COMPARATIVE ANALYSIS OF INVESTMENT IN SENSORY BRAIN TISSUE IN DIURNAL, NOCTURNAL, AND CATHEMERAL RODENTS

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Zoology – Doctor of Philosophy
Ecology, Evolutionary Biology and Behavior – Dual Major

2023

ABSTRACT

Transitions in temporal niche have occurred many times over the course of mammalian evolution and have been associated with changes in sensory stimuli available to animals. This is particularly true of visual cues because levels of light are so much higher during the day than night. For this reason, evolutionary transitions between diurnal, nocturnal, and cathemeral lifestyles are expected to be accompanied by modifications of sensory systems to optimize the ability of animals to receive, process, and react to important stimuli in the environment.

In chapter one, I examine the influence of temporal niche on investment in sensory brain tissue of diurnal and nocturnal rodents by measuring the size of five sensory brain regions that process olfactory (olfactory bulbs), visual (lateral geniculate nucleus, superior colliculus) and auditory information (medial geniculate nucleus, and inferior colliculus). A phylogenetic framework was used to assess the influence of temporal niche on the relative sizes of these brain structures. Compared to nocturnal species, diurnal species had larger visual regions, whereas nocturnal species had larger olfactory bulbs than their diurnal counterparts. Of the two auditory structures examined, one (medial geniculate nucleus) was larger in diurnal species, while the other (inferior colliculus) did not differ significantly with temporal niche. Our results suggest possible tradeoffs of investment between olfactory and visual areas of the brain, with diurnal species investing more in processing visual information and nocturnal species investing more in processing olfactory information.

In chapter two, I investigate investment in sensory brain tissue of cathemeral species by measuring five sensory brain regions that process olfactory (olfactory bulbs), visual (lateral geniculate nucleus, superior colliculus) and auditory information (medial geniculate nucleus and

inferior colliculus). Using a phylogenetic framework, I assessed the influence of temporal niche on the relative sizes of these brain structures. My data reveal that sensory structures in the brains of cathemeral rodents are not simply intermediate in size between those of diurnal and nocturnal rodents. Rather, cathemeral species were either distinctly nocturnal-like or diurnal-like. Cathemeral species had olfactory bulbs similar in size to diurnal species, and smaller than nocturnal species. One visual structure was not influenced by temporal niche, whereas the other visual structure was larger in diurnal species compared to both nocturnal and cathemeral species. The two auditory structures showed different patterns of investment. The inferior colliculus of the cathemeral and nocturnal species was similar in size, both of which were significantly smaller than diurnal species. The medial geniculate nucleus was similar in size between diurnal and cathemeral species, both of which were larger than that of nocturnal species. These results suggest a more complicated scenario than simply partitioning investment to accommodate activity in both day and night.

In chapter 3, I carry out a refined assessment of the lateral geniculate nucleus (LGN) in diurnal, nocturnal, and cathemeral rodents. In chapters one and two, I found the LGN to be largest in diurnal rodents, compared to nocturnal and cathemeral rodents. The LGN is subdivided into three regions which carry out specific functions involved in visual processing and circadian rhythms. The subregions of the LGN were significantly larger in diurnal species, suggesting increased investment in regions that carry out visual processing and circadian functions. When comparing the ratio of the dorsal and ventral LGN, however, there was no influence of temporal niche. This suggests that factors other than temporal niche impact the sizes of these two substructures in relation to one another.

ACKNOWLEDGEMENTS

I would like to thank many people who provided love, support, and guidance as I carried out this work. I would first like to thank my parents, Dave and Aileen VanHouten and Dan and Teressa Taylor, as well as my sons Nathan and Nickolas Morrow, and my sister Kendra Chapman. Without your love, support, and encouragement over the years, this work would not have been possible.

I would like to thank Barbara Lundrigan, my PhD advisor, for allowing me this opportunity and for all of her help, guidance, and patience throughout my graduate education. I would also like to thank Laura Smale, who taught me so much about brain research and methods. Her experience and knowledge was invaluable. I would also like to thank my committee members Heather Eisthen and Catherine Lindell for their expertise, insights, and direction.

I would like to thank Paul Meek, Noga Kronfeld-Schor, Stephen Phelps, Ashley Rowe, and Randy Nelson for providing help with trapping and providing animals and brains used in this work. I would like to thank the following undergraduates who have helped with sectioning and staining of brain tissue; Dena Letot, Ewelina Szewczuk, Jessica Overholser, Manekya Sumithrarachchi, and Kylee Voorhis.

Lastly, I would like to thank Michigan State University, particularly the Department of Integrative Biology, the Ecology, Evolutionary Biology, and Behavior Program, and BEACON, for funding of this dissertation. I am also indebted to Michigan State University Museum for loaning traps and trapping gear. This research was supported by NSF award DBI-0939454.

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
AICc	Sample-size Corrected Akaike's Information Criterion
dLGN	Dorsal Lateral Geniculate Nucleus
EB	Early Burst Evolutionary Model
IC	Inferior Colliculus
IGL	Intergeniculate Nucleus
LGN	Lateral Geniculate Nucleus
MGN	Medial Geniculate Nucleus
ML	Maximum Likelihood
OB	Olfactory Bulb
OU	Ornstein-Uhlenbeck Evolutionary Model
PGLS	Phylogenetic Generalized Least Squares
SC	Superior Colliculus
vLGN	Ventral Lateral Geniculate Nucleus

CHAPTER 1: TRADEOFFS IN THE SENSORY BRAIN BETWEEN DIURNAL AND NOCTURNAL RODENTS

1 | INTRODUCTION

Over a 24-hour period there are predictable patterns of change in temperature and light levels. There are also temporal differences in biotic factors, such as the presence of predators or competitors and resource availability. These differences between day and night result in distinct sensory environments, which most animals have evolved to exploit by concentrating their activity to specific times. An animal's temporal niche, or daily activity pattern, refers to the time in which the animal is most likely to be awake and active. There is great diversity in the types of daily activity patterns seen in vertebrates. Animals may be active during the night (nocturnal), during the day (diurnal), at dusk and dawn (crepuscular), or during both night and day (cathemeral). In addition to these discrete categories, there are varying levels of flexibility in patterns of activity (Refinetti 2008; Castillo-Ruiz et al. 2012; Helm et al. 2017). Flexibility exists in multiple forms. Even within strict temporal niche categories, patterns of activity can vary in a variety of ways, e.g. the number of activity peaks exhibited (unimodal, bimodal, or polymodal). Some species switch their most active period from one time of the day to another in response to stressors, such as predators or competitors (Castillo-Ruiz et al. 2012), and some display predictable seasonal shifts in activity patterns (Meek et al. 2012; Ikeda et al. 2016). Furthermore, intraspecific variability in daily activity patterns occur in many species (Gutman and Dayan 2005; Refinetti 2006; Hertel et al. 2017).

Despite the diversity of daily activity patterns currently observed in mammals, there is strong evidence that the earliest mammals were nocturnal (Hall et al. 2012; Anderson and

Weins 2017; Maor et al. 2017). This was likely a mechanism to avoid the dominant diurnal dinosaurs of that period (Gerkema et al. 2013). Nocturnal behavior was apparently conserved in mammals through most, or all, of the Mesozoic, but with the empty niches resulting from the extinction of dinosaurs at the end of that era (circa 66 million years ago), the range of mammalian temporal niches expanded (Maor et al. 2017). Daily activity patterns are constrained phylogenetically, with closely related species more likely to occupy similar temporal niches (Roll et al. 2006; Andersen and Weins 2017). However, a study including nearly half of the approximately 6,450 extant mammalian species found that several orders include species that occupy different temporal niches (Bennie et al. 2014). In addition, many families include both diurnal and nocturnal species (Curtis and Rasmussen 2006), and even within genera, there can be interspecific variability in daily activity patterns. This indicates that, despite phylogenetic conservatism, evolutionary transitions in temporal niche have occurred many times within Class Mammalia.

How and why these transitions occur is a subject of ongoing research. Ankel-Simons and Rasmussen (2008) suggest that some temporal niche transitions simply reflect the opportunistic filling of an empty niche. Temporal niche transitions might also be driven by changes in food availability (van der Vinne et al. 2019; Wu and Wang 2019), or the appearance of new competitors (Gutman and Dayan 2005; Gliwicz and Dabrowski 2008; Meek et al. 2012), or predators (Gliwicz and Dabrowski 2008; Wu et al. 2018; Wu and Wang 2019). It is likely that all of these have played a role in shifting temporal niches in different lineages.

Regardless of the selective forces driving temporal niche transitions, adaptation to a new one requires physiological and sensory system changes. A vital component of an animal's

fitness is the ability to sense and respond to the external environment. Different sensory modalities capitalize on different forms of stimuli and those stimuli vary across a 24-hr day. Photic stimuli, for example, are abundant during the day but limited at night, and it is generally thought that diurnal species rely more heavily on vision for foraging, hunting, and predator avoidance, than nocturnal species (Hut et al. 2012). If diurnal species have more robust visual systems, it raises the question of whether other sensory systems, e.g. olfactory and auditory, might be better developed in nocturnal species to help guide their activity in the dark. Moreover, if diurnal and nocturnal species rely differently on these senses, what adaptations to the sensory system should be expected?

While sense organs receive sensory stimuli and may do the initial processing, the brain functions to do the more sophisticated and refined processing to extract crucial information from those signals, thus enabling an animal to respond appropriately to external cues. The quantity and quality of different types of sensory information available should therefore impact how an animal invests, not only in the collection of sensory information through sensory organs, but also in the processing of that information by structures within the brain. Since neural tissue is among the most energetically expensive there is (Niven and Laughlin 2008), selection would be expected to optimize the relative investment in the various components of the sensory brain (i.e., areas of the brain that receive, integrate, and process sensory information) with the caveat that different regions may not be developmentally independent.

The influence of developmental constraints on brain evolution has received considerable attention, much of it focused on the relative importance of concerted processes (i.e., change in one structure is accompanied by proportional changes in other structures)

versus mosaic ones (i.e., one structure evolves independently from other structures) in evolutionary change. Many studies have shown that brain size and certain brain divisions exhibit distinct allometry and scale in a phylogenetically conserved pattern in vertebrates (Chalfin et al. 2007; Yopak et al. 2010; Finlay et al. 2011; Finlay et al. 2014), supporting the concerted evolution hypothesis. Other studies have provided evidence for mosaic patterns of evolutionary changes in the brain (Barton and Harvey 2000; Safi and Dechmann 2005; Corfield et al. 2012; Montgomery et al. 2016). It seems likely that concerted and mosaic evolution have taken place concurrently and been shaped by the unique history and demands of the structures under selective pressure. Indeed, Moore and DeVoogd (2017) found clear evidence that both concerted and mosaic processes have shaped the evolution of song circuits in the brains of passerine birds.

Several studies have examined the relationship between daily activity pattern and sensory investment in vertebrates. Differences in olfactory investment between nocturnal and diurnal species have been found in birds (Healy and Guilford 1990), insectivores, and primates (Barton et al. 1995), with nocturnal species possessing larger olfactory bulbs than diurnal species. Iglesias et al. (2018) found that shifts diurnal activity to more nocturnal activity correspond with a decrease in the size of the optic tectum in teleosts. Studies of primates have shown that diurnal species have a larger visual cortex relative to hindbrain volume (Barton 2007) than nocturnal species. Campi and Krubitzer (2010) described differences in visual and somatosensory/motor regions of the cortex in two species of diurnal squirrels relative to the nocturnal Brown Rat (*Rattus norvegicus*). The diurnal squirrels had significantly larger visual regions and smaller somatosensory regions in the cortex, compared to the Brown Rat. Campi et

al. (2011) had similar findings when comparing visual and somatosensory cortices between the Brown Rat and the diurnal African Grass Rat (*Arvicanthis niloticus*), in that the primary visual cortex is larger, and the primary somatosensory cortex smaller, in the diurnal rat, compared to the nocturnal rat. Shuboni-Mulligan et al. (2019) found two visual brain structures, the lateral geniculate nucleus and superior colliculus, to be larger in the diurnal African Grass Rat, compared to the nocturnal Brown Rat. This contrasts with the findings of Finlay et al. (2014) that the volume of the lateral geniculate nucleus is not correlated with daily activity pattern in mammals. Many components of the visual system are highly variable, even between individuals of the same species. Ankel-Simons and Rasmussen (2008) have suggested that this variability makes the visual system highly susceptible to evolutionary changes. It may be that this variation enables temporal niche transitions to occur more readily than they would otherwise be.

Taken together, these studies suggest that an animal's daily activity pattern may influence how it uses and invests in vision and olfaction. However, studies connecting activity pattern and auditory function are lacking. To determine how sensory system evolution relates to temporal niche, it is important to focus on investment in multiple sensory modalities, which would permit identification of possible energetic trade-offs. This approach also ensures standardization of methods and thus has the potential to clarify contradictions in the literature that may reflect differences in experimental design, including studies that combine data from multiple sources.

In this study, we investigate the influence of temporal niche on investment in sensory brain regions supporting olfaction, vision, and audition across 13 rodent species (eight nocturnal, five diurnal), representing at least five independent transitions in temporal niche

(Figure 1.1). We test the hypothesis that evolutionary transitions from nocturnality to diurnality, or vice versa, are accompanied by changes in regions of the brain that process sensory information. We predict trade-offs between vision and olfaction and vision and audition, with diurnal species devoting proportionally more neural tissue to processing visual information, and nocturnal species investing more to processing olfactory and auditory information as a reflection of limited visual cues. Additionally, we investigate if, and to what extent, brain size and the size of these sensory regions are phylogenetically constrained. To accomplish this, we estimate phylogenetic signal in each measure and model different modes of evolution for each area of interest. Lastly, we will discuss the extent to which these sensory areas have evolved in a mosaic or concerted fashion.

2 | MATERIALS AND METHODS

2.1 | Specimens

We collected data from 13 rodent species, representing three extant families: Sciuridae, Cricetidae, and Muridae (Table 1.1, Figure 1.1). The sample includes eight nocturnal species [Southern Flying Squirrel (*Glaucomys volans*), Social Vole (*Microtus socialis*), Striped Desert Hamster (*Phodopus sungorus*), Southern Grasshopper Mouse (*Onychomys torridus*), Northeast African Spiny Mouse (*Acomys cahirinus*), House Mouse (*Mus musculus*), Australian Bush Rat (*Rattus fuscipes*), and Brown Rat (*Rattus norvegicus*)] and five diurnal species [North American Red Squirrel (*Tamiasciurus hudsonicus*), Eastern Chipmunk (*Tamias striatus*), Short-tailed Singing Mouse (*Scotinomys teguina*), Golden Spiny Mouse (*Acomys russatus*), and African Grass Rat (*Arvicanthis niloticus*)]. The ancestral rodent was almost certainly nocturnal as are most extant rodent species (Roll et al. 2006; Maor et al. 2017). The families Cricetidae and Muridae likely had nocturnal ancestors as well, but within both clades diurnality has evolved several times independently, including in 3 lineages that are examined here (Figure 1.1). The earliest sciurids, in contrast, were probably diurnal, as are most extant members of this family (Roll et al. 2006). A single transition back to nocturnality appears to have occurred approximately 18 million years ago at the origin of Tribe Pteromyini, today represented by 58 species of flying squirrels (Mercer and Roth 2003; Burgin et al. 2020).

Southern Flying Squirrels, North American Red Squirrels, Eastern Chipmunks, and House Mice were live-trapped in and around East Lansing, Michigan, between October 2015 and December 2017 (Table 1.1). Australian Bush Rats were live-trapped in New South Wales, Australia, in August 2015. Brown Rats were purchased from Charles River Laboratories.

Southern Grasshopper Mice and African Grass Rats were obtained from Michigan State University laboratory colonies. Striped Desert Hamsters were obtained from a laboratory colony at Ohio State University. Intact whole brains from Social Voles, Northeast African Spiny Mice, and Golden Spiny Mice were obtained from Tel Aviv University and those from Short-tailed Singing Mice were obtained from a University of Texas at Austin laboratory.

The number of individuals of each species ranged from 3 to 6 (Table 1.1). We used only adult individuals and tried to sample both males and females. However, the Southern Flying Squirrels were all female in this study. All animals were handled according to protocols approved by the following institutional and regional authorities: American Society of Mammalogists (Sikes et al. 2016), MSU (Michigan State University) Institutional Animal Care and Use (protocol # 07/16-116-00), Office of Environment and Heritage of New South Wales (NSW), Australia (License #SL100634), and NSW Department of Industry and Investment Animal Research Authority (ORA 14/17/009).

2.2 | Regions of Interest

The structures chosen to estimate investment in olfaction, vision, and audition, respectively, included the olfactory bulbs (OB); the lateral geniculate nucleus (LGN) and superior colliculus (SC); and the medial geniculate nucleus (MGN) and inferior colliculus (IC).

Olfaction. The main olfactory bulbs receive input directly from the olfactory neurons of the olfactory epithelium, along with the Grueneberg ganglion and septal organ, all of which are in the nasal cavity (Gruneberg 1973; Tian and Ma 2004). The accessory olfactory bulbs in rodents receive input from the vomeronasal organ and lie dorsocaudally on the main olfactory

bulbs (Halpern and Martinez-Marcos 2003). Estimation of investment in olfaction was accomplished by measuring the combined mass of the main and accessory olfactory bulbs.

Vision. The LGN is a visual brain structure located in the thalamus. The LGN receives direct afferents from the retina, sends and receives projections from the SC (Baldwin et al. 2011), and sends projections out to the primary visual cortex (Horng et al. 2009). It is comprised of three distinct subdivisions (i.e., dorsal LGN, intergeniculate leaflet, and ventral LGN), the latter two of which are also known to function in the patterning of activity across the day (Harrington 1997). All three subdivisions of the LGN were included in our measurement.

The SC is a visual structure in the midbrain that, in mammals, is divided into seven functionally distinct layers (May 2006). In addition to sending and receiving projections to the LGN, the SC has reciprocal connections with the pulvinar complex, another thalamic visual structure (Baldwin et al. 2011). In this study, we measured only the three superficial layers (i.e., the zonal layer, superficial gray layer, and optic nerve layer), as they function almost exclusively in processing visual information, directing eye movements, and receive most of the retinal input to this structure. The deeper layers of the SC function in multiple forms of sensory processing, including auditory and somatosensory (Gaese and Johnen, 2000; McHaffie et al. 1989).

Audition. The MGN of the thalamus plays an important role in auditory processing and it conveys information between the inferior colliculus (IC) and the auditory cortex (Hu et al. 1994). While the MGN is the main target of projections from the IC, it also receives and integrates information from the auditory nuclei in the brain stem, and projects to the amygdala and frontal cortex (Winer and Schreiner 2005). In mammals, the MGN is subdivided into three

functionally distinct parts: dorsal MGN, medial MGN, and ventral MGN (Winer and Schreiner 2005; Najdzion et al. 2011). All three subdivisions of the MGN were included in our measurement.

The IC has the most diverse connections of the regions measured here and is an important site of convergence within the auditory pathway (Kulesza et al. 2002). In addition to its connections with the MGN, it functions to integrate auditory information from the brain stem and the auditory cortex (Winer and Schreiner 2005). The IC is subdivided into three distinct parts: the central nucleus, dorsal cortex, and lateral cortex. All three subdivisions of the IC were included in our measurement.

2.3 | Brain collection and histology

Fresh, unfixed tissue was used to avoid issues of uneven shrinkage of brain tissues. Each animal was euthanized with a lethal dose of sodium pentobarbital, administered intraperitoneally. Immediately after death, the animal was weighed to the nearest gram, and its brain was extracted, placed in powdered dry ice for 2-5 minutes, and transferred to a -80° freezer until further processing. After removal from the freezer, each brain was trimmed immediately caudal to the medulla oblongata and weighed to the nearest milligram. OBs were then separated from the brain just anterior to the olfactory peduncles, then weighed to the nearest milligram. The portion of the brain extending from the anterior thalamus to just caudal to the auditory tectum was coronally sectioned at 40µm thickness on a cryostat, except for the House Mice brains which, because of their small size, were sectioned at 20µm thickness. Three alternate series of brain tissue sections were mounted onto slides and one was stained for acetylcholinesterase as follows: slides were incubated for 5 hours in a solution of 0.0072%

ethopropazine HCl, 0.075% glycine, 0.05% cupric sulfate, 0.12% acetylthiocholine iodide, and 0.68% sodium acetate (pH 5.0); rinsed 2 times (3 minutes each) with distilled H_2O; and developed in a 0.77% sodium sulfide solution (pH 7.8) for 45 minutes. Slides were then rinsed with 2 changes of distilled H_2O (3 minutes each), then run through a series of ascending ethanol concentrations (70%, 95%, 100%, and 100%) for 1 minute each (to dehydrate the adhering tissue), cleared through 2 changes of xylenes for 5 minutes each, and coverslipped using DPX mounting medium. The two remaining series were set aside for future work.

2.4 | Measurements

Estimation of investment in olfaction was accomplished by measuring the combined mass of the main and accessory olfactory bulbs. For the other regions of interest (LGN, SC, MGN, and IC), photomicrographs of AChE-stained sections (Figure 1.2) were taken with a digital camera (MBF Bioscience CX9000) attached to a Zeiss light microscope (Carl Zeiss, Gottengen, Germany, 5x objective), using the 2D slide scanning module on Stereo Investigator 2017 (MBF Bioscience). The Cavalieri method was used (100 x 100 um grid, every third section) to calculate volumetric measurements in Stereo Investigator 2017 (MBF Bioscience). Boundaries of each brain structure were determined according to the rat brain atlas (Paxinos and Watson 2014). For each structure, only one side was measured, and that value was doubled to obtain total volume.

While neuronal density, including neuron/glial proportions, would provide a more accurate indicator of investment in brain tissue, it is more difficult to measure, and thus many studies, including this one, have used size (i.e., mass or volume) as an alternative proxy for

investment. Neuron density scales closely with volume in sensory brain structures of rodents (Herculano-Houzel et al. 2011; Najdzion et al. 2009; 2011).

2.5 | Data analysis

Variables and transformations. Continuous variables used in the analyses include body, brain, and OB mass, as well as LGN, SC, MGN, and IC volume. All analyses were carried out in R Studio (RStudio 2020) using log-transformed data. All species were assigned to one of two categorical states, diurnal or nocturnal, based on descriptions of daily activity patterns gleaned from field studies reported in the literature (Table 1.1). Laboratory studies were not considered in determining activity patterns.

