A short face mutation on Chromosome 11

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Mutation (allele) symbol: *Sofa*

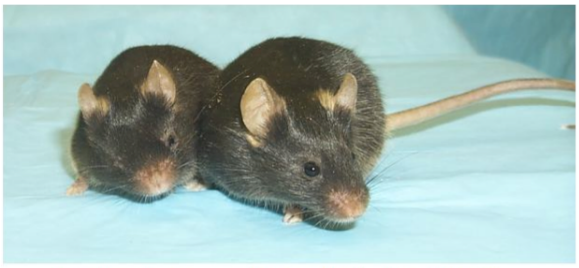
Mutation (allele) name: short face

Strain of Origin: C57BL/6J-*Apc*^{Min}/J

Current Strain Name: B6(AKR)-*Pfas*^{Sofa}/J

Stock Number: 004235 (jaxmice.jax.org)

Phenotype Category: Craniofacial



Sofa heterozygote on the left
and littermate control on the right

Abstract
We report a new spontaneous mutation with a short nose.

Origin and description
The dominant *Sofa* mutation arose spontaneously in the C57BL/6J-*Apc*^{Min}/J (Stock #002020) production colony of The Jackson Laboratory in 2000. The *Apc*^{Min} strain arose from an AKR/J x C57BL/6J mating and had been subsequently backcrossed to C57BL/6J for 47 generations when the *Sofa* mutation arose in the *Apc*^{Min} colony. The *Apc*^{Min} mutation is no longer in the *Sofa* colony, as was confirmed by genotyping breeder mice from the *Sofa* strain on two separate occasions; all typed as wild types.

The *Sofa* mutation affects skull shape and is characterized by a short nose, domed skull and wide set eyes. Both males and females are affected and have normal fertility. The mutation has varied penetrance ranging from an obvious to a subtler short nose phenotype. For this reason, mice that are phenotypically categorized as controls may occasionally be low penetrance carriers of the *Sofa* mutation. The strain is maintained by mating a +/+ to a +/*Sofa* sibling.

Genetic analysis
Using the Mouse Mutant Resource standard mapping protocol, *Sofa* was mapped by mating a female from the A/J inbred strain to a male *Sofa* heterozygous mutant. Affected mice were then backcrossed to normal A/J mice to produce N2 offspring. Spleen and tail tips were collected from the affected N2 mice and stored at -70C. DNA was extracted from either spleen or tail tips using standard phenol extraction methods. PCR was done with MIT or Research Genetics primer pairs (MapPairs, from Research Genetics, Huntsville, Ala., or from Integrated DNA Technologies, Coralville, IA). According to scoring of 180 meioses, *Sofa* maps to Chromosome 11 with flanking markers of *D11Mit4* (1/180) and *D11Mit364* (1/180), or from 68.4 Mb to 72.0 Mb. There is no recombination at *D11Mit298* (69.3 Mb) or *D11Mit29* (69.6 Mb).

The *Sofa* mutation is homozygous lethal. After outcrossing an A/J inbred to a +/*Sofa* and scoring F2s at the *Sofa* critical region, no homozygotes were observed in embryos at stages ranging from E 18.5 down to E 7.5. Thirty-seven percent (15/41) were wild type and 63% (26/41) were heterozygotes.

Biological characterization

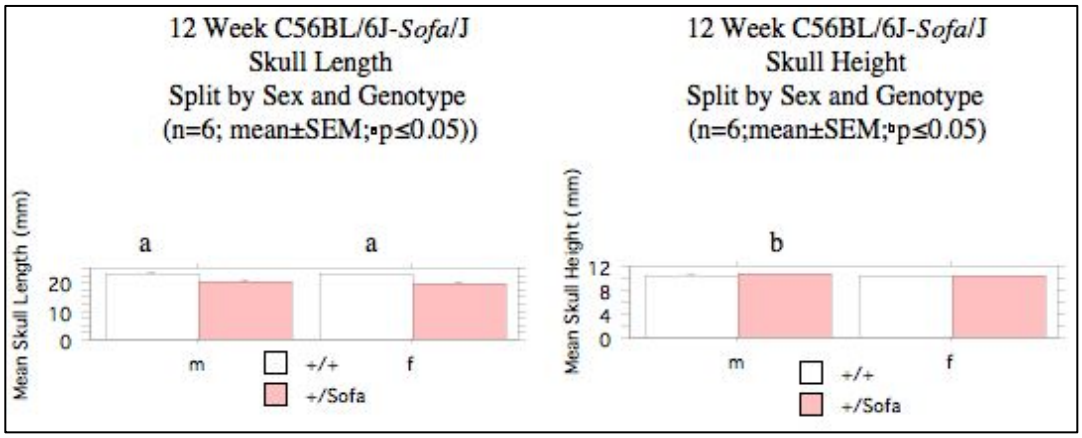
Craniofacial morphology of twelve-week-old mice[1]

