5-2014

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Recommended Citation

Robinson, Christopher D., "Convergence in communication behavior and brain morphology in lizards: An evolutionary approach" (2014). *Biology Honors Theses*. 16.
http://digitalcommons.trinity.edu/bio_honors/16

CONVERGENCE IN COMMUNICATION BEHAVIOR AND BRAIN MORPHOLOGY IN LIZARDS: AN EVOLUTIONARY APPROACH

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A DEPARTMENT HONORS THESIS SUBMITTED TO THE
DEPARTMENT OF BIOLOGY AT TRINITY UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR GRADUATION WITH
DEPARTMENTAL HONORS

DATE 4/16/2014

[redacted]

THESIS ADVISOR DEPARTMENT CHAIR

ASSOCIATE VICE PRESIDENT FOR ACADEMIC AFFARIS,
CURRICULUM AND STUDENT ISSUES

Abstract

Animal communication allows information to be transferred from a sender to a receiver, and can occur via visual, chemical, auditory, and tactile modalities. Communication behaviors are known to be generally associated with specific brain regions, but it is currently unknown how the cellular morphology of these regions differs in species that quantitatively differ in the use of particular communication modalities. Further, these relationships are rarely considered in an evolutionary context. In this thesis, I investigated the relationship between the use of communication modalities and neural morphologies (in particular, soma size and density) in the brains of six species of lizards: *Anolis carolinensis*, *Aspidoscelis gularis*, *Hemidactylus turcicus*, *Leiocephalus carinatus*, *Sceloporus olivaceus*, and *Scincella lateralis*. I performed behavioral observations of these species, quantifying their rates of visual and chemical communication behaviors. I then collected brains from 10 males of each species and measured soma size and density in two brain regions associated with visual behaviors (the lateral geniculate nucleus, and the optic tectum), one brain region associated with chemical behaviors (the nucleus sphericus), and one brain region generally associated with social behaviors (the preoptic area). I found that species that communicate with higher rates of visual displays had a denser lateral geniculate nucleus, and that species that communicate with a higher percentage of chemical displays had larger somas in the nucleus sphericus. These relationships between communication behaviors and neural morphologies suggest that structures within the brain have evolved convergently in species with similar communication behaviors.

Acknowledgments

I have so many people that I would like to thank, because without them I would have lost my mind a long time ago. First, I’d like to thank Dr. Michele Johnson for her unwavering support during my time in the lab, for teaching me how to be an effective scientist both in the lab and in the field, and for giving me the initial opportunity to work with her. I am forever grateful for her encouragement and advice that allowed me to complete such a great project. I’d like to thank my committee, Dr. David Ribble and Dr. Troy Murphy, for the help that they provided in finalizing my thesis, and for advising me during my wonderful time here at Trinity. Additionally, I would be nowhere without all of the fantastic technicians that have worked for Michele – Bonnie Kircher, Michael Patton, Alisa Dill, and Tara Whittle. Bonnie and Michael are two of the hardest working people I have ever had the pleasure of working with in my life, and I can’t thank them enough for all that they did to help me, or for being such great friends during that time. Alisa taught me most of the valuable skills needed for this project – behavioral observations, lizard catching, and cryostating. None of what I’ve done would have been possible if it wasn’t for her. And as for Tara, she was the person who first introduced me to the idea of research when she was my Bio 1 lab professor. I remember as a young freshman in my first ever lab looking up to her for all that she had done, and it was through her that I got into contact with Michele about helping out in the lab (after I naively asked Tara if I could join the research lab that she ran, which promptly made her laugh at me). Thank you, Tara, for believing in a young me. Furthermore, I want to thank all of the rest of the members of the lab, for it was you all that helped me collect my many hours of behavioral data, provided me with encouragement through rough times, and gave me friends with whom I could laugh and joke around. You all are great.

And finally, I wouldn’t be where I am today without a long list of friends that have helped me in their own unique ways. I would like to thank (in no particular order): Ellee Cook, Cade Bradshaw, Erin Tansey, George Kazen, Juan Carlos Gutierrez, Julian Garza, Sierra French, Valerie Martinez, Vanessa Ortiz, Meghan Tang, Aparna Gomes, Manuel Pallarez, Mark Lindeman, Adam Silva, Dr. Kelly Lyons, Dr. Thom Sanger, Ultimate Frisbee, Avenged Sevenfold, milk, and the Dallas Cowboys.

This thesis was generously funded by many sources: Sigma Xi Grant-in-Aid of Research, Trinity University’s Mind Science Foundation, Trinity University’s Howard Hughes Medical Institute Undergraduate Research Fellowship, Trinity University’s Biology Summer Undergraduate Research Fellowship, Ecolab, and the National Science Foundation (IOS 1257021 to Michele Johnson).

TABLE OF CONTENTS

Introduction

Communication is crucial for the survival and the reproductive success of almost all animals. One central function of the behaviors used to send or receive messages between conspecifics and/or heterospecifics is to provide important information about the social environment. For example, signals can be used to communicate the size and strength of a competitor, a predator's level of aggression, or a potential mate’s availability for copulation. These signals can be sent and received using several modalities, including visual, chemical, auditory, and tactile sensory mechanisms (Dangles et al., 2009). In each case, specialized morphologies are needed to detect messages communicated using a specific modality. For example, among species that utilize visual communication, there must be light-sensitive cells, such as in an eye, so the receiver of the message can sense visual stimuli. For chemical modalities, there must be morphologies that allow chemical signals to be sensed and secreted. Further, all messages must be processed by the brain so that appropriate responses can be produced. Each modality is associated with certain brain regions, and species that rely more heavily on certain modalities over others may exhibit enhanced neural morphologies in these regions. In this thesis, I studied a group of six lizard species to explore the relationships between behaviors that facilitate communication using two modalities (visual and chemical), and the morphology in brain regions associated with these modalities. I used phylogenetically-informed comparative analyses to test for convergent evolution in brain morphology in species with similar communication behaviors.