Phylogenetic signal estimations. To estimate the influence of phylogeny on each variable, we calculated Blomberg's *K* (based on 1000 randomizations for p-value) and Pagel's λ (based on likelihood ratio tests) using the PHYTOOLS 0.7-70 package in R (Revell 2012). Estimations were carried out using the residuals from linear regressions. Brain size was regressed on body size, and the size of each sensory region (OB, LGN, SC, MGN, and IC) was regressed on brain size.

Modes of Evolution and ANCOVA. For each brain structure of interest, different modes of evolution were modeled using phylogenetic general least squares (PGLS) in the package PHYLOLM 2.6.2 in RStudio (Ho and Ane 2014). Most models incorporated one of three different branch-length transformations: lambda (λ), delta (δ), or kappa (κ). For a λ transformation, the internal branch lengths are multiplied by a constant, but the tip branches are left unaffected. A λ value of 0 is equivalent to no phylogenetic effect, whereas a λ value of 1 is equivalent to a fixed Brownian motion model (Harmon 2019). In a Brownian motion model, biological traits

accumulate random, incremental changes. For a δ transformation, all the values of the phylogenetic covariance matrix are raised to the power of δ. This transforms the sum of the length of shared branches between two tips (Harmon 2019). For a κ transformation, all branch lengths are raised to the power of κ. In this case, the elements of the phylogenetic covariance matrix are the sum of the individually transformed branch lengths (Harmon 2019).

We compared the following models for each brain region measurement: λ set to 0 (equivalent to no phylogenetic effect), λ set to 1 (equivalent to a fixed Brownian model), λ set to maximum likelihood (ML), δ set to ML, and κ set to ML. We also modeled early burst (EB) evolution and the Ornstein-Uhlenbeck (OU) evolutionary model. We then compared the seven models using sample-size corrected Akaike's Information Criterion (AICc). An ANCOVA was performed using the best linear model for each brain region as a function of total brain size and activity pattern (nocturnal vs. diurnal). The ANCOVAs that were performed using a phylogenetic regression were carried out in the CAPER package in RStudio (Orme et al., 2018).

3 | RESULTS

3.1 | Phylogenetic Signal

Blomberg's *K* was marginally significant for brain size, and significant for size of the OB and SC, indicating a modest phylogenetic signal in brain size and a strong signal in OB and SC size (Table 1.2). Pagel's λ, in contrast, detected a significant phylogenetic signal only in relative brain size. These differences in detected phylogenetic signal may reflect the small sample size used for this study. Blomberg's *K* is typically more reliable than Pagel's λ when working with smaller sample sizes (Munkemuller et al. 2012). The results of the Blomberg's *K* estimations were consistent with the models of evolution when we compared each brain region.

3.2 | Brain Size

Brain mass ranged from 0.61% of body mass in the Brown Rat to 2.81% in the Southern Flying Squirrel (Figure 1.3). The optimal linear model for brain size was Brownian motion, which explained 51.5% of the variation ($F_{3,8} = 4.893$, $p = 0.032$). Body size is a significant predictor of brain size (Table 1.3). Brain size increases with body size in both diurnal and nocturnal species. Activity pattern does not have a significant influence on relative brain size.

3.3 | Olfactory System

OB. OB size ranged from 2.02% of brain mass in the North American Red Squirrel to 5.35% of brain mass in the Australian Bush Rat (Figure 1.4). The three squirrel species exhibited the smallest relative OB size compared to all other species. The Brownian Motion model of evolution performed best for OB data, explaining 93.1% of the variation in OB size ($F_{3,9} = 54.6$, $p < 0.001$). The phylogenetic ANCOVA shows that brain size and activity pattern are both

significant predictors of relative OB size (Table 1.3), with nocturnal species possessing larger OBs than diurnal species.

3.4 | Visual System

LGN. The volume of the LGN ranged from 0.2% of brain mass, in the Australian Bush Rat, to 0.41% of brain mass in Eastern Chipmunk (Figure 1.5). The LGN showed phylogenetic independence when comparing the different regression models of evolution, with the non-phylogenetic linear model explaining 93.6% of the variation in LGN size ($F_{3,55} = 284.5$, $p < 0.001$). The ANCOVA found that brain size and activity pattern are both significant predictors of LGN volume and there is also a significant interaction between the two (Table 1.3). Diurnal species have a larger LGN than nocturnal species.

SC. Compared to the LGN, the SC exhibited a greater range of variation in relative size, with two sciurids, the North American Red Squirrel and Eastern Chipmunk, exhibiting much larger values for SC than the other species (Figure 1.6). The SC showed a strong phylogenetic component and the Brownian motion model performed best, explaining 78.1% of the variation in SC size ($F_{3,9} = 15.24$, $p < 0.001$). The phylogenetic ANCOVA of the SC showed that brain size and activity pattern are both significant predictors of SC size (Table 1.3), with diurnal species possessing a larger SC than nocturnal species.

3.5 | Auditory System

MGN. Volume of the MGN ranged from 0.14% of brain mass in the Striped Desert Hamster, to 0.46% of brain mass in the Eastern Chipmunk (Figure 1.7). The non-phylogenetic regression performed best for the MGN data, explaining 85.9% of the variation in MGN size ($F_{3,54}=115.8$, $p < 0.001$). The ANCOVA results showed that brain size and activity pattern are

both statistically significant predictors of MGN size (Table 1.3), with diurnal species exhibiting a larger MGN than nocturnal species.

IC. Volume of the IC ranged from 0.71% of brain mass in the North American Red Squirrel, to 1.97% of brain mass in the African Grass Rat (Figure 1.8). The model that best fit the IC size data was a non-phylogenetic regression. That model explained 87.6% of the variation in IC size ($F_{3,50}$=125.6, $p < 0.001$). The ANCOVA of the IC showed that brain size is a significant predictor of IC size (Table 1.3). While activity pattern alone is not a significant factor influencing IC size, there is a highly significant interaction between brain size and activity pattern, reflected in the different slopes of the linear regressions.

4 | DISCUSSION

The overall size of the brain had the largest impact on the sizes of the sensory regions within it, as expected, however, the sizes of sensory regions were also influenced by temporal niche (Table1.3), and there appear to be trade-offs between investment in visual and olfactory regions of the brain. Specifically, nocturnal species had significantly larger OBs than their diurnal counterparts, while diurnal species had larger LGNs and SCs, the two visual areas examined. These findings suggest a mosaic pattern of change that may be influenced by tradeoffs in investment in some tissues associated with temporal niche. There was also a significant difference between diurnal and nocturnal species in the size of the MGN, but in a direction that was the reverse of what we predicted. Specifically, the MGN, like the LGN, was larger in diurnal than nocturnal species. This may reflect a tendency for these two thalamic structures to evolve in a concerted manner. Below we discuss some of the issues raised by these data.

4.1 | Brain size

A transition to nocturnality is thought to have occurred as mammals evolved from their diurnal synapsid ancestors (Walls 1942; Gerkema 2013) and with it came a major expansion in brain size (Jerrison 1973). It has been suggested that this temporal niche transition contributed to overall enlargement of the brain in early mammals, as they developed sensory systems that went well beyond those of their ancestors to guide their behavior in the darkness of the night (Jerrison 2002). This raises the question of whether subsequent transitions back to diurnality might have been accompanied by changes in brain size. Our data, collected from animals representing at least four of these transitions, did not find evidence for this in Rodentia, i.e.

nocturnal and diurnal species did not differ significantly in brain size relative to body size (Table 1.3, Figure 1.3). However, although unrelated to the size of the brain overall, temporal niche was associated with sizes of different sensory structures within it.

Brain size varied quite drastically among species sampled (Figure 1.3). The brain size of the Brown Rat, relative to body size, was the smallest. This may be due to the fact that the Brown Rats in this study were lab-reared. Body size, in some cases, may not be the best metric to determine relative brain size. Lab-reared animals spent significantly less time foraging and have a constant availability of food, which can lead to an animal being overweight, which can lead to unreliable estimates for brain size. It is important to consider which species are wild-caught vs lab-reared when interpreting relative brain size.

4.2 | Olfactory System

Olfactory Bulbs (OB)

OBs, which exhibited a high level of phylogenetic signal, were significantly larger in nocturnal than in the diurnal species (Figure 1.4). These results are consistent with Barton et al. (1995) findings for insectivores and primates. OBs receive chemical stimuli, via olfactory epithelium, which may contain information about the presence of food, predators and competitors, as well as reproductive condition of a male or female conspecific. Olfactory stimuli can be detected from the three-dimensional world around an animal, and the olfactory bulbs play a role in mapping odorants in space (Jacobs 2012). Unlike visual or auditory cues, chemical ones can last for several days and thus provide information about the recent past as well as the present, and they may do this at all phases of the day-night cycle. The relatively large size of

OBs in nocturnal species could reflect a history of more intense selection for animals with abilities of these sorts.

The smallest relative OBs were in the three sciurids. Diurnality was likely present in the first sciurids, which appeared in the fossil record approximately 36 million years ago (Mercer and Roth 2003), thus the members of this lineage have had a long time to adapt to a day-active way of life in which the increase in visual information may have made olfactory processing less crucial. The transition back to nocturnality of the flying squirrel lineage approximately 18 mya (Mercer and Roth 2003) might be expected to result in selection for increasing OB size. Indeed, the OB of flying squirrels was 47.9% larger than that of the other tree squirrel (i.e., the North American Red Squirrel), raising the possibility that processing of olfactory information became more important as these animals branched off from other tree squirrels and returned to their ancestral, nocturnal, condition. However, the OBs of the ground-dwelling diurnal sciurid, the Eastern Chipmunk, was similar to that of the flying squirrel (Fig. 1.4). The relatively small OBs of the two tree squirrels raises the possibility that the terrestrial (vs arboreal) lifestyle is a driver of OB evolution. Regardless of how the differences evolved, they suggest that nocturnal species may be able to use olfactory cues more effectively than their diurnal relatives.

4.3 | Visual systems

Lateral Geniculate Nucleus (LGN)

The relative size of the LGN was significantly larger in diurnal than nocturnal species (Figure 1.5). As a part of the visual pathway, the LGN receives direct input from the retina, receives projections from the SC, and projects to the primary visual cortex. This enables animals to extract different kinds of information about the surrounding world from light, such as

information about form, distance, location, movement and reflection of different wavelengths (e.g. Glickfeld et al. 2014). Our data suggest that selection for diurnality among rodents may have been accompanied by increases, to varying degrees, in the ability to use light to obtain such information.

The relative size of the LGN was notably high in the two diurnal squirrels, which is consistent with behavioral evidence that the visual systems of diurnal sciurids are especially well developed (e.g. Jacobs and Birch 1982; Van Hooser and Nelson 2006). The only nocturnal sciurid examined here, the Southern Flying Squirrel, presents an interesting case in that its LGN, though smaller (relative to brain weight) than in diurnal sciurids, is substantially larger than in the other nocturnal species examined here (Figure 1.5). This might reflect the history of this lineage, which is thought to have evolved from diurnal sciurid ancestors approximately 18 mya (Mercer and Roth 2003). Also, visual information may be of greater value to animals that glide than to those that use other forms of locomotion at night. Wavelength information (i.e., color) is limited in flying squirrels as they have mutations that have rendered short wavelength sensitive photopigments non-functional (Carvalho et al. 2006). The reduction of functional cones may manifest as a diminution of tissue within the LGN where cells involved in wavelength discrimination have been described in primates (De Valois and Abramov 1966). Examination of the LGN and sensory behavior in other rodents, both diurnal and nocturnal, is needed to better understand both the differences between flying squirrels and the other nocturnal species examined, and between them and other sciurids.

Interestingly, when Finlay et al. (2014) analyzed the size of the LGN in 31 species of mammals (5 primates and 26 non-primate species), they saw no overall effect of temporal

niche. The differences between those results and ours likely reflects the species examined: their analysis included only five rodents and only one of those was diurnal. Their results and ours together suggest the possibility of a link between temporal niche and LGN volume that exists in Rodentia but is absent in primates, and perhaps other mammals.

One issue to consider is that the three subdivisions of the LGN (dorsal, intergeniculate, and ventral) were combined into a single metric. While the dorsal LGN functions in primary visual processing, the intergeniculate leaflet and ventral LGN contribute to additional processes, including the patterning of daily activity. There is some evidence of differences between these subregions in one diurnal rodent, the African Grass Rat, compared to some nocturnal rodents (Gall et al. 2014; Langel et al. 2018). Separate measurements of LGN subregions could shed further light on the differences between diurnal and nocturnal species with respect to this structure.

Superior Colliculus (SC)

The SC, which exhibited a notable phylogenetic effect (Table 1.2), was significantly larger in our diurnal species compared to the nocturnal ones (Figure 1.6). The SC was strikingly large in the diurnal squirrels, which could reflect the long diurnal history of this lineage. The SC receives direct input from the retina, and it provides information about light to the cortex through parallel, though interconnected, output pathways (May 2006). One of these is indirect, via its projection to the LGN; the other pathway the SC takes part in is via its projection to the pulvinar complex in the thalamus, which then projects to multiple extra-striate regions of the cortex (Baldwin et al. 2017). The photic information appears to be processed in different ways along these pathways and to serve somewhat different functions. In addition to processing

visual information, the region of the SC that we measured (i.e., outer three layers) plays a major role in directing movements of the eyes, and consequently what an animal sees (May 2006). The 'decisions' that an individual makes about, effectively, visual attention are complex and their effects can have a major impact on the information that is processed by the larger visual system (May 2006). The data here raise the possibility that diurnal species may be better able to respond to visual cues in a manner that impacts the movement of their eyes.

The fact that both the LGN and SC were larger (relative to brain size) in diurnal species, compared to nocturnal species, could mean these two visual regions are linked and selection on one leads to changes in the other. If that were the case, we would expect to see species rank similarly (i.e., largest to smallest) in the size of the LGN and SC. While species are similarly ranked, the magnitudes of species' differences in the sizes of these regions are not consistent. The fact that the SC functions in two parallel visual pathways, whereas the LGN functions in only one, could explain this imbalance. Comparing the size of the pulvinar complex, which is involved in the extrageniculate pathway, could provide clarification. It is also possible that, due to their different roles within the visual system, each structure was independently expanded by somewhat different selective pressures that may have arisen as diurnal species adapted to rely more heavily on light information.

4.4 | Auditory Systems

Medial Geniculate Nucleus (MGN)

Diurnal species had a significantly larger MGN than nocturnal species (Figure 1.7). The MGN acts to process and relay auditory information between the inferior colliculus (IC) and the auditory cortex; it also receives projections from several auditory nuclei in the brainstem (Hu et

al. 1994). More specifically, it functions in processing frequency, intensity, and location of sounds (Winer and Morest 1983). It also acts as a selection filter, as it is the last opportunity for auditory information to be processed before reaching the auditory cortex (Blundon and Zakhorenko 2013).

Our findings for MGN are of particular interest in that two diurnal species, the Eastern Chipmunk and African Grass Rat, exhibited values at least twice those of any other species (Figure 1.7). It is unclear why this should be the case, but one possibility is that it reflects the importance of vocal communication in these species. Although the Eastern Chipmunk is solitary, it is known to be extremely vocal, particularly in the context of territorial communication and alarm calls (Burke da Silva et al. 2002; Baack and Switzer 2000). The African Grass Rat is a social species and begins to develop vocal communication very early after birth; African Grass Rats are known to be highly vocal as adults (Delaney and Monro 1985). On the other hand, the Short-tailed Singing Mouse, which is also a vocal species, does not have a particularly large MGN. While our data do not allow us to evaluate this hypothesis, future studies could test for such an association by comparing components of the auditory system between species with varying levels of communicative complexity.

Inferior Colliculus (IC)

The size of the IC was not significantly different between diurnal and nocturnal species, but it was affected by a strong interaction between temporal niche and brain size (Table 1.3). In species with smaller brains, diurnal species had a larger IC, whereas in species with larger brains, nocturnal species had a larger IC (Figure 1.8). The IC organizes inputs from auditory nuclei in the brainstem and projects to the SC and the MGN, which in turn projects to the

auditory cortex (Winer and Schreiner 2005). It is a convergence point in which sensory, motor, and cognitive information are integrated to carry out higher-order auditory functions, such as localizing sounds, distinguishing between important and insignificant sounds, and perceiving and generating vocal communication (Gruters and Groh 2012).

The African Grass Rat had a very large MGN, and the largest IC relative to brain size. The IC of the chipmunk, although not as extreme as in the African Grass Rat, was also notably large (Figures 1.7 and 1.8). Both species are highly vocal, but while African Grass Rats are social and live in colonial burrows (Senzota 1990), Eastern Chipmunks are solitary, with only one adult individual per burrow (Snyder 1982). The Short-tailed Singing Mouse, a social species, has the second largest IC of the diurnal species here. It could be that sociability requires more integration of other forms of information to carry out complex social behaviors.

4.5 | Variation in the MGN and IC

Another interesting pattern seen in our auditory data is the degree of interspecific variation. Diurnal species showed very high levels of interspecific variation in both the MGN and IC, whereas nocturnal species exhibited much less interspecific variation in both structures (Figures 1.7 and 1.8). Could this suggest that there are a greater variety of selection factors acting on auditory systems of animals that are active during the day?

Shelley and Blumstein (2005) investigated the relationship between sociality, vocal alarm calls, and diurnality in rodents. They established an association between sociality and diurnality, and sociality and alarm calls, but there was a stronger, directional relationship between diurnality and alarm calls. They demonstrated that the evolution of diurnality preceded the evolution of vocal alarm communication in rodents. It has also been suggested

that prey typically alarm call only when there is enough light to detect and track predators (Blumstein and Armitage 1997). If diurnality is indeed predominantly responsible for the evolution of alarm calls, this suggests a coupling of visual cues with auditory communication. Such coupling could have created selection acting on the auditory system of diurnal animals that is not present, or present to a lesser degree, in night-active animals.

5 | CONCLUSIONS

The species differences observed in the size of the olfactory and visual areas support the hypothesis of a trade-off related to temporal niche, specifically that with the evolution of diurnality, and concomitant increase in available visual cues, overall investment in visual processing increases, and reliance on olfactory cues decreases. The brain components of these two sensory modalities appear to have evolved in a segregated manner, separate from other brain structures, which represents a mosaic pattern of evolutionary change. These results support earlier work by Finlay and Darlington (1995) and Finlay et al. (2001) which found that OBs do not change in concert with other brain structures. It is possible that olfactory bulbs may have fewer constraints compared to other brain regions. Our data also suggest there may be some level of coevolution between the visual and auditory systems. The evolution of diurnality may have enabled certain types of communicative behaviors that involve visual and auditory components to evolve.

While our data do support mosaic evolution of specific brain regions, overall brain size did not differ with activity pattern, suggesting that brain size is conserved to some degree. Finlay et al. (2001) have shown that the size of larger regions of the brain, such as the neocortex, diencephalon, cerebellum, and medulla are highly conserved and change in concert with one another. It is likely that while larger regions of the brain may be constrained, smaller regions within those may show dissociative changes, as is seen in our data.

The species included in this study exhibit either strictly diurnal or nocturnal behavioral rhythms. If the differences found in the olfactory and visual structures reflect evolutionary transitions in temporal niche, then the magnitude of differences in these structures may be

smaller in species that have more intermediate or flexible daily activity patterns. These species would be active, to some degree, during daytime and nighttime hours and may therefore exhibit an intermediate level of investment in both olfaction and vision, compared to strictly diurnal or nocturnal species.

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APPENDIX

Table 1.1: Family, common name, genus and species, source, sample size (N) with numbers of males (m) and females (f) used, activity pattern, and references for activity pattern.

Family	Common Name	Genus & Species	Source	N (m, f)	Activity Pattern	References
Sciuridae	Southern Flying Squirrel	*Glaucomys volans*	Live-trapped, East Lansing	3 (0,3)	nocturnal	Aschoff 1966; Muul 1968
	North American Red Squirrel	*Tamiasciurus hudsonicus*	Live-trapped, East Lansing	3 (1,2)	diurnal	Pauls 1978
	Eastern Chipmunk	*Tamias striatus*	Live-trapped, East Lansing	4 (2,2)	diurnal	Elliot 1978
Cricetidae	Social Vole	*Microtus socialis*	Kronfeld-Schor Lab, Tel Aviv University	3 (2,1)	nocturnal	Shalmon et al. 1993
	Striped Desert Hamster	*Phodopus sungorus*	Nelson Lab, *Ohio State University	6 (3,3)	nocturnal	Wynne-Edwars et al. 1999
	Short-tailed Singing Mouse	*Scotinomys teguina*	Phelps Lab, University of Texas; Austin	6 (3,3)	diurnal	Hooper & Carleton 1975
	Southern Grasshopper Mouse	*Onychomys torridus*	Rowe Lab, **Michigan State University	3 (1,2)	nocturnal	O'Farrell 1974; Upham & Hafner 2013

Table 1.1 (cont'd)

Family	Common Name	Genus & Species	Source	N (m, f)	Activity Pattern	References
Muridae	Northeast African Spiny Mouse	*Acomys cahirinus*	Kronfeld-Schor Lab, Tel Aviv University	5 (4,1)	nocturnal	Weber & Hohn 2005
	Golden Spiny Mouse	*Acomys russatus*	Kronfeld-Schor Lab, Tel Aviv University	4 (2,2)	diurnal	Shargal et al. 2000; Gutman & Dayan 2005; Levy et al. 2012
	House Mouse	*Mus musculus*	Live-trapped, Lansing	6 (3,3)	nocturnal	Robbers et al. 2015
	African Grass Rat	*Arvicanthis niloticus*	Smale Lab, *Michigan State University	6 (3,3)	diurnal	Blanchong & Smale 2000
	Australian Bush Rat	*Rattus fuscipes*	Live-trapped, NSW Australia	5 (2,3)	nocturnal	Wood 1971; Meek et al. 2012
	Brown Rat	*Rattus norvegicus*	Lab, Charles River	5 (3,2)	nocturnal	Taylor 1978

*Currently at West Virginia University

**Currently at University of Oklahoma

Table 1.2: Phylogenetic signal estimates (Blomberg's κ and Pagel's λ) for brain mass, olfactory bulb mass (OB), and volumes of lateral geniculate nucleus (LGN), superior colliculus (SC), medial geniculate nucleus (MGN), and inferior colliculus (IC). Each measure is size-independent, i.e. based on the residuals from a linear regression.

Measure	κ	**p-value**	**λ**	**p**
Brain	0.603	0.065	0.594	0.031
OB	0.850	0.009	1	0.110
LGN	0.534	0.175	<0.001	1
SC	0.861	0.011	1	0.118
MGN	0.489	0.235	<0.001	1
IC	0.448	0.339	<0.001	1

Table 1.3: ANCOVA results examining the effects of temporal niche on total brain mass as a proportion of body mass; olfactory bulb (OB) mass as a proportion of total brain mass; and volumes of the lateral geniculate nucleus (LGN), superior colliculus (SC), medial geniculate nucleus (MGN), and inferior colliculus (IC) as proportions of total brain mass. $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***.