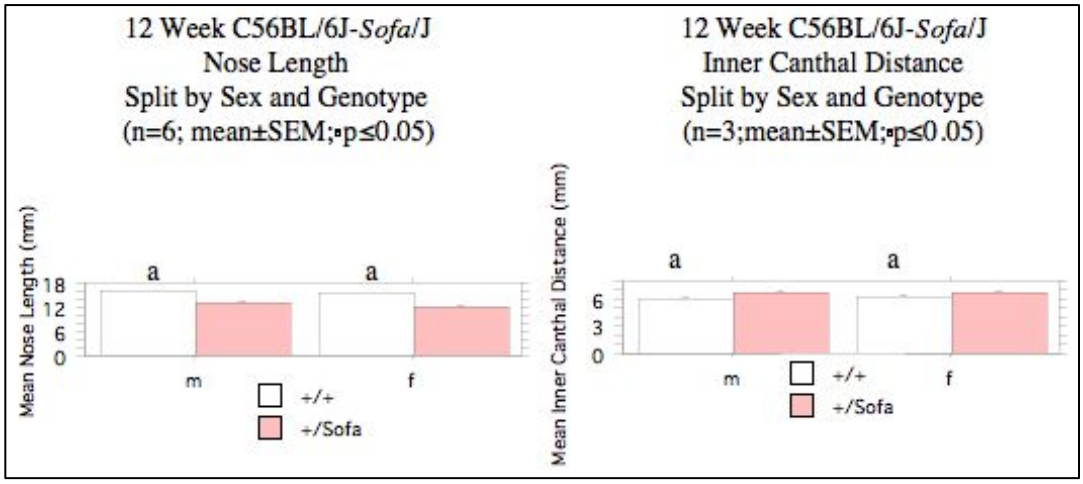
Skulls were prepared by incomplete maceration in potassium hydroxide, stained with alizarin red, and stored in undiluted glycerin (Green, 1952). Morphological measurements of the skull were made using digital calipers (Stoelting, Wood Dale, Ill) with previously established landmarks (Richtsmeier, 2000).

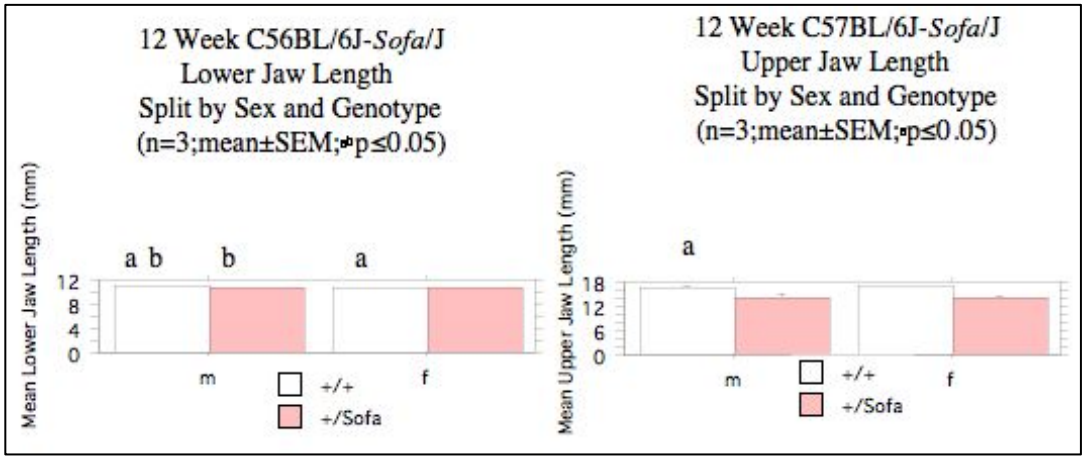
Table 1: Digital Caliper Measurements and Calculated Ratios of Twelve-Week-Old C57BL/6J-*Sofa*/J Skulls Stained with Alizarin Red (n=6; mean±SEM;$^{ab}p \leq 0.05$))

