Brain, Behavior and Evolution

Behavioral Flexibility Positively Correlated with Relative Brain Volume in Predatory Bats

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Abstract

We investigated the potential relationships between foraging strategies and relative brain and brain region volumes in predatory (animal-eating) echolocating bats. The species we considered represent the ancestral state for the order and approximately 70% of living bat species. The two dominant foraging strategies used by echolocating predatory bats are substrate-gleaning (taking prey from surfaces) and aerial hawking (taking airborne prey). We used species-specific behavioral, morphological, and ecological data to classify each of 59 predatory species as one of the following: (1) ground gleaning, (2) behaviorally flexible (i.e., known to both glean and hawk prey), (3) clutter tolerant aerial hawking, or (4) open-space aerial hawking. In analyses using both species level data and phylogenetically independent contrasts, relative brain size was larger in behaviorally flexible species. Further, relative neocortex volume was significantly reduced in bats that aerially hawk prey primarily in open spaces. Conversely, our foraging behavior index did not account for variability in hippocampus and inferior colliculus volume and we discuss these results in the context of past research.

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Introduction

Brain and brain region size and differentiation are positively related to sensory, cognitive, and motor activities [Streidter, 2004]. Species with relatively larger brains might be better able to cope with complex and fluctuating environments as a result of enhanced capacity for information processing and storage, decision-making, and initiation of appropriate behavioral responses [Reader and Laland, 2002; Streidter, 2004]. In mammals, the overall expansion of the brain can be partially explained by the evolution and elaboration of the forebrain [Streidter, 2004]. In the forebrain, the neocortex provides the neural substrate for internal representations of the external world and uses these templates for risk-assessment, decision-making and initiation of behavioral responses [Kaas, 1989; Dear et al., 1993; Streidter, 2004]. Among mammals and birds that have been studied, the hippocampus plays an important role in learning and memory, particularly of spatial information [O'Keefe and Nadel, 1978; Healy et al., 2005] and receives most of its input from the neocortex [Streidter, 2004]. The relative size of both neocortex and hippocampus [i.e., volume relative to body mass and/or to the rest of the brain; see Harvey and Krebs 1990] is typically associated with the cognitive demands of ecologically relevant behaviors. In birds and primates, degree of behavioral flexibility is positively correlated with relative brain and neocortex volume [Lefebvre et al.,

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1997; Reader and Laland, 2002]. In birds and rodents, exceptional species- or sex-specific needs for spatial information processing are associated with enhanced hippocampus volume [Healy et al., 2005].

Bats are an intriguing group for further tests of the association between the cognitive demands of foraging style or habitat use and the relative size of specific brain areas. Bats are the most ecologically diverse and geographically widespread group of mammals, second only to rodents in sheer number of species [Jones et al., 2002; Jones and MacLarnon, 2004]. This success has been attributed to the evolution of flight and echolocation, which allowed early bats to exploit nocturnal flying insects as prey [Griffin, 1958; Neuweiler, 1990]. Unlike the exceptional neocortices of most primates, and with the exception of regions devoted to the processing of sound, the neocortices of microbats are typically mammalian [Pollack and Casseday, 1989; O'Neill, 1995; Pollak et al., 1995; Baron et al., 1996; Radtke-Schuller, 2001]. Bats have been intensively studied with respect to ecology and foraging behavior [Neuweiler, 1989; Fenton, 1990; Arita and Fenton, 1997; Schnitzler et al., 2003], and an extensive database on brain and brain region size now exists within a single publication [Baron et al., 1996]. Moreover, a molecular phylogeny of bats complementing the fossil record has recently been reported [Teeling et al., 2005].

In our report we use this available data to test the hypothesis that relative brain and neocortex volume are correlated with behavioral flexibility in foraging. To our knowledge, this is the first investigation of a potential link between behavioral flexibility in foraging strategy and relative brain and brain region volumes in bats or any other group of mammals except primates [see Reader and Laland, 2002]. Because relative hippocampus volume has been associated with ecological demands on spatial memory in various other mammals and birds [for a recent discussion see Healy et al., 2005], we also tested whether relative hippocampus volume is correlated with size of foraging area or home range size or with spatial complexity of preferred foraging habitat in bats. Finally, because audition is a predatory bats' primary modality for localizing prey (whether by echolocation or by listening to prey-generated sounds), we tested the hypothesis that relative inferior colliculus volume is associated with the type of foraging behavior. At the neurophysiological level the inferior colliculus, located in the midbrain, is the most intensively studied of all auditory processing regions in echolocating bats and other mammals [Pollak and Park, 1995; Neuweiler, 2000]. It integrates auditory information from all lower auditory nuclei and outputs to the neocortex a synthetic representation of the various forms of information [Pollak and Park, 1995].

Bats have been previously studied with respect to how aspects of their ecology are related to relative brain and brain region size [Eisenberg and Wilson, 1978; Barton et al., 1995; Hutcheon et al., 2002; Jones and MacLarnon, 2004; Safi and Dechmann, 2005]. To date, however, most comparative studies have used diet [e.g., frugivorous versus predatory species; Hutcheon et al., 2002; Jones and MacLarnon, 2004], sensory information processing [i.e., vision, echolocation, olfaction; Barton et al., 1995; Hutcheon et al., 2002; Jones and MacLarnon, 2004; Safi and Dechmann, 2005] or ecological niche [Safi and Dechmann, 2005] for categorizing species. Here we focus on echolocating, predatory microbats that comprise about 750 of the 1,131 extant bat species [Jones et al., 2002], thus allowing a fine scale investigation of the potential relationship between foraging behavior and brain and brain region size controlling for diet (animals), activity timing (nocturnal), and key sensory modality for orientation (echolocation).