Regions	Factors	DF	Mean Sq	F Value	Pr(>F)
Total brain	Temporal Niche	1	0.00001	0.0125	0.914
	Body Size	1	0.01183	14.2612	0.005**
	Temporal Niche*Body	1	0.00033	0.4040	0.543
	Residuals	8	0.00083		
Olfactory Structure					
OB	Temporal Niche	1	0.00064	5.1631	0.049*
	Brain Size	1	0.01911	154.4564	<0.001***
	Temporal Niche*Brain	1	0.00052	4.1801	0.071
	Residuals	9	0.00012		
Visual Structures					
LGN	Temporal Niche	1	0.2294	40.269	<0.001***
	Brain Size	1	4.6037	808.313	<0.001***
	Temporal Niche*Brain	1	0.0281	4.925	0.031*
	Residuals	55	0.0057		
SC	Temporal Niche	1	0.00433	6.8836	0.028*
	Brain Size	1	0.02269	36.1529	<0.001***
	Temporal Niche*Brain	1	0.00168	2.6839	0.136
	Residuals	9	0.00063		
Auditory Structures					
MGN	Temporal Niche	1	0.1824	10.3912	0.002**
	Brain Size	1	5.9126	336.9143	<0.001***
	Temporal Niche*Brain	1	0.0007	0.0397	0.843
	Residuals	54	0.0175		
IC	Temporal Niche	1	0.0284	2.782	0.102
	Brain Size	1	3.6597	358.493	<0.001***
	Temporal Niche*Brain	1	0.1573	15.407	<0.001***
	Residuals	50	0.0102		

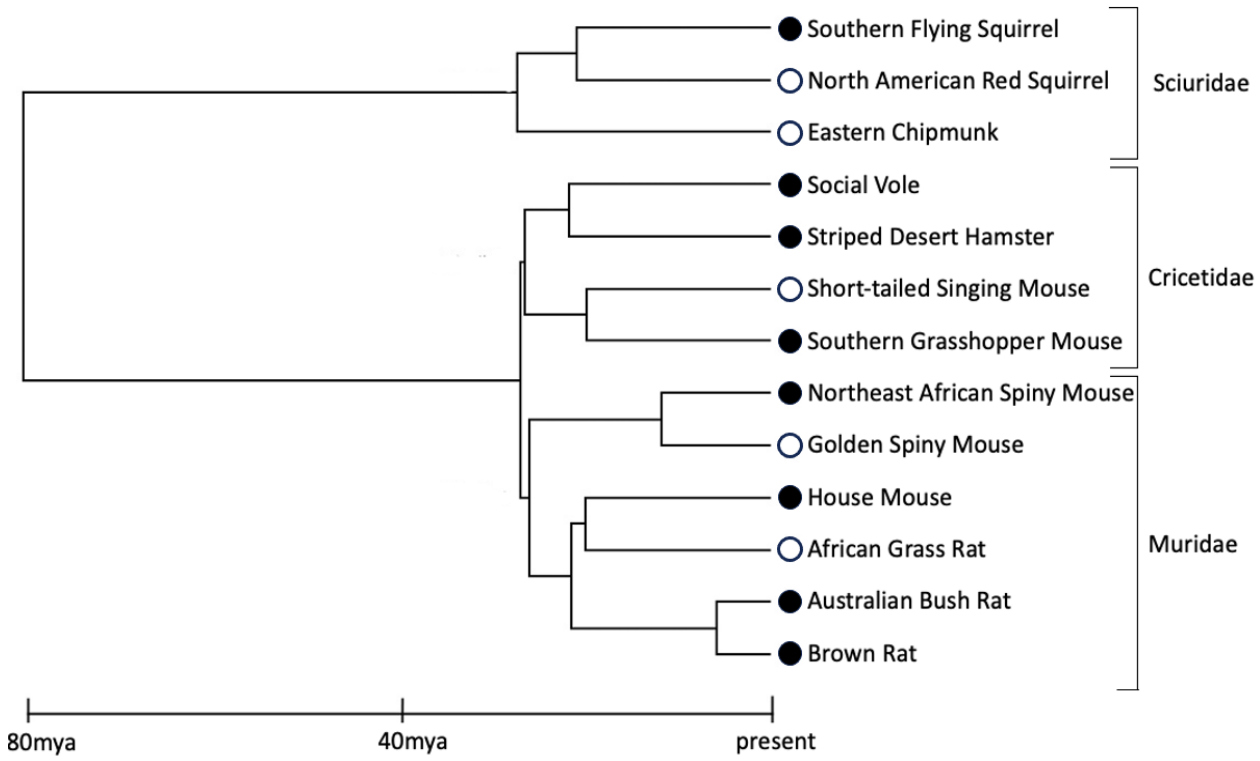

Figure 1.1: Phylogeny and temporal niche of 13 rodent species (open: diurnal, black: nocturnal). Phylogenetic relationships and divergence times were established from Fabre et al. [2012]. Mya = million years ago.

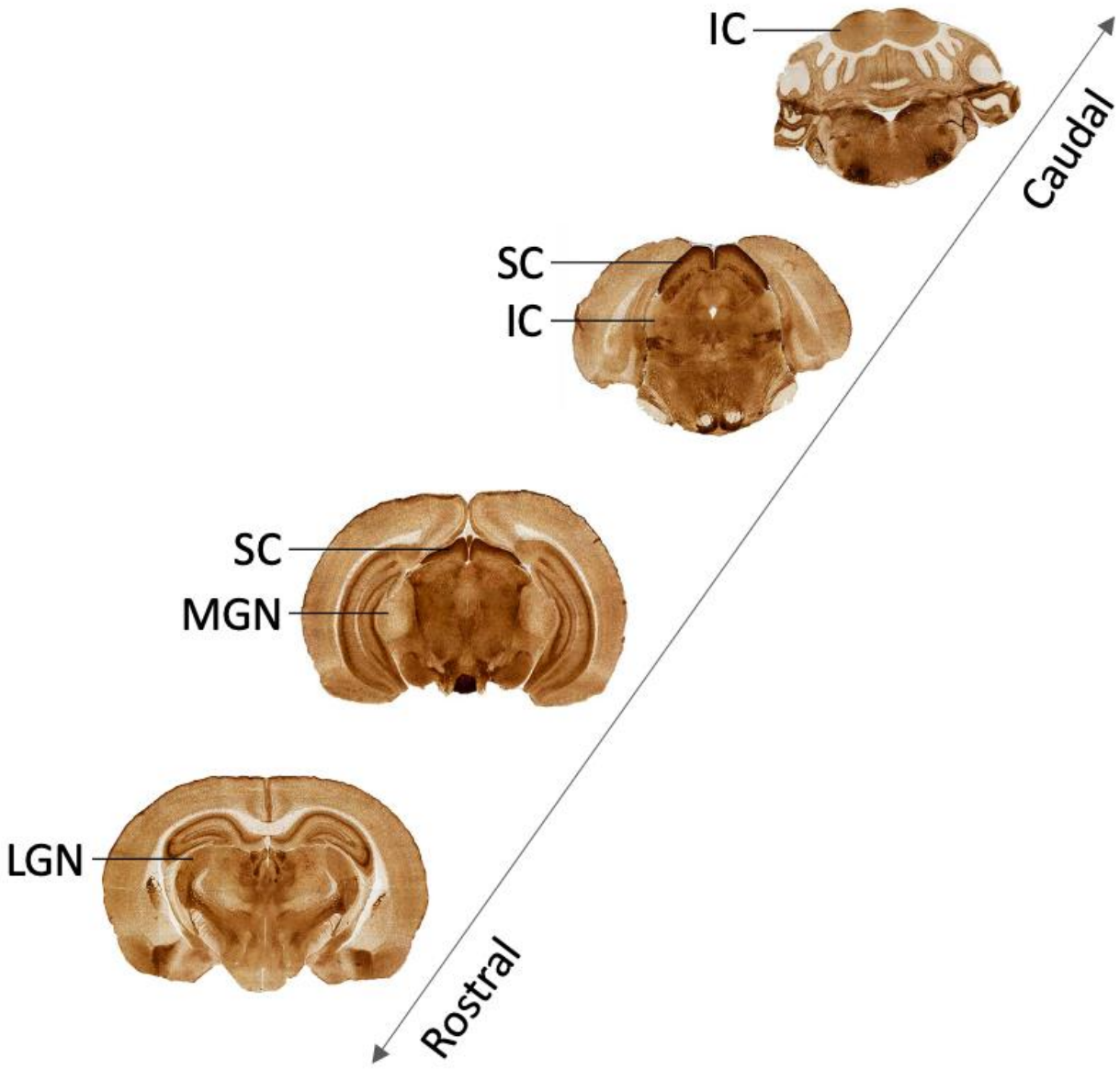

Figure 1.2: Photomicrographs of an AChE-stained African Grass Rat brain, showing the visual and auditory brain regions measured in this study: lateral geniculate nucleus (LGN), superior colliculus (SC), medial geniculate nucleus (MGN) and inferior colliculus (IC).

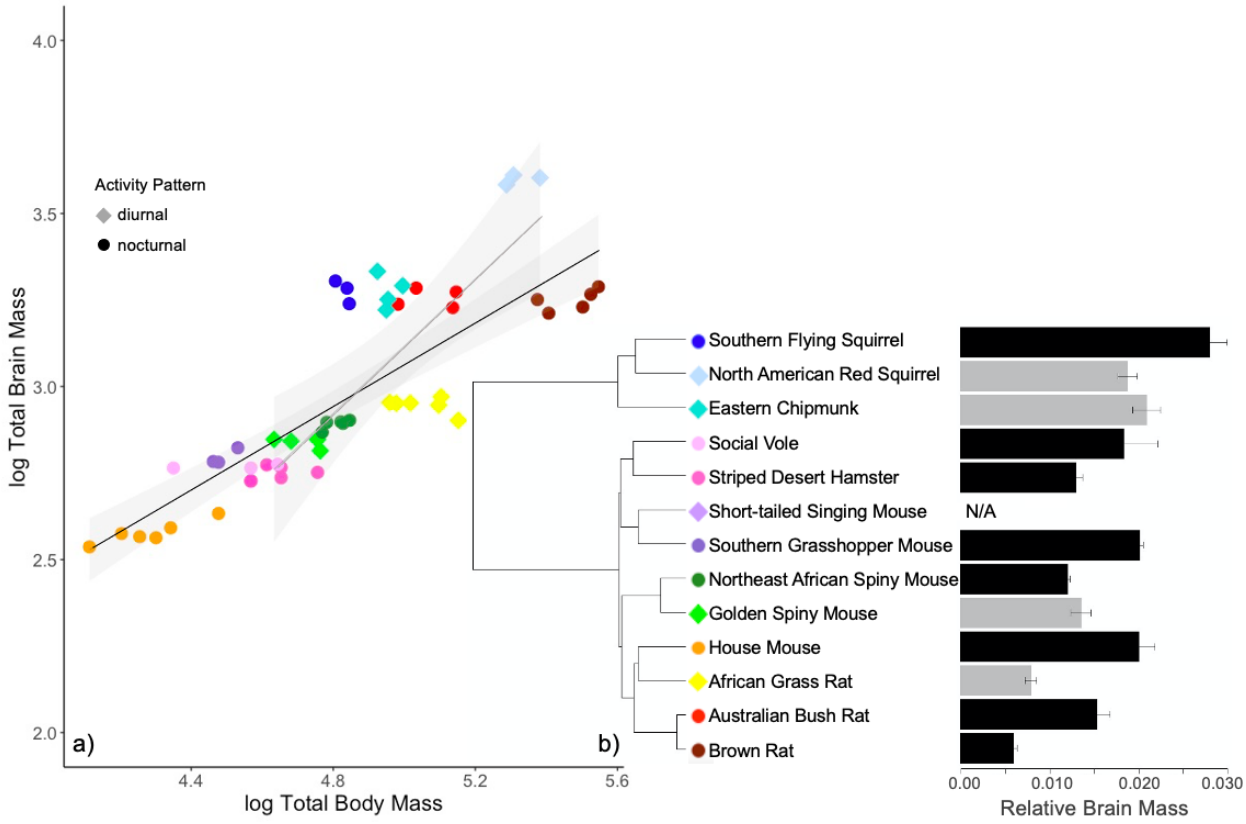

Figure 1.3: Log brain mass regressed against log body mass of 12 rodent species. Shading represents 95% confidence intervals. Mean brain mass relative to body mass, error bars are SEM (grey: diurnal, black: nocturnal).

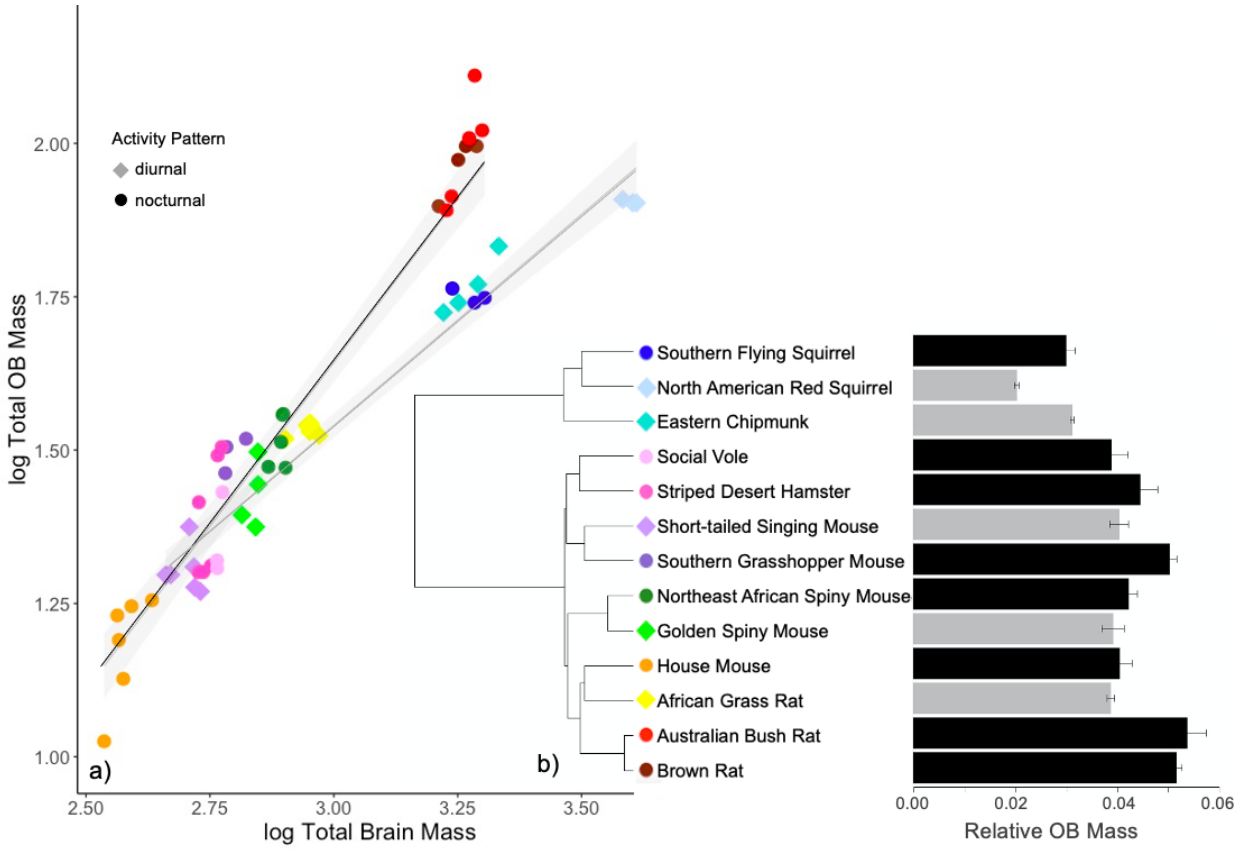

Figure 1.4: Log olfactory bulb (OB) mass regressed against log brain mass of 13 rodent species. Shading represents 95% confidence intervals. Mean OB mass relative to brain mass, error bars are SEM (grey: diurnal, black: nocturnal).

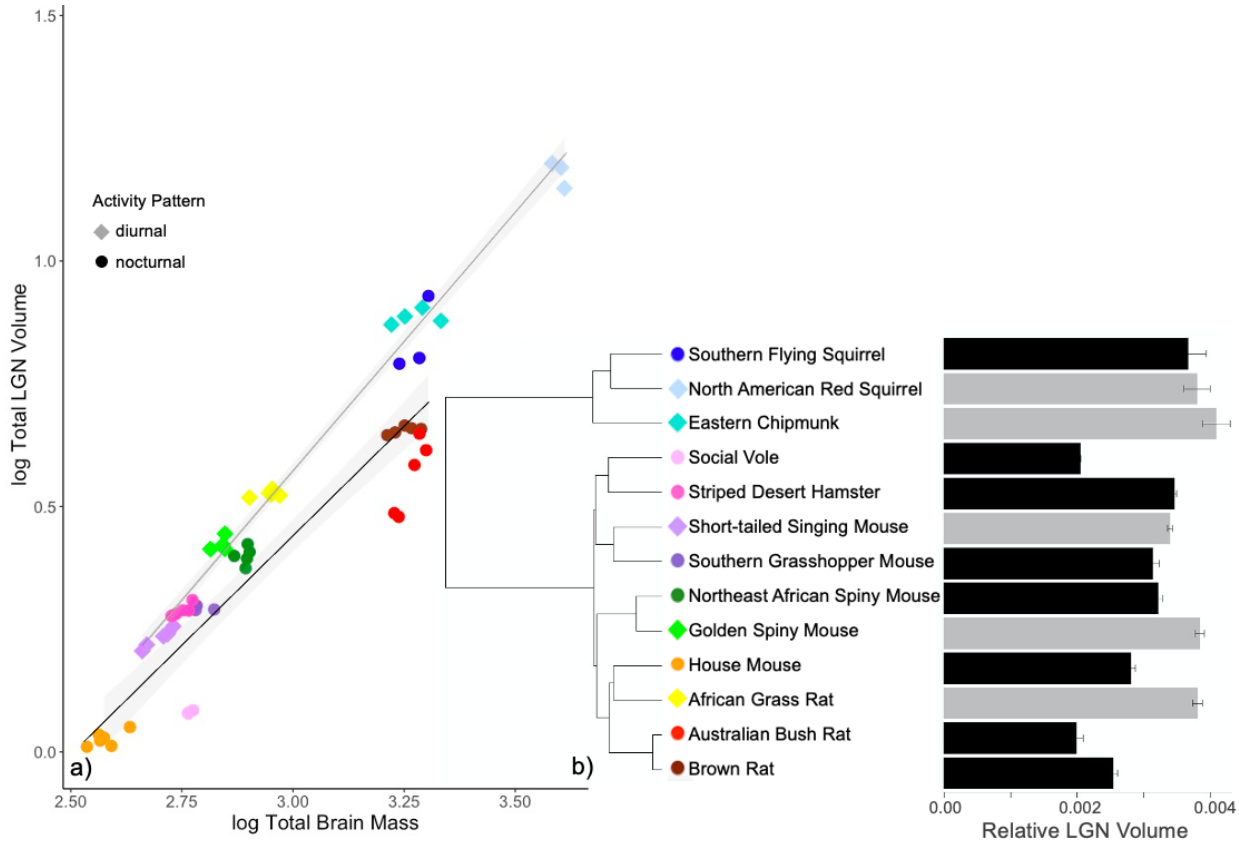

Figure 1.5: Log lateral geniculate nucleus (LGN) volume regressed against log brain mass of 13 rodent species. Shading represents 95% confidence intervals. Mean LGN volume relative to brain mass, error bars are SEM (grey: diurnal, black: nocturnal).

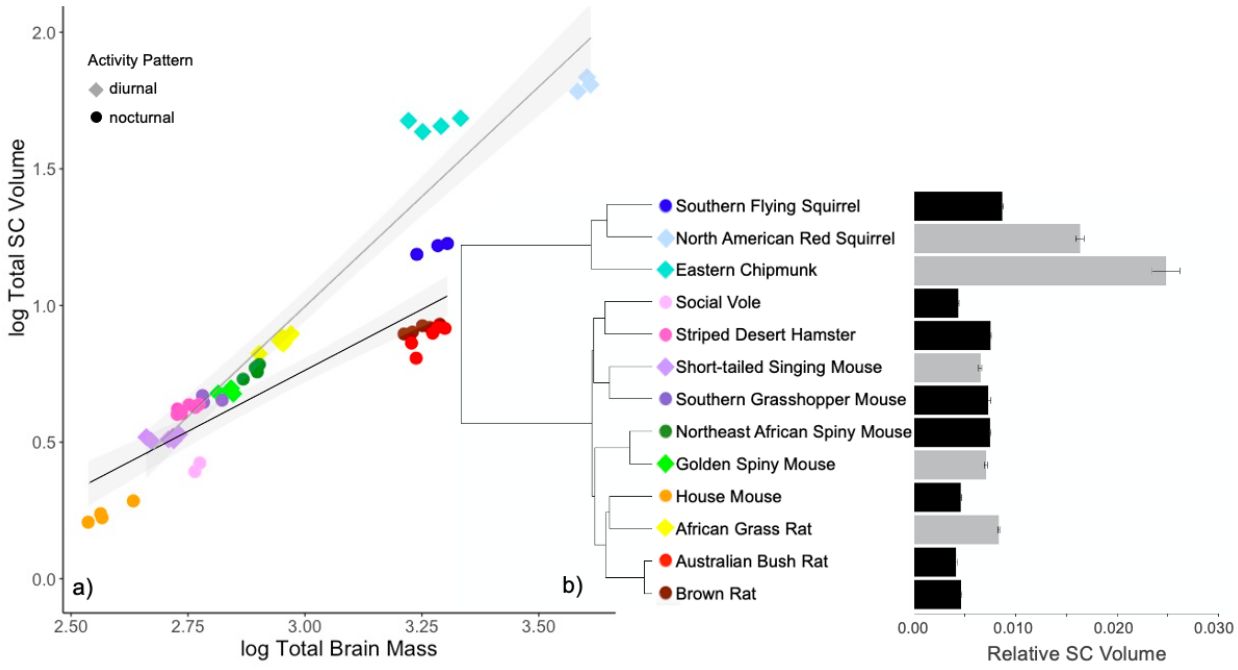

Figure 1.6: Log superior colliculus (SC) volume regressed against log brain mass of 13 rodent species. Shading represents 95% confidence intervals. Mean SC volume relative to brain mass, error bars are SEM (grey: diurnal, black: nocturnal).