Measurements	Male +/+	Male +/*Sofa*	Female +/+	Female +/*Sofa*
Skull Length (mm)	23.19 ± 0.107^{a}	20.37 ± 0.525	23.02 ± 0.147^{a}	19.61 ± 0.248
Nose Length (mm)	15.85 ± 0.080^{a}	12.76 ± 0.505	15.62 ± 0.098^{a}	12.24 ± 0.261
Skull Height (mm)	10.46 ± 0.062	10.53 ± 0.066^{b}	10.27 ± 0.073	10.31 ± 0.053
Skull Width (mm)	10.52 ± 0.049	10.67 ± 0.068	10.45 ± 0.056	10.51 ± 0.066
Inner Canthal Distance (mm)	6.09 ± 0.075^{a}	6.74 ± 0.164	6.31 ± 0.151^{a}	6.78 ± 0.099
Lower Jaw Length (mm)	10.98 ± 0.070^{ab}	10.75 ± 0.066^{b}	10.72 ± 0.034^{a}	10.54 ± 0.069
Upper Jaw Length (mm)	16.60 ± 0.176^{a}	14.25 ± 0.582	13.370 ± 0.0834	13.432 ± 0.1476
Jaw Length Ratio	1.51 ± 0.014^{ab}	1.33 ± 0.054	1.58 ± 0.021^{a}	1.35 ± 0.028
Skull/Nose Length Ratio	1.46 ± 0.012^{a}	1.60 ± 0.023	1.47 ± 0.013^{a}	1.60 ± 0.018
Skull Height/ Length Ratio	0.45 ± 0.004^{a}	0.52 ± 0.016	0.45 ± 0.003^{a}	0.53 ± 0.006
Skull Length/ Width Ratio	2.21 ± 0.015^{a}	1.91 ± 0.057	2.20 ± 0.014^{a}	1.87 ± 0.029
Skull Height/ Width Ratio	0.99 ± 0.009	0.99 ± 0.003	0.98 ± 0.008	0.98 ± 0.006
Right Ear Pinna (mm)	13.785 ± 0.1196	13.553 ± 0.1605	ND	ND

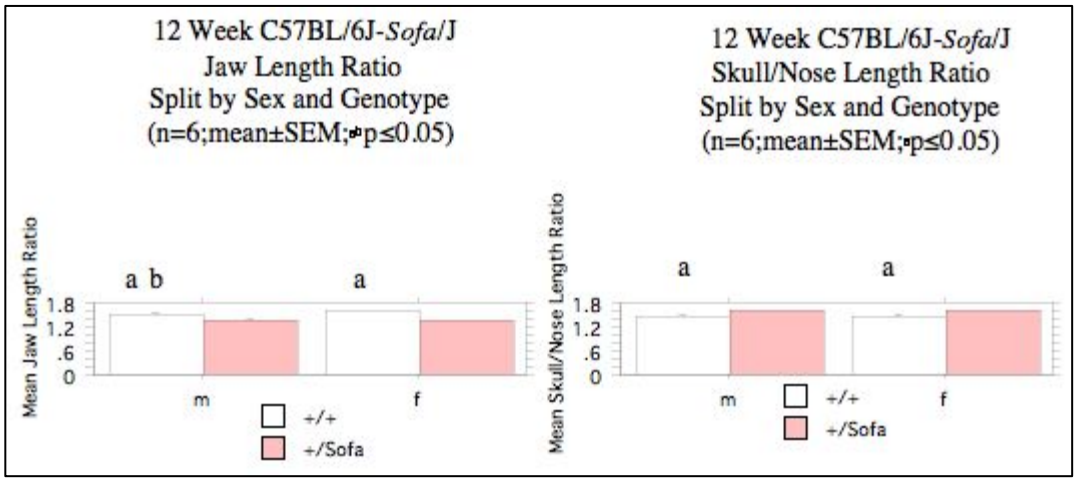
Statistical differences between mutants and controls within both sexes are seen. Mutants have lower measurements for skull length, nose length and lower jaw length. Significantly different ratios within male and female mutants and controls are upper to lower jaw length, skull to nose length, skull height to skull length, and skull length to skull width.

