Arthropod Structure & Development xxx (2011) 521–529

Contents lists available at ScienceDirect

Arthropod Structure & Development

journal homepage: www.elsevier.com/locate/asd

The allometry of CNS size and consequences of miniaturization in orb-weaving and cleptoparasitic spiders

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ARTICLE INFO

Article history:
Received 25 November 2010
Received in revised form
12 May 2011
Accepted 15 July 2011

Keywords:
Miniaturization
Allometry
Orb-web spiders
cleptoparasitic spiders
Araneae

ABSTRACT

Allometric studies of the gross neuroanatomy of adults from nine species of spiders from six web-weaving families (Orbicularia), and nymphs from six of these species, show that very small spiders resemble other small animals in having disproportionately larger central nervous systems (CNSs) relative to body mass when compared with large-bodied forms. Small spiderlings and minute adult spiders have similar relative CNS volumes. The relatively large CNS of a very small spider occupies up to 78% of the cephalothorax volume. The CNSs of very small spiders extend into their coxae, occupying as much as 26% of the profile area of the coxae of an *Anapisona simoni* spiderling (body mass < 0.005 mg). Such modifications occur both in species with minute adults, and in tiny spiderlings of species with large-bodied adults. In at least one such species, *Leucauge mariana*, the CNS of the spiderling extends into a prominent ventral bulge of the sternum. Tiny spiders also have reduced neuronal cell body diameters. The adults of nearly all orbicularian spiders weave prey capture webs, as do the spiderlings, beginning with second instar nymphs. Comparable allometric relations occur in adults of both orb-weaving and cleptoparasitic species, indicating that this behavioral difference is not reflected in differences in gross CNS allometry.

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1. Introduction

Very small animals confront special problems in the structure and function of their nervous systems. Small nervous systems must either be comprised of fewer and/or smaller neurons, or have smaller sub-cellular components such as synaptic boutons or mitochondria, which will affect information processing (Chittka and Niven, 2009; Eberhard and Wcislo, in press). At extremely small body sizes problems arise because there are absolute lower limits to the size of cells and sub-cellular components. For example, the minimum size of neuronal cell bodies is constrained by the size of the nucleus, which is itself constrained by genome size (Rensch, 1959; Gregory, 2001). Another factor that imposes a lower limit of ~0.1 μm on axon diameter is noise generated by neural components such as ion channels, which can prevent reliable signaling (Faisal et al., 2005). A third limitation is imposed by the energy consumption of metabolically expensive neural tissue, which is correlated with the proportionally larger surface area of smaller neurons and the volume of mitochondria they contain (e.g., Niven and Laughlin, 2008).

Both vertebrates and invertebrates conform to Haller's Rule, which holds that the brains of smaller animals are larger relative to body size than large-bodied forms (e.g., Rensch, 1948; Striedter, 2005; Bonner, 2006; Wehner et al., 2007; Polilov and Beutel, 2009; Seid et al., 2011; Eberhard and Wcislo, in press). The minute first instar larva of a strepsipteran, *Mengenilla chobauti*, for example, has a CNS (supra- and sub-oesophageal ganglia) that is proportionally more than 200 times larger than that of a large water beetle, relative to their respective body volumes (Beutel et al., 2005). The brains of tiny ants, *Brachymyrmex* spp. (~0.04 mg body mass), and a tiny ptiliid beetle, *Mikado* sp. (~0.0016 mg body mass), constitute approximately 15% and 16% of their respective

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1467-8039/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.asd.2011.07.002

biomasses (Seid et al., 2011; Polilov and Beutel, 2009), which is far greater, for example, than the 2–3% of humans. In free-living miniature insects with body lengths as small as 390 μm, neuron size is severely reduced (down to diameters of 2 μm), and up to 90% of the cell volume is comprised of the nucleus (summarized in Grebennikov, 2008; Eberhard and Wcislo, in press). In addition, neurons may be more densely packed at small sizes (Beutel et al., 2005).

Such scaling trends imply that miniaturization has negative consequences for an animal's energy budget because the metabolic costs of relatively large CNSs in tiny animals would be substantially higher relative to larger animals (Niven and Laughlin, 2008). There are several possible, non-exclusive, solutions to miniaturization problems (reviewed in Eberhard and Wcislo, in press). A size limitation hypothesis (Eberhard, 2007) posits that very small animals evolve life histories that require less demanding behavioral capabilities, minimizing the need for expensive brain tissue, or they suffer from reduced performance. Manifestations of reduced performance could be increased error rates, slower execution of behavior, or higher response thresholds, but there is a dearth of data to assess this hypothesis (e.g., reviewed in Healy and Rowe, 2007; Eberhard and Wcislo, in press). Studies on tiny orb-weaving spiders suggest that their behavioral capabilities are neither less demanding nor inferior to those of larger individuals (Eberhard, 2007, 2011; Hesselberg, 2010). Others have hypothesized that extremely small invertebrates suffer no behavioral impairments due to the small size of their CNSs, but did not provide supporting data (e.g., Beutel et al., 2005; Polilov and Beutel, 2009). A second possible solution is that very small animals specialize and sacrifice behavioral flexibility *per se*, eliminating the information processing needed to trigger the expression of alternative phenotypes (Levins and MacArthur, 1969; Bernays and Wcislo, 1994; Bernays, 2001). The use of filters or tuned receptors would also minimize the need for processing, and evolutionary innovations in the peripheral sensory system may lead to decreased demands on CNS processing (e.g., Wehner, 1987; Fratzl and Barth, 2009). In addition to these peripheral processes, small animals might use neural mechanisms more efficiently, including an increased reliance on analog signals to transmit information, which are more efficient energetically than digital ones (e.g., Sarpeshkar, 1998; Laughlin et al., 1998), or increasingly rely on multifunctional neurons (see Anderson, 2010; also Niven and Chittka, 2010). Finally, a third possible solution is the "oversized brain" hypothesis: very small animals could maintain disproportionately large CNSs and pay disproportionately high energetic costs, to enable behavioral capabilities comparable to those of larger animals.

This paper uses web-building and cleptoparasitic spiders to describe CNS scaling relationships among individuals that vary in body mass by 400,000 times (Table 1). Web-weaving spiders provide excellent opportunities to address questions of behavioral and neural system trade-offs, because their webs provide detailed records of a series of subtle behavioral decisions, which are readily comparable among spiders that vary enormously in size (Vollrath, 1992; Eberhard, 2007, 2011; Hesselberg, 2010). In addition, the higher phylogeny of spiders, especially the Orbicularia, is relatively well-resolved (Scharff and Coddington, 1997; Griswold et al., 1998; Kuntner et al., 2008), so it is possible to identify cases in which miniature body size is hypothesized to be secondarily derived in groups that retain comparable web construction behaviors.