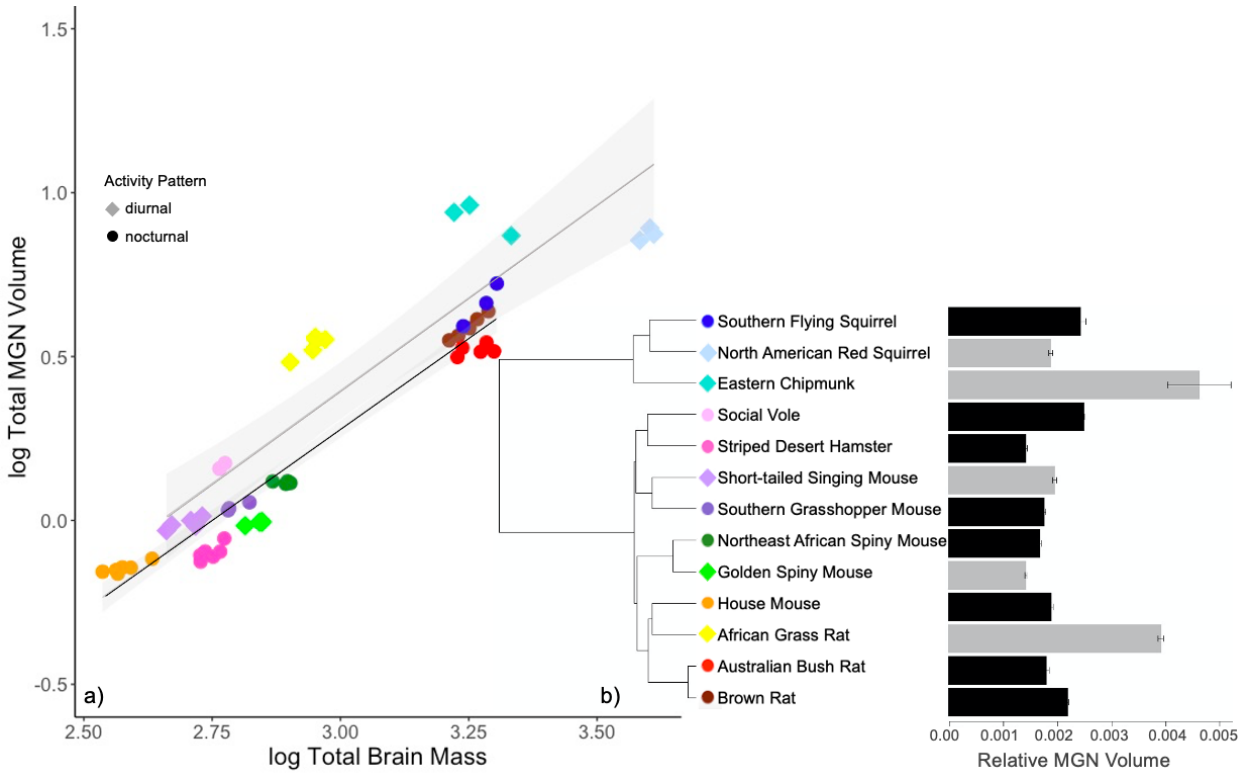

Figure 1.7: Log medial geniculate nucleus (MGN) volume regressed against log brain mass of 13 rodent species. Shading represents 95% confidence intervals. Mean MGN volume relative to brain mass, error bars are SEM (grey: diurnal, black: nocturnal).

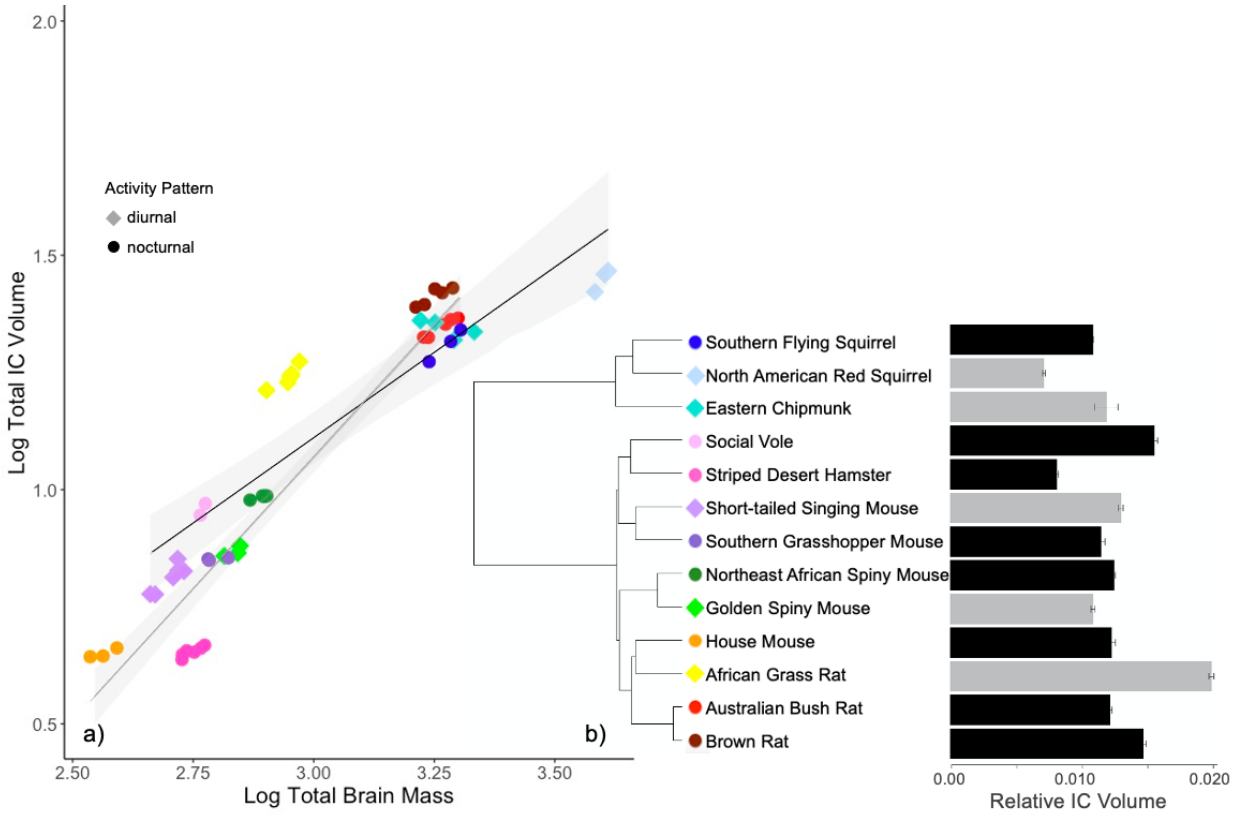

Figure 1.8: Log inferior colliculus (IC) volume regressed against log brain mass of 13 rodent species. Shading represents 95% confidence intervals. Mean IC volume relative to brain mass, error bars are SEM (grey: diurnal, black: nocturnal.

CHAPTER 2: COMPARATIVE ANALYSIS OF INVESTMENT IN VISION, OLFACTION, AND AUDITION IN CATHEMERAL RODENTS

1 | INTRODUCTION

While the earliest mammals were almost certainly nocturnal (i.e., active at night), mammals today exhibit a range of activity patterns (Martin 1990; Hall et al. 2012; Anderson and Weins 2017; Maor et al. 2017). In addition to nocturnal and diurnal (i.e., active during the day), some species do not concentrate their activity during either daytime or nighttime but exhibit similar amounts of activity during light and dark phases, a pattern referred to as cathemeral (Tattersall 1987). A study by Maor et al. (2017) suggests that cathemerality first appeared in mammals roughly 75 million years ago, 10 million years before diurnality. Today, cathemerality is widespread across Mammalia, having been identified in more than half of the extant orders (reviewed by Curtis and Rasmussen 2006). The complex phylogenetic patterns in temporal niche among extant mammals suggests that mammals have evolved to exploit the environment on a temporal level.

For a mammal to respond optimally to the external world, two fundamental actions must occur within the sensory system: sensory stimuli must be received via sensory organs and nerves, and the signals coming in must be processed in sensory organs and the brain to extract relevant information. Neural tissue is energetically expensive to develop and maintain (Niven and Laughlin 2008); therefore, selection is expected to optimize investment in components of the sensory system that are the most beneficial. Since some forms of sensory stimuli vary between day and night (e.g., availability of photic cues), the benefits of optimizing different sensory modalities should differ between day-active and night-active species. Indeed, a

relationship between temporal niche and olfactory, visual, and auditory sensory system development has been established in studies of nocturnal and diurnal mammals (reviewed below).

Cathemeral species, though common in nature, have been largely overlooked in this research, and the work that has been done is focused primarily on primates and on fully fossorial species, i.e. species that spend most, or all, of their time underground. The aim of this study is to extend understanding of the relationship between investment in sensory brain regions and temporal niche by adding cathemeral rodents that are not fully fossorial to a larger data set that includes nocturnal and diurnal ones. In some ways, these cathemeral species can be thought of as temporal generalists. While nocturnal and diurnal species have adapted to very specific sensory worlds, these cathemeral species navigate and exploit, but must also survive, both dark and light environments.

Several studies have reported a correlation between activity patterns and the olfactory system of mammals. Barton et al. (1995) found larger olfactory bulbs in nocturnal primates and insectivores compared to their diurnal relatives and Morrow et al. (in review) found the same pattern in rodents. Hughes et al. (2018) found that nocturnal and crepuscular mammals have a greater number of functional olfactory receptor genes than diurnal species. These studies demonstrate the important role olfaction plays in species that are active during periods with limited light availability. However, data on the olfactory system of cathemeral species is lacking.

Links between the visual system and daily activity patterns have also been well documented. Nocturnal primates exhibit traits in eye morphology that increase sensitivity to visual cues (e.g., increased curvature of the cornea and lens, increased retinal summation, and

a relatively high proportion of rods to cones), while the eyes of diurnal primates have characteristics that increase visual acuity at the expense of sensitivity (e.g., flatter cornea and lens, decreased retinal summation, and a relatively high proportion of cones to rods) (Detwiler 1939; 1940; 1941; Walls, 1942; Prince 1956; Duke-Elder 1958; Tansley, 1965). Multiple studies have shown that cathemeral primates typically possess intermediate forms of these features of the eye (Walls 1942; Ahnelt and Kolb 2000; Kay and Kirk 2000; Kirk and Kay 2004).

Several studies have examined relationships between regions of the brain that process visual information and temporal niche. Barton (2007) found that diurnal primates have a larger visual cortex relative to hindbrain volume than nocturnal primates. In addition, Campi et al. (2011), Shuboni-Mulligan et al. (2019), and Morrow et al. (in review) found that diurnal rodents have larger visual areas of the brain (i.e., primary visual cortex, lateral geniculate nucleus, and superior colliculus) than their nocturnal relatives. Notably, Finlay et al. (2014) did not find a relationship between activity pattern and the volume of the lateral geniculate nucleus in primates, indicating that some taxonomic groups do not follow the pattern seen in rodents. Overall, the great majority of studies examining brain structures that process visual information have found a difference between diurnal and nocturnal mammals. Although visual brain structures have been examined in cathemeral mole-rats that live underground (Cooper et al. 1993; Crish et al. 2006; Nemec et al. 2008), we are not aware of any studies that have investigated these structures in cathemeral species that are regularly active above ground.

The relationship between activity pattern and audition in mammals is not as well studied as that of olfaction and vision. Auditory cues work well in dark and light and could, theoretically, help compensate for the absence of visual cues available to nocturnal species.

However, the use of auditory signals might prove detrimental to nocturnal species that rely on darkness to avoid being detected by predators or prey. An analysis of two auditory brain structures in rodents, the medial geniculate nucleus and inferior colliculus, found that the medial geniculate nucleus was significantly larger in diurnal than nocturnal rodents, while the size of the inferior colliculus was unrelated to temporal niche (Morrow et al., in review). No work has been done, to our knowledge, on auditory structures in the brains of cathemeral species.

In this study, we use a phylogenetic framework to examine investment in brain structures known to process visual, olfactory, and auditory stimuli in 15 rodent species, representing nocturnal, diurnal, and cathemeral activity patterns. We expect cathemeral species, like nocturnal ones, to invest significantly more in olfaction than diurnal species because they are active during times when photic cues are not available. We further predict that cathemeral species will invest more than nocturnal species, but possibly less than diurnal species, in brain regions that process visual information because they are often active during the day when visual cues are available to them (as well as to their predators). The latter would be consistent with earlier work (reviewed above) establishing that cathemeral primates exhibit characteristics of the eye that are intermediate between those of diurnal and nocturnal primates. Relationships between auditory structures and cathemerality are more challenging to predict. Our earlier work (Morrow et al. in review) found one auditory structure (medial geniculate nucleus) to be larger in diurnal than nocturnal rodents and the other (inferior colliculus) to be unrelated to temporal niche. For these reasons, we investigate if either, or both, of these structures is associated with cathemerality.

2 | MATERIALS AND METHODS

2.1 | Specimens

Data were collected from 15 rodent species, representing two extant families: Cricetidae and Muridae (Table 2.1, Figure 2.1). Our sample includes seven nocturnal species [Social Vole (*Microtus socialis*), Striped Desert Hamster (*Phodopus sungorus*), Southern Grasshopper Mouse (*Onychomys torridus*), Northeast African Spiny Mouse (*Acomys cahirinus*), House Mouse (*Mus musculus*), Australian Bush Rat (*Rattus fuscipes*), and Brown Rat (*Rattus norvegicus*)], three diurnal species [Short-tailed Singing Mouse (*Scotinomys teguina*), Golden Spiny Mouse (*Acomys russatus*), African Grass Rat (*Arvicanthis niloticus*)], and five cathemeral species [Eastern Meadow Vole (*Microtus pennsylvanicus*), Southern Red-Backed Vole (*Myodes gapperi*), Hispid Cotton Rat (*Sigmodon hispidus*), Mongolian Jird (*Meriones unguiculatus*), and Australian Swamp Rat (*Rattus lutreolus*)].

Eastern Meadow Voles, Southern Red-backed Voles, and House Mice were live-trapped in Michigan between October 2015 and December 2017 (Table 2.1). Australian Bush Rats and Australian Swamp Rats were live-trapped in New South Wales, Australia, in August 2015. Mongolian Jirds and Brown Rats were purchased from Charles River Laboratories. Hispid Cotton Rats were purchased from Harlan Laboratories. Southern Grasshopper Mice and African Grass Rats were obtained from Michigan State University laboratory colonies. Striped Desert Hamsters were obtained from a laboratory colony at Ohio State University. Intact whole brains from Social Voles, Northeast African Spiny Mice, and Golden Spiny Mice were obtained from Tel Aviv University and those from Short-tailed Singing Mice were obtained from a University of Texas at Austin laboratory.

We included from 2 to 6 individuals per species (Table 2.1). Only adult animals were sampled, and we attempted to sample both males and females of each species. However, the Eastern Meadow Voles were all female in this study. All animals were handled according to protocols approved by the following institutional and regional authorities: American Society of Mammalogists (Sikes et al. 2016), Michigan State University Institutional Animal Care and Use Committee (protocol # 07/16-116-00), Office of Environment and Heritage of New South Wales (NSW), Australia (License #SL100634), and NSW Department of Industry and Investment Animal Research Authority (ORA 14/17/009).

2.2 | Regions of Interest

We selected two structures in the midbrain (i.e., superior colliculus and inferior colliculus) and three structures in the forebrain (i.e., olfactory bulb, lateral geniculate nucleus, and medial geniculate nucleus) to serve as indicators of investment in olfaction, vision, and audition.

Midbrain structures

The superior colliculus (SC) consists of seven distinct layers in mammals (May 2006). Only the three most superficial layers (i.e., the zonal layer, superficial gray layer, and optic nerve layer) were included in our measurement, as they receive most of the retinal input and function nearly exclusively in processing visual information, whereas the deeper layers also play a role in auditory and somatosensory processing (Gaese and Johnen, 2000; McHaffie et al. 1989). The SC sends and receives projections from the LGN and the pulvinar complex, both of which are visual thalamic nuclei (Baldwin et al. 2011).

The inferior colliculus (IC) is ventral and posterior to the SC. It consists of three subdivisions (i.e., central nucleus, dorsal cortex, and lateral cortex), all of which were included in our measurement. The IC has connections with the medial geniculate nucleus and integrates auditory information from the brain stem and auditory cortex (Winer and Schreiner 2005). It is an important area of convergence within the auditory pathway (Kulesza et al. 2002).

Forebrain structures

Investment in olfaction was estimated by combining the mass of the main and accessory olfactory bulbs (OB). The main olfactory bulbs receive input from olfactory neurons interspersed in the olfactory epithelium, the septal organ, and the Gruenberg ganglion, all of which are in the nasal cavity (Gruneberg 1973; Tian and Ma 2004). Accessory olfactory bulbs are located dorsocaudally to the main olfactory bulbs and receive input from the vomeronasal organ (Halpern and Martinez-Marcos 2003).

In addition to the SC, we measured a second visual structure, the lateral geniculate nucleus (LGN), which consists of three smaller subdivisions (i.e., dorsal LGN, intergeniculate leaflet, and ventral LGN), all of which were included in our measurement. The LGN receives input from the retina, delivers and receives projections from the SC (Baldwin et al. 2011), and sends projections to the primary visual cortex (Horng et al. 2009).

Our other forebrain region, the medial geniculate nucleus (MGN), is another auditory structure. It is located in the thalamus, posterior to the LGN, and is subdivided into three distinct parts in mammals (i.e., dorsal MGN, medial MGN, and ventral MGN), all of which were included in our measurement (Winer and Schreiner 2005; Najdzion et al. 2011). The MGN

receives projections from the IC and auditory nuclei in the brainstem and sends projections to the amygdala and frontal cortex (Winer and Schreiner 2005).

2.3 | Brain Collection and Histology

Only fresh, unfixed tissue was used in this study. All individuals were euthanized via an intraperitoneal injection of sodium pentobarbital. After death, the individual was weighed to the nearest gram and the brain removed and placed in powdered dry ice. After 2-5 minutes in dry ice, the brain was moved to a -80° freezer where it was stored until further processing. After removal from the freezer, the brain was cut just caudal to the medulla oblongata and weighed to the nearest milligram. The OBs were then cut from the brain just anterior to the olfactory peduncles and weighed to the nearest milligram. The part of the brain between the anterior thalamus and just caudal to the IC was coronally sectioned at 40µm thickness on a cryostat. Due to the very small size of the House Mice brains, they were sectioned at 20µm thickness. Three alternate series of brain tissue sections were mounted directly onto slides. Two series were set aside for future work and the third was stained for acetylcholinesterase using the following protocol: slides were incubated for 5 hours in a solution of 0.0072% ethopropazine HCl, 0.075% glycine, 0.05% cupric sulfate, 0.12% acetylthiocholine iodide, and 0.68% sodium acetate (pH 5.0); rinsed 2 times (3 minutes each) with distilled H_2O; and developed in a 0.77% sodium sulfide solution (pH 7.8) for 45 minutes. Slides were then rinsed with 2 changes of distilled H_2O (3 minutes each) and run through a series of ascending ethanol concentrations (70%, 95%, 100%, and 100%) for 1 minute each (to dehydrate the adhering tissue), cleared through 2 changes of xylenes for 5 minutes each, and coverslipped using DPX mounting medium.

2.4 | Measurements

The OBs, which included main and accessory bulbs, were weighed to the nearest milligram. The visual and auditory regions (SC, IC, LGN, and MGN) were measured by taking photomicrographs of acetylcholinesterase-stained sections (Figure 2.2) using a digital camera (MBF Bioscience CX9000) attached to a Zeiss light microscope (Carl Zeiss, Gottengen, Germany, 5x objective), using the 2D slide scanning module on Stereo Investigator 2017 (MBF Bioscience). Volumetric measurements were calculated using the Cavalieri method (100 x 100 um grid, every third section) in Stereo Investigator 2017 (MBF Bioscience). Boundaries of each brain structure were determined according to the rat brain atlas (Paxinos and Watson 2014). For each structure, one side (left or right) was measured, and that value was doubled to obtain total volume.

While neuronal density would likely provide a more accurate estimation of investment in brain tissue, it is difficult to measure. Therefore, many studies, including this one, have used mass and/or volume as an alternative proxy for investment. Neuron density scales closely with volume in brain structures of rodents (Herculano-Houzel et al. 2011; Najdzion et al. 2009; 2011).

2.5 | Data Analysis

Variables and transformations. Continuous variables used in the analyses include body, brain, and OB mass, as well as SC, IC, LGN, and MGN volume. All continuous variables were log-transformed prior to phylogenetic signal estimations and linear model comparisons. ANOVAs were carried out using arcsine transformed relative sizes (brain region divided by overall brain size). All data transformations and analyses were carried out in R Studio (RStudio 2020). Each

species was assigned to one of three categorical states, diurnal, nocturnal, or cathemeral, based on descriptions of daily activity patterns from field studies reported in the literature (Table 2.1).

Phylogenetic signal estimations. We calculated Blomberg's *K* (based on 1000 randomizations for p-value) and Pagel's λ (based on likelihood ratio tests) using the PHYTOOLS 0.7-70 package in R (Revell 2012) to assess phylogenetic signal in each measurement of interest. To calculate size-independent estimations, we used the residuals from linear regressions. The size of each sensory region (OB, SC, IC, LGN, and MGN) was regressed on overall brain size.

Modes of Evolution. We compared different evolutionary models for the size of each region of interest using phylogenetic generalized least squares (PGLS) in the PHYLOLM 2.6.2 package in RStudio (Ho and Ane 2014). Four models incorporated one of three different branch-length transformations: lambda (λ), delta (δ), or kappa (κ). For a λ transformation, internal branch lengths are multiplied by lambda. A λ equal to 0 indicates no phylogenetic effect, while a λ value of 1 is equivalent to a Brownian motion model of evolution. In a Brownian motion model, biological traits accumulate random, incremental changes. A δ transformation affects the phylogenetic tree by raising the node heights of the tree to the power of δ. A δ value greater than 1 would model an increase in the rate of evolution over time, whereas a δ value less than 1 would model the rate of evolution decreasing over time. A κ transformation occurs by raising each branch length to the power of κ. A κ value equal to zero indicates a punctuated model of evolution and a value of 1 for κ indicates Brownian motion.