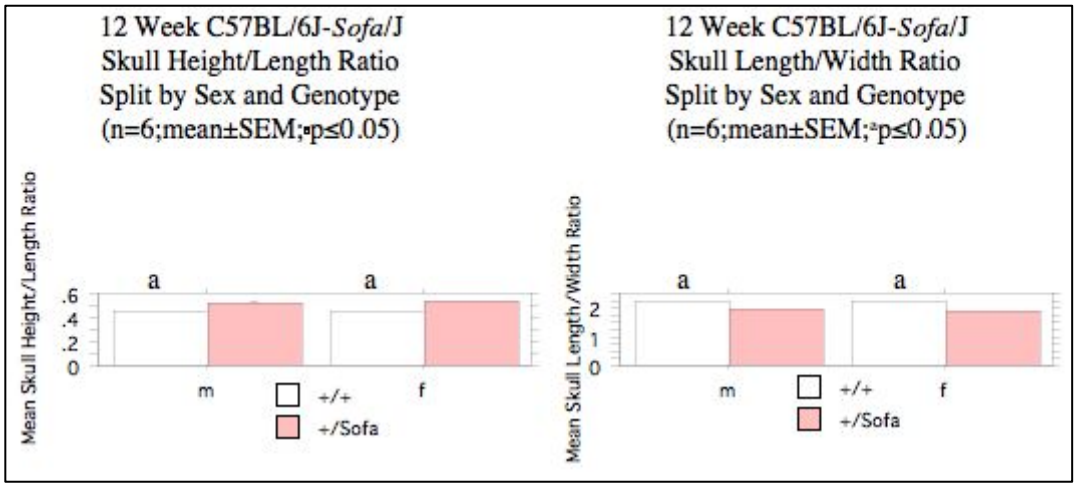






[a] +/+ vs. +/*Sofa* within sex
[b] male vs. female within genotype





[a] +/+ vs. +/*Sofa* within sex
[b] male vs. female within genotype

Overall, skull shape seems to be the primary characteristic of the *Sofa* mutation affecting male and female mutants equally. Furthermore, the face of the *Sofa* skull is more affected than the back of the skull.

DEXA analysis of whole body aBMD and body composition of twelve-week-old mice
Whole body, areal bone mineral density (aBMD), bone mineral content (BMC) and body composition (lean, fat and % fat mass) were assessed by PIXImus densitometry (GE LUNAR, Madison, WI) (Table 2). Significant differences varied between sexes. Most notable is whole body fat and percent fat that is significantly reduced in heterozygote males compared to wild type males.

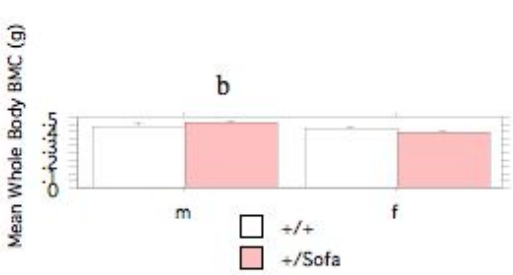
Table 2: PIXImus Densitometric Measurements of Twelve-Week-Old C57BL/6J-*Sofa*/J
(n=6; mean±SEM; [ab]p≤0.05)

