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Genetic and Environmental Control of Variation in Retinal Ganglion Cell Number in Mice

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How much of the remarkable variation in neuron number within a species is generated by genetic differences, and how much is generated by environmental factors? We address this problem for a single population of neurons in the mouse CNS. Retinal ganglion cells of inbred and outbred strains, wild species and subspecies, and F_1 hybrids were studied using an unbiased electron microscopic method with known technical reliability.

Ganglion cell numbers among diverse types of mice are highly variable, ranging from 32,000 to 87,000. The distribution of all cases ($n = 252$) is close to normal, with a mean of 58,500 and an SD of 7800. Genetic factors are most important in controlling this variation; 76% of the variance is heritable and up to 90% is attributable to genetic factors in a broad sense.

Strain averages have an unanticipated bimodal distribution, with distinct peaks at 55,500 and 63,500 cells. Three pairs of closely related strains have ganglion cell populations that differ by >20% (10,000 cells). These findings indicate that different alleles at one or two genes have major effects on normal variation in ganglion cell number.

Nongenetic factors are still appreciable and account for a coefficient of variation that averages ~3.6% within inbred strains and isogenic F_1 hybrids. Age- and sex-related differences in neuron number are negligible. Variation within isogenic strains appears to be generated mainly by developmental noise.

Key words: aging; brain evolution; brain weight; developmental noise; heritability; inbred mice; natural variation; neuron number; quantitative trait loci; retinal ganglion cell; sex differences; wild mice

Neuron number within a species can be highly variable. This is true for both large and small populations. For example, the number of neurons in the lateral geniculate nucleus of monkeys ranges from 1.0 million to 1.8 million (Williams and Rakic, 1988; Ahmad and Spear, 1993), whereas the number of giant interneurons in the spinal cord of lampreys ranges from 12 to 22 (Selzer, 1979). The adaptive significance of this twofold variation has not yet been explored in detail (Williams and Herrup, 1988), but there is an approximate relationship between neuron number and an animal's behavioral capacity (Wimer and Wimer, 1985; Ellis and Horvitz, 1986; Purves, 1988; Crusio et al., 1989a,b; Lipp, 1989; Lipp et al., 1989; Williams et al., 1993; Legendre et al., 1994; Tejedor et al., 1995). For this reason, variation in neuron number is likely to be a key target of natural selection and a major influence on the pace of brain evolution. Variation in neuron number is attributable to both genetic and environmental factors. But a key question is how much of the variance in single populations is attributable to heritable factors versus environmental and other epigenetic factors?

One effective way to partition the total variance in neuron number is to analyze groups of genetically identical animals. When technical error is eliminated, the remaining variation among these isogenic animals is generated by environmental factors and developmental noise. Goodman (1976, 1979) found some remarkable differences within isogenic grasshoppers reared in a tightly regulated environment. In one clone, half of the animals had six ocellar interneurons, whereas the other half had seven to nine. This finding illustrates the importance of nongenetic factors in generating variation. In contrast, in a systematic survey of optic nerves from a set of >100 isogenic crustaceans, Macagno (1980) found only a single animal that did not have precisely 176 axons. This finding provides a counterexample of the precision with which genetic and epigenetic factors can regulate final cell number.

Comparable studies have not been carried out in mammals, primarily because counting large neuron populations can be difficult. Sampling error and counting bias, ambiguities in distinguishing cell types, and complex geometries can make counts unreliable and obscure genetic and environmental sources of variation. To overcome some of these technical problems, we have used an electron microscopic method to count retinal ganglion cells in mice. These cells are the sole projection neurons of the vertebrate retina. As we have shown in several studies, an unbiased census of the entire population can be obtained by counting axons in a single cross-section of the optic nerve (Chalupa et al., 1984; Lia et al., 1986; Williams et al., 1986; Rice et al., 1995a). Our main aim in this study is to provide a genetic dissection of variation for a single well-defined neuron population in a mammal. To get robust estimates of variation and its sources, we collected data from the following variety of mice: (1) different species and subspecies belonging to the genus *Mus*, (2) inbred and

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isogenic laboratory mice, (3) isogenic but heterozygous F_1 progeny generated by crossing inbred strains, and (4) groups of genetically heterogeneous mice that have a level of genetic variability closer to that found in natural populations.

MATERIALS AND METHODS

Tissue was taken from 252 mice belonging to 31 different strains (Fig. 1). Most animals were shipped directly from the Jackson Laboratory (Bar Harbor, ME). The male:female ratio of all cases was 0.9 but varied considerably among strains, from 1:5 in strain AKR/J to 5:1 in PLSJF1. The age of mice ranged from 21 to 329 d. Animals were fed a 6% fat National Institutes of Health 31 diet at the Jackson Laboratory and a 5% fat Agway Prolab 3000 rat and mouse chow at the University of Tennessee. Colonies were maintained at 20–24°C on a 14:10 hr light/dark cycle in a pathogen-free environment.

Standard inbred strains. Standard inbred laboratory strains of mice (Fig. 1, *yellow group*) are derived from domesticated hybrids generated from crosses between *Mus musculus domesticus* (also known as *M. domesticus*) and *M. musculus molossinus* (Bonhomme, 1992). The standard strains we studied have been inbred by successive sibling matings for >80 generations (Festing, 1993). Animals are therefore homozygous at essentially all loci. C57BL/6JAx1 is a provisional, nonstandard nomenclature we have introduced (see Results) to designate animals obtained from the Jackson Laboratory Annex 1 colony between 1994 and 1995.

Wild strains (Fig. 1, bottom panel*).* We studied the following four species of mice: (1) *M. musculus*, the common house mouse, a wide-ranging and highly adaptable commensal species from which laboratory mice are derived (Bronson, 1984); (2) *M. spretus* (SPRET/Ei), a short-tailed field mouse distributed around the western Mediterranean; (3) *M. spicilegus* (PANCEVO/Ei), a colonial mound-building species from the Balkans and Ukraine; and (4) *M. caroli* (CARL/ChGo), a small tropical East Asian species (Fig. 1, *bottom group*) (see Table 2). We also studied the following four subspecies of *M. musculus*: (1) *M. m. castaneus* (CAST/Ei and CASA/Rk), a South East Asian subspecies; (2) *M. m. musculus* (CZECHII/Ei), the commensal Eastern European and Asiatic mouse; (3) *M. m. molossinus* (MOLD/Rk), a Japanese hybrid subspecies; and (4) *M. m. domesticus* (WSB/Ei), the commensal and very widely dispersed subspecies of Western Europe and the Americas (Fig. 1, *bottom group*) (see Table 2). Seven of these wild strains have been inbred for >16 generations at the Jackson Laboratory. The exception is CARL/ChGo, an outbred wild sample of *Mus caroli* maintained since the mid-1970s as a colony of 5 to 10 breeding pairs with specific avoidance of sibling mating (V. Chapman, personal communication). The evolutionary relations and ecological characteristics of these species are reviewed in Bonhomme and Guénet (1989), Nowak (1991), and Bonhomme (1992).

Genetically heterogeneous mice. Two types of mice included in this study are genetically heterogeneous. One is referred to as CD-1 or ICR (Hsp:ICR). This strain has been bred for high fecundity and fitness (Eaton, 1980) and is maintained by random nonsibling matings at Harlan Sprague Dawley (Indianapolis, IN). The other is CARL/ChGo, described above in Wild strains.

Isogenic F_1 *hybrids.* We studied six sets of isogenic F_1 hybrids (Fig. 1, *light green*). Four of these were hybrids between a BALB/cJ parent and either an A/J, C57BL/6J, C57BL/6JAx1, or CAST/Ei parent. They are referred to as $CB6F_1$/J (a BALB/cJ female crossed to a C57BL/6J male), $B6AxCF_1$ (the cross between a C57BL/6JAx1 female and a BALB/cJ male), CAF_1 (BALB/cJ female by A/J male), and BCF_1 (BALB/cJ female by CAST/Ei male). We also examined the $PLSJF_1$, the progeny of a cross between PL/J and SJL/J. The final F_1 is called $32CASF_1$ and is from a cross between a female BXD32 and a male CAST/Ei. BXD32 was selected for this cross, because it has an extremely high ganglion cell number (75,700 ± 2200) (Williams et al., 1995). The CAF_1, $CB6F_1$, and $PLSJF_1$ were obtained from the Jackson Laboratory. The $B6AxCF_1$, BCF_1, and $32CASF_1$ progeny were generated in our colony. In this paper, we treat all of the F_1 hybrids and the fully inbred strains as isogenic.