A drawback to using spiders in such studies is that the functional anatomy and development of the nervous systems is well characterized for only a few species (e.g., Babu, 1975; Weltzien and Barth, 1991). A previous study of the spider *Argiope aurantia* showed that relative size of the brains of tiny spiderlings is more than 10 times that of adults, and the proportion of spiderling brain mass dedicated to the cellular cortex (or rind) *versus* the neuropil was double that for the adult brain (Babu, 1975; note that Babu used "brain" to refer only to the supra-oesophageal ganglion). Babu (1975) studied only a single species, however, and it is uncertain whether these changes are associated with maturation, changes in size, or both. We resolve this ambiguity by measuring the volumes of the CNSs (supra- and sub-oesophageal ganglia; the latter included all ganglia below the esophagus) of adults of nine species, and second instar nymphs of six of these species, from six orbicularian families. We include spiders that are substantially smaller than those studied by Babu (1975), and show how CNS volume scales with body mass over six orders of magnitude. We identify two morphological changes that occur in very small spiders to accommodate their relatively large CNSs in their cephalothoraces: the CNS extends into the coxae, and deforms the ventral surface of the cephalothorax. We also show that the reduction in CNS volume in small spiders is accompanied by a reduction in the diameter of neuronal cell bodies. Finally, we assess whether species with different behaviors (orb-weaving and cleptoparasitism) follow the same scaling relationships.

Table 1
Body masses of spiders used in this study.

Species	Developmental stage	Body mass (mg)
Mysmena sp.	Nymph 2	<0.005
	Adult female	0.1
Mysmenopsis tengellacompa	Nymph 2	0.02
	Adult female	0.3
Anapisona simoni	Nymph 2	<0.005
	Adult female	0.8
	Adult male	0.8
Faiditus elevatus	Adult female	3
	Adult male	2.4
Faiditus sp.	Adult male	2.7
Eustala illicita	Adult male	14
Leucauge mariana	Nymph 2	0.1
	Adult female	60
	Adult male	20
Argiope argentata	Nymph 2	0.14
	Adult female	250
Nephila clavipes	Nymph 2	0.7
	Adult female	2000
	Adult male	11.2

2. Methods

2.1. Study taxa and voucher specimens

We studied species that span the full range of body size in orbicularian spiders, with mature females that weigh from 0.1mg to 2000 mg (Table 1). These species are distributed in six families and include, in order of increasing sizes of adults, *Mysmena* sp. (Mysmenidae), *Anapisona simoni* (Anapidae), *Mysmenopsis tengellacompa* (Mysmenidae), *Faiditus elevatus* (Theridiidae), *Faiditus* sp. (Theridiidae), *Leucauge mariana* (Tetragnathidae), *Eustala illicita* (Araneidae), *Argiope argentata* (Araneidae), and *Nephila clavipes* (Nephilidae) (see Fig. 1 for their phylogenetic relationships). All species build orb webs or orb-like webs, except for *Mysmenopsis* and *Faiditus*, which are independently derived cleptoparasites that live in the webs of larger spiders and do not build prey capture webs (see Discussion for references). All species pass one immature stage within the egg sac before second instar spiderlings (*sensu* Foelix, 1996) emerge to build prey capture webs. At least superficially their webs are similar to those of the adults (Hesselberg, 2010; WGE, pers. obs.), so nymphs require behavioral capabilities comparable to those of adults with respect to prey capture. Adult

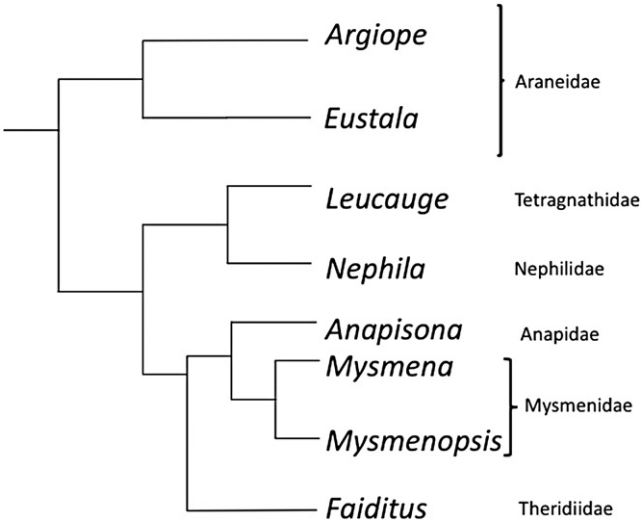

Fig. 1. Hypothesis of the phylogenetic relationships among the taxa in this study (after Griswold et al., 1998 and Scharff and Coddington, 1997).

spiders or their egg sacs were collected in the field in central Panamá or near San José, Costa Rica. Second instar spiderlings were reared from egg sacs, except for those of *Mysmena* sp., *M. tengellacompa*, *A. simoni*, and *L. mariana*, for which we used the smallest individuals found on prey capture webs in the field, and were assumed to be in the second instar based on their sizes. Voucher specimens are deposited in the Museo de los Invertebrados de la Universidad de Panamá.

2.2. Histology and light microscopy

For anatomical observations, and for all quantitative measurements including volume reconstructions, spiders were prepared histologically using the following standard procedures. Spiders were killed by immersion in fixative (2% paraformaldehyde, 6% glutaraldehyde, 0.1 M Na-cacodylate, pH 7.4) at room temperature. To promote penetration of fixative, the legs (except the coxae), abdomen and cheliceres were removed immediately. In larger spiders, a small part of the dorsal cuticle of the cephalothorax was removed; in the largest spiders (*A. argentata*, *N. clavipes*) the CNS was completely dissected out of the cephalothorax in fixative. Fixation continued overnight at 4 °C, followed by two 10-min washes in 0.1 M cacodylate buffer and 2 h post-fixation in 1.5% OsO_4. Tissues were briefly rinsed with cacodylate buffer and water, then dehydrated with 2,2-dimethoxypropane followed by 100% acetone (20 min each) before being washed with an acetone/EPON solution (50:50) overnight, then infiltrated with 100% EPON for 2 h at room temperature and 1 h at 50 °C. Finally CNS tissue in 100% EPON was positioned in a Beem® capsule and cured at 60 °C overnight. Horizontal sections were cut using a Microm® HM 355s microtome with stainless steel disposable knives. The section thickness varied from 3 to 10 μm producing between 47 and 121 sections, depending upon the size of the cephalothorax. The sections were mounted in order on glass slides, stained using methylene blue (Richardson's dye), and then photographed using a Nikon® 8700 camera, or a Nikon® DS-Ri1 camera, attached to a Nikon® Eclipse E600 compound microscope.