For each brain region of interest, we compared the following seven models: λ set to 0 (no phylogenetic effect), λ set to 1 (Brownian model), maximum likelihood (ML) of λ, ML of δ,

ML of κ, early burst (EB) model of evolution, and Ornstein-Uhlenbeck (OU) fixed-root model of evolution. We compared the models using the sample-size corrected Akaike's Information Criterion (AICc). Using the best model for each measure, we carried out ANOVAs and Tukey's HSD post-hoc tests to compare each variable as a function of activity pattern (nocturnal, diurnal, cathemeral). Phylogenetic ANOVAs and post-hoc tests were carried out using the PHYTOOLS 0.7-70 package in R (Revell 2012).

3 | RESULTS

3.1 | Phylogenetic Signal

Blomberg's *K* and Pagel's λ estimates were both significant for SC size, indicating a strong phylogenetic signal for that brain region (Table 2.2). None of the other brain regions reached significance for either indicator of phylogenetic signal. The results of the Blomberg's *K* and Pagel's λ estimations were consistent with the models of evolution selected (based on AICc) for the ANCOVAs.

3.2 | Midbrain Structures

SC. Volume of the SC ranged from 0.33% of brain mass in the Eastern Meadow Vole to 1% of brain mass in the Mongolian Jird (Figure 2.3). The SC showed a strong phylogenetic component and the Brownian motion model performed best, explaining 62.2% of the variation in SC size ($F_{1,13} = 24.02$, $p < 0.001$). While the Mongolian Jird and Hispid Cotton Rat stand out as having the largest SCs, the three vole species and three *Rattus* species have rather small SCs, reflecting the strong phylogenetic influence on this structure. The phylogenetic ANOVA of the SC found no significant differences between diurnal, nocturnal, and cathemeral species in relative SC size (Table 2.3).

IC. Volume of the IC ranged from 0.81% of brain mass in the Striped Desert Hamster, to 1.97% of brain mass in the African Grass Rat (Figure 2.4). The model that best fit the IC size data was a non-phylogenetic regression. That model explained 85.2% of the variation in IC size ($F_{1,62}$=362.1, $p < 0.001$). The ANOVA of the IC showed that activity pattern is a significant predictor of relative IC size (Table 2.3). The Tukey's HSD posthoc analysis showed diurnal species to have a significantly larger IC than cathemeral and nocturnal species.

3.3 | Forebrain Structures

OB. OB size ranged from 2.52% of brain mass in the Eastern Meadow Vole to 5.35% of brain mass in the Australian Bush Rat (Figure 2.5). The non-phylogenetic model performed best for OB data, explaining 88.6% of the variation in OB size ($F_{1,66} = 523$, $p < 0.001$). The results of the ANOVA show that activity pattern is a significant predictor of relative OB size (Table 2.3). A Tukey's HSD posthoc test revealed significant differences in relative OB size between nocturnal and diurnal species, as well as between nocturnal and cathemeral species. Nocturnal species have significantly larger OBs than diurnal and cathemeral species.

LGN. Volume of the LGN ranged from 0.20% of brain mass in the Australian Bush Rat to 0.40% of brain mass in the Hispid Cotton Rat (Figure 2.6). The non-phylogenetic model performed best, explaining 82.5% of variation in size ($F_{1,67} = 321.1$, $p < 0.001$). The ANOVA found that activity pattern is a significant predictor of relative LGN size (Table 2.3). A Tukey's HSD post hoc test shows that diurnal species have a significantly larger LGN than cathemeral and nocturnal species, whereas there is no significant difference in relative LGN size between cathemeral and nocturnal species.

MGN. Volume of the MGN ranged from 0.14% of brain mass in the Striped Desert Hamster and the Golden Spiny Mouse, to 0.39% of brain mass in the African Grass Rat (Figure 2.7). The non-phylogenetic regression performed best for the MGN data, explaining 82% of the variation in MGN size ($F_{1,67}=310.4$, $p < 0.001$). The ANOVA results showed that activity pattern is a statistically significant predictor of MGN size (Table 2.3). Tukey's HSD pairwise comparisons found a significant difference in MGN size between nocturnal and diurnal species, as well as

nocturnal and cathemeral species. Diurnal and cathemeral species exhibit a larger MGN than nocturnal species.

4 | DISCUSSION

Issues related to the evolution of the sensory brain in relation to temporal niche have been examined previously in diurnal and nocturnal species but not, to our knowledge, in species that are active above ground both day and night. At a very general level, our data reveal that sensory structures in the brains of these cathemeral rodents are not simply intermediate in size between those of diurnal and nocturnal rodents. Rather, we found a complex mosaic of patterns, and where differences in the size of sensory brain structures were associated with temporal niche, cathemeral species were either like those of diurnal species or like those of nocturnal ones. Below we discuss our findings related to cathemerality and investment in the five regions of the sensory brain investigated in this study.

4.1 | Midbrain Structures

We compared the relative sizes of five brain regions, two of which are found in the midbrain: superior colliculus (SC) and inferior colliculus (IC). The SC, a visual structure, was the only structure that exhibited a significant phylogenetic effect and the only structure for which there was no significant effect of temporal niche on volume (Figure 2.3). Interestingly, a cathemeral rodent, the Mongolian Jird, had the largest SC relative to brain size of any species in this study. One possible explanation comes from consideration of a study of jirds by Kui et al. (2022) in which individual differences in the size of the SC were shown to be correlated with differences in head-bobbing, a behavior that Mongolian Jirds employ to gather depth information prior to making leaps. This raises the possibility that the SC of the Mongolian Jird is larger than in our other species because it engages in these behaviors. It is unclear, however, whether other species sampled here carry out similar behaviors. We measured only the three

most superficial layers of the SC, which are primarily visuosensory. The deeper layers receive inputs from other sensory modalities and play a role in motor- and attention-related responses. It is unclear whether the entire SC (i.e. our three superficial layers plus the intermediate and deeper layers) would be larger in jirds than in the other species we examined. Measuring all layers of the SC in species that exhibit differences in head-bobbing/ jumping, and in temporal niche, would provide meaningful information about how this structure evolved in relation to behavior. It would also address the question of whether individual layers of the SC change independently in response to lineage-specific selection pressures or whether they change in concert with one another.

The other midbrain structure investigated here, the IC, is involved in audition. The mean relative volume of the IC in cathemeral species was indistinguishable from that of nocturnal species, while the IC of diurnal species was significantly larger than that of both cathemeral and nocturnal species. Although auditory cues are effective in the light and dark, some nocturnal species, and to a lesser degree cathemeral ones, rely on darkness to avoid detection while foraging. Such species might be expected to invest less than their diurnal relatives in auditory forms of communication because sounds can attract unwanted attention. Another factor that could influence investment in use of auditory cues is sociality. The IC is an important structure for species-specific vocalizations in mice (Peterson and Hurley 2017). In the IC, acoustic responses are more selective to species-specific calls than are the nuclei of the brainstem (Klug et al. 2002; Xie et al. 2005). The three species with the largest ICs in this study, the Social Vole, Brown Rat, and African Grass Rat, are all social species (Gromov 2022; Schweinfurth 2020; Senzota 1990), whereas the solitary Striped Desert Hamster has the smallest IC. It is possible

that sociality plays a significant role in investment of specific components of the auditory system, such as the IC. Without better data on forms and levels of auditory communication of all species sampled here, it is difficult to test this hypothesis. For example, the Short-tailed Singing Mouse is quite vocal, but does not have a particularly large IC. Sociality and vocal communication may play a role in investment in the size of some auditory structures in the brain, but adaptations of the auditory system are likely far more complex.

4.2 | Forebrain Structures

Diurnal and nocturnal species are specialized for activity in different sensory worlds, which is reflected in their investment in forebrain regions that process olfactory, visual, and auditory information. As cathemeral species are active both night and day, we expected they would invest in the LGN and SC at levels intermediate to those seen in diurnal and nocturnal species, similar to the patterns seen in the eye morphology of cathemeral primates. As nocturnal species rely heavily on olfaction to operate in a dark environment, we expected cathemeral rodents to invest in olfactory regions similar to that of the nocturnal condition. The patterns we found were complex and do not support this hypothesis.

The OBs of diurnal and cathemeral species were similar in size and both were significantly smaller than those of nocturnal species. The difference between diurnal and nocturnal species was expected and is consistent with other evidence that olfaction in primates is especially important for activity at night (e.g., Barton et al. 1995). However, these data beg the question of why cathemeral species, which are also active at night, invest so much less in OBs than their nocturnal relatives. Our data on the LGN, a visual structure, raise a parallel question. Here, we expected cathemeral species to invest more than nocturnal species, as the

former are regularly active when the sun is up. We found, instead, that diurnal species have a significantly larger LGN than both nocturnal and cathemeral species. Taken together, it appears that cathemeral rodents do not invest in either olfactory or visual processing by the forebrain structures we examined in a manner that reflects their activity in both night and day. We do not know if this pattern would be true of cathemeral species in other mammalian clades as, to our knowledge, this issue has not been examined.

These data raise the question of what enables cathemeral species to be active at night with only the OBs of diurnal species, or during the day with only the LGN of nocturnal species. One possibility is that cathemeral species have foraging or antipredator strategies that are not as dependent on the olfactory and visual processing that these structures permit in the more temporally specialized species. It is also possible that adaptations or brain regions that we did not examine here have evolved to capitalize on visual and olfactory information. Finally, there may be other sensory stimuli that cathemeral mammals are able to exploit. This possibility is suggested by the fact that the cathemeral species, like the diurnal ones, have significantly larger MGNs than nocturnal species.

The IC and MGN are both involved in audition, yet our cathemeral species resemble nocturnal species with respect to the size of the IC, but diurnal ones with respect to the size of the MGN. This may reflect differing roles and connections within the auditory circuits. The IC receives and organizes information from multiple nuclei in the brainstem and relays information to the MGN, which in turn projects to the auditory cortex (Winer and Schreiner 2005). The IC is also a convergence point where motor, sensory, and cognitive information are integrated to carry out auditory functions such as perceiving and generating vocal signals,

localizing sounds via interaural time differences, and distinguishing between essential and irrelevant sounds (Gruters and Groh 2012). The MGN, in addition to providing information to the auditory cortex, sends signals to the amygdala (Hut et al. 1994; Winer and Schreiner 2005) which may influence learning and memory (Edeline 1990; McIntosh and Gonzalez-Lima 1995; 1998). The MGN also processes information about frequencies and intensities of sounds (Winer and Morest 1983), and acts as a selection filter before projecting to the cortex (Blundon and Zakhorenko 2013). These differences in the pathways and functions of the IC and MGN may be reflected in the differences in these structures that we see when we compare diurnal, cathemeral, and nocturnal rodents.

4.3 | Cathemerality and the Sensory Brain

Diurnal and nocturnal species are adapted to very specific temporal environments and likely invest in the senses that provide the best cost/benefit ratio. Our results, as well as earlier work, suggest that diurnal and nocturnal rodents have evolved to exploit the information available during their active periods (i.e., through visual cues during the day and olfactory cues during the night). However, the pattern of investment in different regions of the sensory brain of cathemeral species was not as we predicted. That is, where these regions differed in nocturnal and diurnal species, they were either distinctly nocturnal-like or diurnal-like in cathemeral species. This suggests a more complicated scenario than simply partitioning investment to accommodate activity in both day and night.

A potentially confounding factor in this analysis is the interspecific variation in activity pattern within temporal niche categories. The cathemeral species, in particular, do not exhibit identical patterns of activity or identical degrees of plasticity in those patterns. While they are

neither strictly diurnal nor nocturnal, the times at which they are active can vary between species and, in some cases, between populations of the same species. Small sample sizes, like that for our diurnal category, could exacerbate this issue, potentially masking patterns that would otherwise be apparent. In looking at interspecific variation for each brain region, cathemeral species appear to exhibit higher levels of interspecific variation than diurnal and nocturnal species in the size of the SC and OBs (Figures 2.3 and 2.5). A data set incorporating a greater number of species, with finer categories than nocturnal/ diurnal/ cathemeral could further illuminate the relationship between temporal niche and evolution of the sensory brain.

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APPENDIX

Table 2.1: Family, common name, genus and species, source, sample size (N) with numbers of males (m) and females (f) used, activity pattern, and references for activity pattern designation. MI = Michigan; NSW = New South Wales.

Family	Common Name	Genus & Species	Source	N (m, f)	Activity Pattern	References
Cricetidae	Eastern Meadow Vole	*Microtus pennsylvanicus*	Live-trapped, Middleville MI	3 (0,3)	cathemeral	Reich 1981
	Social Vole	*Microtus socialis*	Kronfeld-Schor Lab, Tel Aviv University	3 (2,1)	nocturnal	Shalmon et al. 1993
	Southern Red-backed Vole	*Myodes gapperi*	Live-trapped, Sugar Island MI	3 (2,1)	cathemeral	Merritt 1981
	Striped Desert Hamster	*Phodopus sungorus*	Nelson Lab, *Ohio State University	6 (3,3)	nocturnal	Wynne-Edwards et al. 1999
	Short-tailed Singing Mouse	*Scotinomys teguina*	Phelps Lab, University of Texas, Austin	6 (3,3)	diurnal	Hooper & Carleton 1975
	Southern Grasshopper Mouse	*Onychomys torridus*	Rowe Lab, **Michigan State University	3 (1,2)	nocturnal	O'Farrell 1974; Upham & Hafner 2013
	Hispid Cotton Rat	*Sigmodon hispidus*	Harlan Laboratories Inc.	2 (1,1)	cathemeral	Cameron & Spencer 1981

Table 2.1 (cont'd)

Family	Common Name	Genus & Species	Source	N (m, f)	Activity Pattern	References
Muridae	Northeast African Spiny Mouse	*Acomys cahirinus*	Kronfeld-Schor Lab, Tel Aviv University	5 (4,1)	nocturnal	Weber & Hohn 2005
	Golden Spiny Mouse	*Acomys russatus*	Kronfeld-Schor Lab, Tel Aviv University	4 (2,2)	diurnal	Shargal et al. 2000; Gutman & Dayan 2005; Levy et al. 2012
	Mongolian Jird	*Meriones unguiculatus*	Charles River Laboratories	6 (3,3)	cathemeral	Gulotta 1971
	House Mouse	*Mus musculus*	Live-trapped, Lansing MI	6 (3,3)	nocturnal	Robbers et al. 2015
	African Grass Rat	*Arvicanthis niloticus*	Smale Lab, Michigan State University	6 (3,3)	diurnal	Blanchong & Smale 2000
	Australian Bush Rat	*Rattus fusicpes*	Live-trapped, NSW Australia	5 (2,3)	nocturnal	Wood 1971; Meek et al. 2012
	Australian Swamp Rat	*Rattus lutreolus*	Live-trapped, NSW Australia	6 (3,3)	cathemeral	Taylor & Calaby 1988
	Brown Rat	*Rattus norvegicus*	Charles River Laboratories	5 (3,2)	nocturnal	Taylor 1978

*Currently at West Virginia University

**Currently at University of Oklahoma

Table 2.2: Phylogenetic signal estimates: Blomberg's κ and Pagel's λ for olfactory bulb mass (OB), and volumes of superior colliculus (SC), inferior colliculus (IC), lateral geniculate nucleus (LGN), and medial geniculate nucleus (MGN). Each measure is size-independent, i.e., based on the residuals from a linear regression.

Measure	κ	**p-value**	**λ**	**p-value**
SC	1.221	0.011	0.955	0.025
IC	0.765	0.221	<0.001	1
OB	0.377	0.865	<0.001	1
LGN	0.793	0.149	0.669	0.140
MGN	0.430	0.775	<0.001	1

Table 2.3: ANOVA results examining effects of temporal niche on relative sizes of the superior colliculus (SC), inferior colliculus (IC), olfactory bulb (OB), lateral geniculate nucleus (LGN), and medial geniculate nucleus (MGN) with pairwise comparisons between nocturnal and diurnal species, diurnal and cathemeral species, and nocturnal and cathemeral species. P < 0.05 *, P < 0.01 **, P < 0.001 ***.

	ANOVA results	**Pairwise comparisons**	**p-value**
SC		N vs D	0.789
	F: 0.670	D vs C	0.926
	p: 0.485	N vs C	0.926
IC		N vs D	<0.001***
	F: 8.28	D vs C	0.006**
	p: <0.001***	N vs C	0.867
OB		N vs D	0.041*
	F: 12.95	D vs C	0.116
	p: <0.001***	N vs C	<0.001***
LGN		N vs D	<0.001***
	F: 15.82	D vs C	<0.001***
	p: <0.001***	N vs C	0.281
MGN		N vs D	0.002**
	F: 15.44	D vs C	0.409
	p: <0.001***	N vs C	<0.001***

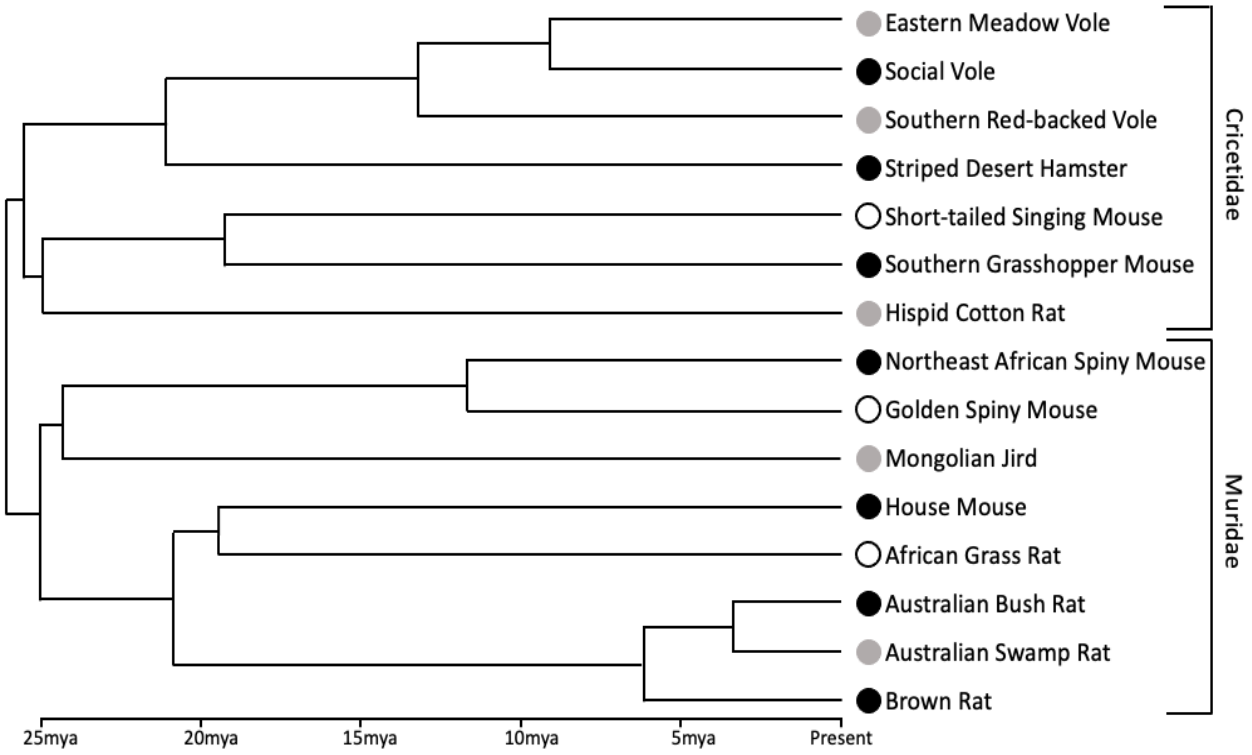

Figure 2.1: Phylogeny and temporal niche of the 15 rodent species examined in this study. Black = nocturnal, White = diurnal, Gray = cathemeral. Phylogenetic relationships and divergence times were established from Fabre et al. (2012). Mya = million years ago.

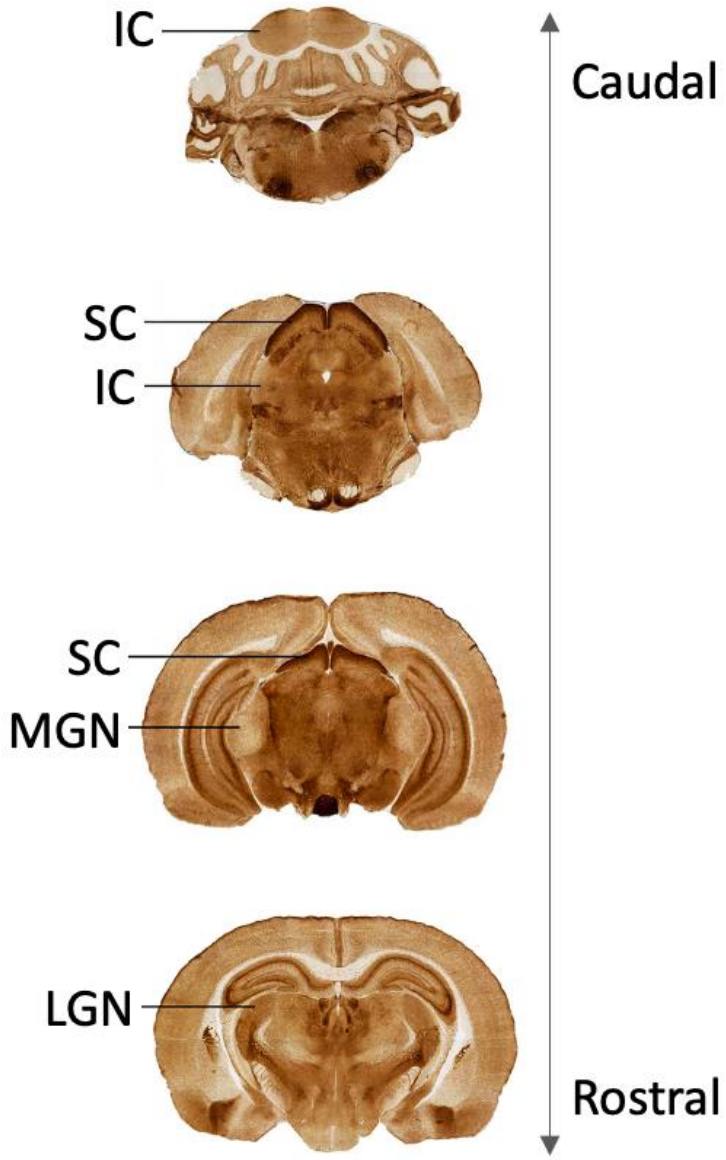

Figure 2.2: Photomicrographs of an AChE-stained African Grass Rat brain, showing the visual and auditory brain regions measured in this study: lateral geniculate nucleus (LGN), superior colliculus (SC), medial geniculate nucleus (MGN), and inferior colliculus (IC).