Mutants. Several strains carry mutations that affect the retina. Five strains that we studied (C3H/HeJ, CD-1, PL/J, SJL/J, and MOLD/Rk) carry the retinal degeneration allele, *rd*, at the β-phosphodiesterase locus. These strains lose virtually all photoreceptors by 2 months of age. With the exception of MOLD/Rk, all *rd* strains have normal nerves, and several have high ganglion cell populations. Eight of the strains we used are albinos and have a reduced proportion of retinal ganglion cells with uncrossed projections (A/J, AKR/J, BALB/cBy, BALB/cJ, CD-1, 129/J, NZW/LacJ, SJL/J) (see Rice et al., 1995a). We have not found any correlation between pigmentation and total ganglion cell number.

Fixation and processing of tissue. Mice were anesthetized with Avertin (0.5–0.8 ml, i.p.) and were perfused transcardially with 0.9% saline followed by fixative. Approximately 15 ml of 1.25% glutaraldehyde and 1.0% paraformaldehyde in 0.1 M phosphate buffer was injected for 2–4 min. An additional 10 ml of double-strength fixative (2.5% glutaraldehyde and 2.0% paraformaldehyde in the same buffer) was injected for 1–2 min at an increased flow rate. The head was removed and put in fixative overnight and in 0.1 M phosphate buffer the following morning. Optic nerves were dissected and were subsequently osmicated and embedded in Spurr's resin. For most cases, the brains, including olfactory bulb, were dissected free, trimmed just behind the cerebellum, and weighed. Thin sections of either or both nerves were placed on Formvar-coated slot grids and stained with uranyl acetate and lead citrate. The nerves were examined and photographed on a JEOL EX2000II microscope using a systematic sampling protocol (Fig. 2*A*) (Deming, 1950).

Magnification was calibrated by photographing a grid replica (# 80051, 2160 lines/mm, EMS, Fort Washington, PA) at the conclusion of every photography session. The procedure was performed in the following sequence. (1) A set of 20 or more high-magnification micrographs were usually taken at 12,000× magnification (Fig. 2*C*) in a square lattice pattern. No adjustments in position were made with respect to blood vessels or glial cells. (2) The calibration grid was photographed at the same high magnification (Fig. 2*D*). (3) The calibration grid was rephotographed at a low magnification, usually 200 or 250× (Fig. 2*B*). (4) The thin section of the entire nerve was photographed at the same low power (Fig. 2*A*).

Counting. A counting frame (63 × 86 mm) was traced on the 75 × 100 mm negatives with a fine marker pen, and all axons within the frame and intersecting the upper and right edges were marked and counted on the negative using standard unbiased counting rules (Fig. 2*C*) (Gundersen, 1977). The typical sample area gave a count of 25 axons, and the typical set of samples from one session gave a total count of 500 axons. From 2 to 50% of axons in the adult mouse optic nerve are unmyelinated (higher percentages are found only in sections cut close to the lamina cribrosa). All negatives were counted on a light box with the aid of magnifying glasses (2.5× Optivisor, Donegan Optical). The effective magnification was therefore above 25,000×. All counts were double-checked. To estimate the total axon population, the average density of axons was multiplied by the area of the nerve cross-section. The SE of each estimate was computed from the variance of the set of micrograph counts. When two or more independent samples were obtained from adjacent sections, they often had surprisingly different sampling errors. These independent samples were weighted by the inverse of their variance; samples with low variance were given more weight (Bevington and Robinson, 1992). This was done using the equations:

$$\bar{x} = \frac{\sum_{i=1}^{n} (x_i/s_i^2)}{\sum_{i=1}^{n} (1/s_i^2)} \quad \text{and} \quad s^2 = \frac{1}{\sum_{i=1}^{n} (1/s_i^2)},$$

where $\bar{x}$ is the best estimate of the average, and s^2 is the cumulative estimate of the variance based on n independent samples, each with an average x_i and variance s_i^2.

Quantitative genetics. The variation of a complex trait such as neuron number is the sum of genetic and environmental variation (Roderick and Schlager, 1966; Falconer, 1989; Kearsey and Pooni, 1996). The genetic component V_g can be split into three parts: (1) additive gene effects (V_a, the heritable component); (2) dominance effects among alleles at single loci (V_d); and (3) epistatic interactions between alleles at unlinked loci (V_i). The environmental variance V_e can be divided into a component associated with external environmental factors (nutrition, temperature, health, etc.) and a component associated with internal differences in the cellular environment that is often called developmental noise (Waddington, 1957; Gavrilets and Hastings, 1994). There are also effects that arise from interactions between genetic and environmental factors (Wahlsten, 1989). These are especially important in natural populations that occupy a wide range of habitats (Bronson, 1984). But in a tightly controlled laboratory environment, interaction effects and covariances between genetic and nongenetic variables can be minimized and are usually ignored. Finally, much of the variation may be caused by technical and sampling

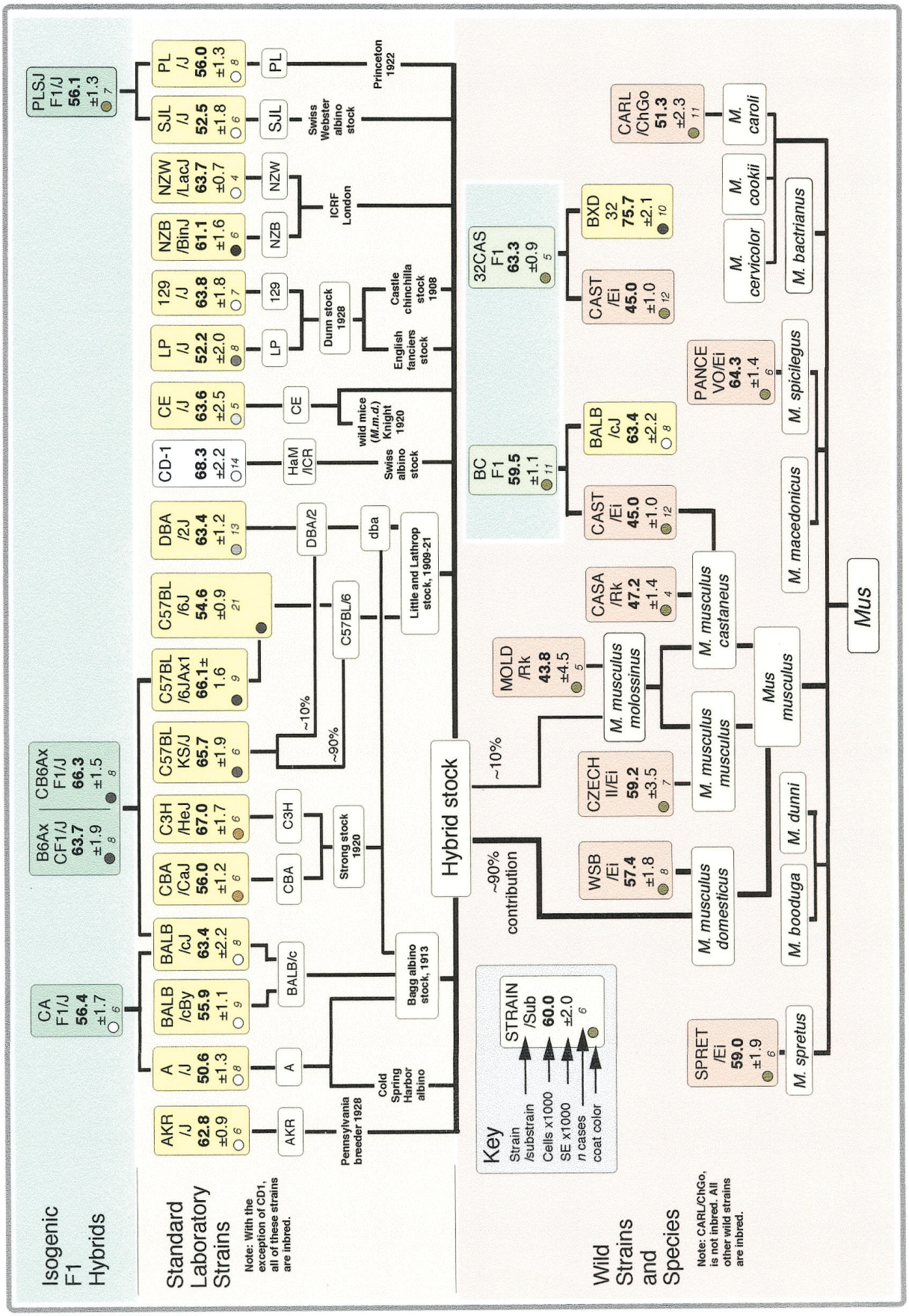

Figure 1. Lineage chart of strains, species, and subspecies of mice in relation to variation in retinal ganglion cell number. Major categories of mice are indicated on the *left* and are described briefly in Materials and Methods. Numbers associated with each strain are the average, SEM, and number of cases. For other features refer to the *Key*.