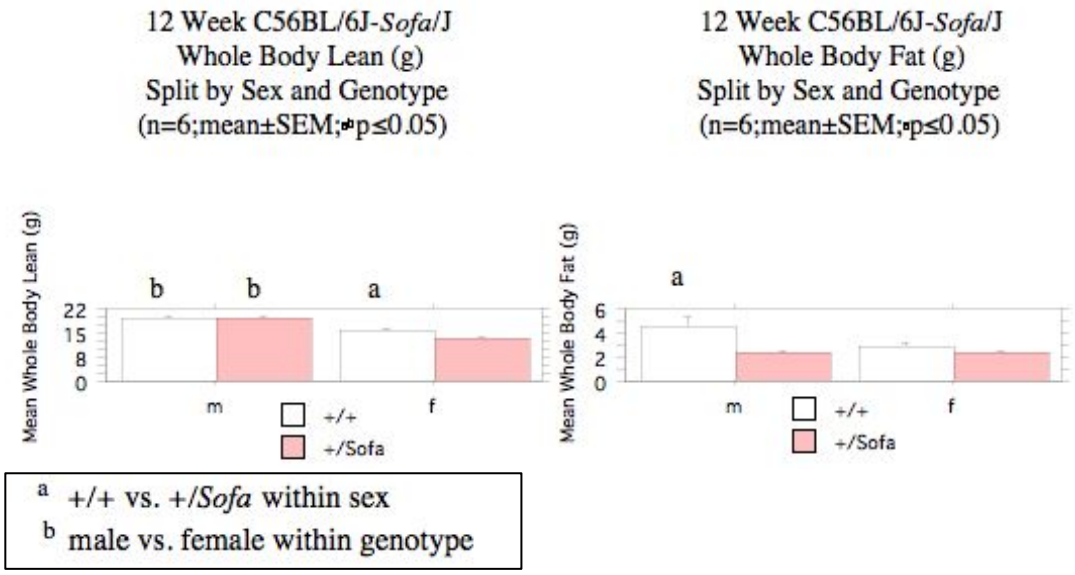
Measurements	Male +/+	Male +/*Sofa*	Female +/+	Female +/*Sofa*
Whole Body BMD (g/cm^2)	0.0456 ± 0.00088	0.458 ± 0.00069[b]	0.0441 ± 0.00064[a]	0.0421 ± 0.00051
Whole Body BMC (g)	0.437 ± 0.0143	0.463 ± 0.0086[b]	0.420 ± 0.0141	0.393 ± 0.0112
Whole Body Lean (g)	19.6 ± 0.50[b]	19.2 ± 0.88[b]	15.6 ± 0.47[a]	13.4 ± 0.32
Whole Body Fat (g)	4.5 ± 0.77[a]	2.4 ± 0.10	2.8 ± 0.32	2.3 ± 0.24
Total Mass (g)	24.1 ± 1.05[b]	21.6 ± 0.93[b]	18.5 ± 0.79[a]	15.8 ± 0.50
% Fat	18[a]	11[b]	15	15
Skull BMD (g/cm^2)	0.1075 ± 0.00131	0.1040 ± 0.00119[b]	0.1110 ± 0.00127	0.1083 ± 0.00082
Skull BMC (g)	0.248 ± 0.0049	0.230 ± 0.0062	0.254 ± 0.0048[a]	0.224 ± 0.0034
Skull BMD/ Body BMD	2.3618 ± 0.05807	2.2738 ± 0.03120	2.5194 ± 0.01297	2.5719 ± 0.03708

Among females, controls have significantly higher whole body BMD, body lean, total mass and skull BMC than mutants. Overall, mutants may be smaller than controls and the results vary depending on gender.

12 Week C56BL/6J-*Sofa*/J
Whole Body BMD (g/cm2)
Split by Sex and Genotype
(n=6;mean ± SEM;[a]p≤0.05)

12 Week C56BL/6J-*Sofa*/J
Whole Body BMC (g)
Split by Sex and Genotype
(n=6;mean±SEM;[a]p≤0.05)





Using our standard pathological screen[1] no lesions were found in four, six and nine-week-old mutant mice. Hearing was assessed by auditory brainstem response (ABR). Mutants and controls were examined at two, three and six months of age and no hearing loss was observed. Several sporadic cases of otitis media were found but did not appear to segregate with the mutation. A clinical eye exam of mutants and controls ranging from 1 to 2 months old revealed no abnormalities.

Acknowledgements
We wish to thank: Coleen Kane for preparation of tissues for histological assessment; Rod Bronson, Ph.D, pathological evaluation; Stephen Murray, Ph.D, with instruction for embryo collection; Heping Yu for ABR analysis, Qing Yin Zheng for the Otitis Media screen, Norm Hawes for clinical eye evaluation, and Pat Ward-Bailey for assistance with manuscript preparation and web posting.

Addendum
This mutation was found to be a 15 base pair deletion in the phosphoribosylformylglycinamidine synthase gene (*Pfas*). Please see Fairfield et al., Genome Biology 2011, Sept 14; 12(9):R86.