Reduced silver Bodian preparations of adult females and nymphs of *A. simoni* and *Mysmena* sp. were used to verify the distinctions between neuropil and leg nerves in the coxae. Specimens were immersed in acetic acid-alcohol-formalin, all appendages were removed except the basal portions of the legs, and small holes were made in the dorsum of the cephalothorax, to promote penetration of the fixative. Fixation and staining procedures followed Sinakevitch et al. (2003). Serial sections then were rehydrated and incubated for 24 h at 50 °C in 2% silver proteinate (Polysciences, Inc.) with 2 g of copper wire fragments/200 ml of solution. The tissue was then treated following methods in Bodian (1937).

2.3. Criteria for volumetric data analyses

Previous volumetric studies of spider brains omitted the criteria used to identify different brain regions for analyses, which impedes comparative studies. Consequently, we describe our techniques and criteria in detail. The boundaries of the central neuropil were easily discerned, but the limits of the cortex were clear in only some sections. We thus report estimates of the volumes of both the cortex + neuropile, and the neuropile alone (see Table 2 of Supplementary Materials [SM]). When the outer limits of the cortex were poorly defined, we used conservative estimates of their extensions. When calculating total volumes we excluded cell bodies in the coxae of some specimens that may have been part of the cortex. Thus our measurements are likely to underestimate CNS volume.

The division between the neuropils of the sub-oesophageal (sub-OG) and the supra-oesophageal ganglion (sup-OG) was traced, enabling separate measurements of their volumes. This division was not planar; instead it included a downward projection of the anterior portion of the sup-OG neuropil (apparently corresponding to the cheliceral ganglia) into the sub-OG neuropil. To differentiate these regions we first identified a section in the sub-OG in which the 5 pairs of neuromeres (associated with the pedipalps and legs) were clearly visible. Starting from this section we continued tracing upward and downward in other sections, following the dividing line between the sub-OP and sup-OG. In some sections the dividing line was not clear, so we interpolated from adjacent sections. All intra-specific comparisons of spiders of different sizes were based on data taken by the same person to minimize inter-observer bias in defining the outer limits of different regions.

Tracings of cephalothorax profiles excluded the bases of the coxae, the endites and the chelicere, but did include the labia. Most of the cephalothorax volume was measured directly from reconstructions, but it was usually necessary to estimate the volume of a small, final portion, because a portion fell from the EPON block near the end of sectioning (see SM Fig. 9). We thus report two estimates of cephalothorax volume in our calculations (SM Table 2). The volume of the cephalothorax was measured up to, and including, the dorsal-most section of the sup-OG, which is an underestimate. Alternatively, we included the missing volume extrapolated from the data already obtained (see SM Fig. 9), which is an overestimate. Comparison of the underestimated volumes with complete cephalothorax volumes from the same species showed that the volume of the underestimate was about 3% less than the volume of the complete specimen. In a few cases in which a given section was folded or otherwise not measurable, we interpolated the probable outlines from those in the preceding and the following sections.

2.4. CNS reconstructions and weighing body mass

We identified CNS regions and their subdivisions using specific criteria (see Section 2.3), and then traced their outlines, and those of the cephalothorax, in successive digital photographic images of the serial sections using the program PC-Reconstruct (Fiala, 2005). The same program was used to align the tracings, and reconstruct

three-dimensional volumes. CNS measurements were plotted against body mass, which was the fresh weight of spiders, measured using either an AND® GR-202 microbalance or a Sartorius® CPA2P microbalance.

2.5. Invasion of leg coxae by CNS neuropil

The degree to which the CNS neuropil invaded the coxae was estimated indirectly. Many sections were relatively thick in comparison with the diameters of the coxae, so we did not calculate the volume of the coxa nor the volume of the extension. Instead, the section of a coxa with the greatest extension of neuropil was chosen, and the area occupied by neuropil was compared with the total area occupied by the coxa in that section. The extension of the neuropil into the coxa represented a minimum estimate of the CNS extension because the cortex was excluded.

2.6. Measuring neuronal cell body diameter

Measurements of the dimensions of apparent neuronal cell bodies were made from digital photographs by sampling cells in rectangular boxes (the height of the box being the thickness of the section). Each box contained approximately 0.1% of the estimated volume of the sub-OG or sup-OG: one box was just below the ventral margin of the sub-OG neuropil, one just above the dorsal margin of the sup-OG neuropil, and one near the abdominal neuromere of the sub-OG. In each case a section was chosen in which cell limits were particularly clear. Within each rectangle, we measured the maximum diameter and the diameter perpendicular to this for each apparent neuronal cell body.

2.7. Statistical analyses

We did not attempt to correct quantitative analyses for phylogenetic biases, as several of the larger contrasts were between young and old spiders of the same species. Statistical analyses are indicated in the text, and were done using PASW Statistics v.18.0 (SPSS, Inc); comparisons of regression coefficients followed Zar (1984). Allometric studies often avoid combining immature and adult stages in the same analyses in an effort to ensure that comparisons are made between homologous structures of comparable developmental stages, so we calculated separate regressions using adults or nymphs only, as well as combining adults with nymphs (see Discussion for rationale). Several individuals of each species and developmental stage were processed and qualitatively analyzed. We selected the best preparation of each for quantitative analyses, except for *Mysmena* sp. and *Mysmenopsis tengellacompa* for which we measured three adults and two nymphs, and present mean values for them. We used $\log_{10}$ transformed data in our analyses.

3. Results

3.1. Gross morphology and neuroanatomy

As in other spiders (Babu, 1975; Babu and Barth, 1984; Hill, 1975), the cephalothorax of all the species in our study contained the supra-oesophageal ganglion (sup-OG), and the sub-oesophageal ganglion (sub-OG), which includes the pedal, palpal and abdominal neuromeres (Fig. 2). The central portion of each ganglion is comprised of a neuropil (N) consisting of a tangled fibrous mass that is surrounded by a cellular cortex (C) (Figs. 2 and 3). The cortex is relatively thick near the upper and lower boundaries of the neuropil, which are readily identified, but in some places the outer limits of the cortex are difficult to discern.