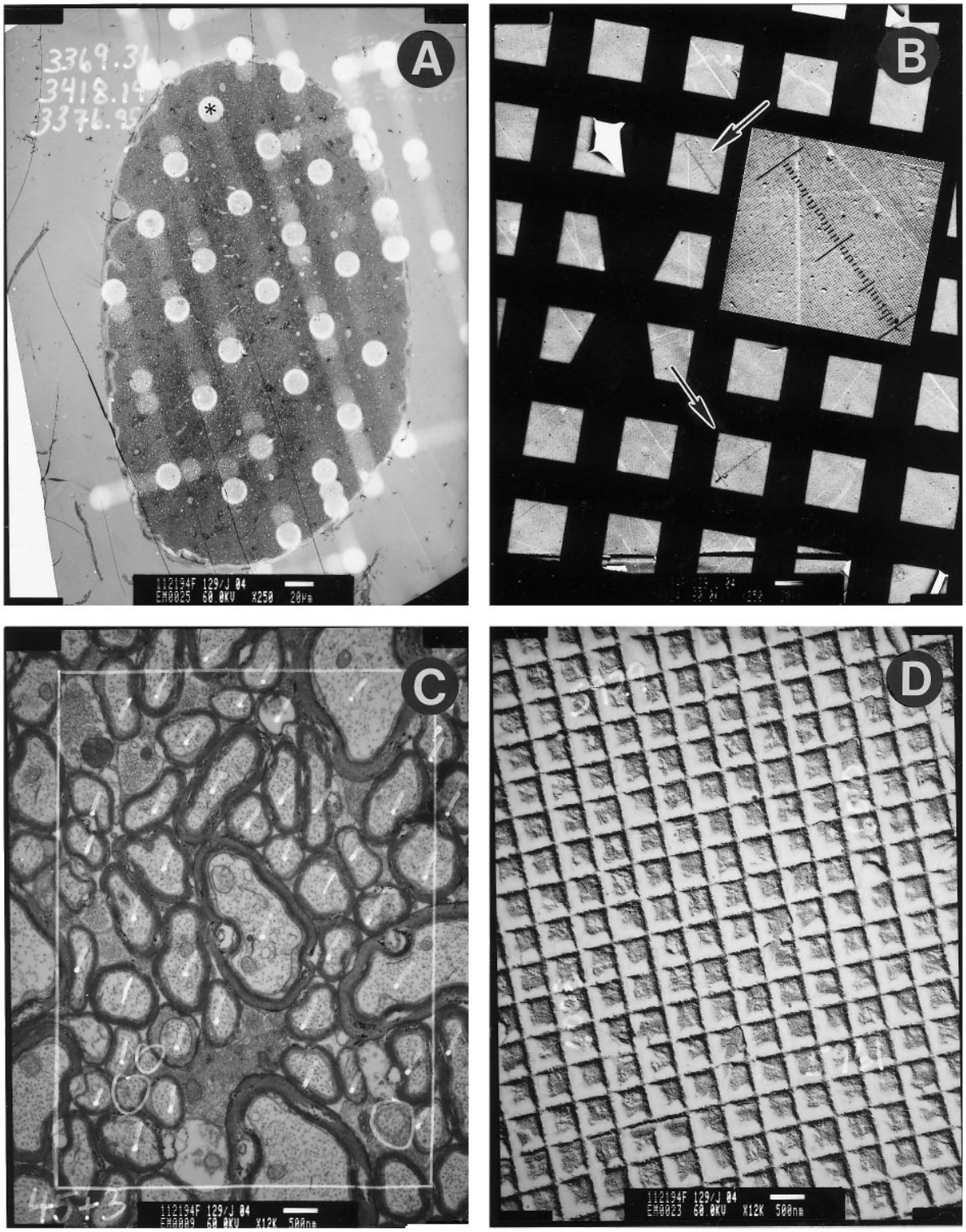

Figure 2. A set of four marked contact prints (1:1 reproduction) of negatives used to generate estimates of ganglion cell number. *A* and *B* are matched low-power electron micrographs of the ultrathin section (*A*) and the calibration grid (*B*). *C* and *D* are matched high-power micrographs of a sample site (see *asterisk* in *A*) and a high-magnification calibration. The series of *white spots* in *A* are regions bleached during the high-magnification sampling. The outline of the nerve was traced on the negative (*A*) under a dissecting microscope. The boundary was drawn across the outer rank of axons, even if that involved an occasional intrusion into the nerve. The area of the nerve was computed by tracing this boundary using a calibrated digitizing tablet two or more times (see faint numbers, *top left* in *A*). The *asterisk* in *A* marks the site illustrated at higher magnification in *C*. Two sites marked by *arrows* on the calibration negative (*B*) have been measured. The top site (*inset* in *B*) is illustrated at higher magnification. A series of 41 marks, spanning 80 grid units, were made with a small scalpel. The separation between endpoints was measured under a dissecting microscope with a digital caliper accurate to 10 μm. Distances on all calibration negatives were measured two or four times in the two orthogonal axes of the grid. *C* is one of the sample negatives that illustrates the counting frame (*faint white rectangle*) in which 45 myelinated and 3 unmyleinated fibers were counted. *D* is the calibration micrograph used to compute the sample area counted in *C*. The grid dimensions in *D* are 0.463 $\times$ 0.463 μm.

errors (V_t). In sum, the total phenotypic variance in neuron number, V_p, can be expressed as $V_p = V_a + V_d + V_i + V_e + V_t$.

In this study, we have generated estimates of V_p, V_a, V_e, V_t, and V_g (the sum of V_a, V_d, and V_i). Calculation of variance estimates follows methods outlined in Falconer (1989) and Stansfield (1991). V_p for the 17 inbred strains is the variance of the 136 cases listed in Table 3. The environmental variance, V_e, is equal to the average variance within isogenic and inbred strains, V_w, minus the technical variance, V_t. The variance terms in the equation, $V_e = V_w - V_t$, can be calculated directly, or they can be calculated as the squares of coefficients of variation. The latter method is often preferred, because it is insensitive to large differences in absolute cell number. V_g is equal to V_p minus all nongenetic variance ($V_e + V_t$). To calculate V_a, we first estimate the variance of the 17 strain averages, V_b. V_a equals $V_b - (V_e + V_t)/n$, where n is the harmonic average of cases per strain. V_t was estimated by replicating counts. Gene dominance, heterosis, and inbreeding depression were estimated from the F_1 heterozygotes and by comparing inbred and outbred populations (Falconer, 1989).

Heritability. We have used a method advanced by Hegmann and Possidente (1981) in which heritability in the narrow sense, h^2, is computed from data collected from a randomly selected set of inbred strains. The 17 inbred strains included in this study were chosen without reference to any preexisting information on ganglion cell population size, brain weight, or even body size. To compute heritability, we solved the equation $h^2 = 1/2(V_a)/[1/2(V_a) + V_e]$, in which V_a is the variance between inbred strains and V_e is the average environmental variance within isogenic stains. V_a is divided by 2, because the heterozygotes are eliminated during the process of inbreeding. This loss of the intermediate heterozygotes leads to a twofold increase in the additive genetic variance compared with the founder population. The variance and SD of the estimate of h^2 was computed using a jackknife procedure (Mosteller and Tukey, 1977). This involved calculating heritabilities for 17 subsets of data, each missing data from one strain ($h_{(-i)}$). The jackknife variance is

$$V_{JK}(h^2) = \frac{n-1}{n} \sum_{i=1}^{n} (h_{(-i)} - \bar{h}_{(\cdot)})^2,$$

where $\bar{h}_{(\cdot)}$ is the mean heritability of the 17 subsets.

An estimate of broad-sense heritability was computed by taking the ratio of the average variance within the two outbred groups of mice (CD-1 and CARL/ChGo) to the average variance, V_e, within all isogenic laboratory strains (Vogel and Motulsky, 1986; Crusio, 1992). As explained in Results, coefficients of variation were used to compute this value. Total genetic determination, ω^2, was also estimated using the formula:

$$\omega^2 = \frac{F-1}{F + (df_w + 1)/df_b},$$

where F is the F ratio and df_w and df_b are the degrees of freedom within and between inbred strains (Wahlsten, 1992).

Heritability estimates are based on ratios between genetic and nongenetic variance. Consequently, minimizing environmental variance increases measured heritability. In this study, all mice were reared in a pathogen-free laboratory environment, a situation that eliminates many environmental differences and almost certainly increases estimates of genetic control compared with dispersed populations of mice in the wild. We did include mice having a wide range of ages and both sexes and taken from different litters and different mothers within strains. Therefore, the samples of mice we studied will be representative of most laboratory colonies.