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Protocols

[1] Standard Protocols of The Jackson Laboratory Craniofacial Resource

PIXImus Densitometry

PIXImus scans (PIXImus, LUNAR, Madison, WI) which provide skeletal and body composition data such as Bone Mineral Density (BMD, g/cm2), Bone Mineral Content (BMC, g/cm2), body mass (g), lean mass (g), fat mass (g), and % fat mass, are completed on groups of 6 male and 6 female 12 week old mutant and control mice. The skulls and bodies are scanned separately to provide independent data on skull BMD and BMC and body BMD and BMC. The PIXImus small animal densitometer (DEXA) has a resolution of 0.18 x 0.18 mm pixels and is equipped with software version 1.46 . The PIXImus is calibrated routinely with a phantom utilizing known values, and a quality assurance test is performed daily. The variability in precision for measuring total body BMD is, less than 1%, and approximately 1.5% for specialized regions such as the skull. The correlation between PIXImus BMD measurements of 614 lumbar vertebrae compared to peripheral quantitative computerized tomography (pQCT) measurements was found to be significant ($p<0.001$; $r=.704$) (Donahue, 1999) .

Skull Preparation

Skulls of 6 male and 6 female mutants and controls are collected at 12 weeks of age, prepared by incomplete maceration in potassium hydroxide, stained with alizarin red, and stored in undiluted glycerin (Green, 1952). During the collection process, right ear pinnae are measured with digital hand calipers (Stoelting, Wood Dale, IL, USA).

Hand Caliper Skull Measurements

Seven measurements taken with hand held digital calipers are used routinely to define skull morphology at the Lab's craniofacial resource. These measures have a high degree of accuracy and precision in our hands and are able to discriminate differences between mutant and control skull characteristics. Our linear measures have been added to those defined in Richtsmeier et al. (2000) Dev Dyn 217(2) p140, which were used for characterizing craniofacial differences in mouse models of Down Syndrome using three dimensional anatomical landmarks. Skulls are cleared with potassium hydroxide and stained with alizarin red dye in preparation for caliper measurements to be taken.

Skeletal Preps

In many cases whole skeletons of mutant and control mice are cleared in 1% KOH, stained with alizarin, stored in glycerin (Green, 1952) and then evaluated for skeletal malformations. Malformations found can indicate that the craniofacial phenotype is part of a greater syndrome.

Data Analysis

Hand caliper skull measurements and PIXImus skeletal and body composition data are evaluated using StatView 4.5 software (Abaccus Cary, NC) for Macintosh computers. Differences are considered significant when $p < 0.05$.

[2] Standard Histology Protocol used in the Mouse Mutant Resource

For fixation of tissues, mice were deeply anesthetized with tribromoethanol (avertin) until they no longer displayed a withdrawal reflex in the hind limbs and then perfused intracardially with Bouin's fixative following a flush of the vasculature with saline solution. After soaking in Bouin's for one week to demineralize bones, tissues were dissected. Six segments of spine with axial muscles and spinal cord in situ, representing cervical, thoracic and lumbar spinal segments, were dissected. The brain was removed and sliced into 6 cross sectional pieces at the levels of olfactory lobes, frontal cortex, striatum, thalamus, midbrain, rostral and caudal medulla with cerebellum. Midsagittal slices of hind leg through the knees were prepared. Slices of basal skull through the pituitary and inner ears were taken. Both eyes, salivary glands and submandibular lymph node, trachea plus thyroid and sometimes parathyroid were removed and cassetted. A longitudinal slice of skin from the back was removed. The thymus, slices of lung, and a longitudinal slice of heart were cassetted. Similarly slices of liver through gall bladder, kidney with adrenal attached, pancreas and spleen were prepared. The stomach was sliced longitudinally to include both squamous and glandular portions. Loops of small intestine from 3 levels and slices of large intestine and cecum were removed, as were slices of urinary bladder. The whole uterus, with ovaries attached, was taken. In males testes were sliced longitudinally. The accessory male organs including seminal vesicles, coagulating gland and prostate were removed en block. Altogether in most cases all tissue fit into a total of 10 cassettes. The cassettes were processed in an automatic tissue processor to dehydrate tissues which were then embedded in paraffin. Six micron sections were cut and stained with hematoxylin and eosin (H&E). Sections of brain and spinal cord in vertebral bones also were stained with luxol fast blue (LFB) for myelin and cresylecht violet (CV) for cellular detail.