The cortex contains a mass of apparent neuronal cell bodies (Fig. 3), which belong to neurons or glia, though these are often difficult to distinguish from neighboring tissue, especially in very small spiderlings. Additional preparations using DAPI staining and intracellular staining with Alexafluor showed that the regions we identified as cortex contained neuronal cell bodies (G. Vargas and J.K. Douglass, unpubl. obs.). We did not confirm all the cell bodies in the cortex were neuronal cell bodies, so we use the phrase "apparent neuronal cell bodies" throughout.

3.2. Distension of the sternum of L. mariana nymph

When viewed externally, the second instar spiderling of *L. mariana* has a conspicuous bulge in its sternum, whereas the adult sternum is flat (see photos in Eberhard and Wcislo, in press). Sagittal sections of these spiderlings reveal that this ventral bulge houses a mass of apparent neuronal cell bodies (SM Fig. 10). This mass was coherent and did not easily peel away from the CNS when dissected

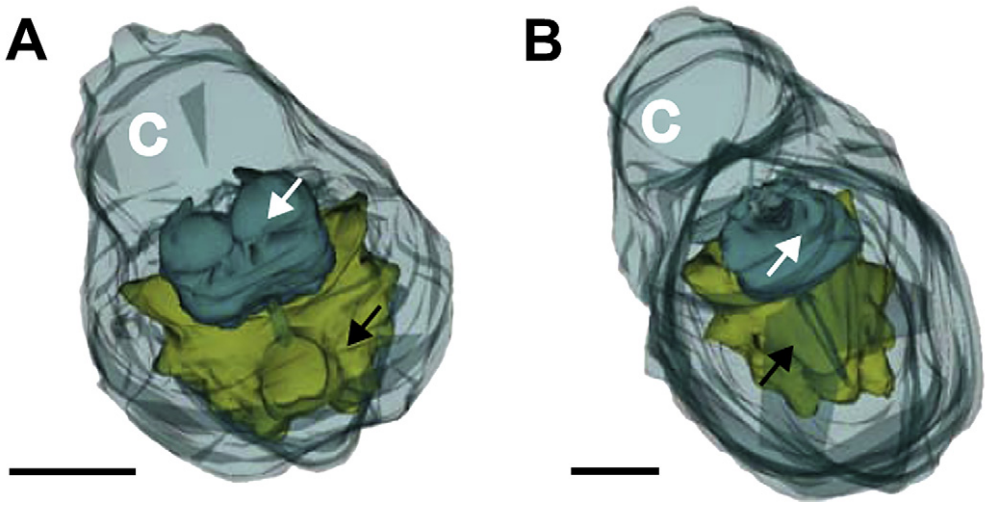

Fig. 2. Three-dimensional reconstructions (dorsal views) showing the cephalothorax, the supra-oesophageal (*white arrows*) and sub-oesophageal (*black arrows*) ganglia, of two spiders. (A) An adult female of an orb-weaver *Anapisona simoni*; (B) An adult female of a cleptoparasite *Faiditus elevatus*. Both scale bars are 200 um. C = cephalothorax. Anterior is to the top of the page.

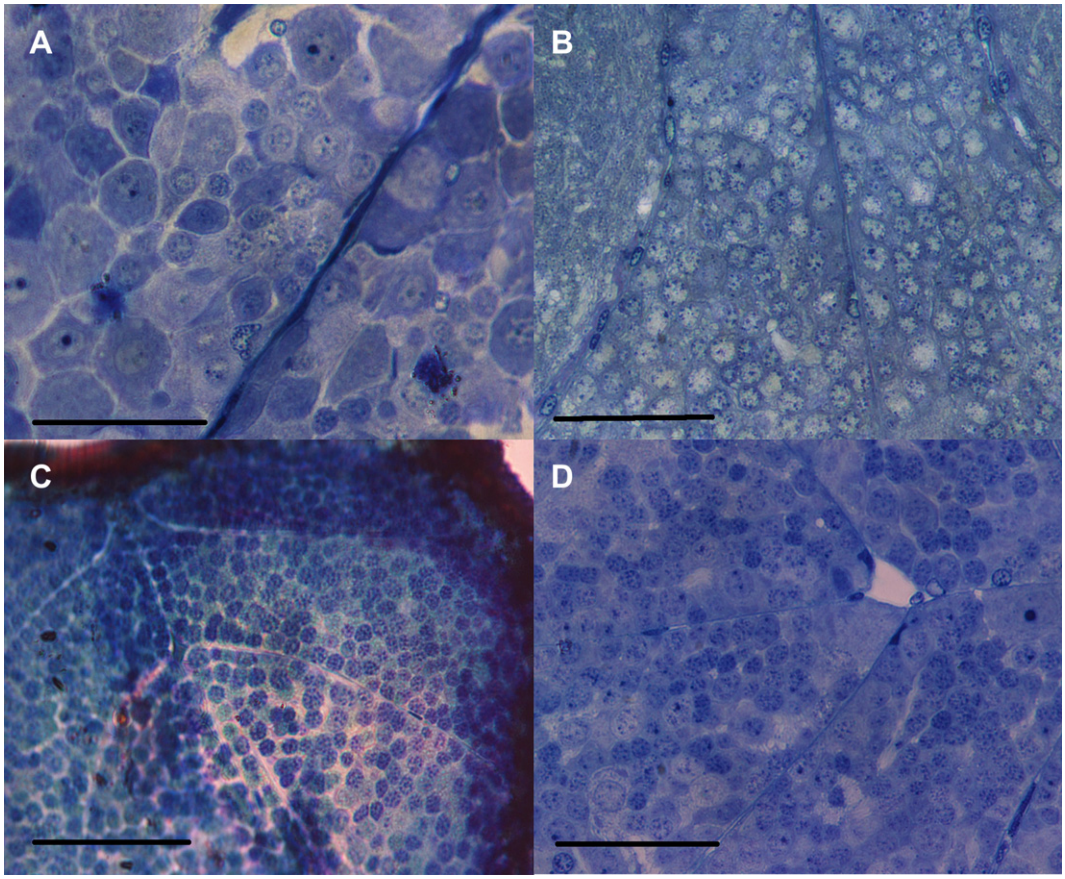

Fig. 3. Light micrographs of semi-thin sections of neuronal cell bodies in the cortex of different spider species. (A) *Argiope argentata*, adult female. (B) *Nephila clavipes*, adult male. (C) *Anapisona simoni*, adult female. (D) *Leucauge mariana*, nymph. All scale bars are 50 μm.

in Ringer's solution, in contrast to the much flatter layer of tissue on the lower side of the adult's sub-OG, which peeled away easily.