Reliability and accuracy of estimates. A set of 69 nerves were counted two to four times to estimate the cumulative error of our estimates of ganglion cell number. All of these replicate counts are listed in Table 1 within parentheses. Usually, an adjacent thin section was photographed and counted months to several years after the original sample, often by different personnel. The average of all first counts was 58,967 ± 1145, whereas that of second counts was 58,667 ± 1361. The test–retest reliability coefficient, r_{TX}, was 0.83. The mean difference between pairs of estimates was 4080, and the average SD of sets of replicates was 3116. SDs based on samples of two or three replicates underestimate population SDs by 25 and 13%, respectively (Sokal and Rohlf, 1981). The corrected technical SD is 3780. The technical coefficient of variation for individual samples of ~25 micrographs averaged 6.3 ± 0.5% SE. We estimate that 60–70% of all technical variance is associated with sampling error and the relatively small number of micrographs used to determine average axon density, whereas most of the remaining technical variance was attributable to calibration and measurement error.

Independent confirmation of count accuracy. Counts of peroxidase-labeled ganglion cells in 17 cases (for methods, see Rice et al., 1995a) confirm the accuracy of the electron microscopic method for estimating ganglion cell number. The average axon count for these cases was 57,474 ± 1766 SE; the estimates based on counting peroxidase-labeled ganglion cell bodies in these same cases averaged 55,850 ± 2007, an insignificant difference.

RESULTS

Neuron number, age, sex, and brain and body weight

Distribution of individual values

The ganglion cell population of mice averages 58,500 ± 500 SE (n = 252). The distribution is unimodal and has an SD of 7800 (Fig. 3). We have included a wide diversity of types of mice (Fig. 1) and for this reason, the distribution might have been expected to have extended tails. However, near normality extends over a range of ~4 SDs. There is a small but significant deficit in the expected number of cases with populations close to the average (Fig. 3, *asterisk*) that gives the distribution a slightly flattened shape compared with the expected Gaussian distribution. This deviation has a straightforward explanation; we sampled many homozygous mice (Table 1) that tend to have extreme phenotypes.

Age and the ganglion cell population

The average longevity of mice from different strains ranges from 300 to 850 d (Green and Witham, 1992). Our estimates were taken from mice averaging 85 d old but with a range extending from 21 to 329 d. The 82 youngest mice (<48 d old) had an average population of 59,124 ± 686 (SE), whereas the 84 oldest animals (>180 d old) had an average population of 58,545 ± 1027 (SE). Both of these groups contained a wide mix of strains. As expected from the insignificant difference between young and old groups, the correlation coefficient across all cases between neuron number and age was close to zero ($r = -0.08$, $n = 234$, the 95% confidence interval of r extends from −0.21 to +0.05).

Sex and the ganglion cell population

There are no detectible sex differences in retinal ganglion cell number. The average population for 133 females is 58,522 ± 674 SE (average age, 87 ± 6 d; body weight, 18 ± 1 gm; brain weight, 427 ± 6 mg), whereas that for 119 males is 58,503 ± 714 SE (average age, 82 ± 6 d; body weight, 20.7 ± 1 gm; brain weight, 431 ± 5 mg).

Brain and body weight and the ganglion cell population

Are differences in ganglion cell numbers among mice associated with differences in brain weight? The correlation between neuron number and brain weight across all individual cases for which both parameters were measured is 0.34 (n = 176, 95% confidence interval of r extends from 0.20 to 0.46). The corresponding correlation between neuron number and body weight is only 0.17 (range of r from +0.02 to +0.31). Correlations between brain weight and ganglion cell number are somewhat higher when strain averages are analyzed. The correlation is +0.56 for the group of 7 species and subspecies in Table 2 and +0.59 for the group of 17 standard inbred strains in Table 3. Correlations between neuron number and body weight are +0.25 (wild strains) and +0.45 (standard strains). An important conclusion from this analysis is that ~30% of the variance in ganglion cell number may be associated, directly or indirectly, with differences in brain weight.

Table 1. Individual estimates of ganglion cell number with replication[a]

Key. All values are in thousands. The data are organized by strain from lowest case to highest case as follows:

Strain case 1 ± SE (first estimate for case 1 ± SE, second estimate for case 1 ± SE), case 2 ± SE, ...