The two dominant foraging strategies used by echolocating predatory bats are substrate-gleaning (taking prey from surfaces) and aerial hawking (taking airborne prey) [Norberg and Rayner, 1987; Arlettaz et al., 2001]. These strategies differ with respect to echolocation call design requirements [Schnitzler and Kalko, 2001], information acquisition and processing [Fuzessery, 1997], and flight mechanics and behavior [Norberg and Rayner, 1987; Ratcliffe and Dawson, 2003]. Many species are specialized with respect to one strategy; however, those species that we refer to as behaviorally flexible use both strategies to good effect. During an aerial hawking attack, the positions of both predator and would-be prey are constantly changing in dynamic and often unpredictable ways until the point of capture [Neuweiler, 1990; Moss and Surlykke, 2001]. Call sequences recorded during these attacks stereotypically consist of three distinct phases with respect to echolocation behaviors: search, approach and buzz [Griffin et al., 1960; Kalko, 1995; Surlykke and Moss, 2000; Schnitzler and Kalko, 2001]. In contrast to hawking, during substrate gleaning attacks bats attempt to capture stationary or slow moving prey from the ground, foliage or tree trunks. During these attacks bats do not typically produce a buzz [Schnitzler and Kalko, 2001], and although it is clear that echolocation alone is sufficient for the detection and localization of airborne targets [Fenton, 1995], for gleaning bats prey-generated sounds also appear necessary for the successful capture of

prey [e.g., Fuzessery, 1997; Arlettaz et al., 2001; Ratcliffe and Dawson, 2003]. It has been suggested that gleaning bats have relatively larger inferior colliculi because of the demands of processing both echoic and prey-generated sounds simultaneously [Baron et al., 1996; Reep and Bhatnagar, 2000].

We placed predatory bats in four categories: (1) ground gleaning bats, (2) behaviorally flexible bats (i.e., species that both glean and aerial hawk), (3) clutter tolerant aerial hawking bats, and (4) open space aerial hawking bats. Clutter tolerant bats differ from open space aerial hawking bats in two respects: their wing morphology allows them to maneuver in and at the edge of vegetation with relative ease [Norberg and Rayner, 1987] and their echolocation calls are designed to reduce call-echo overlap and increase target and background resolution [Schnitzler et al., 2003]. All ground-gleaning bats in our data set are thought to take airborne prey at times. All clutter tolerant aerial hawking species, to our knowledge, have not been reported to glean prey. Behaviorally flexible species are highly maneuverable and able to glean prey like some ground-gleaning bats, while also agile and able to take airborne prey like clutter-tolerant aerial hawking bats [e.g., *Hipposideros ruber*, Bell and Fenton, 1984; *Myotis emarginatus*, Schumm et al., 1991; *M. evotis*, Faure and Barclay, 1994; *Rhinollophus blasii*, Siemers and Ivanova, 2004]. Accordingly, a behaviorally flexible species' wing morphology and echolocation behavior need to be consistent with both modes of foraging, but by themselves, these characteristics are not reliable proxies for behavioral flexibility [Ratcliffe and Dawson, 2003; Stoffberg and Jacobs, 2004; U. M. Lindhe-Norberg, personal communication]. In our classification, behaviorally flexible species were identified as such because they had been observed to both aerial hawk and glean prey and had wing morphology and echolocation behavior (if known) consistent with these observations. We recognize, however, that there is more than one feasible solution to the problem of categorizing echolocating bats [e.g., Schnitzler et al., 2003].

In summary, we hypothesized that the behaviorally flexible species would have relatively larger brains/neocortices than would species relying primarily on either hawking or gleaning alone, consistent with findings from birds and primates. Based on published literature, we hypothesized that the inferior colliculus would be larger in bats that glean some or all of their prey. Lastly, given the paucity of data on the function(s) of the hippocampus in bats we were interested to see if and how it related to our four foraging categories.

Materials and Methods

Data Assembly

Ecomorphological measures of wing design and body mass were taken from Norberg and Rayner [1987] and Norberg and Fenton [1988]. Relative wing loading (an index independent of body mass) for each species was recalculated using formulae provided in Norberg [1998]. Body mass and brain, hippocampus, inferior colliculus, and neocortex volume data were taken from Baron et al. [1996]. A total of 70 predatory species is found in both data sets. After excluding several species for reasons explained below, our data set consisted of 59 species for foraging behavior, relative brain, neocortex, and hippocampus volumes, and 37 species for foraging behavior and relative inferior colliculus volumes (see table 1). Body mass varies between, and fluctuates within, individuals of the same species [Harvey and Krebs, 1990]. We therefore used the matched and averaged body masses and brain and brain region volumes from the tables presented in Baron et al. [1996] for our comparative analyses, but validated the body mass values used by comparing reported body masses from Baron et al. [1996] to those from Norberg and Rayner [1987] and Norberg and Fenton [1988], and averaged capture data (n = 5/species for 58 species; one species, *Craseonycteris thonglongyai*, was coded as missing data) from the collections of the Royal Ontario Museum (Toronto, Canada) using repeated-measures ANOVAs for all species and for each foraging behavior category (see below). At the level of species and foraging behavior category, we found no significant differences between these three independent data sets (n = 59, p > 0.05 all tests).

Foraging Behavior Categorization

Using morphological (see above) and behavioral data (compiled from 200+ references from the literature, references available from corresponding author), we assigned each bat species to one of four foraging behavior categories.