3.3. Expansion of CNS into the coxae

The sub-OG neuropil extends into the leg coxae in nymphs of five species from four families, all of which have a body mass <0.7 mg (SM Table 3; also Figs. 4 and 5). Expansions also occur in the adults of four small species from three families (SM Table 3). The tangled fibrous neuropil extending into the coxae was contiguous with the CNS neuropil in the cephalothorax and was distinguished from the leg nerves that contained the parallel, longitudinal organization typical of a peripheral nerve (Figs. 4 and 5). This organization is discernible in methylene blue stained sections (Fig. 4) and in reduced silver-stained sections (Fig. 5). Proportional extensions into the coxae are larger in smaller spiders, as in all four intra-specific comparisons involving tiny nymphs *versus* larger adults the extensions were proportionally greater in nymphs (SM Table 3). The largest extension occurs in the smallest spiderling in our sample (second instar of *A. simoni*—Table 1), and occupies 26.4% of the area of the coxa (SM Table 3).

3.4. CNS volume relative to body mass and cephalothorax volume

The volumes of both sup-OG and sub-OG were larger in spiders with greater body mass, but spiders with smaller body mass had relatively larger CNS volumes (Fig. 6). Based on adult males and females, CNS volume scaled with body mass$^{0.51}$ [log (CNS volume) = 0.51*log (body mass) + 1.506; $R^2 = 0.922$; $F_{1,10} = 188.01$, $P < 0.0001$] (Fig. 6, dashed line). The CNS volume of the nymphs from six species scaled with body mass$^{0.36}$ [log (CNS volume) = 0.36*log (body mass) + 1.52; $R^2 = 0.828$, $P = 0.01$)], but assumptions of the model were violated. This nymph-only regression coefficient was not significantly different from that for adults ($t = 1.58$, $P > 0.1$). Using the full data set of nymphs and adults (see Discussion for justification), CNS volume scaled with body mass$^{0.45}$ [log (CNS volume) = 0.45*log (body mass) + 1.585; $R^2 = 0.929$; $F_{1,16} = 208.437$, $P < 0.0001$] (Fig. 6, solid line). Thus, smaller spiders had relatively larger CNS volumes, irrespective of whether they were nymphs or adults.

The relatively larger CNSs of small spiders occupied proportionally more of the cephalothorax and extended into the coxae (Sections 3.3 and 3.4). The fraction of cephalothorax volume occupied by the CNS, estimated from histological sections and using a conservative estimate for cephalothorax volume (see Section 2.3), was larger in smaller spiders (SM Table 2). At the extremes, the CNS constituted 77.8% of the cephalothorax volume in *A. simoni* nymphs, but only 14.3% in *Leucauge* females. Considering only adults, the volume of cephalothorax occupied by the CNS scaled with body mass in grams$^{-10.3}$ [log (cephalothorax volume occupied by CNS) = −10.304*log (body mass) + 30.169; $R^2 = 0.539$; $F_{1,12} = 8.17$, $P = 0.024$] (Fig. 7, dashed line). The slope from the adult-only regression was not significantly different from that for nymphs only ($b = -12.257$) (small sample sizes violated assumptions of the model). Combining data from both adults and nymphs, the fraction of the cephalothorax occupied by the CNS scaled with body mass in grams$^{-13.7}$ [log (fraction of the cephalothorax occupied by the CNS) = −13.749*log (body mass) + 32.767; $R^2 = 0.791$;

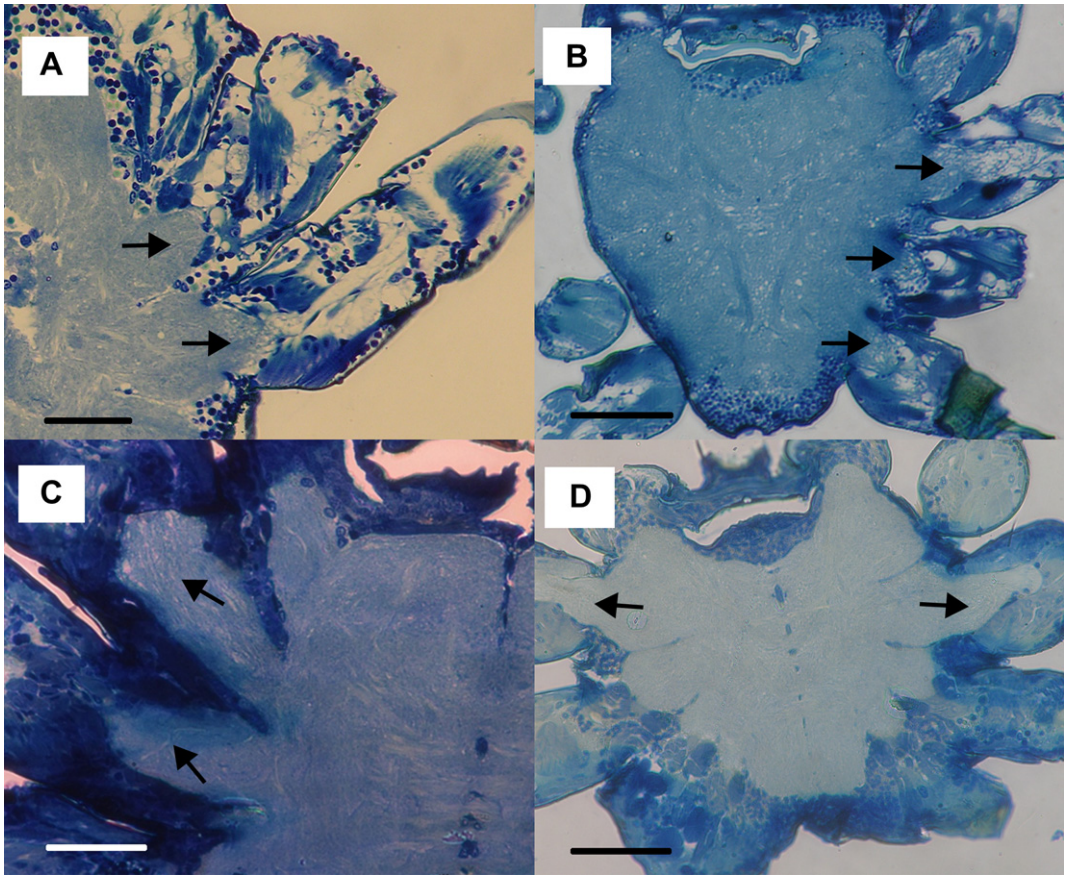

Fig. 4. Horizontal sections of several spider species showing extension of neuropil (*black arrows*) into coxae in tiny individuals. (A) *Leucauge mariana*, nymph 2. (B) *Mysmena* sp., nymph 2; (C) *Nephila clavipes*, nymph 2; (D) *Anapisona simoni*. adult female. All scale bars are 50 μm.