Standard Inbred Strains	
129/J	57.2±1.8 (57.3±2.1, 57.0±3.2), 59.3±2.8, 62.8±3.1, 65.7±5.9, 66.3±2.5, 66.7±4.7, 68.4±2.4 (68.2±3.3, 68.7±3.4)
A/J	44.9 ±1.7 (46.3±2.1, 42.3±2.8), 49.3±2.8, 49.5±2.6, 49.8±3.1, 50.5±2.8, 51.3±2.3 (49.0±2.6, 46.6±5.7, 56.7±3.3), 52.9±2.2 (56.4±4.9, 52.0±2.5), 56.7±2.0 (55.5±2.8, 58.0±2.9)
AKR/J	60.1 ±1.9 (62.7±4.6, 60.0±2.1, 58.4±3.5, 60.3±3.1), 61.1±2.3 (65.1±4.4, 59.3±2.8, 61.4±4.3), 62.5±4.7, 63.2±2.3 (66.1±4.2, 63.8±2.8, 62.1 ±2.3), 64.7±4.5, 65.1±2.8 (69.0±4.5, 65.9±3.5, 61.3±3.9)
BALB/cBy	51.3±2.0 (49.2±3.4, 52.3±2.4), 52.4±4.0, 54.4±2.4, 54.4±2.1 (57.4±7.0, 51.3±2.2), 55.0±3.0, 56.6±3.8, 57.7±2.6 (63.9±3.8, 51.7±3.7), 59.8±4.0 (64.7±6.8,57.3±4.9), 61.0±2.2 (57.7±5.0, 61.8±2.5)
BALB/cJ	53.3±2.7 (53.8±3.3, 52.9±5.1, 53.0±4.1), 58.7±3.1, 58.8±6.2, 63.5±2.4 (57.2±3.2, 60.3±3.8, 68.6±4.3), 67.5±3.2, 68.1±3.0, 68.4±2.6 (69.3±3.3, 67.0±4.2), 68.8±4.6
C3H/HeJ	60.8±2.4 (59.1±3.2, 60.9±3.7, 64.1±4.4), 65.6±8.0, 66.8±4.1, 68.3±4.7, 69.5±4.7, 71.2±3.0 (71.3±4.3, 71.1±4.2)
C57BL/6JAx1	58.6±2.9 (59.8±4.3, 57.6±4.0), 59.3±3.4, 64.2±5.0, 66.9±2.7 (67.5±3.7, 66.4±3.8), 67.2±3.5, 67.8±3.7, 69.4±3.2 (72.9±5.5, 67.6±3.9), 69.5±3.4, 71.8±5.0
C57BL/6J	47.1±1.9 (48.4±3.3, 46.3±2.4), 49.0±4.2, 49.4±3.4, 49.8±3.6, 50.1±3.1 (47.8±4.9, 51.5±3.9), 51.1±3.5, 52.8±3.3, 53.6±4.2, 54.3±2.4 (56.6±4.3, 53.2±2.9), 54.9±2.8, 55.2±5.1, 56.0±3.2, 56.8±2.5 (64.7±3.8, 50.4±3.4), 57.3±5.6, 57.5±3.6, 58.1±3.1, 58.2±4.4, 58.2±4.3, 58.6±3.3 (65.7±5.5, 54.6±4.1), 59.0±3.8, 60.7±3.6
C57BLKS/J	62.0±3.8, 62.2±3.2 (58.6±4.7, 65.2±4.3), 63.9±2.8 (65.2±3.6, 61.9±4.5), 66.0±3.4, 67.3±4.7, 72.6±3.5 (75.5±4.6, 68.7±5.4)
CBA/CaJ	52.8±2.6 (47.7±4.0, 56.2±3.3), 54.2±3.2 (48.2±4.5, 60.6±4.6), 55.7±3.1, 56.3±3.1, 56.9±4.6, 60.3±3.2, (62.4±4.9, 58.6±4.3)
CE/J	55.2±2.4 (59.2±4.4, 57.5±2.8, 52.5±4.9), 64.7±4.0, 64.8±3.7, 66.3±2.2 (68.9±3.9, 68.9±4.1, 63.7±3.4), 66.9±3.4 (63.0±4.6, 71.7±5.1)
DBA/2J	57.6±2.5 (56.8±3.3, 58.7±3.7), 57.9±4.0, 59.0±3.7, 60.0±4.7, 61.7±2.2 (61.0±2.6, 63.8±4.4), 62.0±4.2, 63.0±3.8, 64.7±3.4, 65.5±3.5 (68.7±4.7, 61.4±5.2), 66.0±5.8, 66.9±3.9 (64.3±4.8, 71.5±6.4), 69.4±4.8, 69.8±2.7
LP/J	46.2±2.6, 46.2±2.8, 50.2±3.6, 50.4±2.6, 51.7±3.7, 55.3±3.5, 58.8±3.3, 59.1±4.8 (54.7±6.8, 63.5±6.9)
NZB/BinJ	54.9±2.1 (56.1±2.5, 51.8±4.0), 60.4±3.1 (65.4±4.1, 55.5±4.7), 60.7±2.3 (55.3±3.0, 59.3±3.5, 64.3±4.4), 62.5±5.1, 64.0±3.9, 64.0±2.2 (61.4±2.4, 66.6±4.5)
NZW/LacJ	62.6±2.3 (67.8±5.3, 61.4±2.6), 62.9±3.6, 64.2±3.6, 65.1±2.3 (62.1±3.1, 68.9±3.5),
PL/J	50.5±2.0 (52.7±3.1, 49.4±3.6, 49.7±3.6), 52.5±3.4, 54.6±3.6, 56.2±2.1 (55.3±2.6, 57.9±3.5), 56.8±4.2, 57.2±1.8 (63.7±3.4, 62.7±3.4, 51.2±2.7), 59.6±4.4, 60.3±3.1
SJL/J	45.6±3.2, 51.8±3.0, 52.4±2.9, 53.9±2.7, 54.7±4.2, 56.4±3.7
Wild species of mice	
CASA/Rk	45.1±2.3, 45.5±2.9, 48.9±3.6, 49.3±2.4 (56.2±3.6, 43.2±3.4)
CAST/Ei	40.2±2.9, 41.3±3.3, 41.6±3.1, 43.2±4.7, 44.0±2.6, 44.5±4.1, 44.9±4.4, 44.9±3.2, 46.3±4.3, 49.4±3.5, 50.0±4.8, 50.2±3.4
CZECHII/Ei	49.3±3.6, 54.0±4.0, 54.4±3.8, 56.8±5.6, 61.0±3.4, 65.3±3.6, 73.6±3.7
MOLD/Rk	32.3±2.2 (35.5±3.0, 32.1±3.4, 31.5±1.6), 37.7±1.8 (40.3±2.5, 34.9±2.5), 47.2±2.3, 49.2±3.1, 52.4±3.2
PANCEVO/Ei	61.3±3.4, 61.5±7.4, 62.3±6.4, 65.7±4.5, 67.3±5.8, 67.7±5.3
SPRET/Ei	53.3±3.8, 55.8±2.5 (54.6±2.7, 62.0±6.3), 57.6±3.5, 58.2±3.2, 61.8±2.1 (63.2±3.1, 60.6±3.0), 64.4±3.6
WSB/Ei	50.8±2.7, 51.2±4.1, 56.9±3.3, 57.1±5.0, 58.6±4.7, 59.1±5.1, 62.3±3.9, 63.0±2.9
F_1 hybrids	
32CASTF$_1$	60.6±3.7, 63.0±5.3, 63.5±2.8, 63.9±4.9, 65.3±7.0
B6AxCF$_1$	56.7±2.4 (57.4±4.8, 56.5±2.8), 59.3±2.7 (59.0±3.0, 60.3±6.1), 59.7±3.6, 63.6±4.4, 64.9±3.0, 66.5±3.2 (76.1±4.8, 58.2±4.5), 69.5±5.2 (69.6±7.0, 69.3±7.7), 70.8±4.5 (61.0±8.7, 74.3±5.2)
BCF$_1$	55.2±3.1 (54.5±5.8, 55.6±3.7), 53.3±4.9, 56.0±5.2, 56.1±4.0, 57.8±5.6, 60.7±3.5, 61.2±4.3 (60.5±6.1, 62.0±6.1), 61.7±4.3, 61.8±4.7, 63.7±3.1 (68.5±5.4, 61.3±3.7), 64.4±3.3
CAF$_1$	52.3±2.9, 53.5±2.4 (48.4±3.0, 58.6±4.2), 55.6±3.1, 56.4±3.8, 58.2±3.6, 62.6±3.2 (62.8±4.2 62.4±5.0)
CB6F$_1$	61.1±2.8 (63.4±4.2, 59.3±3.6), 65.4±3.8, 66.0±2.8 (61.1±4.3, 69.5±3.7), 66.2±3.7, 69.0±3.9, 70.1±2.6 (71.2±3.7, 68.9±3.7)
PLSJF$_1$/J	50.0±2.8 (50.0±4.0, 50.1±4.0), 54.0±3.1, 56.1±2.5 (51.0±3.3, 63.2±3.9), 57.5±2.5, 57.9±3.4, 58.2±2.9, 59.0±4.1
Outbred Strains	
CARL/ChGo	39.5±1.6 (38.9±1.8, 41.9±3.5), 43.5±1.3 (43.6±1.4, 43.5±2.9), 46.7±3.5, 46.9±3.6, 47.3±2.3 (45.8±2.8, 50.4±4.0), 52.5±4.4, 54.8±3.6, 54.9±2.5, 57.5±2.9, 57.5±4.2, 62.8±2.1 (64.1±2.5, 59.6±3.9)
CD-1	57.2±2.7 (59.9±3.6, 53.6±4.2), 61.8±2.6 (58.6±3.5, 65.3±3.8), 62.4±2.5, 62.6±4.5, 64.9±3.0, 65.4±2.8, 65.7±3.2, 65.9±3.1, 69.1±2.4, 69.2±2.4, 73.6±3.7, 74.0±2.7 (74.5±3.1, 74.8±5.7, 73.6±2.4), 77.7±2.2, 87.2±2.5 (88.8±3.3, 85.2±3.8)

[a]Data on sex, brain and body weights of these mice are available at http://mickey.utmem.edu/neuron.html. Cases that were replicated were not chosen randomly but often represented the highest and lowest cases in each strain or those with unusually high sampling error (Table 1). This nonrandom selection could inflate estimates of technical error. A separate comparison with other cases that were selected randomly for replication (data not shown) demonstrates that any such bias is negligible.

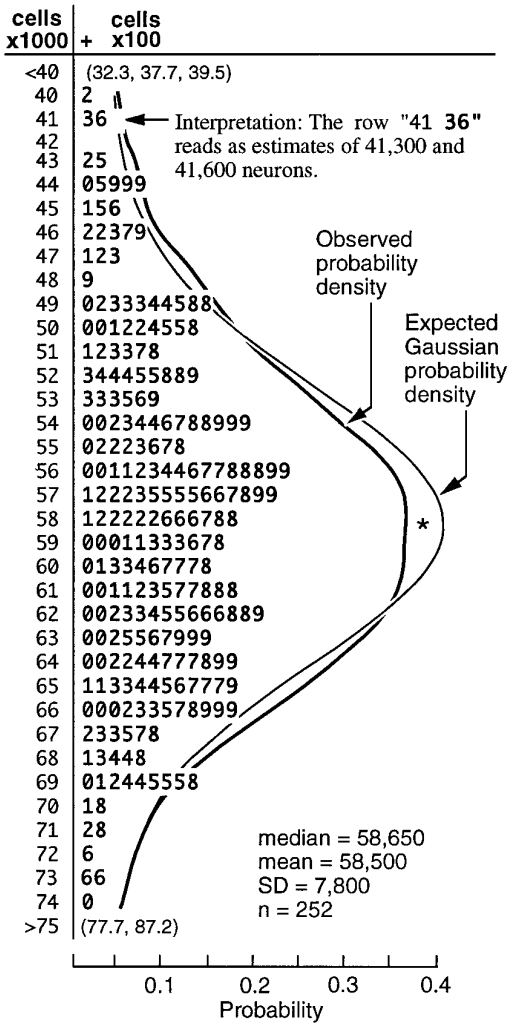

Figure 3. Distribution of individual counts. In this stem and leaf display, each of 252 cases is encoded as a single digit. The figure can be read as a vertical histogram with bins of 1000 cells and bars made up of rows of digits. The *bold black curve* is the observed probability density calculated from the sum of the 252 individual Gaussian probability functions. In contrast, the predicted Gaussian probability density (*fine line*) is based on the sample average and SD of 58,500 ± 7790. The median is 58,650, and the quartiles are at ~52,500 and 64,200. The *asterisk* highlights the deficit of expected cases close to the mean. Values <40,000 and >75,000 are enclosed within parentheses. Excluding the 23 animals that do not belong to the *M. musculus* complex (Fig. 1) (CARL/Go, SPRET/Ei, and PANCEVO/Ei) does not alter the distribution in any significant way.