Category 1 consists of ground gleaning predatory bat species as defined by criteria outlined in Norberg and Fenton [1988]. Briefly, these bats are all low-duty cycle echolocating species that consistently use short duration, low intensity, broadband calls to prevent call-echo overlap and self-deafening and increase target-background resolution [Ratcliffe et al., 2005]. These bats tend to be large and take primarily large surface-bound prey, often including vertebrates in addition to arthropods, using prey-generated sounds for the detection and localization of prey. Wing morphology (low aspect ratio = broad wings with rounded tips) and low wing loading (large wing surface area to body mass ratio) corroborate these observations and suggests that these large bats are capable of lifting relatively large terrestrial prey, prey that should be more energetically beneficial than are airborne prey for these slow flying predators [Norberg and Fenton, 1988]. We refer to species in this category as ground gleaning bats.

Category 2 consists of species that have been observed to both glean and hawk prey and are considered gleaning and hovering species or slow hawking species according to Norberg and Rayner [1987] based on wing morphology and wing loading. Briefly, these bats include high and low duty-cycle echolocators. Most importantly they glean their surface bound arthropod prey in a manner similar to ground gleaning bats (Category 1), but hawk their airborne insect prey like clutter tolerant aerial hawking bats (see Category 3 below). These species are thought to hunt successfully in both cluttered and open environments [Fenton, 1990]. We follow

Table 1. Family, genus, species name and foraging behavior categories for the 59 predatory bats used in our comparative analyses

Family	Species	Category	Family	Species	Category
Rhinolophidae	*Rhinolophus hipposideros*	2	Vespertilionidae	**Chalinolobus gouldii**	3
	R. landeri	3		*C. morio*	3
	R. megaphyllus	2		*Eptesicus fuscus*	4
Hipposideridae	*Hipposideros caffer*	2		**E. serotinus**	2
	H. speoris	2		**Lasiurus borealis**	4
	Triaenops persicus	3		**Miniopterus australis**	3
Megadermatidae	*Cardioderma cor*	1		*M. inflatus*	3
	Lavia frons	2		*M. schreibersii*	3
	Macroderma gigas	1		*Myotis bechsteini*	2
	Megaderma lyra	1		**M. myotis**	1
	M. spasma	2		*M. nattereri*	2
Craseonycteridae	**Craseonycteris thonglongyai**	2		*M. nigricans*	3
Rhinopomatidae	**Rhinopoma hardwickii**	4		**Nyctalus noctula**	4
	R. microphyllum	4		*Nyctophilus geoffroyi*	2
Nycteridae	**Nycteris grandis**	1		**N. timoriensis**	2
	N. hispida	2		**Pipistrellus mimus**	3
	N. macrotis	2		**Scotophilus dinganii**	3
	N. thebaica	2		**S. heathii**	4
Emballonuridae	**Emballonura monticola**	3		**Scotozous dormeri**	3
	E. semicaudata	3		**Tylonycteris robustula**	4
	Rynchonycteris naso	3	Molossidae	*Chaerephon plicata*	4
	Saccopteryx bilineata	2		**C. pumila**	4
	Taphozous mauritianus	4		*Eumops glaucinus*	4
	T. melanopogon	4		**Molossus ater**	4
Phyllostomidae	**Micronycteris megalotis**	2		**M. molossus**	4
	Mimon crenulatum	2		**Otomops martiensseni**	4
	Lophostoma silvicolum	2		*Tadarida aegyptiaca*	4
	Trachops cirrhosus	1		**T. condylura**	4
	Vampyrum spectrum	1			
Mormoopidae	**Pteronotus gymnonotus**	3			
	Mormoops megalophylla	4			

Species for which we had inferior colliculus volume data are indicated in bold.

1 = Ground gleaning species; 2 = behaviorally flexible species; 3 = clutter-resistant aerial hawking species; 4 = open space aerial hawking species.

Ratcliffe and Dawson [2003] and Siemers and Ivanova [2004] and refer to species in this category as behaviorally flexible bats.

Category 3 consists of species that have only been reported to aerially hawk their prey and are considered to be slow hawking species, according to Norberg and Rayner [1987], based on wing morphology and wing loading. These species have not, to our knowledge, been reported to glean their prey and have been observed to hunt insects in both cluttered and open environments. We refer to these species as clutter tolerant aerial hawking bats.

Category 4 consists of species that have been reported to aerially hawk their prey in open spaces, not observed to hunt in cluttered environments and are considered fast hawking species according to Norberg and Rayner [1987] based on wing morphology and wing loading. We follow Fenton [1990] and refer to these species as open space aerial hawking bats.

Five species were difficult but not impossible to categorize and, due to their ambiguous status, our classifications of these species deserve comment. According to the measurements reported in Norberg and Rayner [1987], *Miniopterus schreibersii* falls into the slow hawking category whereas *M. inflatus* falls into the fast hawking category. On the basis of visual inspection both *M. schreibersii* and *M. inflatus* captured from the wild by one of us (M.B.F.) seemed to have more similar wing morphology and wing loading. Measurements of *M. inflatus* taken from the collections of the Royal Ontario Museum confirmed this impression and we therefore placed *M. inflatus* with its congener *M. schreibersii* into Category 3.

Norberg and Rayner [1987] classify *Craseonycteris thonglongyai* as a gleaning and hovering species. We classify this species as behaviorally flexible (Category 2). *C. thonglongyai* is a small (<2 g), rare, and relatively unstudied species endemic to Thailand. Al-

though there are no confirmed reports of gleaning behavior, all other data strongly suggest that this hovering species, which uses short duration, high frequency, broadband echolocation calls, can both glean and hawk prey [Hill and Smith, 1981; Norberg and Rayner, 1987; Surlykke et al., 1993].