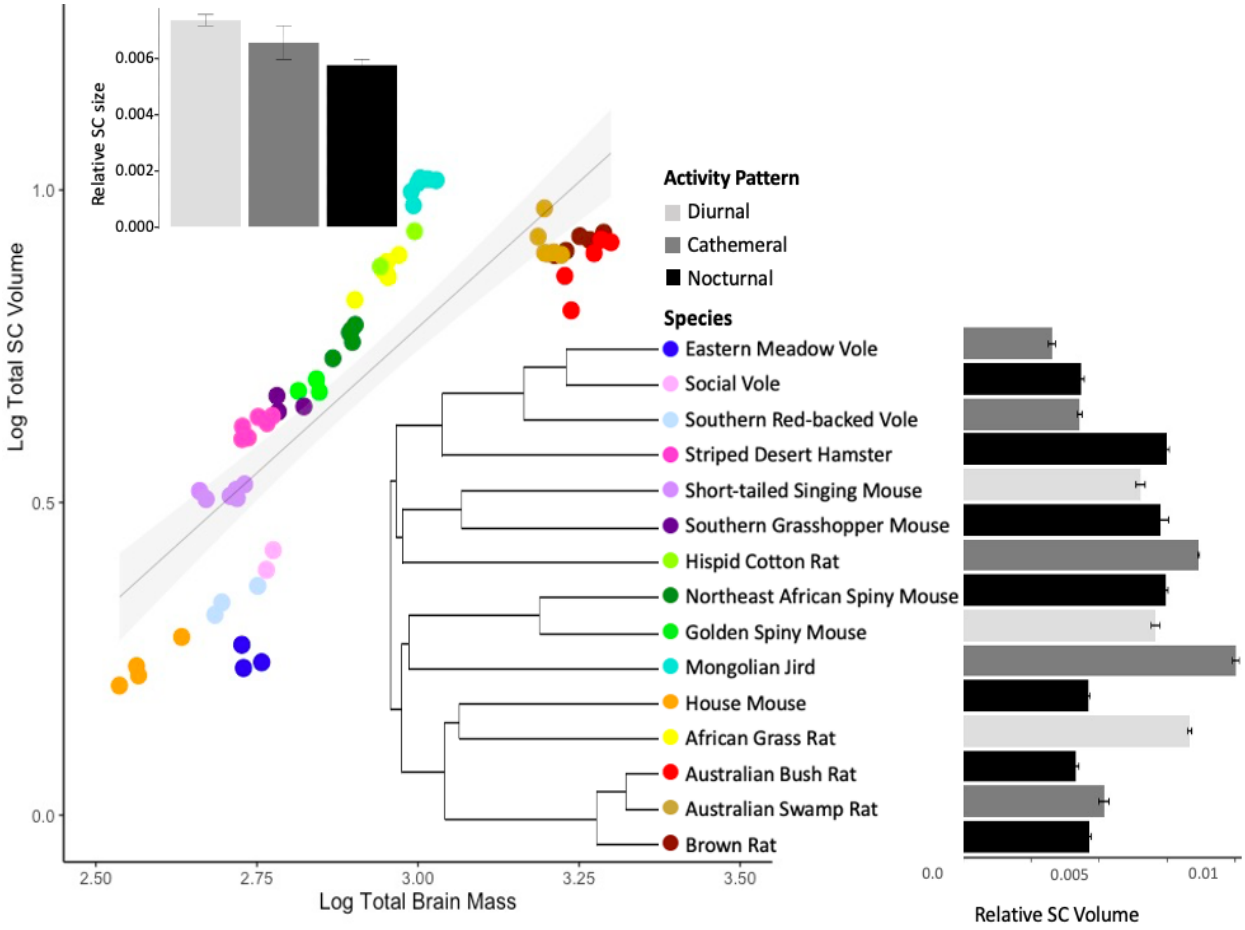

Figure 2.3: Plot of log superior colliculus (SC) volume regressed against log brain mass for 15 rodent species. Shading represents 95% confidence intervals. Bar plots are mean SC volume relative to brain mass; error bars are SEM.

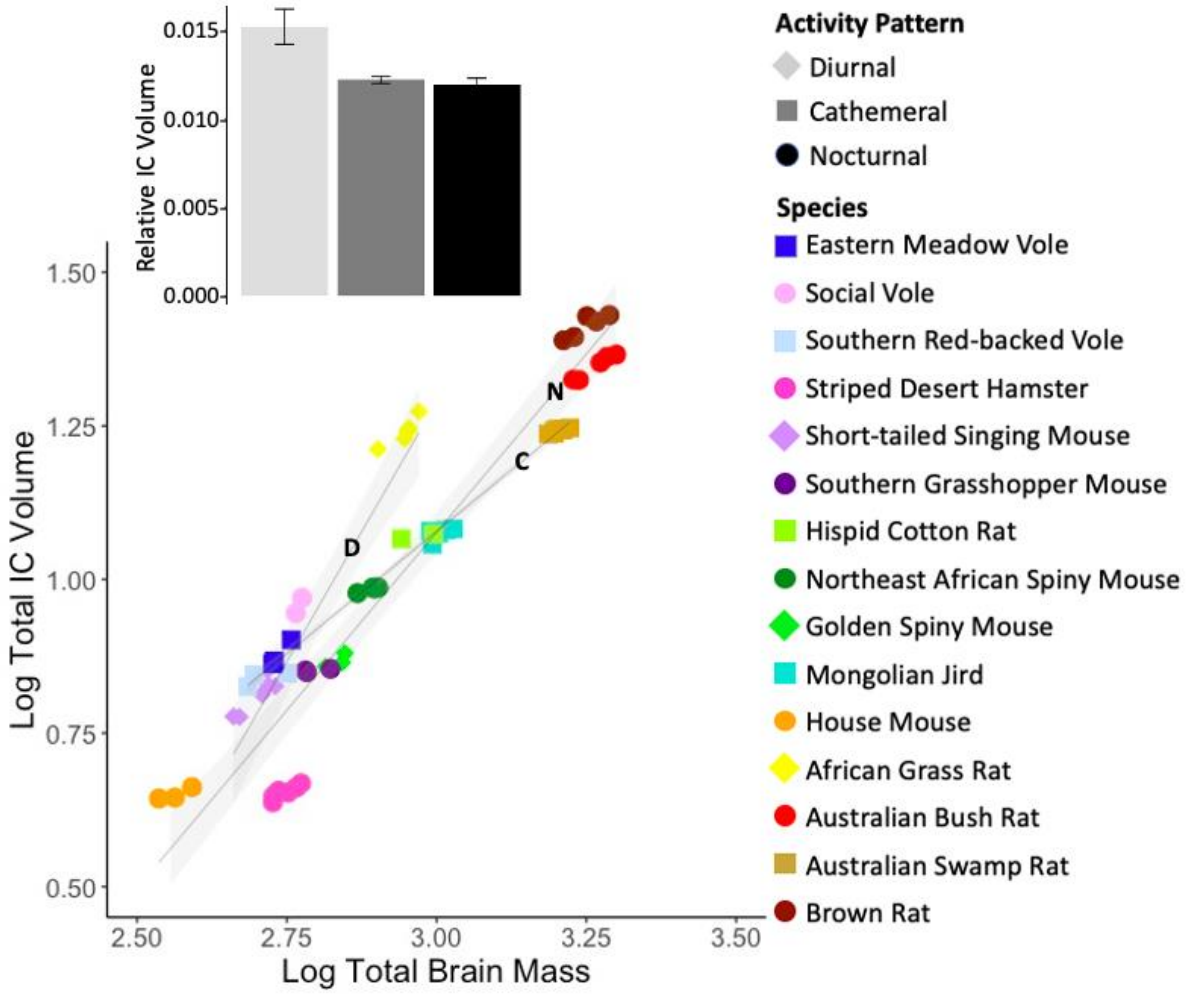

Figure 2.4: Plot of log inferior colliculus (IC) volume regressed against log brain mass for 15 rodent species. Shading represents 95% confidence intervals. Regressions lines labeled by activity pattern (D=diurnal, C=cathemeral, N=nocturnal). Bar plot is mean IC volume relative to brain mass; error bars are SEM.

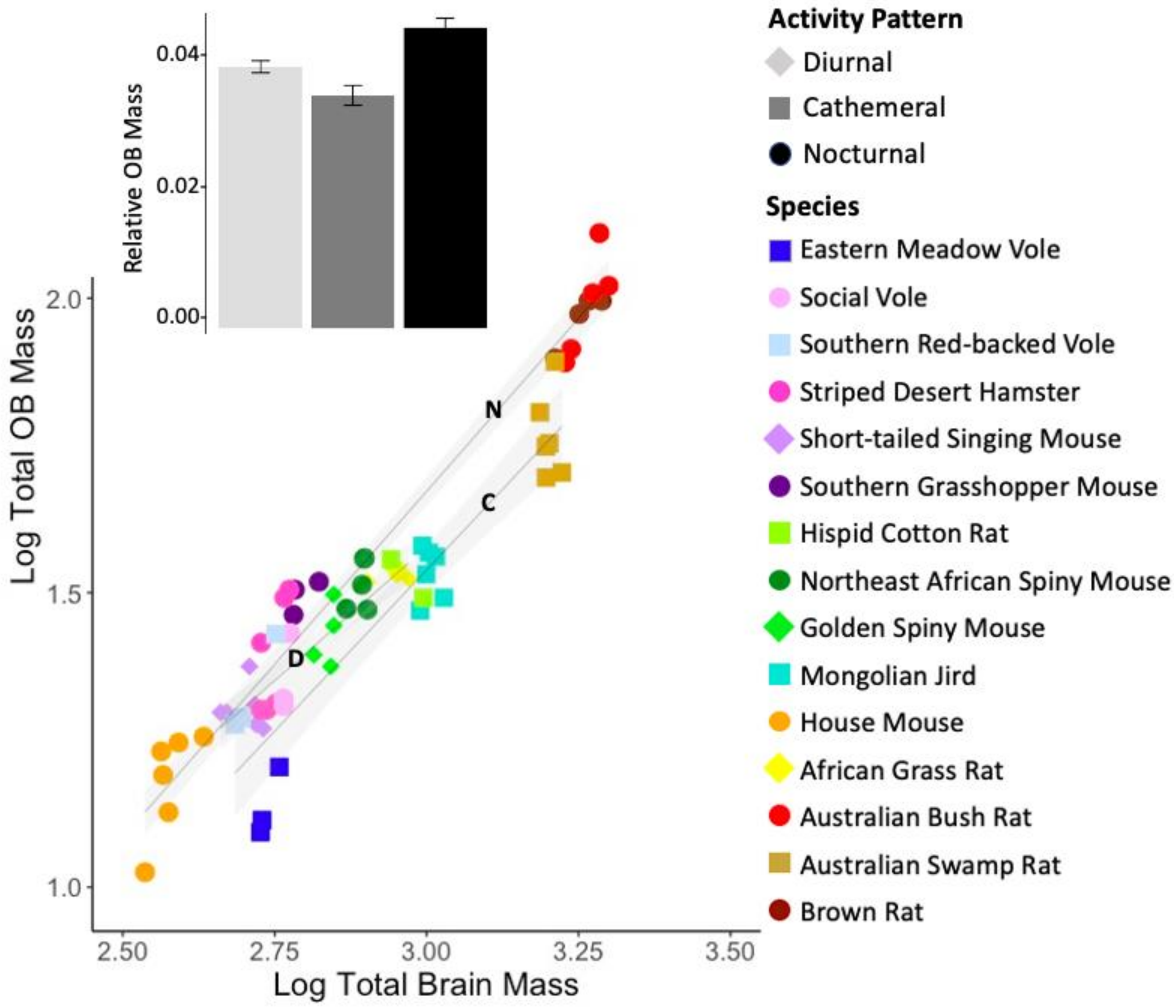

Figure 2.5: Plot of log olfactory bulb (OB) mass regressed against log brain mass for 15 rodent species. Shading represents 95% confidence intervals. Regressions lines labeled by activity pattern (D=diurnal, C=cathemeral, N=nocturnal). Bar plot is mean OB mass relative to brain mass; error bars are SEM.

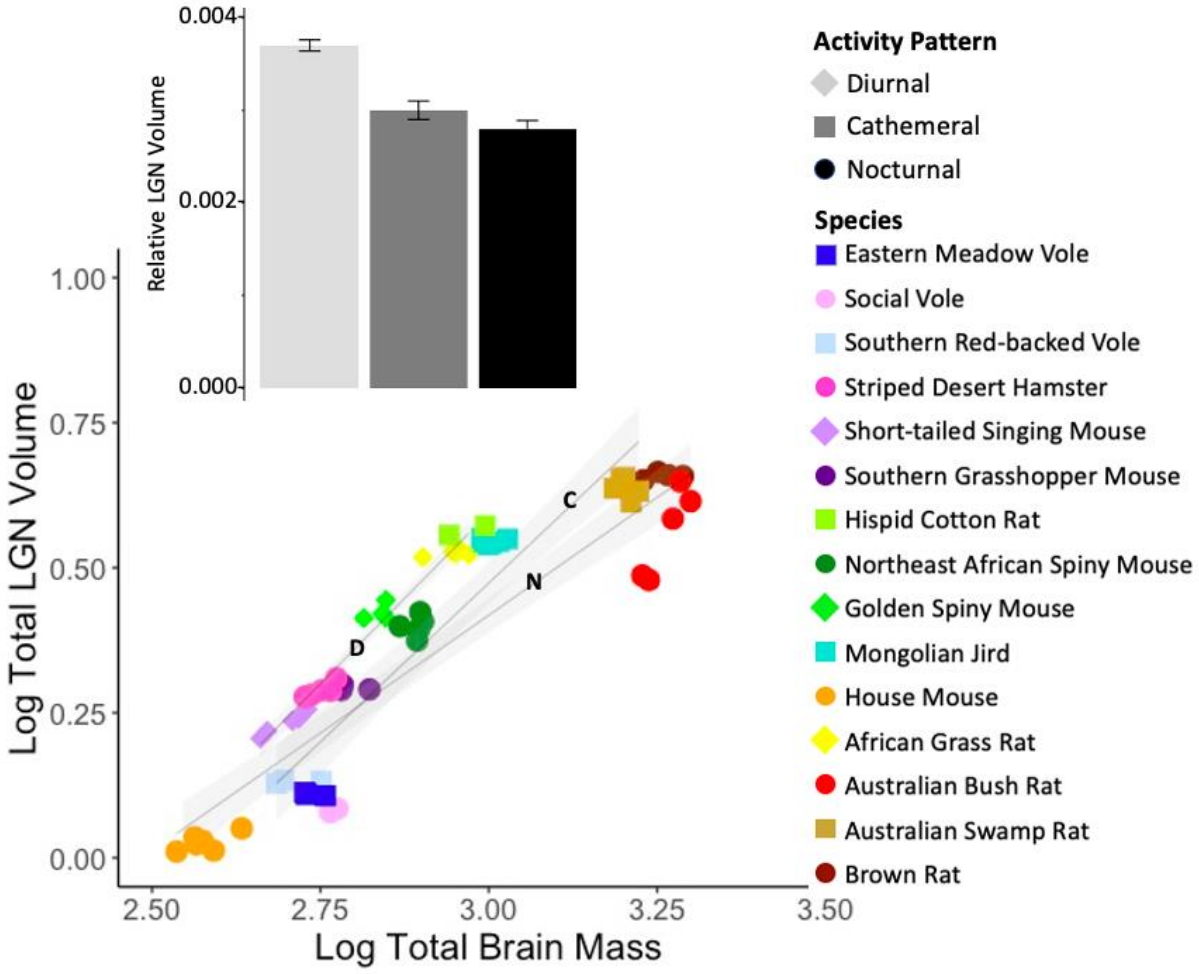

Figure 2.6: Plot of log lateral geniculate nucleus (LGN) volume regressed against log brain mass for 15 rodent species. Shading represents 95% confidence intervals. Regressions lines labeled by activity pattern (D=diurnal, C=cathemeral, N=nocturnal). Bar plot is mean LGN volume relative to brain mass; error bars are SEM.

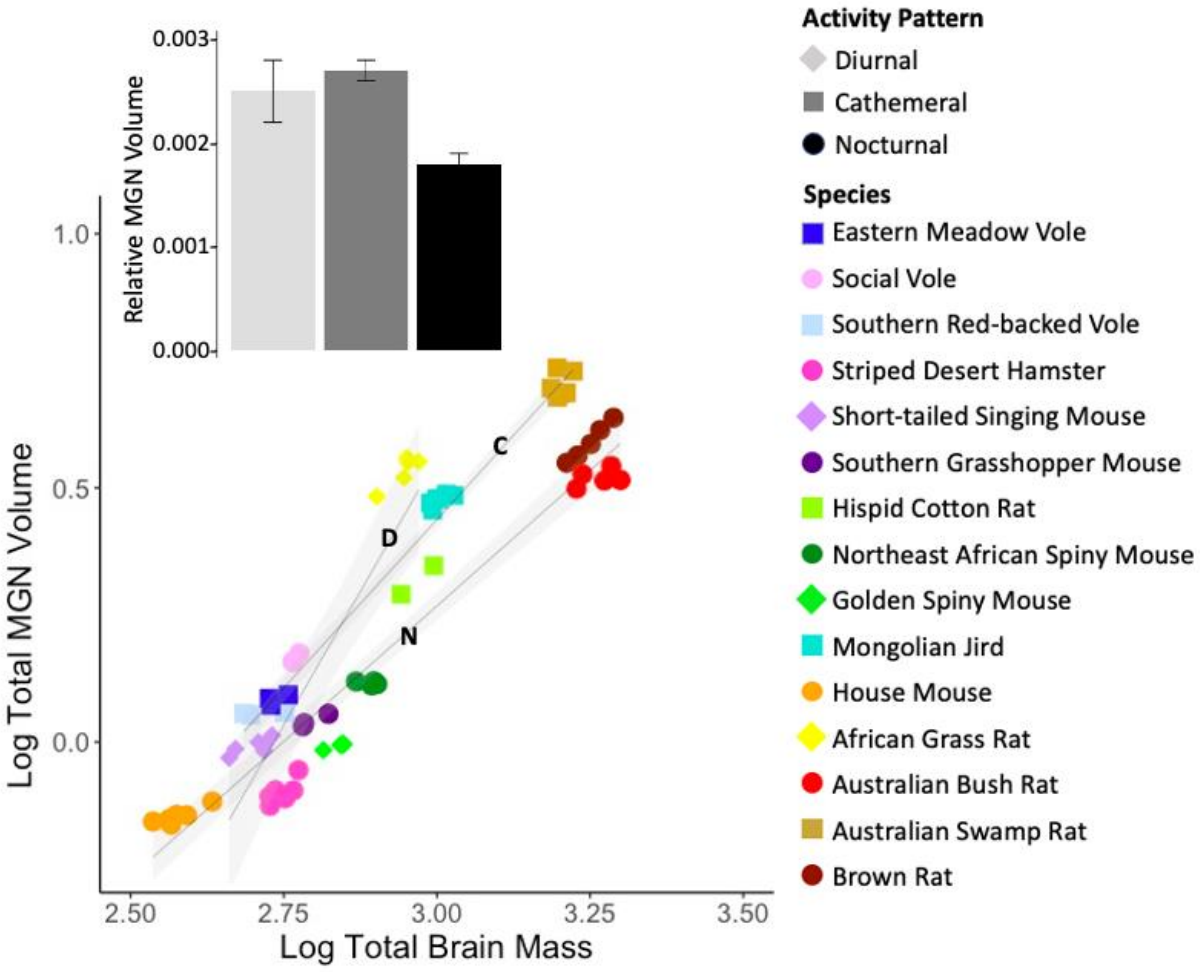

Figure 2.7: Plot of log medial geniculate nucleus (MGN) volume regressed against log brain mass for 15 rodent species. Shading represents 95% confidence intervals. Regressions lines labeled by activity pattern (D=diurnal, C=cathemeral, N=nocturnal). Bar plot is mean MGN volume relative to brain mass; error bars are SEM.

CHAPTER 3: COMPARATIVE ANALYSIS OF THE SUBDIVISIONS OF THE LATERAL GENICULATE NUCLEUS IN DIURNAL, CATHEMERAL, AND NOCTURNAL RODENTS

1 | INTRODUCTION

The lateral geniculate nucleus (LGN) is a brain structure in the thalamus of vertebrates that receives input from the retina, has connections with many parts of the brain, and carries out several complex functions related to processing of photic information and to modulation of daily rhythms (Weyand 2016; Brock et al 2022).

Given its role in visual processing, one might expect LGN size to be correlated with daily activity patterns, i.e., better developed in diurnal (day-active) than in nocturnal (night-active) species. In a study that included representatives from 8 orders of extant mammals, Finlay et al. (2014) found that LGN size scales strongly with brain size and is influenced by phylogeny. However, they did not find a significant relationship between LGN size (relative to brain size) and activity pattern. Their sample, however, was sparse for orders other than Primates, and included just 5 rodent species, only one of which was diurnal.

In a subsequent study focused on rodents, Morrow et al. (in review) examined 13 species, representing 3 families, and found no significant influence of phylogeny on LGN size. Moreover, they and others (Shuboni-Mulligan 2019) reported a significant relationship between LGN size (relative to total brain size) and activity pattern in this group, with the LGN larger in diurnal than nocturnal species. The relationship between LGN size and access to visual cues in rodents is also evident from studies of species that are rarely above ground, such as subterranean European water voles (*Arvicola amphibius*), blind mole-rats (*Spalax ehrenbergi*),

and African mole-rats (Bathyergidae), where the LGN is poorly developed (Compoint-Monmignaut 1983; Cooper et al. 1993; Nemec et al. 2008).

In this study, we look more closely at the relationship between temporal niche and LGN size in rodents by assessing the relative influence of different LGN subregions on this relationship. The LGN is composed of three subregions: the dorsal LGN (dLGN), ventral LGN (vLGN), and intergeniculate leaflet (IGL). While the overall size of the LGN, relative to brain size, is known to be larger in diurnal than nocturnal rodents (Shuboni-Mulligan et al. 2019; Morrow et al. in review), the relative contributions of the three subdivisions, which all differ functionally, has not been examined.

The dLGN is the main thalamic relay between the retina and primary visual cortex and sends and receives projections from the superior colliculus, a visual structure in the midbrain (Murphy et al. 2000). This subdivision of the LGN has mainly visuosensory functions and participates in processes related to spatial cognition, color discrimination, location, distance, and movement (Mohr et al., 2011; Glickfeld et al. 2014, Hok et al., preprint).