$F_{1,12} = 45.489$, $P < 0.0001$] (Fig. 7, solid line). Thus, the CNS occupied a greater proportion of the cephalothorax in small spiders than in large ones.

3.5. Relative volumes of CNS cortex and neuropil

The proportion of the CNS that was comprised of cortex varied among nymphs, adult females, and adult males ($\bar{x} \pm$ SE = 40.98 ± 1.83; 34.46 ± 2.05; and 28.52 ± 2.35 respectively) and tended to be higher in younger spiders (ANOVA, $F_{2,15} = 13.52$, $P < 0.0001$); Scheffé *post-hoc* tests showed that the overall significance was due to significant differences between nymphs and both adult females ($P = 0.032$) and adult males ($P < 0.0001$), and the *post-hoc* comparison between adult females and males was not significant ($P = 0.067$). A regression analysis of the relative volume of cortex *versus* body mass was not significant when using adults only ($P > 0.4$), nymphs only ($P > 0.4$), or the pooled data set ($P = 0.08$). The sup-OG of nymphs constituted a large proportion of total CNS volume ($\bar{x} = 0.41$, S.D. = 0.033, $N = 6$), and this relative percentage was nearly identical in tiny adults (body mass < 1 mg)

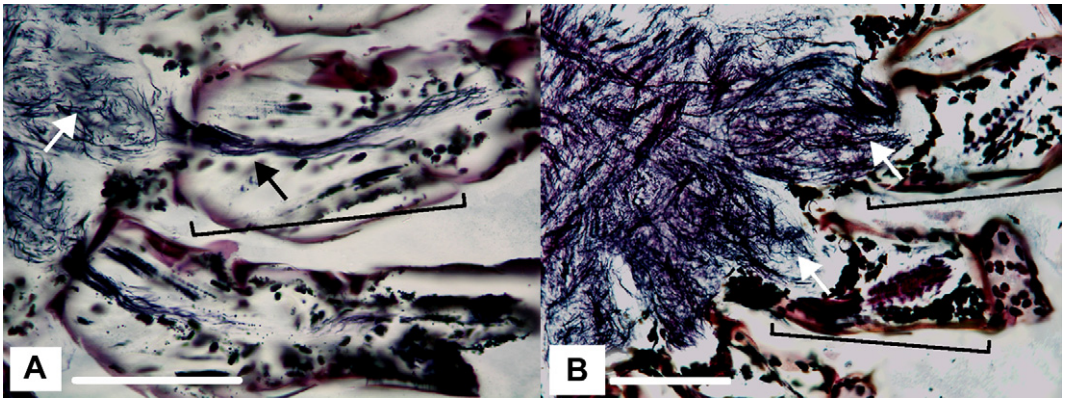

Fig. 5. Reduced silver-stained preparations showing the internal organization of the leg nerve and neuropil in legs of an adult female of *Mysmena* sp. (A) Leg nerve (*black arrow*) and neuropil (*white arrow*) of leg II. Scale bar is 100 μm. (B) Extension of the neuropil into legs I and II (*white arrows*). Scale bar is 100 μm. Solid black brackets indicate the approximate limits of the coxae.

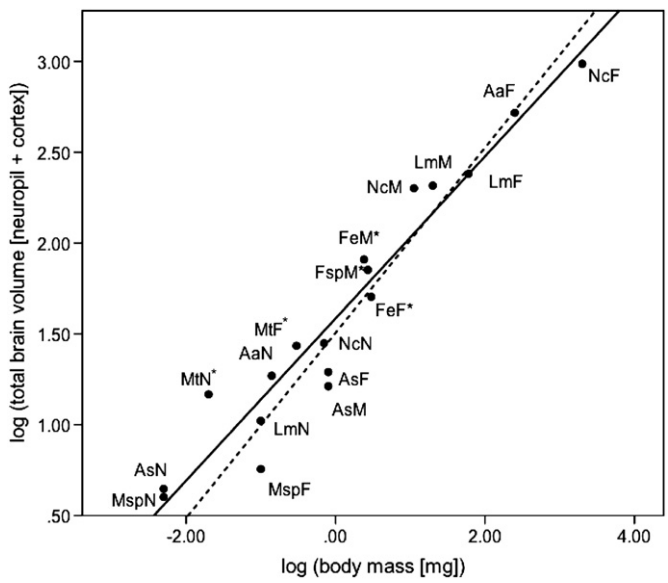

Fig. 6. Allometric relation between log of total brain volume (neuropil + cortex) and log (body mass) for adults and nymphs of orb-weaving spiders. The black line is the regression line for the pooled data set, and the dashed line is the line for adults only. Species names are coded as follows: Msp = *Mysmena* sp.; As = *Anapisona simoni*; Mt = *Mysmenopsis tengellacompa*; Fe* = *Faiditus elevatus*; Fsp* = *Faiditus* sp.; Lm = *Leucauge mariana*; Aa = *Argiope argentata*; and Nc = *Nephila clavipes*. M = adult male; F = adult female; N = nymph; * = cleptoparastic species.

($\bar{x}$ = 0.4, S.D. = 0.04, N = 4); for large-bodied adults (body mass > 1 mg) this proportion was smaller ($\bar{x}$ = 0.345, S.D. = 0.074, N = 8) (SM Table 4), but these differences were not significant.