Eptesicus fuscus could have been assigned to either category 3 or 4. We assigned this bat to category 4 based on field observations of their preferred habitat type [Brigham and Fenton 1991] and echolocation behavior in a cluttered flight room [D.S. Jacobs and J.M. Ratcliffe, unpublished observations]. Similarly, *Scotophilus heathii* could also have been assigned to either category 3 or 4. We assigned this species to category 4 because of its relatively large size [mass = 34.5 g, wingspan = 37.2 cm; measures taken from Norberg and Rayner, 1987] and overall morphological similarity to the open space aerial hawking bat *Lasiurus cinereus* [Barclay, 1985; Norberg and Rayner, 1987].

Tadarida aegyptiaca has been reported to hunt on the ground [Advani, 1982]. However, by all other measures this species appears to be an open space aerial hawking bat. Therefore, we assigned *T. aegyptiaca* to category 4 just as all other molossids in our study.

Species identified as trawling bats *(Noctilio leporinus, N. albeventris* and *Myotis adversus, M. albescens, M. bocagei, M. dasyncheme)* in Norberg and Rayner [1987] were excluded from further analysis because the sensorimotor demands of this strategy differ from those of substrate gleaning and aerial hawking [Siemers et al., 2001]. *Hipposideros diadema* and *H. commersoni* defied categorization and so were also excluded (see table 1 for species-specific classifications).

Comparative Methods

Species level comparisons do not control for the potentially confounding effects of common ancestry [Felsenstein, 1985; Harvey and Pagel, 1991; Price, 1997; Harvey and Rambault, 2000]; however, species level comparisons do allow for the effect of covariance of ecology and phylogeny [Brown et al., 1996; Willig et al., 2003]. Conversely, phylogenetically independent contrasts remove the effects of hypothetical evolutionary relationships [Felsenstein, 1985; Harvey and Pagel, 1991], but in doing so might also remove relevant variation due to shared ecological histories [Brown et al., 1996; Price, 1997; Harvey and Rambault, 2000; Willig et al., 2003]. Both methods can thus include and/or omit biologically relevant information [Price, 1997; Harvey and Rambault, 2000], and, as in Pitcher et al. [2005], we therefore performed both types of analysis. For both, we log transformed the data on body mass, brain volume, and, for inferior colliculus, hippocampus, and neocortex, brain region volume and brain remainder volumes (total brain volume – brain region of interest volume) and took standardized residuals [Harvey and Pagel, 1991] from the log-log regressions as species-specific measures of relative brain and brain component volumes (fig. 1).

We compared residuals at the species level and, to control for possible phylogenetic effects [Felsenstein, 1985; Harvey and Pagel, 1991], calculated standardized independent linear contrasts based on these residuals using the program Comparative Analysis of Independent Contrasts (CAIC) v. 2.6.8 [Purvis and Rambault, 1995]. Independent contrasts were standardized assuming equal branch lengths reflecting a speciation model of evolution [Harvey and Rambault, 2000]. In CAIC, we used the Crunch procedure to analyze all variables [Dunn et al., 2001; Pitcher et al., 2005]. Our independent variable (foraging behavior) was categorical (discontinuous) and delineated into the four levels using phylogenetically transformed dummy variables [Winquist and Lemon, 1994; Martin, 1995; Martin and Badyaev, 1996; Dunn et al., 2001; Pitcher et al., 2005]. All dependent variables (relative brain and brain region volumes) were continuous.

Potential Behavioral/Anatomical Confound

Most predatory bats use frequency-modulated, downward sweeping signals, produced at a low duty-cycle (i.e., <<20% of the time they are echolocating). Low duty-cycle species separate pulse and echo in time and appear unable to tolerate overlap between call and echo [Fenton, 1995; Grinnell, 1995]. However, bats belonging to the families Rhinolophidae and Hipposideridae and the mormoopid *Pteronotus parnellii* are high duty-cycle echolocators, separating pulse and echo in frequency. They use longer, primarily constant frequency calls up to and beyond 80% of the time they are echolocating. High duty-cycle echolocators tolerate call-echo overlap and use Doppler-shift compensation to detect and assess potential prey [Schnitzler and Henson, 1980; Bell and Fenton, 1984]. These strikingly different approaches to echolocation are reflected in the neural structures responsible for auditory processing [Pollak and Park, 1995; O'Neill, 1995]. Fossil evidence suggests that both approaches have been present since the Eocene [Simmons and Geisler, 1998]. Because high duty-cycle species (6 in total) are found only in categories 2 and 3 (table 1), we re-ran all of our analyses after removing these species from our data set to control for the potentially confounding effects of this approach to echolocation on relative brain and brain component volumes.

Our phylogeny was based on the molecular phylogeny of Teeling et al. [2005] for inter-familial relationships. When more than two species represented a family (8 of 11 families, table 1) we used the phylogeny of Jones et al. [2002] for species level relationships (including relevant polytomies) within Emballonuridae, Hipposideridae, Megadermatidae, Molossidae, Nycteridae, and Phyllostomidae and the complementary molecular phylogenies of Hoofer and Van Den Bussche [2003] and Ruedi and Mayer [2001] for species level relationships within Vespertilionidae. With the exception of *Pipistrellus mimus*, we excluded pipistrelles *(P. ceylonicus, P. nanus, P. subflavus)* because this group is paraphyletic [Hoofer and Van Den Bussche, 2003]. Similarly, *Eptesicus* species might represent a paraphyletic group [Hoofer and Van Den Bussche, 2003]. Therefore, we excluded *Eptesicus (= Vespadelus) pumilus* because *E. pumilus* does not appear in any phylogeny known to us. We used the molecular phylogeny of Guillén et al. [2003] for species-level relationships within Rhinolophidae.