The ventral LGN (vLGN) has connections with multiple subcortical visual areas but does not project to the visual cortex (Conley et al., 1993). This subdivision has both visuosensory and visuomotor functions. It integrates visual and ocular motor systems (Livingston and Fedder 2003), which contributes to brightness discrimination and modulates saccade eye movement and pupillary reflexes (Conley et al., 1993). The vLGN also plays a crucial role in integrating photic and nonphotic cues that contribute to entrainment of circadian rhythms (Harrington 1997; Brock et al 2022).

Located between the dLGN and vLGN is the intergeniculate leaflet (IGL). Though it is distinct from the vLGN in some respects, the IGL shares many neurochemicals and physiological properties with the vLGN (Brock et al. 2022) and it plays a clear role in modulation of daily activity rhythms (Redlin et al. 1999; Lewandowski and Usarek 2002; Gall et al. 2013). The IGL receives direct retinal input and projects to the suprachiasmatic nuclei, which is the primary circadian pacemaker in mammals (Edelstein and Amir 1999). Due to the similar developmental origins, patterns of connectivity, and close anatomic proximity, many functional studies group the IGL and vLGN together (Brock et al. 2022), as we do in this study.

While the subregions of the LGN of mammals have been well examined cytoarchitecturally, few studies have quantified these regions, and little is known about which behavioral or ecological factors influence the relative proportions of these subregions. Brauer et al. (1982) compared the volumes of the dLGN and vLGN in 16 mammalian species and found that species with a larger neocortex had larger dLGNs in relation to vLGNs. Najdzion et al. (2009) examined the dLGN and vLGN of four distantly related mammalian species: a common shrew, bank vole, rabbit, and a fox. They found that the relative sizes of these subregions were very similar in the common shrew and bank vole, with the dLGN slightly larger than the vLGN. This contrasts with the rabbit and fox, both of which had a much larger dLGN than vLGN. The fox, in fact, had a dLGN approximately 20 times larger than the vLGN. Based on these results, and those of Brauer et al. (1982), Najdzion et al. (2009) suggests that mammals with high levels of neocorticalization, binocular vision, and/or carnivorous diets have larger dLGNs relative to vLGNs. However, very little is known about the extent to which the different LGN subregions vary with temporal niche.

Here we use a phylogenetic framework to assess the influence of temporal niche on the dLGN and vLGN in 18 rodent species. We sampled five day-active (i.e., diurnal), five day- and night-active (i.e., cathemeral), and eight night-active (i.e., nocturnal) rodent species. As there is some evidence that the visual cortex, a major target of the dLGN, is expanded in diurnal rodents relative to nocturnal ones (Campi and Krubitzer 2010; Campi et al. 2011), we expect a significantly larger dLGN in diurnal species. It is more difficult to make predictions for the vLGN as some functions are related directly to daily activity patterns and others are not. The responses of internally driven rhythms (circadian rhythms) to light are very similar in nocturnal and diurnal species and the vLGN plays a role in the mediation of these responses (Smale et al. 2008). Light also has direct effects on activity in both diurnal and nocturnal species and these appear to be mediated by circuits that include the vLGN. Though these direct effects of the light are not the same, (i.e., light increases activity in diurnal species and suppresses activity in nocturnal species) (Shuboni et al. 2012) they may involve the same circuits. For these reasons, we do not expect the size of the vLGN to differ significantly between diurnal and nocturnal species. It is also difficult to make predictions for cathemeral species, as little is known about their visual brain or their behavioral responses to light. However, since activity pattern appears to mask underlying circadian processes in cathemeral species (Curtis and Rasmussen 2006), we do not expect the size of their vLGN to differ from those of diurnal and nocturnal species.

2 | MATERIALS AND METHODS

2.1 | Specimens

Measurements were taken from 18 rodent species, representing three extant families: Sciuridae, Cricetidae and Muridae (Table 3.1, Figure 3.1). Our sample includes five diurnal species [North American Red Squirrel (*Tamiasciurus hudsonicus*), Eastern Chipmunk (*Tamias striatus*), Short-tailed Singing Mouse (*Scotinomys teguina*), Golden Spiny Mouse (*Acomys russatus*), and African Grass Rat (*Arvicanthis niloticus*)], five cathemeral species [Eastern Meadow Vole (*Microtus pennsylvanicus*), Southern Red-Backed Vole (*Myodes gapperi*), Hispid Cotton Rat (*Sigmodon hispidus*), Mongolian Jird (*Meriones unguiculatus*), and Australian Swamp Rat (*Rattus lutreolus*)], and eight nocturnal species [Southern Flying Squirrel (*Glaucomys volans*), Social Vole (*Microtus socialis*), Striped Desert Hamster (*Phodopus sungorus*), Southern Grasshopper Mouse (*Onychomys torridus*), Northeast African Spiny Mouse (*Acomys cahirinus*), House Mouse (*Mus musculus*), Australian Bush Rat (*Rattus fuscipes*), and Brown Rat (*Rattus norvegicus*)].

Southern Flying Squirrels, North American Red Squirrels, Eastern Chipmunks, Eastern Meadow Voles, Southern Red-backed Voles, and House Mice were live-trapped in Michigan between October 2015 and December 2017 (Table 3.1). Australian Bush Rats and Australian Swamp Rats were live-trapped in New South Wales, Australia, in August 2015. Mongolian Jirds and Brown Rats were purchased from Charles River Laboratories. Hispid Cotton Rats were purchased from Harlan Laboratories. Southern Grasshopper Mice and African Grass Rats were obtained from Michigan State University laboratory colonies. Striped Desert Hamsters were obtained from a laboratory colony at Ohio State University. Intact whole brains from Social

Voles, Northeast African Spiny Mice, and Golden Spiny Mice were obtained from Tel Aviv University and those from Short-tailed Singing Mice were obtained from a University of Texas at Austin laboratory.

We included from 2 to 6 individuals per species (Table 3.1). Only adult animals were sampled, and we attempted to sample both males and females of each species. However, the Southern Flying Squirrels, North American Red Squirrels, and Eastern Meadow Voles were all female in this study. All animals were handled according to protocols approved by the following institutional and regional authorities: American Society of Mammalogists (Sikes et al. 2016), Michigan State University Institutional Animal Care and Use Committee (protocol # 07/16-116-00), Office of Environment and Heritage of New South Wales (NSW), Australia (License #SL100634), and NSW Department of Industry and Investment Animal Research Authority (ORA 14/17/009).

2.2 | Data Collection and Histology

Measurements included in this study include total brain mass, and volumes of the total LGN, dLGN, and vLGN (including IGL). Due to obscure boundaries between the vLGN and IGL, and their similar functions in circadian rhythms, we treated the vLGN and IGL as a single structure, which will be referred to as the vLGN from here on.

Only fresh, unfixed tissue was used in this study. All individuals were euthanized via an intraperitoneal injection of sodium pentobarbital. After death, the individual was weighed to the nearest gram and the brain removed and placed in powdered dry ice. After 2-5 minutes in dry ice, the brain was moved to a -80° freezer where it was stored until further processing. After removal from the freezer, the brain was cut just caudal to the medulla

oblongata and weighed to the nearest milligram. The thalamus was coronally sectioned at 40µm thickness on a cryostat. Due to the very small size of the House Mice brains, they were sectioned at 20µm thickness. Three alternate series of brain tissue sections were mounted directly onto slides and one was stained for acetylcholinesterase using the following protocol: slides were incubated for 5 hours in a solution of 0.0072% ethopropazine HCl, 0.075% glycine, 0.05% cupric sulfate, 0.12% acetylthiocholine iodide, and 0.68% sodium acetate (pH 5.0); rinsed 2 times (3 minutes each) with distilled H_2O; and developed in a 0.77% sodium sulfide solution (pH 7.8) for 45 minutes. Slides were then rinsed with 2 changes of distilled H_2O (3 minutes each) and run through a series of ascending ethanol concentrations (70%, 95%, 100%, and 100%) for 1 minute each (to dehydrate the adhering tissue), cleared through 2 changes of xylenes for 5 minutes each, and coverslipped using DPX mounting medium. The two unstained series of slides were set aside for future work.

2.3 | Measurements

The volumes of the LGN, dLGN, and vLGN were measured by taking photomicrographs of acetylcholinesterase-stained (AChE) sections using a digital camera (MBF Bioscience CX9000) attached to a Zeiss light microscope (Carl Zeiss, Gottengen, Germany, 5x objective), using the 2D slide scanning module on Stereo Investigator 2017 (MBF Bioscience). Volumetric measurements were calculated using the Cavalieri method (100 x 100 um grid, every third section) in Stereo Investigator 2017 (MBF Bioscience). Boundaries of the LGN, dLGN, and vLGN (including IGL) were determined according to the rat brain atlas (Paxinos and Watson 2014) (Figure 3.2). For each structure, one side was measured, and that value was doubled to obtain total volume.

2.4 | Data Analysis

Variables. Continuous variables used in the analyses include brain mass and volumes of the LGN, dLGN, and vLGN. Each species was assigned to one of three categorical states: diurnal, cathemeral, or nocturnal based on descriptions of daily activity patterns from field studies reported in the literature (Table 3.1).

Phylogenetic signal estimations. Phylogenetic signal of the LGN, dLGN, vLGN, and vLGN/dLGN ratio was estimated using Blomberg's *K* (based on 1000 randomizations for p-value) and Pagel's λ (based on likelihood ratio tests) in the PHYTOOLS 0.7-70 package in R Studio (Revell 2012). To calculate size-independent estimations of the LGN, dLGN, and vLGN, we used the residuals from linear regressions of each region regressed on overall brain mass. All continuous variables were log-transformed prior to phylogenetic signal estimations.

ANOVAs. ANOVAs and Tukey's HSD post-hoc tests were performed, as a function of activity pattern, using arcsine-transformed relative sizes (region of interest divided by overall brain size) of the LGN, dLGN, and vLGN. We also carried out non-phylogenetic and phylogenetic ANOVAs followed by Tukey's HSD post-hoc tests on the arcsine transformed vLGN/dLGN ratio as a function of activity pattern. All analyses were carried out in R Studio (RStudio 2020) with the phylogenetic ANOVA carried out using the PHYTOOLS package 0.7-70 (Revell 2012).

3 | RESULTS

3.1 | Phylogenetic Signal

Blomberg's *K* and Pagel's λ estimates detected no significant phylogenetic signal in the size of the LGN, dLGN, and vLGN, relative to brain size (Table 3.2). Blomberg's *K* was significant for the vLGN/dLGN ratio, indicating a modest phylogenetic signal, while Pagel's λ was not significant for that ratio. The difference in these estimates may reflect our small sample size. Blomberg's *K* is usually more reliable than Pagel's λ when working with small sample sizes (Munkemuller et al. 2012).

3.2 | Volumetric Analyses

Total LGN volume, relative to brain mass, ranged from 0.20% in the Australian Bush Rat to 0.41% in the Eastern Chipmunk (Table 3.3). As expected, activity pattern is a significant predictor of total LGN volume ($F=18.47$, $p<0.001$), with diurnal species possessing a larger LGN (relative to brain size) than cathemeral and nocturnal species (Table 3.4; Figure 3.3a).

The dLGN volume ranged from 0.12% of total brain mass in the Social Vole to 0.32% in the Eastern Chipmunk (Table 3.3). As brain size increases, the dLGN increases linearly, accounting for most of the variation in dLGN size ($R^2 = 0.85$) (Figure 3.4a). However, the two male Australian Bush Rats are far below the goodness-of-fit line and have a surprisingly smaller dLGN than their female counterparts. The relative volume of the dLGN is also significantly influenced by activity pattern ($F=11.88$, $p<0.001$), with diurnal species possessing a larger dLGN than cathemeral and nocturnal species (Table 3.4; Figure 3.3b).

The volume of the vLGN, relative to brain mass, ranged from 0.07% in the Australian Bush Rat to 0.15% in the African Grass Rat. The vLGN increases linearly with an increase in brain

mass, similar to the dLGN, but with a slightly flatter slope (R^2 = 0.86) (Figure 3.4b). The North American Red Squirrel and African Grass Rat, both diurnal species, are notably above the regression line. Activity pattern is a significant predictor of the relative size of the vLGN ($F=14.27$, $p<0.001$) with the same pattern as is seen in the overall LGN and dLGN; diurnal species have a larger vLGN compared to cathemeral and nocturnal species (Table 3.4; Figure 3.3c).

3.3 | vLGN/dLGN Ratio

In contrast to the findings of the LGN, dLGN, and vLGN, the ratio of the vLGN to dLGN did have a significant phylogenetic signal (Table 2) and was not significantly influenced by activity pattern (Table 4; Figure 5). The results of both the non-phylogenetic ANOVA ($F=2.13$, $p=0.126$) and phylogenetic ANOVA ($F=0.14$, $p=0.840$) showed no difference between diurnal, cathemeral, and nocturnal species. However, the vLGN/dLGN ratio varied markedly among species ($F=27.64$, $p<0.001$) (Figure 3.6), ranging from 0.300 in the Eastern Chipmunk to 0.831 in the House Mouse (Table 3.3). It appears that the proportion of the vLGN in relation to the dLGN is the only metric that is phylogenetically constrained and not influenced by activity pattern.

4 | DISCUSSION

4.1 | Phylogenetics of the LGN

Our results suggest (based on Blomberg's κ and Pagel's λ estimations) that phylogeny does not significantly influence the volumes of the LGN, dLGN, or vLGN, relative to brain size (Table 3.2). In contrast, Finlay et al. (2014) estimated Pagel's λ for size of the LGN in 82 mammalian species and found a strong phylogenetic component. This may reflect the broader taxonomic sampling and larger sample size in the Finlay et al. (2014) study. Differences between species that are more distantly related may be more pronounced and hence easier to identify. Finlay et al. (2014) also carried out a more refined analysis by comparing the numbers of magnocellular and parvocellular neurons in the LGNs of 25 primate species. Magnocellular neurons process information about motion and luminance contrast, whereas the parvocellular neurons convey information about color (Hendry and Calkins 1998). They found that the number of parvocellular neurons, regressed against brain size, had a significant phylogenetic component, while the number of magnocellular neurons, regressed against brain size, was not significantly related to phylogeny. They further determined that the number of parvocellular neurons regressed against magnocellular neurons in the LGN showed a strong, significant, phylogenetic signal. These results, in conjunction with our finding of no phylogenetic signal for LGN but a significant phylogenetic signal for the ratio of vLGN to dLGN, suggests that some components of the rodent LGN, such as certain types of neurons, or certain subregions, may be strongly influenced by phylogeny, while others are less constrained.

4.2 | Activity Pattern and the LGN

As predicted, diurnal species have larger total LGN and dLGN, relative to brain size, than cathemeral and nocturnal species. Diurnal species consistently have ample photic cues available during their active periods and would presumably benefit (more than nocturnal species) from investing in visual systems. The finding of a larger LGN in diurnal than nocturnal species is consistent with many other studies showing that diurnal teleosts (Iglesias et al 2018), rodents (Campi and Krubitzer 2010; Campi et al. 2011; Shuboni-Mulligan et al. 2019; Morrow et al. in review), and primates (Barton 2007) have larger visual regions of the brain than nocturnal relatives. Our data also suggest that the LGN of diurnal rodents is significantly larger than that of cathemeral ones, whose LGN is similar to that of nocturnal species. This might seem surprising, as cathemeral species are also active during daylight hours. However, it may be related to the fact that the eyes of nocturnal species are adapted to optimize visual sensitivity, whereas those of diurnal species are better adapted for visual acuity and often for discrimination of different wavelengths. It is possible that eyes of rodents can't be optimized for both. Cathemeral species may thus have eyes better adapted for night vision and consequently devote less brain tissue to processing of visual information than do diurnal species.

As the dLGN is devoted almost entirely to processing visual information, we predicted that it would be relatively large in the diurnal species, contributing to the overall increase in volume of the LGN. This does appear to be the case as the patterns in size of the LGN and dLGN in diurnal, cathemeral, and nocturnal species are nearly identical (Figure 3.3). These results,

when taken together with the absence of phylogenetic signal in this subregion, suggest that the dLGN is responsive to selection in species that rely heavily on vision (i.e., diurnal species).

The vLGN results were somewhat surprising. One of the major functions of the vLGN (and IGL, which was included with it in our measurement) is to modulate circadian rhythms (Harrington 1997; Brock et al. 2022). While diurnal, cathemeral, and nocturnal species do react differently to light, we did not expect to see those differences reflected in the overall size of the vLGN. Yet, diurnal species exhibited a significantly larger vLGN than cathemeral and nocturnal species. It may be that functions of the vLGN not related to circadian rhythms are responsible for this finding. While the vLGN, in contrast to the dLGN, does not share direct connections with the primary visual cortex, it does contribute to the processing of visual information, including both visuosensory and visuomotor functions (Conley et al. 1993; Livingston and Fedder 2003). Possibly the groups of neurons that carry out these functions have increased in species with well-developed visual systems. Quantification of the vLGN and IGL separately might shed light on this issue. The IGL is an important modulator of the suprachiasmatic nucleus, which is responsible for generating rhythms (Moore and Card 1994). Separate analyses of the vLGN and IGL could help determine whether the volumetric differences between diurnal versus cathemeral and nocturnal species is related to the visual functions of the vLGN, or circadian modulation by the vLGN and IGL. Unfortunately, the boundary between the IGL and vLGN is obscured in AChE staining, so we were not able to measure these regions separately.

Another possible explanation for the relatively large size of the vLGN in diurnal species is that substructures of the LGN change in a concerted manner (i.e., a change in the dLGN is accompanied by similar changes in the vLGN). Patterns of concerted evolutionary changes have

been documented in several regions of the brain (Chalfin et al. 2007; Yopak et al. 2010; Finlay et al. 2011; Finlay et al. 2014; Moore and DeVoogd 2017). However, the fact that we see considerable, statistically significant interspecific variation in the ratio of vLGN to dLGN in this study (Figure 3.6), as did Najdzion et al. (2009) and Brauer et al. (1982), indicates that the vLGN and dLGN have not always changed in concert with one another.

4.3 | Interspecific Differences in vLGN/dLGN Ratio

The vLGN to dLGN ratio did not differ significantly with activity pattern, in contrast to the findings for those regions individually. However, there was a moderate phylogenetic signal and significant interspecific variation in this ratio. It is not clear why different species have different proportions of vLGN and dLGN. Brauer et al. (1982) found that mammals with high levels of neocorticalization have a larger dLGN relative to vLGN, which makes sense given the connections between the dLGN and visual cortex. Additional studies suggest that primates and carnivores have particularly large dLGN to vLGN ratios (Niimi et al. 1963; Madarasz et al. 1978; Babb 1980; Brauer et al. 1982; Najdzion et al. 2009), possibly because they are highly visual mammals with binocular vision and high levels of neocorticalization.

The diurnal rodents in this study invested more in each subregion of the LGN than did their nocturnal relatives, but there was not a significant difference between diurnal and nocturnal species in the vLGN to dLGN ratio. In contrast to our other measures, this ratio is influenced by phylogeny; it is also surprisingly variable in our sample of rodents. Brauer et al. (1982), based on a much broader sampling of mammal species, reported a positive relationship between neocorticalization and dLGN/vLGN. If the level of neocorticalization is correlated to the vLGN/dLGN ratio, this could explain the phylogenetic signal observed, as neocorticalization

varies among taxonomic groups (Finlay et al. 2001). Najdzion et al. (2009) suggested that binocular vision and a carnivorous diet might influence this metric, but the number and diversity of species examined is not yet sufficient to rigorously evaluate these hypotheses. Further studies of possible factors (e.g., developmental timing, habitat, locomotion, diet), with better sampling within and between taxonomic groups, are needed to better understand variation in the vLGN to dLGN ratio.

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APPENDIX

Table 3.1: Family, common name, genus and species, source, sample size (N) with numbers of males (m) and females (f) used, activity pattern, and references for activity pattern designation. MI = Michigan; NSW = New South Wales.

Family	**Common Name**	**Genus & Species**	**Source**	**N (m, f)**	**Activity Pattern**	**References**
Sciuridae	Southern Flying Squirrel	*Glaucomys volans*	Live-trapped, East Lansing	3 (0,3)	nocturnal	Aschoff 1966; Muul 1968
	North American Red Squirrel	*Tamiasciurus hudsonicus*	Live-trapped, East Lansing	2 (0,2)	diurnal	Pauls 1978
	Eastern Chipmunk	*Tamias striatus*	Live-trapped, East Lansing	3 (2,1)	diurnal	Elliot 1978
Cricetidae	Eastern Meadow Vole	*Microtus pennsylvanicus*	Live-trapped, Middleville MI	3 (0,3)	cathemeral	Reich 1981
	Social Vole	*Microtus socialis*	Kronfeld-Schor Lab, Tel Aviv University	3 (2,1)	nocturnal	Shalmon et al. 1993
	Southern Red-backed Vole	*Myodes gapperi*	Live-trapped, Sugar Island MI	3 (2,1)	cathemeral	Merritt 1981
	Striped Desert Hamster	*Phodopus sungorus*	Nelson Lab, *Ohio State University	6 (3,3)	nocturnal	Wynne-Edwards et al. 1999
	Short-tailed Singing Mouse	*Scotinomys teguina*	Phelps Lab, University of Texas, Austin	6 (3,3)	diurnal	Hooper & Carleton 1975
	Southern Grasshopper Mouse	*Onychomys torridus*	Rowe Lab, **Michigan State University	3 (1,2)	nocturnal	O'Farrell 1974; Upham & Hafner 2013
	Hispid Cotton Rat	*Sigmodon hispidus*	Harlan Laboratories Inc.	2 (1,1)	cathemeral	Cameron & Spencer 1981

Table 3.1 (cont'd)

Family	Common Name	Genus & Species	Source	N (m, f)	Activity Pattern	References
Muridae	Northeast African Spiny Mouse	*Acomys cahirinus*	Kronfeld-Schor Lab, Tel Aviv University	5 (4,1)	nocturnal	Weber & Hohn 2005
	Golden Spiny Mouse	*Acomys russatus*	Kronfeld-Schor Lab, Tel Aviv University	4 (2,2)	diurnal	Shargal et al. 2000; Gutman & Dayan 2005; Levy et al. 2012
	Mongolian Jird	*Meriones unguiculatus*	Charles River Laboratories	4 (2,2)	cathemeral	Gulotta 1971
	House Mouse	*Mus musculus*	Live-trapped, Lansing MI	6 (3,3)	nocturnal	Robbers et al. 2015
	African Grass Rat	*Arvicanthis niloticus*	Smale Lab, Michigan State University	6 (3,3)	diurnal	Blanchong & Smale 2000
	Australian Bush Rat	*Rattus fuscipes*	Live-trapped, NSW Australia	5 (2,3)	nocturnal	Wood 1971; Meek et al. 2012
	Australian Swamp Rat	*Rattus lutreolus*	Live-trapped, NSW Australia	4 (1,3)	cathemeral	Taylor & Calaby 1988
	Brown Rat	*Rattus norvegicus*	Charles River Laboratories	5 (3,2)	nocturnal	Taylor 1978

*Currently at West Virginia University

**Currently at University of Oklahoma

Table 3.2: Phylogenetic signal estimates: Blomberg's κ and Pagel's λ for volumes of lateral geniculate nucleus (LGN), dorsal lateral geniculate nucleus (dLGN), ventral lateral geniculate nucleus (vLGN), and the vLGN/dLGN ratio. Each individual measure (LGN, dLGN, vLGN) is size-independent, i.e., based on the residuals from a linear regression.