3.6. Diameters of neuronal cell bodies

Comparison of transverse sections from the cortex of large and small spiders revealed differences in the diameters of apparent neuronal cell bodies (Fig. 3). The cortex of adult spiders with a greater body mass contained larger diameter apparent neuronal cell bodies than the cortex of nymphs from the same species or adults of species with lower body mass (Fig. 8). Based on adult males and females, neuronal cell body diameter scaled with body mass$^{0.19}$ [log (cell body diameter) = 0.19*log (body mass) + 0.69; R^2 = 0.82; $F_{1,11}$ = 51.60, P < 0.00001] (Fig. 8, dashed line). The CNS volume of the nymphs from six species scaled with body mass$^{0.17}$ [log (cell body diameter) = 0.17*log (body mass) + 0.84; R^2 = 0.95; $F_{1,5}$ = 102.16, P < 0.001]. Using the full data set of nymphs and adults (see Discussion for justification), CNS volume scaled with body mass$^{0.13}$ [log (cell body diameter) = 0.13*log (body mass) + 0.74; R^2 = 0.80; $F_{1,17}$ = 69.27, P < 0.00001] (Fig. 8, solid line). Thus, the smaller spiders had smaller apparent neuronal cell body diameter, irrespective of whether they were nymphs or adults.

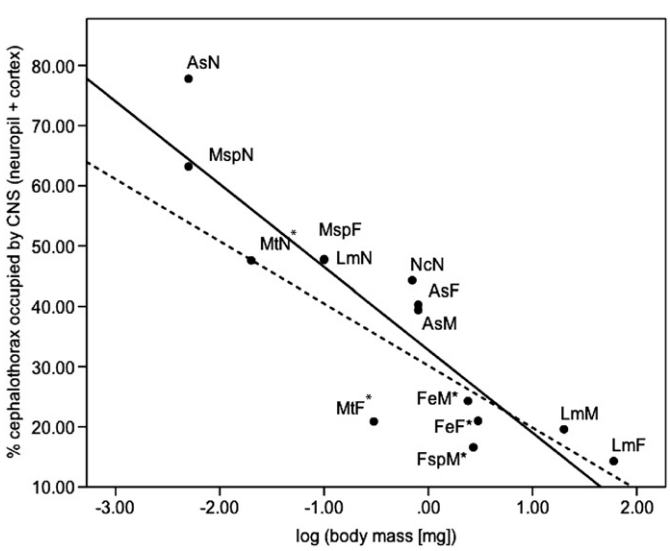

Fig. 7. Allometric relation between the percentage of the cephalothorax volume that is occupied by the CNS (cortex + neuropil), using a conservative estimate of cephalothorax volume, and log (body mass). The black line is the regression line for the pooled data set, and the dashed line is the line for adults only. Msp = *Mysmena* sp.; As = *Anapisona simoni*; Mt = *Mysmenopsis tengellacompa*; Fe* = *Faiditus elevatus*; Fsp* = *Faiditus* sp.; Lm = *Leucauge mariana*; Aa = *Argiope argentata*; and Nc = *Nephila clavipes*. M = adult male; F = adult female; N = nymph; * = cleptoparastic species.

4. Discussion

In general, spiders show CNS allometric patterns similar to those of vertebrates (Striedter, 2005) and insects (Rensch, 1948; Beutel et al., 2005; Seid et al., 2011) (reviewed in Eberhard and Wcislo, in press). Strikingly, the relatively large CNSs of tiny spiders "overflow" from the cephalothorax into the coxae of their legs and pedipalps. This overflow was only observed in the smallest spiders (both spiderlings and adults), and showed the highest values in the smallest species or developmental stages. Similar extensions of the CNS to the coxae also occur in some mites (GV and R. Madrigal-Brenes, unpubl. data). The oversized CNSs may also be related to a heretofore overlooked association in spiders between the external morphology of the sternum and the CNS. In lateral view, the sternum of a *L. mariana* mature female is nearly flat, while that of the spiderling bulges ventrally (see photo in Eberhard and Wcislo, in press). Internally this bulge contains an extensive mass of the sub-OG consisting of numerous apparent neuronal cell bodies. Similar bulges occur in the sterna of nymphs, but not adults, of the spiders *Nephila clavipes* and *Argiope argentata* (GV, pers. obs.). Assuming no other structural role for the bulge, it implies that the cephalothorax of some very small spiders may be deformed to provide additional space for their relatively oversized CNSs.

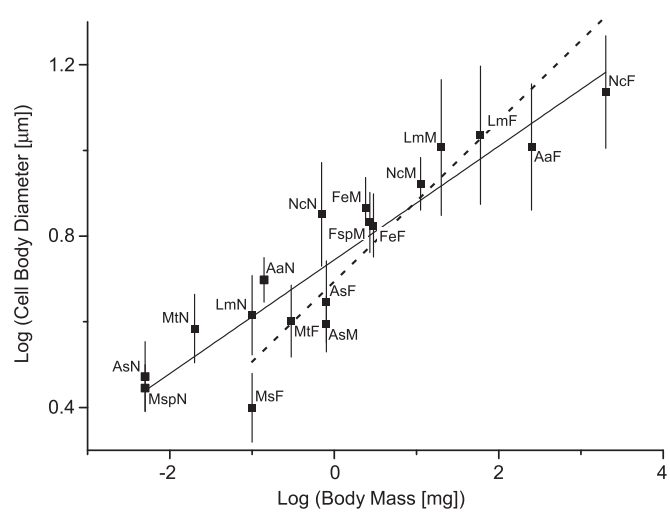

Fig. 8. Allometric relation between log (neuronal cell body diameter) and log (body mass). The black line is the regression line for the pooled data set, and the dashed line is the line for adults only. Species names are coded as follows: Msp = *Mysmena* sp.; As = *Anapisona simoni*; Mt = *Mysmenopsis tengellacompa*; Fe = *Faiditus elevatus*; Fsp = *Faiditus* sp.; Lm = *Leucauge mariana*; Aa = *Argiope argentata*; and Nc = *Nephila clavipes*. M = adult male; F = adult female; N = nymph.

The distortions of CNS shape resemble those in some tiny insects, in which portions of the brain extend into the prothorax and even the abdomen (e.g., Beutel et al., 2005; Polilov and Beutel, 2009; Grebennikov, 2008). Such morphological distortions suggest that tiny insects and spiders go to extreme lengths to provide space for neural tissue, presumably to minimize behavioral deficits that might be associated with very small CNS size. These modifications are analogous to those observed in miniature salamanders, which lost certain skull bones, providing additional room in the head for their relatively large brains (Hanken and Wake, 1993; Roth et al., 1995). Whether the small spiders in our study have also lost structures within their cephalothorax to accommodate their proportionally large CNS is not known. Comparing adults and nymphs of the jumping spider, *Phidippus clarus*, there is an increase in the digestive diverticula in the adult cephalothorax (Hill, 1975). It is not known if this difference is associated with any dietary changes between nymphs and adults, but this observation raises the possibility that nymphs may possess a relatively large CNS at the expense of the digestive diverticula. Whether such a reduction in the digestive diverticula occurs in the adults of small species is also unknown.