Statistical Analyses

Comparative analyses of standardized residuals [Harvey and Pagel, 1991] for our four behavioral categories were run using ANOVA at the species level (considering each species as an independent sample) and phylogenetically independent contrasts level (controlling for assumed non-independence due to hypothetical evolutionary relationships). Additionally, we set foraging behavior as the independent variable, log brain or log brain region volumes as the dependent variable and log body mass or log total brain volume as the covariate and reanalyzed the species level data using GLM [Darlington and Smulders, 2001]. Species level GLM and ANOVA results did not differ qualitatively (i.e., differ in significance or relationships of category means); to facilitate comparison between species level and independent contrasts level analyses, we include only ANOVA results here.
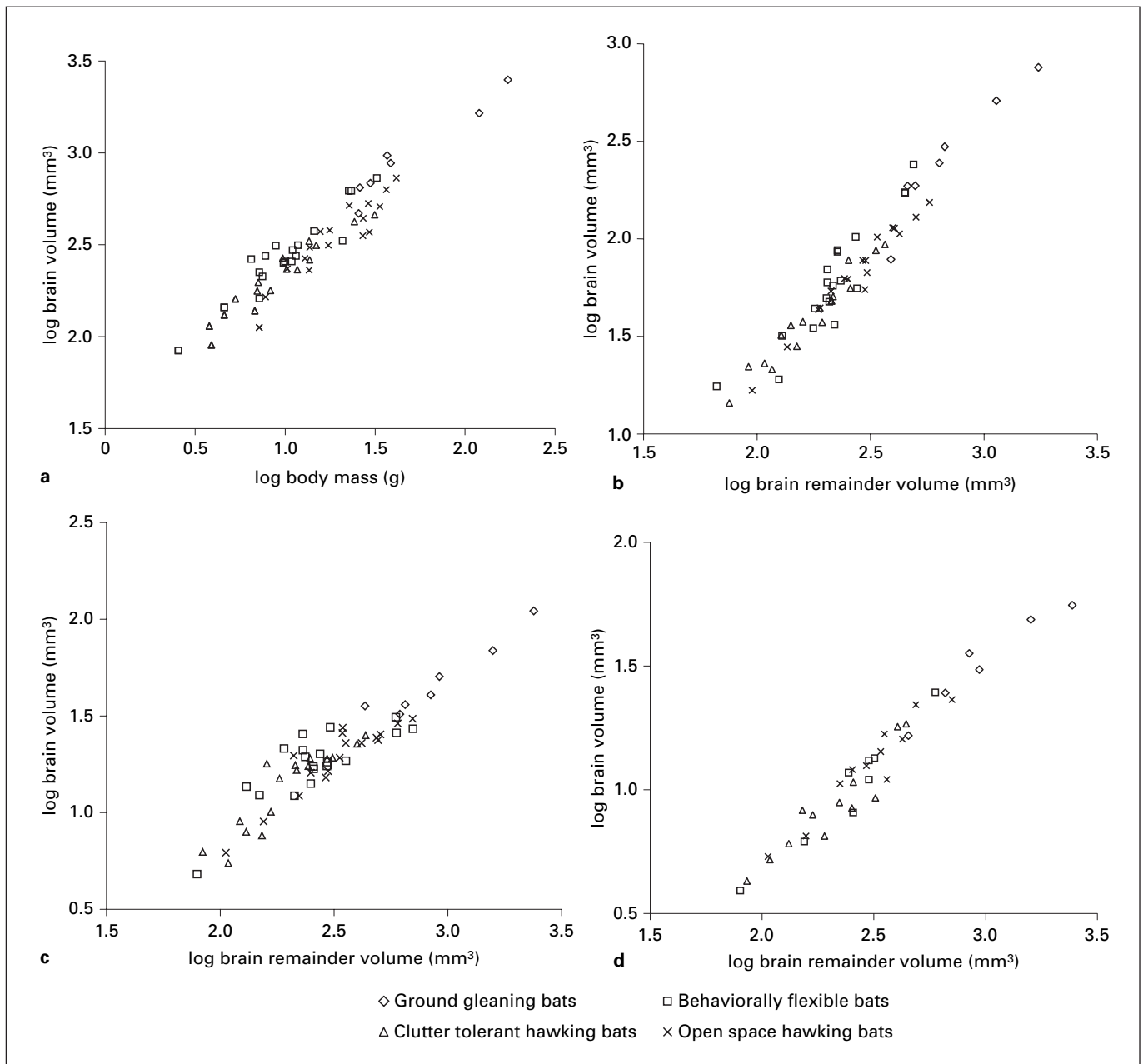
Fig. 1. Log-log regressions of **a** brain volume (mm³) vs. body mass (g), **b** neocortex volume (mm³) versus brain remainder volume (mm³), **c** hippocampus volume (mm³) versus brain remainder volume (mm³), and **d** inferior colliculus volume (mm³) versus brain remainder volume (mm³).