Table 2. Ganglion cell population size in wild strains

Type[a]	Species	Mean[b]	SE[c]	SD[c]	*n*
CASA/Rk	*M. m. castaneus*	47,205	±1380	±2390	4
CAST/Ei	*M. m. castaneus*	45,047	±1040	±3451	12
CZECHII/Ei	*M. m. musculus*	59,207	±3482	±8530	7
MOLD/Rk*	*M. m. molossinus*	43,758	±4476	±8952	5
WSB/Ei	*M. m. domesticus*	57,380	±1759	±4655	8
PANCEVO/Ei	*M. spicilegus*	64,300	±1383	±3093	6
SPRET/Ei	*M. spretus*	59,049	±1896	±4240	6
CARL/ChGo	*M. caroli*	51,263	±2270	±7177	11

[a]All types are inbred, with the exception of CARL/ChGo, an outcrossed strain.

[b]The last two or three digits in strain estimates are not significant and are given only to minimize rounding errors in any subsequent analysis.

[c]SEs, SDs, and coefficients of variation throughout this paper are corrected for bias because of the small sample size (Sokal and Rohlf, 1981).

*The low value for *M. molossinus* is suspect because of the high incidence of necrotic axons in the optic nerve of the MOLD/Rk inbred strain. Estimates from the two youngest MOLD/Rk cases (49,200 and 47,200) are probably more representative.

Table 3. Ganglion cell population size in homozygous inbred laboratory strains

Type	Mean	SE	SD	*n*
129/J	63,772	±1771	±4339	7
A/J	50,615	±1319	±3490	8
AKR/J	62,788	±935	±2091	6
BALB/cBy	55,859	±1178	±3331	9
BALB/cJ	63,393	±2290	±6058	8
C3H/HeJ*	67,029	±1696	±3793	6
C57BL/6**	54,630	±874	±3910	21
C57BL / 6JAx1	66,082	±1655	±4684	9
C57BLKS/J	65,667	±1886	±4217	6
CBA/CaJ	56,028	±1203	±2691	6
CE/J	63,593	±2536	±5072	5
DBA/2J	63,351	±1208	±4186	13
LP/J	52,225	±1989	±5262	8
NZB/BinJ	61,063	±1600	±3579	6
NZW/LacJ	63,711	±727	±1259	4
PL/J	55,976	±1309	±3462	8
SJL/J	52,473	±1770	±3958	6
Averages	59,897	±1526	±3846	8

*Pooled data from 3 C3H/HeJ and 3 C3H/HeSnJ mice.

**Pooled data from pigmented and coisogenic albino mice.

Relationship between brain weight and neuron number within isogenic strains

The correlations calculated above are strongly affected by the genetic differences among mice. This genetic component can be eliminated by examining correlations within isogenic strains. Differences in environmental factors, particularly differences in the quality of maternal care, might be expected to produce common effects on brain weight and neuron number even within a single inbred strain. To perform this analysis across all isogenic cases, we computed individual *z* scores for brain weight and cell number using strain averages and SDs. This effectively eliminates genetic sources of correlation between these variables, leaving only the environmental factors to produce a correlation. The resulting correlation between normalized scores of brain weight and neuron number is very close to zero ($r = 0.017$, $n = 178$). This demonstrates convincingly that the environmental factors to which laboratory-reared mice are exposed do not have common effects on retinal ganglion cell number and brain weight. The moderate positive correlation between brain weight and neuron number noted above when strains are compared is therefore attributable to genetic factors that affect both parameters.

Survey of differences among species, subspecies, and strains of mice

Variation among species and subspecies

We examined animals belonging to four different species of the subgenus *Mus* (Nowak, 1991; Bonhomme, 1992). The ganglion

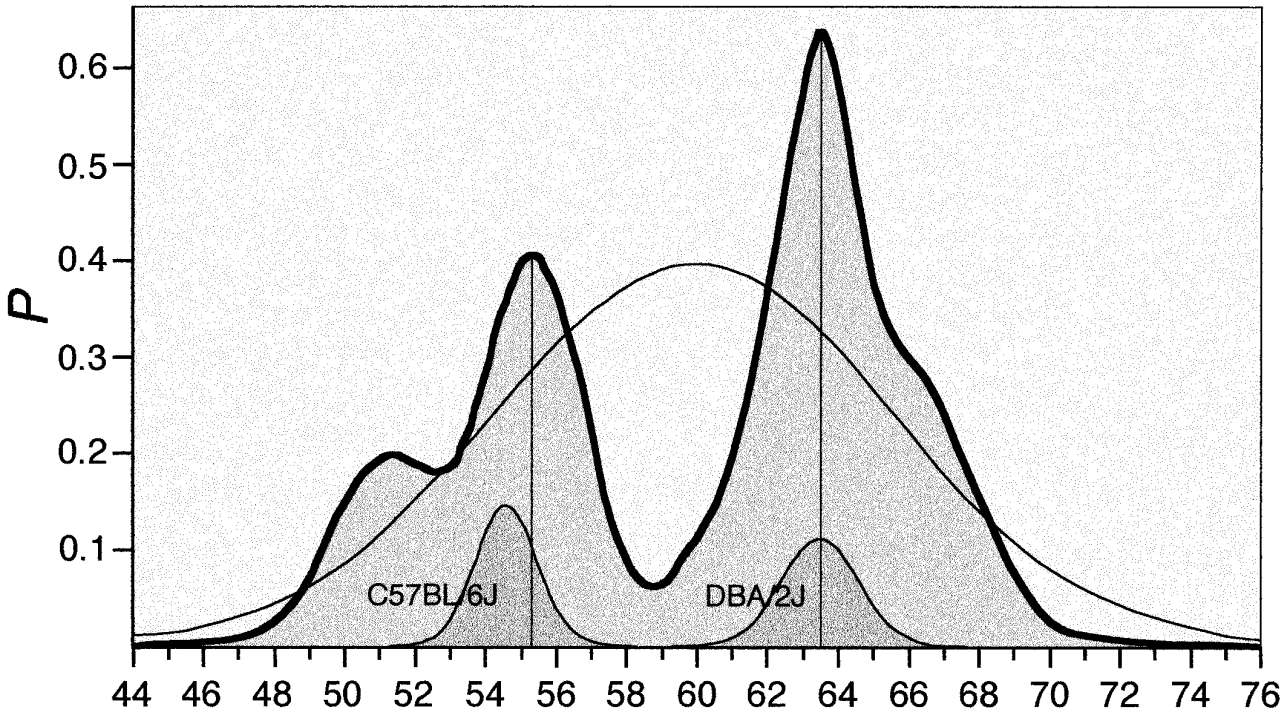

Figure 4. Bimodal distribution of the ganglion cell numbers among 17 inbred strains. The Gaussian probability density of the sample mean was computed at 500 cell intervals for each strain listed in Table 3. Two examples of these functions are shown (strains C57BL/6J and DBA/2J). The 17 functions were summed to obtain a cumulative probability density. The modes are at ~55,500 and 63,500. The expected normal distribution based on the 17 strain averages is also illustrated as a *light line* with an average of 59,900 and an SD of 5400.

cell population across this diverse group has a range from 45,000 to 65,000 (Tables 1, 2, Fig. 1, *bottom group*). We also examined several subspecies of *M. musculus* that are known to have contributed to the genome of the common laboratory mouse (Fig. 1, Tables 1, 2). These wild inbred *M. musculus* strains have averages that range between 45,000 and 60,000 cells. An ANOVA demonstrates a highly significant difference among species and subspecies ($F_{(6,41)} = 16.9$, $p < 0.0001$). With the exception of CARL/ChGo, all of the animals we have studied are fully inbred. Consequently, the differences in cell numbers among these inbred strains are probably greater than that among heterozygous samples taken from wild populations.

Variation among standard laboratory strains

Estimates of ganglion cell number in the standard inbred strains range from 50,600 in A/J to 67,000 in C3H/HeJ (Tables 1, 3, Fig. 1). The variance among strains is much greater than that within strains ($F_{(16,119)} = 15.0$, $p < 0.0001$). The inbred strains are in many cases closely related by descent. Yet we find that even closely related strains can have large differences in ganglion cell number (Fig. 1, Table 3). For example, strains 129/J and LP/J originated from a common ancestor in the mid-1920s, but their mean populations now differ by 11,500 cells or ~20% (Scheffé $t = 4.75$, $p < 0.05$ two-tailed for six comparisons). A nearly equal difference of 11,000 cells exists between the closely related strains C3H/HeJ and CBA/CaJ (Scheffé $t = 6.22$, $p < 0.05$).