All the spiderlings we studied had a larger proportion of the cephalothorax filled with CNS than did adults. This relationship also occurs in the orb-weaving spider *Argiope aurantia* in which the proportion of the cephalothorax occupied by the sup-OG is more than ten times larger (46%) in an average 0.4 mg first instar individual, relative to the corresponding value (4%) in an average 140 mg adult female (Babu, 1975). In many of the very small species we studied, the CNS occupied a large proportion of the cephalothorax volume. For example, 63% of the cephalothorax volume is occupied by the CNS in second instar nymphs of *Mysmena* that weigh $<$0.005 mg whereas in 0.1 mg adult females this drops to 48%. Thus, the CNS is proportionately large in early developmental stages in comparison to other tissues. The second instar nymphs in our study are independent, and thus require the necessary neural capacities for web-building. Comparison of adults of species with large disparities in size between the sexes also showed that the CNSs of small-bodied males occupied a proportionally larger volume of the cephalothorax than the larger-bodied females. For example, 20% of the cephalothorax volume is occupied by CNS in adult males of *L. mariana* that weight 20 mg whereas in 60 mg adult females this drops to 14%.

A comparison of spiderlings with adults of *A. aurantia* also showed differences in the relative sizes of the subdivisions of the CNS, and differences in relative size, with smaller spiderlings having relatively larger sup-OG (Babu, 1975). Table 4 of the SM combines our data with those of Weltzien and Barth (1991), and shows that smaller animals do not consistently have relatively larger sup-OG, as stated by Babu (1975). Such comparisons should be interpreted with caution because previous studies of spider CNS size used different methods to measure volumes, so comparisons of the absolute values from different studies may be unreliable.

A reduction in the absolute volume of the CNS must be accompanied by changes in the components of the nervous system, such as neuron size, number, or density. The smaller spiders in our study have smaller cell body diameters in the cortex irrespective of their developmental stage. With diameters between 2 and 3 μm, the cell bodies of the smallest spiders approach the minimum possible because of the diameter of the nucleus (Grebennikov, 2008). The diameters of cell bodies in the CNS of smaller species were less variable than those of larger species, consistent with the idea that they have been reduced to nearly the minimum possible. We did not count neuron numbers in our study, but Babu (1975) observed that the numbers of cells in the adult and second instar spiderlings of *Argiope aurantia* were essentially the same despite a 24-fold difference in absolute volume. Taken together these findings are consistent with the idea that the differences in CNS volumes of spiderlings and adults are due to growth of cell bodies and possible neurites in the intervening developmental stages.

Allometric studies often do not combine immature and adult stages in the same analyses, in an effort to compare homologous structures of comparable developmental stages (see Striedter, 2005). In contrast, here we argue that comparisons including nymphs and adults of web-building spiders are particularly informative, for several reasons. Most importantly, if there are limitations on brain size imposed by body size (see Roth et al., 1990; Bonner, 2006), then organisms must confront them regardless of developmental stage; animals cannot avoid them simply by virtue of being immature. Conspecific nymphs and adults show similar (homologous) web-building behavior, despite significant differences in the degree of neuronal maturity. Finally, intra-specific comparisons eliminate potentially confounding effects arising from phylogenetic differences. Collectively, our data comparing adults and nymphs, males and females, and web-building and cleptoparasitic spiders, show that animals with different behavioral and natural history challenges exhibit similar adjustments to the consequences of small body size that follow from Haller's Rule governing brain size relative to body size. Further studies are needed to assess whether small spiders also economize on CNS volume by utilizing more efficient but less flexible perceptual mechanisms, or more efficient neural ones (references in Introduction).

The hypothesis that there are behavioral deficits associated with extremely small size is contradicted by the limited available data. Very small spiders exhibit no manifestations of inferior behavior, such as errors in web architecture (Eberhard, 2007, 2011; Hesselberg, 2010). It remains to be determined whether small species of other groups are similar in this respect (reviewed in Eberhard and Wcislo, in press). A significant limitation of our study is that we present only volumetric data for the entire CNS, and cannot associate particular behaviors with changes in specific CNS regions. Weltzien and Barth (1991) measured relative volumes of the central body neuropil in four species of spiders that differed significantly in their web-building behavior, and demonstrated that the relative size of the central body is roughly constant across species, and thus not related to behavioral demands associated with web-building. The three cleptoparasitic species in our study (*M. tengellacompa*, *Faiditus* sp. and *F. elevatus*) do not spin webs, yet have CNS volumes comparable to those of similarly sized web-building species. The independent loss (in different families) of web-building behavior shows the lack of an association between CNS volume and behavioral capacities associated with web construction. Such examples point to a difficulty in making precise behavioral comparisons among taxa (Eberhard and Wcislo, in press). Although the behavioral capacities of cleptoparasitic spiders are greatly reduced with respect to web-building behaviors, they are enhanced in others (e.g., Vollrath, 1979; Whitehouse, 1986; Eberhard et al., 1993), and presently there is no basis to assume one or the other requires more neural processing. Analogous examples are known for brood parasitic birds, which have lost their nest-building behavior, but have an enhanced need for spatial learning abilities because they must regularly re-visit multiple host nests; consistent with these behavioral differences, females of the brood parasitic brown-headed cowbird have relatively large hippocampal volumes (Sherry et al., 1993).

Very small orbicularian spiders evolved solutions to the miniaturization problem that involve a reduction in cell body diameter; modifications to the cephalothorax that permit the CNS to nearly fill all available space, even overflowing into the coxae and distorting the shape of their exoskeletons. These structural modifications, and the relative cephalothorax volumes occupied by their CNSs, are

presumably consequences of a need to sustain behavioral capabilities. We now need detailed anatomical studies, and thorough behavioral studies, to more fully understand these scaling patterns.

Acknowledgments

We thank S. Tierney, A. Smith, N. Strausfeld, and two anonymous reviewers for help with discussions and comments on the manuscript. Financial support was provided by the F. H. Levinson Fund, the Smithsonian Institution's Scholarly Studies Program to WTW (PI), the Smithsonian Tropical Research Institute's (STRI) "Adelante" Internship program (to RQ), the Royal Society (to JEN), and general research funds from STRI to WTW and WGE. We are grateful to the STRI staff for logistical support, and to the Autoridad Nacional del Medio Ambiente for collecting and research permits, SE/A-51-10.

Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.asd.2011.07.002.

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