Results

The volume of all three structures we examined increased significantly with brain volume remainder; total brain volume increased significantly with body mass. Overall, log brain volume was positively related to log body mass ($F_{1,57} = 570.1$, $r^2 = 0.91$, $p < 0.001$). Log neocortex volume was positively related to log brain volume remainder ($F_{1,57} = 846.27$, $r^2 = 0.94$, $p < 0.001$). Log hippocampus volume was positively related to log brain volume remainder ($F_{1,55} = 351.1$, $r^2 = 0.86$, $p < 0.001$). Log inferior colliculus volume was positively related to log brain volume

Fig. 2. Relative brain size in predatory bats in relation to four foraging behavior categories (1 = ground gleaning species; 2 = behaviorally flexible species; 3 = clutter-resistant aerial hawking species; 4 = open space aerial hawking species). **a** Based on species-level analysis, **b** based on phylogenetically independent contrasts.
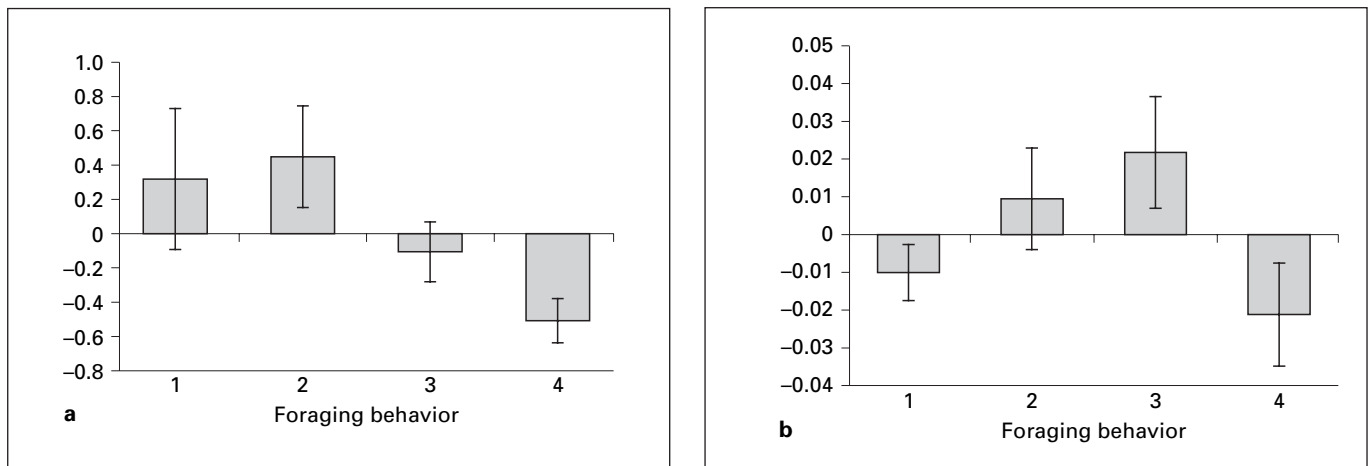

Fig. 3. Relative neocortex size in predatory bats in relation to four foraging behavior categories; otherwise, all else as described for figure 2.

remainder ($F_{1,35} = 696.93$, $r^2 = 0.95$, $p < 0.001$). Figure 1 illustrates these relationships while indicating the distribution of species within our four categories.

At the species level, relative brain size was significantly greater in behaviorally flexible and ground gleaning species than in clutter tolerant and open space aerial hawking species (species data: ANOVA: $F_{3,55} = 11.86$, $p < 0.001$; Tukey HSD post-hoc tests; fig. 2a). For independent contrasts, relative brain volume was significantly greater in behaviorally flexible species than the three remaining classes which did not differ significantly from each other (independent contrasts: ANOVA: $F_{3,54} = 8.73$, $p < 0.001$; Tukey HSD post-hoc tests; fig. 2b). Relative neocortex volume was significantly lower in open space aerial hawking species at the species level than in behaviorally flexible species; no other significant between group differences were found (ANOVA: $F_{3,55} = 3.53$, $p = 0.02$; Tukey HSD post-hoc test; fig. 3a); for independent contrasts, there were no significant differences between groups (ANOVA: $F_{3,54} = 2.32$, $p = 0.08$; fig. 3b).
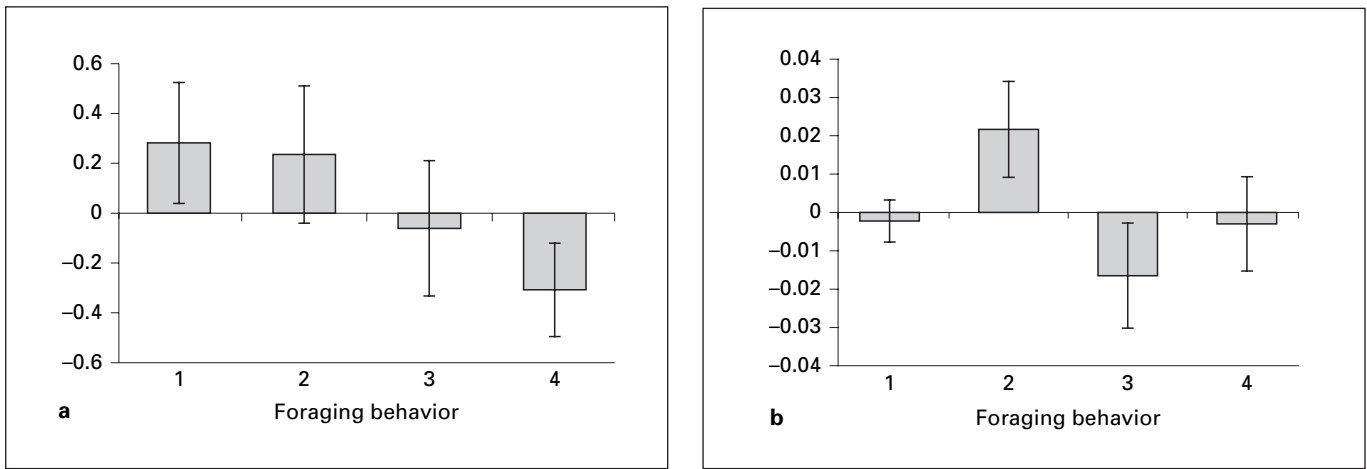
Fig. 4. Relative hippocampus size in predatory bats in relation to four foraging behavior categories; otherwise, all else as described for figure 2.
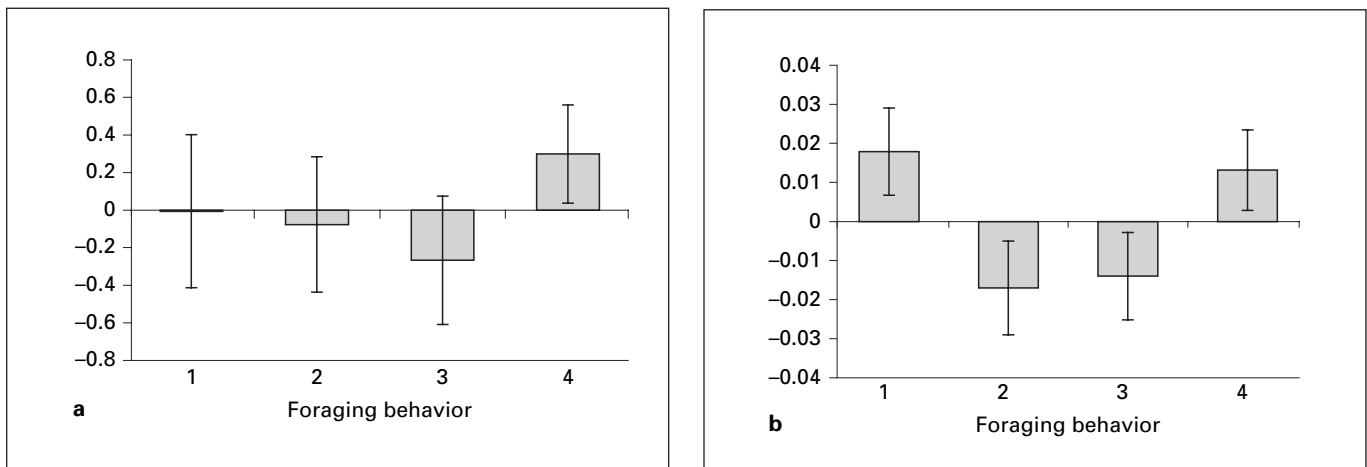

