rhesus)	1500000–1800000	N.P.	N.P.	N.P.	0.50	Unimodal	Potts et al. 1972*a*, *b*
Monkey (rhesus)	1400000	N.P.	0.40–6.00	N.P.	1.20	Unimodal	Ogden & Miller, 1966
Monkey (cynomologus)	1200000	N.P.	0.55–1.55	0.80	N.P.	Trimodal	Sanchez et al. 1986
Cat	85926	N.P.	1.00–10.50	N.P.	2.50	Unimodal	Donovan, 1967
Rabbit	394000±20000 (S.D.)	N.P.	0.25–7.00	N.P.	0.75	Unimodal	Vaney & Hughes, 1976
Rat (albino)	117000	N.P.	N.P.	N.P.	0.90	Unimodal	Forrester & Peters, 1967
Rat (pigmented)	120000±1600 (S.E.M.)	509000 fibres mm^{-2}	0.44–5.20	N.P.	1.00	Unimodal	Hughes, 1977
Chipmunk (*Tamias sibiricus asiaticus*)	565000	N.P.	0.20–3.6	0.83	0.60–0.70	Unimodal	Watakuwa et al. 1987
Mouse C57BL	66155±10052 (S.D.)	N.P.	≤ 3.00	N.P.	0.60–0.80	Trimodal	Gyllensten & Malmfors, 1963; Gyllensten et al. 1966
C57BL	87398±5076 (S.E.M.)	1194±51 (S.E.M.)	≤ 2.00	0.62±0.02	0.48	Unimodal	This study
CBA	79913±2180 (S.E.M.)	1411±36 (S.E.M.)	≤ 1.84	0.55±0.01	0.48	Unimodal	This study
F1	94086±3691 (S.E.M.)	1408±61 (S.E.M.)	≤ 1.84	0.57±0.03	0.48	Unimodal	This study

S.D., standard deviation; S.E.M., standard error of mean; N.P., information not provided.

required in the establishment of an inbred strain of animals. The same modal value was observed in each of the strains studied for the size distribution of myelinated nerve fibres.

Since some of the variables analysed in this study have been investigated by others, this has enabled us to make some tentative observations on species variation, particularly in the mean total myelinated nerve fibre count within the optic nerve, as this has been the area in which most information has been published. In order to facilitate such an exercise, we have presented this information in a tabular form based on the different species studied (see Table 3). The most obvious feature that emerges from the information presented in this Table is the very considerable difference in total number of myelinated fibres present in the primate compared with the nonprimate optic nerves. In the human optic nerve, values of 815000–1000000 (Chacko, 1948) to 1100000–1300000 (Potts et al. 1972*a*, 1976*b*) have been reported, while slightly higher figures were obtained from the analysis of the optic nerve of the rhesus monkey (1400000–1800000; see Ogden & Miller, 1965; Potts et al., 1972*a*, *b*), and 1200000 for the cynomologus monkey (Sanchez et al. 1986). A nerve fibre count of 565000 was reported in the optic nerve of the chipmunk (Watakuwa et al. 1987). In the rabbit, a figure of 394000 fibres was reported (Vaney & Hughes, 1976), and in the cat, Donovan (1967) reported a nerve fibre count of 85926. In the rodents that have been studied, the figures quoted for the total fibres present in the rat of 117000–120000 (Forrester & Peters, 1967; Hughes, 1977) are about 50% greater than those for mice given by Gyllensten & Malmfors (1963) and Gyllensten et al. (1966) and the values cited in the present study, with only a minimum degree of strain variation apparent in this species. The figures indicated here suggest that there might be a relationship between body size and total myelinated fibre count.

Most of the studies cited above provided figures for the size range of the myelinated fibres with, in general terms, the largest fibres being seen in the primates analysed, and in the rabbit and cat. In the latter species, maximum fibre diameters of up to 10.5 μm were reported (see Donovan, 1967) compared with 1.92 μm in this study. It is obvious here that the optic nerve of the mouse, besides containing a lower total myelinated nerve fibre count compared with the rat, has predominantly fibres of smaller diameter.

The difference in the total fibre counts of the C57BL strain of mice in this study and those of Gyllensten & Malmfors (1963), and Gyllensten et al. (1966) are probably most easily accounted for by differences in

the methodology employed to determine this value. In contrast to the present study where transmission electron micrographs of thin sections of the optic nerve were studied, these authors analysed paraffin sections of mouse optic nerves using phase-contrast microscopy. The error factor using the latter approach, both in estimating the total number of fibres present as well as determining their mean diameters, was found to be considerable. When these authors used photomicrographs obtained following transmission electron microscopy of the optic nerve, a modal diameter of 0.6–0.8 μm was reported, compared with 1.0 μm when paraffin sections were analysed. Furthermore, it is unclear why the distribution of fibre diameter should have been trimodal in Gyllensten & Malmfors (1963) and Gyllensten et al. (1966) compared with a unimodal distribution as reported in this study. Whether any clear species specificity exists between total fibre count and the lifestyle of that species has yet to be fully evaluated. Gyllensten & Malmfors (1963) suggested that this may reflect the fact that the mouse is more dependent on other senses than sight for most of its activities, and that this may be the reason why the majority of the myelinated fibres present are of small diameter.

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