Measure	κ	**p-value**	**λ**	**p-value**
LGN	0.476	0.109	0.798	0.345
dLGN	0.486	0.086	0.789	0.281
vLGN	0.395	0.206	<0.001	1
vLGN/dLGN	0.629	0.012	0.503	0.277

Table 3.3: Species means of volumes of lateral geniculate nucleus (LGN), dorsal lateral geniculate nucleus (dLGN), ventral lateral geniculate nucleus (vLGN), relative to brain mass, and ratio of vLGN to dLGN.

Species	**Relative LGN**	**Relative dLGN**	**Relative vLGN**	**Ratio vLGN/dLGN**
Southern Flying Squirrel	0.0037	0.0028	0.0009	0.308
North American Red Squirrel	0.0040	0.0027	0.0013	0.472
Eastern Chipmunk	0.0041	0.0032	0.0009	0.300
Eastern Meadow Vole	0.0026	0.0015	0.0008	0.482
Social Vole	0.0021	0.0012	0.0008	0.672
Southern Red-backed Vole	0.0026	0.0016	0.0010	0.607
Striped Desert Hamster	0.0035	0.0023	0.0012	0.500
Short-tailed Singing Mouse	0.0034	0.0023	0.0011	0.476
Southern Grasshopper Mouse	0.0031	0.0021	0.0011	0.522
Hispid Cotton Rat	0.0040	0.0028	0.0012	0.434
Northeast African Spiny Mouse	0.0032	0.0022	0.0010	0.440
Golden Spiny Mouse	0.0039	0.0025	0.0013	0.535
Mongolian Jird	0.0035	0.0026	0.0009	0.346
House Mouse	0.0028	0.0015	0.0013	0.831
African Grass Rat	0.0038	0.0023	0.0015	0.665
Australian Bush Rat	0.0020	0.0013	0.0007	0.587
Australian Swamp Rat	0.0027	0.0018	0.0009	0.519
Brown Rat	0.0026	0.0017	0.0008	0.484

Table 3.4: ANOVA results examining effects of temporal niche on relative sizes of the lateral geniculate nucleus (LGN), dorsal lateral geniculate nucleus (dLGN), ventral lateral geniculate nucleus (vLGN), and vLGN/dLGN ratio with pairwise comparisons between nocturnal and diurnal species, diurnal and cathemeral species, and nocturnal and cathemeral species. $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***.

	ANOVA results	**Pairwise comparisons**	**p-value**
LGN		N vs D	<0.001***
	F: 18.47	D vs C	<0.001***
	p < 0.001***	N vs C	0.674
dLGN		N vs D	<0.001***
	F: 11.88	D vs C	0.018*
	p: <0.001***	N vs C	0.369
vLGN		N vs D	<0.001***
	F: 14.27	D vs C	<0.001***
	p: <0.001***	N vs C	0.684
vLGN/dLGN Non-phylogenetic		N vs D	0.477
	F: 2.13	D vs C	0.678
	p: 0.126	N vs C	0.121
vLGN/dLGN Phylogenetic		N vs D	1
	F: 0.14	D vs C	1
	p: 0.840	N vs C	1

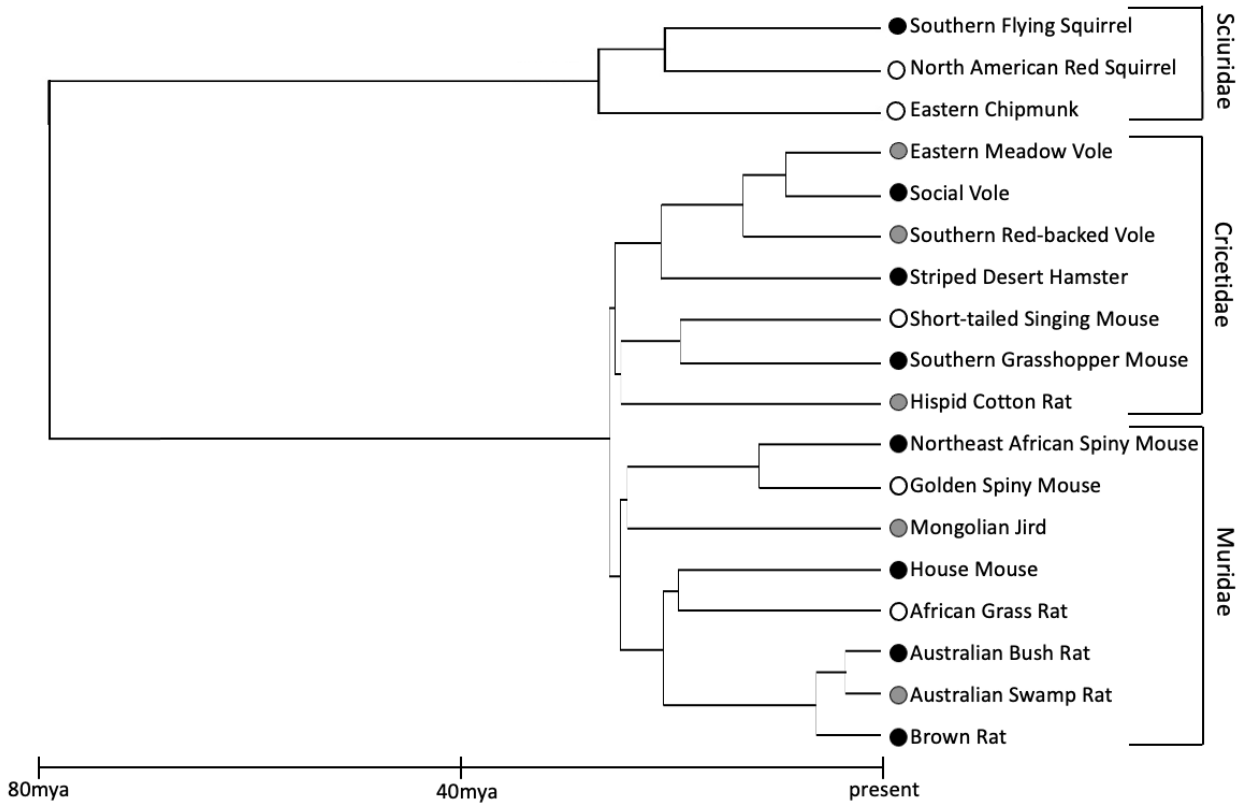

Figure 3.1: Phylogeny and temporal niche of 18 species examined in this study. Black = nocturnal, White = diurnal, Gray = cathemeral. Phylogenetic relationships and divergence times were established from Fabre et al. (2012). Mya = million years ago.

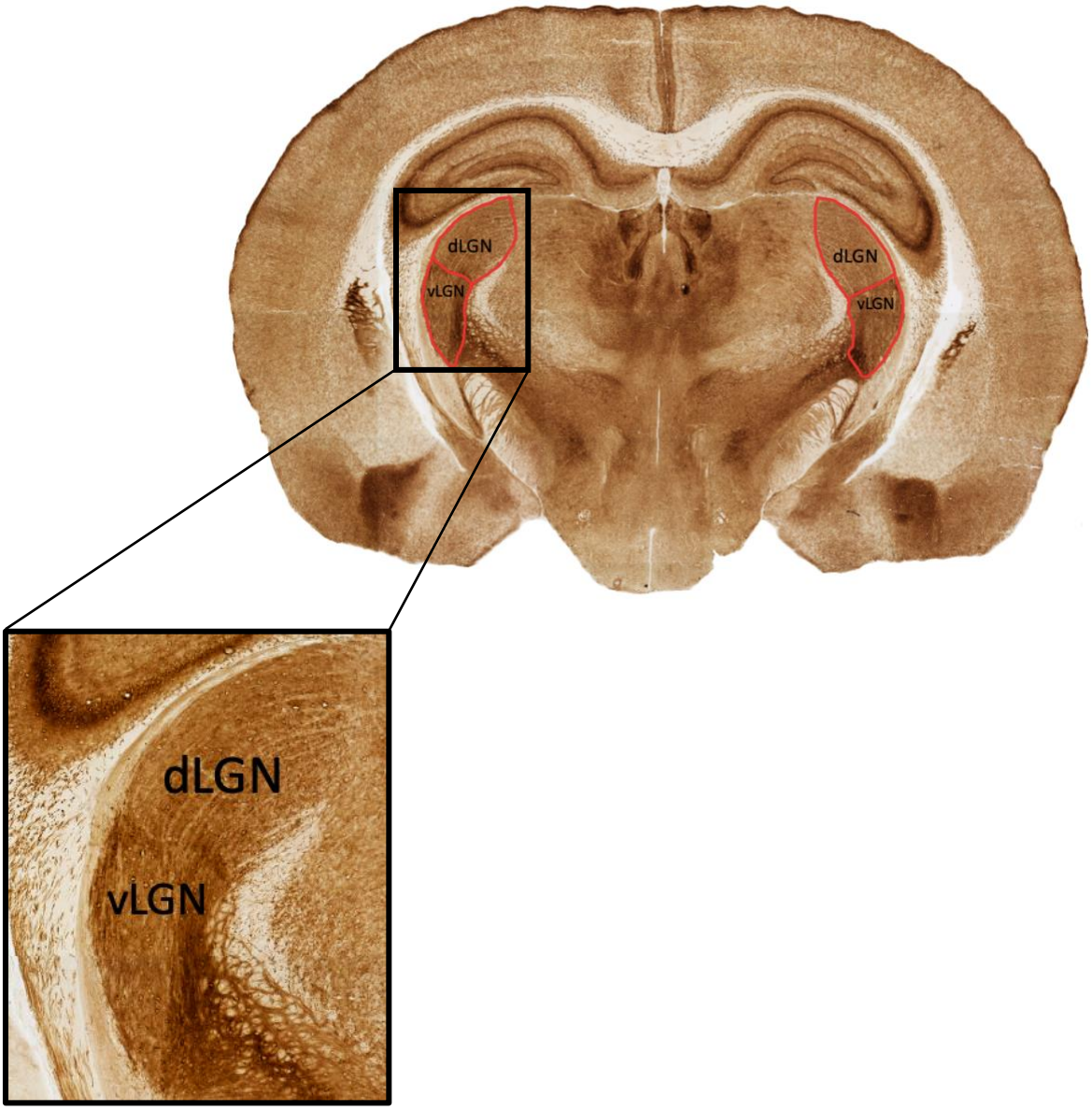

Figure 3.2: AChE-stained section of African Grass Rat brain showing delineations of the dorsal lateral geniculate nucleus (dLGN) and the ventral lateral geniculate nucleus (vLGN), which includes the intergeniculate leaflet (IGL). Boundaries identified using Paxinos and Watson (2014).

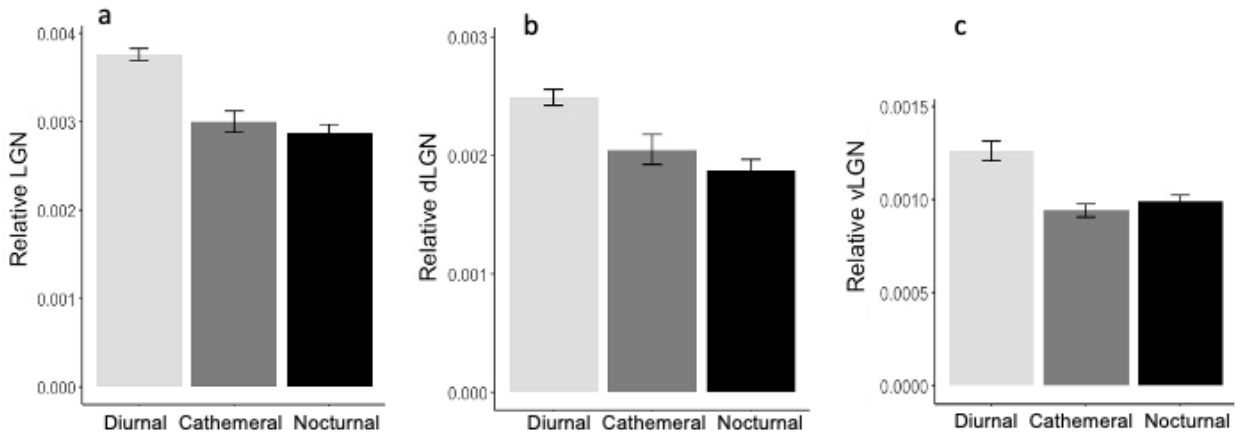

Figure 3.3: Mean volumes of a) LGN, b) dLGN, and c) vLGN, relative to brain mass; error bars are standard error of the mean.

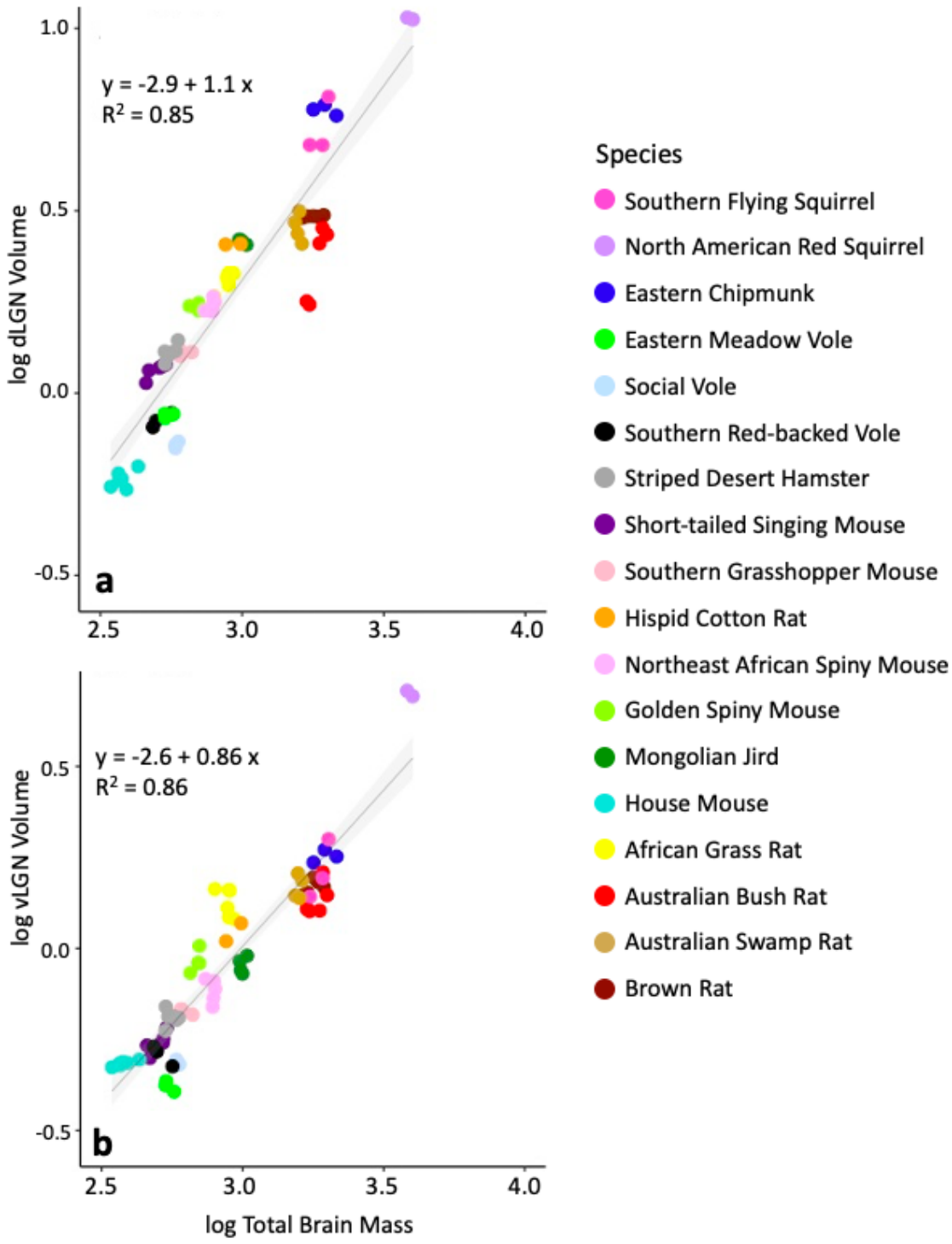

Figure 3.4: Line graphs of a) log dorsal lateral geniculate nucleus (dLGN) and b) log ventral lateral geniculate nucleus (vLGN) regressed against log total brain mass; shading represents 95% confidence intervals. Regression equations and R2 values provided.

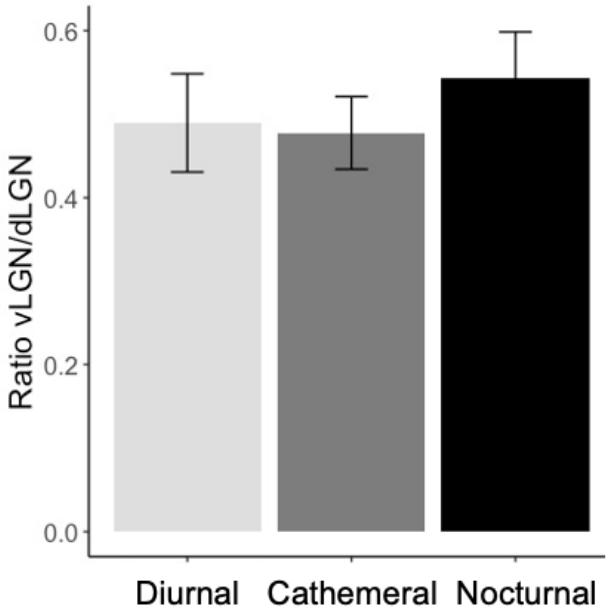

Figure 3.5: Mean ratio of vLGN to dLGN by activity pattern; error bars are standard error of the mean.

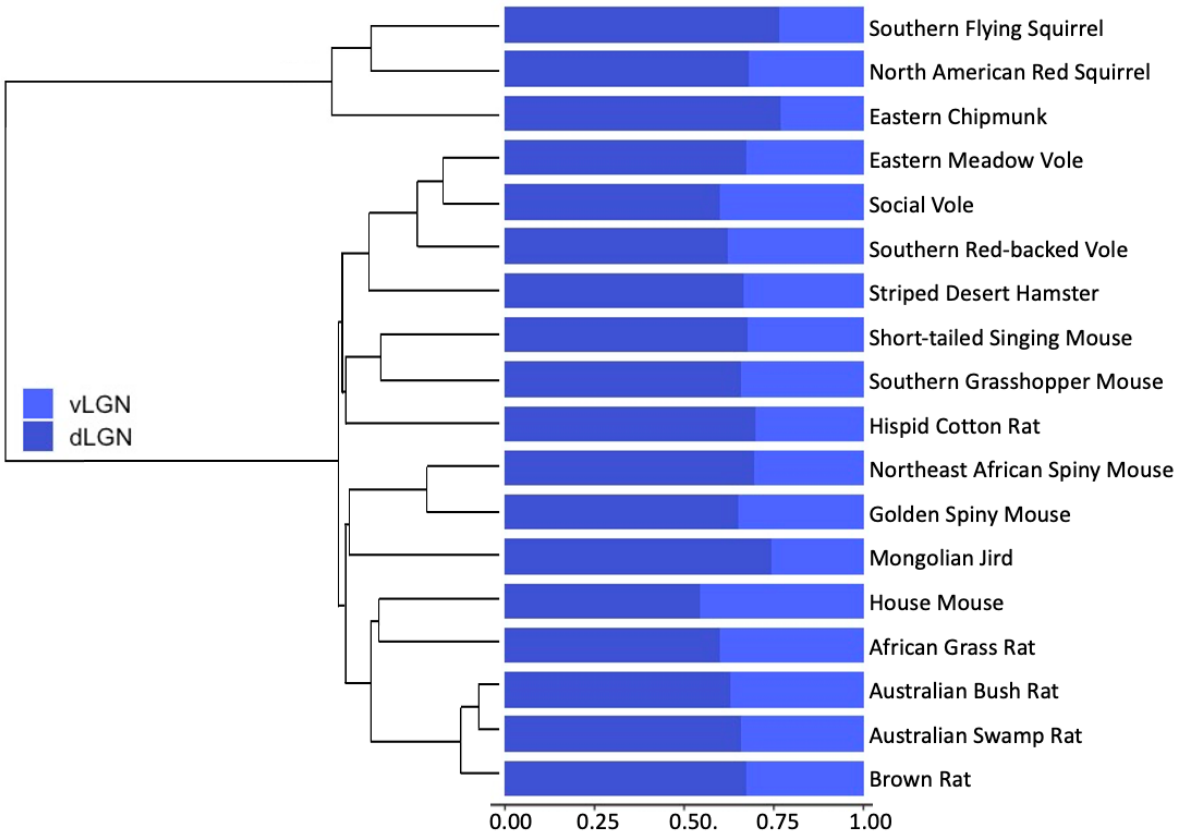

Figure 3.6: Proportions of vLGN and dLGN of total LGN in 18 rodent species. Phylogenetic relationships and divergence times were established from Fabre et al. (2012).

CONCLUSION

The study of how species adapt to their environment is an ongoing and ever-growing field of inquiry. We know that many factors act as selective forces shaping the diversity of life on the planet. Identifying what forces drive adaptation, and how they do so, provide us with a better understanding of how life has changed in the past, and may change in the future. It equips us with the tools to predict the effects different events, such as climate change and urbanization, will have on populations and communities. This, in turn, enables us to act preventatively, or in response, to such events with more effective approaches for stabilizing and conserving natural communities.

Brain evolution has always been of interest to humans, as understanding how and why brains have evolved gives us a framework for pondering scientific and philosophical questions. Even more significant is the acquired knowledge about the nervous system that can be used to help combat human neurological disorders.

One of the aims of this work is to provide evidence as to how our brains change to act optimally in different environments, as well as insight into the role energetics play mechanistically in brain evolution. This will, hopefully, provide us with a better understanding of what drivers act to shape our sensory systems.