Fig. 5. Relative inferior colliculus size in predatory bats in relation to four foraging behavior categories; otherwise, all else as described for figure 2.

At both levels of analysis, we found no significant difference for relative hippocampus volume among the four behavioral classes of predatory bats (species data: ANOVA: $F_{3,55} = 1.13$, p = 0.34; independent contrasts: ANOVA: $F_{3,54} = 1.92$, p = 0.13; fig. 4). Similarly, we found no significant difference for relative inferior colliculus volume (species data: ANOVA: $F_{3,33} = 0.32$, p = 0.814; independent contrasts: ANOVA: $F_{3,32} = 2.61$, p = 0.054; fig. 5).

The only qualitative difference found after removing the high-duty cycle bats from our analyses (6 species belonging to Rhinolophidae and Hipposideridae, see discussion) was for independent contrasts of neocortex residuals. Clutter tolerant aerial hawking species had significantly larger neocortices than open space aerial hawking species and no other significant between group differences were found (ANOVA: $F_{3,48} = 4.51$, p = 0.007). This relationship was apparent as a trend before the removal of high-duty cycle species (see fig. 3b).

Discussion

We found a significant positive relationship between behavioral flexibility and relative brain volume and the species level analysis also provided some evidence for a positive correlation between habitat complexity and relative neocortex volume. These results are consistent with those of Reader and Laland [2002] for primates, Nikolakakis et al. [2003] for passerine birds, and Iwaniuk et al. [2005] for parrots, in that they show a direct connection between relative brain volume and behavioral flexibility. Natural selection could favor increasingly large brains at the expense of increased costs of development and maintenance because these regions confer the ability to use a greater number of foraging strategies and exploit a wider spectrum of habitats [Reader and Laland, 2002; Nikolakakis et al., 2003; Sol et al., 2005]. Similar to Safi et al. [2005] we found that open space aerial hawking bats have relatively smaller brains than do bats that typically hunt in more cluttered/complex habitats. These findings are also consistent with those found for birds that aerially hawk insects in open habitat (e.g., nightjars) versus putatively related birds that hunt substrate-borne prey [e.g., owls; Iwaniuk and Hurd, 2005]. That our analyses including and excluding high-duty cycle bat species produced quantitatively and qualitatively similar results reinforces our confidence in our categories and conclusions.

In the context of foraging behavior, Schnitzler et al. [2003] called attention to the importance of spatial information processing for echolocating bats, but published experimental investigations of spatial memory in bats are few in number [see Schnitzler et al., 2003 and Ratcliffe et al., 2005 for reviews]. Both the gleaning bat *Megaderma lyra* and the aerial hawking bat *Pteronotus parnellii* have well developed spatial memory [Neuweiler and Möhres, 1967; Schnitzler et al., 2003], but the functional significance of the hippocampus for echolocating bats is poorly understood [Duncan and Henson, 1994; Neuweiler, 2000]. Home range size might be positively correlated with hippocampus volume in mustelids and rodents [Galea et al., 1996; Healy et al., 2005]. Foraging and home range in predatory bats is positively related to our four feeding categories [Norberg and Rayner, 1987; Fenton, 1997]. In general, ground gleaning bats have the smallest ranges and open space aerial hawking bats the largest [Fenton, 1997]. Indeed, it is among the open space aerial hawkers that we find species with obvious migratory behavior [Norberg and Rayner, 1987]. However, using foraging behavior as a proxy [Fenton, 1997], range size alone appears to be an unimportant ecological predictor of hippocampus volume in predatory bats (fig. 4).