We also discovered a remarkable difference of ~11,000 cells between groups of C57BL/6 mice (Table 3). The initial 10 animals received from the Jackson Laboratory before the summer of 1994 included 6 standard pigmented C57BL/6J animals (4 females, 2 males) and 4 coisogenic c^{2J} albinos (2 females, 2 males). These two subsets gave averages of 53,800 ± 2000 and 52,800 ± 2600, respectively (see Rice et al., 1995a and Table 1), that are close to the previous estimate of 56,700 ± 3200 obtained by Williams and colleagues (1990) using similar methods. However, C57BL/6J mice obtained from the Annex 1 production colony of the Jackson Laboratory in three separate shipments in the second half of 1994 gave estimates averaging 66,100 ± 1600 (five females, four males) (Table 1). This is far above the average for the first 10 cases ($t = 6.29$, $p < 0.05$). More recent estimates of C57BL/6J animals obtained from several different Jackson Laboratory colonies match the initial low number phenotype (56,026 ± 2928, $n = 6$). Brain weights in the high and low groups do not differ appreciably, 459 ± 5.3 mg for Annex 1 cases versus 471 ± 4.6 mg for the other C57BL/6J mice. We have not identified any nongenetic factors that could have caused this difference. The difference is possibly attributable to the fixation of a mutation or reversion in the Annex 1 colony.

Bimodality of strain averages

The average number of neurons across the 17 homozygous strains listed in Table 3 is 59,900 ± 3700 SD, but this mean corresponds to a central gap in the distribution. No strains have an average between 56,500 and 61,000 (Table 3, Fig. 4). This suggests that the underlying distribution of strain averages is not normally distributed but has two major modes. Bimodality is shown most clearly by adding up the probability densities of the individual strain averages using the function:

$$P(x) = \frac{1}{n}\sum_{i=1}^{n}\frac{1}{s_i\sqrt{2\pi}}\exp\left[-\frac{(x-\bar{X}_i)^2}{2s_i^2}\right],$$

where $P(x)$ is the normalized probability for a particular count x; $\bar{X}_i$ is the average of the ith strain and s_i is the SE of $\bar{X}_i$. In essence,

the resulting cumulative probability density from $x = 44{,}000$–76,000 illustrated in Figure 4 is a histogram in which Gaussian functions rather than single values have been summed. Seven of the 17 inbred strains have populations that range between 50,500 and 56,500. They make up a low-phenotype group that has a mode just above 55,000. Among these low strains, the 95% confidence interval extends up to a maximum of 58,900. A second sharply resolved group is made up of 10 strains, all of which have means between 61,000 and 67,500. In none of these high strains does the lower limit of the 95% confidence interval extend below 57,000. Inflections and shoulders on the two major peaks are probably attributable to sampling error. A Kolmogorov–Smirnov goodness-of-fit test confirms that it is highly unlikely that the population of inbred strains is normally distributed ($D = 0.245$, $p < 0.01$).

Environmental and developmental variation in neuron number

In this section, we show that approximately half of the recorded variation in neuron number within isogenic strains has genuine biological causes. The other half is attributable to technical errors. To estimate technical error, we replicated 69 counts and determined that the SD of counts from single nerves averaged 3780. If we had sampled six adjacent sections from a single nerve, the variation between estimates would still have amounted to ~6.3%. Variation within isogenic strains before replication was 7.2%. To get a realistic estimate of nongenetic environmental effects on the ganglion cell population, we subtracted the technical variance from the total variance before replication. For the inbred strains listed in Table 3, the average corrected environmental coefficient of variation is ~3.6 ± 0.4%.

Coefficients of variation for isogenic strains range from ~2.0% in NZB/LacJ to ~10% in BALB/cJ and LP/J. With an average sample of 8 cases per strain, much of this variation in coefficients is attributable to sampling error, and despite the wide range in coefficients, we have not been able to prove that there is significant nonuniformity of variance across inbred strains (Bartlett's χ^2 (16) = 15.3, $p = 0.5$).

Heritability and gene dominance

Estimates of additive genetic control, h^2

A comparison of the level of variance within and between groups of inbred strains can provide an estimate of the strength of additive genetic control. This parameter is also known as heritability in the narrow sense. The average variance within isogenic laboratory strains, V_w, is 13.7 (variance units are $\times 10^6$ cells^2). When the average technical error, V_t, is subtracted, the average environmental variance, V_e, is reduced to 4.65. In comparison, the additive genetic variance, V_a, computed across strains is 27.9. From these values, we estimate that narrow-sense heritability, h^2, is ~0.76. The jackknife subsamples of h^2 range from 0.73 to 0.77 with an average of 0.76 and an SE of 0.04.

Estimate of broad-sense heritability

The average coefficient of variation in isogenic laboratory mice, CV_i, averages 6.3% ($n = 23$, F_1 and standard inbred strains only); a value that includes both environmental and technical variance. In comparison, the corresponding coefficient, CV_h, in the two groups of genetically heterogeneous mice is much higher, ~12.8% (Table 4). The pronounced difference between isogenic and outbred mice is attributable to genetic factors in a broad sense, including not only the additive component, but also dominance interactions between alleles and epistatic interactions between different genes. Squaring the coefficients of variation of the isogenic and the outbred mice provides values that can be used to estimate the cumulative importance of genetic sources of variance $CV_g^2 = CV_o^2 - CV_i^2$, where CV_g is the coefficient of variation associated with all genetic factors included, and CV_o and CV_i are the coefficients for outbred and isogenic strains, respectively. Heritability in the broad sense is defined as the ratio V_g/V_p (Materials and Methods). This is equivalent to CV_g^2/CV_h^2. From this ratio, we estimate that the fraction of variation that is attributable to genetic factors is ~0.76 (124/163). When the correction is made for technical variance, this estimate increases to ~0.88. The ω^2 estimate of total genetic determination is 0.64. This estimate is generic in the sense that it does not depend on any specific genetic models or assumptions (Wahlsten, 1992). However, the calculation of ω^2 does not account for technical error and therefore underestimates total genetic determination.

Table 4. Ganglion cell population size in genetically heterogeneous groups of mice

Type	Mean	SE	SD	CV%	*n*
CARL/ChGo	51,263	±2270	±7177	14.0	11
CD-1	68,338	±2183	±7869	11.5	14

F_1 *heterosis and inbreeding depression*

To assess the effects of inbreeding and the magnitude of gene dominance effects on neuron number, we compared the population size and its variation between inbred strains and six sets of F_1 hybrids. Each set of hybrids is isogenic (Falconer, 1989), but because these F_1s are generated by crossing very different strains, they have an especially high level of heterozygosity. This often results in quantitative increases in fitness traits compared with the inbred parental strains (Wright, 1978). The crosses listed in Table 5 include low–low, low–high, and high–high parental strain pairs. The ganglion cell population in F_1 hybrids was on average higher (+2600 cells) than the midpoint between parental strains. Large deviations from the midpoints (+7300 and +5200) were noted in two crosses (Table 5). Maternal effects may contribute to some of this apparent dominance. In general, the F_1 results are consistent with a largely additive model of gene action with moderate gene dominance. The level of within-group variation seen in the inbred mice and the isogenic F_1s does not differ significantly (Table 3 vs Table 5) (6.46 ± 0.49% vs 5.77 ± 0.78%). An interesting conclusion from this analysis is that unlike many major fitness traits (cf Waddington, 1957; Leamy, 1982; Wayne et al., 1986), there is no evidence that neuron number in homozygous mice is more easily disturbed by developmental or environmental factors than it is in F_1 heterozygotes.

Traits that are important in fitness tend to have low heritabilities and high levels of directional dominance (Hahn and Haber, 1978; Wright, 1978; Barton and Turelli, 1989). In contrast, traits that are only weakly selected display greater additive genetic variation and comparatively high heritability (Mousseau and Roff, 1987; Roff and Mousseau, 1987). From this perspective, the relatively high heritability of variation in ganglion cell number and the modest directional dominance suggest that the population size does not materially affect the fitness of mice and that polymorphisms having large phenotypic effects have been allowed to accumulate in the population. Given the high variance even in the wild noninbred group of *M. caroli* mice (Table 4), this characteristic is probably not attributable to relaxed selection associated

Table 5. Ganglion cell population size in F_1 hybrids and their maternal and paternal strains

F_1 type	Mean	SE	SD	*n*	Maternal strains		Paternal strains		Mid	Δ
BCF1	59,454	±1117	±3532	11	BALB/cJ	63,393	CAST/Ei	45,047	54,439	5234
32CASF1	63,253	±916	±1832	5	BXD32	75,727	CAST/Ei	45,047	60,387	2866
CAF1/J	56,426	±1736	±3882	6	BALB/cJ	63,393	A/J	50,615	57,004	−578
B6AxCF1/J	63,731	±1944	±5143	8	C57BL/6JAx1	66,082	BALB/cJ	63,393	64,738	−1007
CB6F1/J*	66,293	±1487	±3326	6	BALB/cJ	63,393	C57BL/6J	54,630	59,011	7281*
PLSJF1/J	56,096	±1333	±3266	7	PL/J	55,976	SJL/J	52,473	54,225	1871
Averages	60,875	±1422	±3497	7	Averages	64,661	Averages	51,868	58,264	2611

Mid is the midpoint between maternal and paternal strain averages. Δ is the mean deviation between the midpoint and the F_1 hybrid average.
*The C57BL/6J animals used to generate these F_1s at the Jackson Laboratory may actually have been C57BL/6JAx1 animals. If so, then the dominance deviation in this cross will be much lower than the value given here.

with 400 or more generations of laboratory breeding. Although it would be useful to confirm this by additional analysis of wild populations, this idea is also consistent with the seminocturnal niche of most mice and the reliance that they presumably place on nonvisual sensory modalities (Fuller and Wimer, 1966).