Recently, using wing area corrected for body mass to estimate potential habitat use, Safi and Dechmann [2005] found a positive correlation between wing area and hippocampus volume in bats and suggested that habitat complexity influences hippocampus volume. Behaviorally flexible and clutter tolerant aerial hawking bats should, on average, be expected to operate in more spatially complex ecological conditions than open space aerial hawking bats [Neuweiler, 1989; Fenton, 1990; Schnitzler and Kalko, 2001; Schnitzler et al., 2003]. Our results, however, do not clearly support a coincidental enhancement in hippocampus volume based on the preceding argument (fig. 5). Hutcheon et al. [2002] reported that frugivorous bat species had relatively larger hippocampus volumes. Functionally the hippocampus might serve frugivorous species to enable them to relocate temporally ephemeral but spatially consistent food resources [Hutcheon et al., 2002; Ratcliffe and ter Hofstede, 2005], but the cognitive demands of operating within a complex space could differ from the cognitive demands of remembering the details of complex spaces. The functional significance, if any, of differences in relative hippocampus volume within predatory bats deserves further investigation.

Safi and Dechmann [2005] found that hippocampus and inferior colliculus volume were positively correlated with wing area when all measures were controlled for using body mass. We suggest that the differences between the results of our study and those of Safi and Dechmann [2005] with respect to the hippocampus and inferior colliculus reflect the fact that our categories which were based on wing design, echolocation and observed foraging behavior, are not easily reconciled with Safi and Dechmann's categories that used wing area to body mass ratios as a proxy for habitat complexity. Based on Safi and Dechmann's analysis, habitat complexity appears to be a predictor of relative hippocampus volume in predatory bats, whereas observed foraging strategies used by predatory bats (this study) correlate with relative brain and neocortex volume. The differences between our methods of categorization and/or methods of controlling brain region volume might also account for our different results with respect to relative inferior colliculus volume. Hutcheon et al. [2002] also reported a comparative study of brain and brain region volumes in relation to foraging ecology in bats, including phytophagous as well as predatory species. However, we were unable to reconcile those results with our own due to differences in classifying the species and the brain values used.

When taking prey from surfaces, bats use both echoic and prey-generated sounds, forcing the inferior colliculus and auditory cortex to process both streams of information simultaneously [Fuzessery et al., 1993; Barber et al., 2003; Ratcliffe and Dawson, 2003; Ratcliffe et al., 2005]. Gleaning thus places greater demands on auditory information processing than does aerial hawking, the latter strategy depending solely on echolocation [Faure and Barclay, 1992; Fenton, 1995; Fuzessery, 1997]. The inferior colliculus processes both high frequency (e.g., echoic) and low frequency (e.g., prey-generated) information and is the best studied of the auditory information processing regions in echolocating bats [Pollak and Park 1995; Fuzessery, 1997]. The inferior colliculus is enormous in echolocating bats [Pollak and Park, 1995; Pollak et al., 1995; Neuweiler, 2000] and it has been suggested that it is relatively larger still in gleaning bats, putatively because gleaning bats use both of these streams of auditory information for the detection and localization of prey [Baron et al., 1996; Reep and Bhatnagar, 2000].

However, the specific functions of this central auditory processing region, and how these might differ in different species of bats, are poorly understood [Neuweiler, 1990, 2000]. *Eptesicus fuscus* and *Megaderma lyra*, aerial hawking and gleaning species, respectively, are equally sensitive to low and high frequency sounds, possibly to facilitate social communication and detection of predators [Moss and Schnitzler, 1995]. Our results show that relative inferior colliculus volume is not greater in gleaning or behaviorally flexible species than in open space aerial hawking species and that the opposite relationship might be a more accurate portrait of the correlation between relative inferior colliculus volume and foraging behavior (fig. 5).

Relative sizes of specific brain regions can be related to specific cognitive and behavioral demands that animals face (see Introduction). Such findings are consistent with mosaic evolution [Barton and Harvey, 2000] and modularity of mind [Shettleworth, 2000]. A focus on evolution of specific parts of the brain is often contrasted with views of animal intelligence and its evolution that emphasize overall brain size [e.g., Harvey and Krebs, 1990]. However, it seems most likely that relative size of the whole brain and the development of its components are both important [Streidter, 2004]. This view is consistent with a variety of new comparative evidence for correlations between overall relative brain size and ecologically important variables. Relatively larger brains could have considerable survival value for decision-making and risk assessment in the face of increased predation rates during gleaning attacks resulting from a closer proximity to terrestrial predators [Jones et al., 2003], in addition to pressures imposed by aerial predators [e.g., larger bats, bathawks, and owls; Fenton, 1995]. Large brained, behaviorally flexible species are predicted to be better able to exploit new and complex environments than their relatively smaller brained, less labile relatives of similar body mass [Streidter, 2004; Sol et al., 2005]. This relationship might explain the remarkable radiations of *Rhinolophus* and *Myotis*, two genera that represent more than 20% of all extant predatory bat species (160+ of approx. 750 spp./15 families). These genera likely contain many behaviorally flexible species [Norberg and Rayner, 1987; Ratcliffe and Dawson, 2003; Siemers and Ivanova, 2004] and deserve further attention.

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