DISCUSSION

Environmental variation and the precision of genetic control in isogenic mammals

An analysis of isogenic animals makes it possible to assess the constancy with which the genome can guide the generation of traits such as neuron number. We find that the coefficient of variation in ganglion cell numbers within inbred strains of mice averages ~3.6%. In a strain with an average of 60,000 cells, 10 animals would typically have a range from 56,600 to 63,400 if all technical error could be eliminated. The level of variation in normal outbred mice is substantially higher with a coefficient of variation close to 12.5%. Ten of these animals will generally have a range that is three to four times greater than that of isogenic strains. This comparatively high coefficient of variation is in the range of many other quantitative traits in noninbred mammalian populations (Yablokov, 1974). For example, the coefficients of variation for brain volume in humans is ~9%. Variation for single cytoarchitectonic areas can be much higher, reaching 20–40% in human striate and extrastriate cortex (Gilissen and Zilles, 1996).

The level of variation we have measured is not a reliable estimate for other populations of neurons in mice or other mammals. There are unpredictable differences in the range of phenotypes that single genotypes can generate. For example, the inbred strains BALB/cJ and LP/J have levels of nongenetic variation that reach 10%, even in a very stable environment. An even more convincing example is the belly spot and tail (*Bst*) mutation in mice (Rice et al., 1995b), in which the entire ganglion cell population is often eliminated on just one side. This asymmetry illustrates how important epigenetic developmental factors can be in determining neuron number. The following two conclusions can be drawn: (1) variation itself is variable, and (2) the magnitude of nongenetic variation is under partial genetic control (Scheiner and Lyman, 1989).

Developmental noise

To what extent is the variation noted among isogenic mice attributable to developmental noise in cell production and cell death, and to what extent is this variation attributable to environmental factors such as litter size, maternal care, food, climate, and disease? We favor the idea that this variation is primarily attributable to developmental noise. First, many major sources of environmental variance have been eliminated by rearing mice in a uniform pathogen-free environment. Second, two major residual factors that we allowed to vary, age and sex, do not have any detectible effects on ganglion cell number. Third, the lack of a correlation between neuron number and brain weight within isogenic cases ($r = 0.017$) rules out nutritive or maternal factors that would be expected to have widespread effects. The term *developmental noise* has a negative connotation, and it may be just as valid to consider variation within isogenic mice an adaptive trait that enables single genotypes to exploit a wider range of habitats (Williams, 1992; Scheiner, 1993). With the baseline counts we have generated for numerous inbred strains, it might now be worthwhile to determine just how much change can be evoked by altering the maternal and early postnatal environment.

The significance of genetic variation

The only previous estimate of heritability for neuron number is that for granule cells in the mouse dentate gyrus. Wimer and Wimer (1989) used a sophisticated test cross between two inbred strains and found that ~80% of the variance in this population was generated by autosomal genetic differences. Our estimate of total genetic determination for the ganglion cell population of ~88% is quite close. However, given the vastly different roles that different neuron populations play in generating behavior, there is no reason to suppose that heritability estimates will necessarily cluster close to the 80–90% level. A good analogy is the wide range of heritability (50–90%) for size differences among incisors, premolars, and molars in humans (Altman and Dittmer, 1962).

High estimates of genetic determination and of additive genetic control are of interest for several reasons. The pace of brain evolution is critically dependent on a reservoir of normal allelic variants that modulate brain development (Romer, 1969; Kruska, 1987; Lipp, 1989; Finlay, 1992; Williams et al., 1993). The fact that the size of the ganglion cell population is strongly influenced by additive gene factors indicates that selection could produce rapid change in the mean population (Barton and Turelli, 1989; Falconer, 1989). An interesting question is whether selection for high or low ganglion cell number would be matched by correlated responses in body size, brain weight, or eye size (Lande, 1979; Purves, 1988). Such correlated responses would be consistent with the idea that common gene mechanisms tightly link the proliferation and survival of neurons in different parts of the brain (Finlay and Darlington, 1995). In an analysis of evolutionary change in the cat's visual system, we found that several cell populations were reduced neatly in proportion to the reduction in total brain weight (Williams et al., 1993). However, two other important cell populations, rod photoreceptors and α ganglion cells, did not scale with

brain size. This independence is consistent with the idea that regional and cell-specific differences in gene expression (Lipp, 1989; Rubinstein et al., 1994; Usui et al., 1994) control the size of specific neuron populations.

Implications of a bimodal distribution

All inbred strains that we have studied can be characterized as belonging to either a high- or low-cell-number phenotype. There is no appreciable overlap. This bimodality is surprising, because both cumulative distributions (Fig. 2) and distributions within isogenic strains are close to normal. The bimodality becomes evident only when strain means are compared, because sampling and technical errors are reduced so much when averaging eight cases per strain. Given the constant environment in which all these inbred mice were reared, we can be confident that the bimodality has a genetic rather than an environmental origin, but until developmental studies are complete, we will not know where, when, or how the differences are produced. It will be of interest to determine whether these consistent quantitative differences have detectible effects on visual function.

Candidate mechanisms that generate the bimodality include factors that affect the original number of progenitor cells (Herrup et al., 1984; Herrup, 1986; Williams and Goldowitz, 1992a; Goldowitz et al., 1996), interactions that alter patterns of cell commitment and the kinetics of the cell proliferation (Reh and Kljavin, 1989; Lillien and Cepko, 1992; Williams and Goldowitz, 1992b; Sicinski et al., 1995; Cepko et al., 1996, Gan et al., 1996), and factors that control the loss of 60–70% of the original ganglion cell population that normally occurs shortly after birth (Linden and Pinto, 1985; Williams et al., 1990). In an ongoing analysis of neonatal mice, we have found that some differences between high and low strains are evident at birth, before significant cell loss (Strom et al., 1995) and just after all ganglion cells have been generated (Dräger, 1985).

Whatever molecular and cellular mechanisms are behind the bimodality, it is reasonably safe to conclude that one or more genes controlling ganglion cell number have allelic variants with large effects. The large differences that we noted between pairs of closely related strains of mice (Fig. 1) also support this idea. We have recently mapped a major effect locus that is responsible for as much as 40% of the variance in retinal ganglion cell number among the BXD recombinant inbred strains (Williams et al., 1995). Alleles at this locus may also account for much of the variation we have discovered among standard inbred strains.

Strain variation in neuron number is widespread among rodents. Differences ranging from 25 to 100% have been documented in hippocampus (Wimer et al., 1976, 1978, 1988; Wimer and Wimer, 1989), neocortex (Wimer et al., 1969), forebrain cholinergic regions (Albanese et al., 1985), olfactory bulb (Smith, 1928), substantia nigra (Ross et al., 1976), locus coerulus (Berger et al., 1979), and cerebellum (Wetts and Herrup, 1982). In several cases, the numerical differences have clear biochemical and functional correlates (Berger et al., 1979; Albanese et al., 1985). There are good reasons for renewed interest in these robust quantitative differences in CNS structure. The foremost reason is that is it now practical to map genes associated with complex quantitative traits (Lander and Botstein, 1989; Belknap, 1992; Johnson et al., 1992; Belknap et al., 1993; Plomin and McClearn, 1993; Crabbe et al., 1994; Dietrich et al., 1994; Lai et al., 1994; Lander and Schork, 1994; Takahashi et al., 1994; Kearsey and Pooni, 1996). Given the extraordinarily large numbers of genes expressed in the vertebrate nervous system, no fewer than 30,000 (Sutcliffe, 1988; Adams et al., 1993a,b), it is encouraging that an important subset of genes that have well-defined quantitative effects on the structure of the mammalian CNS can now be identified using this directed approach.

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