The relationship between brain morphology and communication behavior is a prime candidate to explore using phylogenetic comparative methods, because few studies have examined this relationship across multiple species. Under normal statistical assumptions, all data points are independent of one another, while phylogenetic comparative methods consider how species are hierarchically related to one another and use these hierarchical relationships to determine whether similarities among species are derived from evolutionary history alone, or in association with other traits (Felsenstein 1985). Phylogenetic comparative studies are used to hypothesize patterns of evolution (Martins, 1996) and provide a powerful tool for examining morphological or physiological relationships throughout a taxonomic group. However, comparative studies of brain and behavior remain rare in the literature. Brenowitz (1997) is one example of a comparative study that examined brain morphology and communication behaviors across a group of six bird species by studying the relationship between the volume of song control brain regions and song repertoire size, but the data in this study were not analyzed in a phylogenetic context. Thus, the present study is one of the first to directly consider the hypothesis that communication behaviors have evolved in association with brain morphologies using phylogenetically informed statistics.

In this introduction, I first review the modes of communication, behaviors involved in lizard communication, and the role of the brain in communication before I describe the hypotheses tested in the present study.

Modes of communication

The method by which an animal sends a message to another individual can be just as important as the information that is included within the signal. Sensory drive (e.g., Endler and McLellan, 1988) predicts that natural selection promotes the evolution of a signal that will be most easily detectable in the habitat in which it is used. For example, Leal and Fleishman (2004) found that throat fans of the lizard *Anolis cristatellus* vary in color between populations to allow for higher visibility in either mesic or xeric environments.

Visual communication requires the combination of light from the environment and photoreceptors (specialized neurons capable of detecting light) in the receiver to relay messages from a sender to a receiver. There are two types of photoreceptors that characterize the categories of animal vision: rods and cones. Rods are used to perceive brightness and work well in dimly lit environments, while cones are used to detect certain wavelengths of light (ultraviolet, short, medium, and long wavelength). Animals that have only rod photoreceptors, such as nocturnal monkeys in the genus *Aotus* (Jacobs et al., 1993), have monochromatic vision, in which they can only perceive brightness (and so cannot distinguish among colors). Dichromatic vision occurs where there are two types of functioning color receptors (cones) in the eye, and is characteristic of most placental mammals (Bowmaker, 1998), with the exception of primates, who exhibit trichromatic vision, in which there are three types of cone cells (Osorio and Vorobyev, 2008). Finally, animals that possess four types of cones, such as birds, possess tetrachromatic vision (Bowmaker, 1980). Tetrachromatic vision allows for detection of light whose wavelength is in the ultraviolet part of the spectrum. Similarly to birds, many species of lizards are known to see in the ultraviolet spectrum, including some species of anoles, iguanians, and geckos (reviewed in Stoehr and McGraw, 2001).

The evolution of vision, and color vision in particular, has been long associated with the evolution of large or elaborately colored animal morphologies that are used to communicate messages to other organisms. Darwin (1871) hypothesized that extreme sexual dimorphisms, such as the coloration and morphology of bird plumage, evolved for communication in competition and/or mate choice. In competition, these morphological traits are assessed visually to size up a potential mate or rival, such as in roe deer, *Capreolus capreolus*, where an individual can determine his size relative to his opponent's by assessing the size of his opponent's antlers (Vanpé et al., 2007), or in the paper wasp, *Polistes dominulus*, where an individual's agonistic behavior can be predicted by facial coloration patterns (Tibbets and Izzo, 2010). Additionally, females can use these visual characteristics to choose a mate. In three-spined stickleback fish, *Gasterosteus aculeatus*, for example, females are able to assess male quality by the intensity of a male's red abdominal patch. Those individuals that are more highly parasitized and are lower in body condition have less intense red abdominal patches and therefore are selected for copulation by females less often than males of higher condition, which in turn possess a more intense red abdominal patch (Milinski and Bakker, 1990). Since the vast majority of animals have eyes used to assess visual stimuli, visual signals have evolved to convey a wealth of information about an individual to both conspecifics and heterospecifics.

Chemical communication, which is widespread throughout animal taxa and is the oldest form of communication (Steiger et al., 2011), occurs when an individual uses pheromones to convey information to others. Pheromones are species-specific chemical signals (Karlson and Lüscher, 1959) that can be described as either primers or releasers (Wilson and Bossert, 1963). A primer is a pheromone that causes something to happen gradually over time, including over the course of an individual's development. In honeybees, a primer pheromone is crucial for the division of labor among individuals in a nest, such that 'brood pheromone' (a collective term for a mixture of ten fatty-acid esters on honeybee larvae) regulates behavioral development and the transition from a nurse bee to a foraging bee (Le Conte et al., 2001). A releaser, on the other hand, is a pheromone that causes an immediate behavioral response. In the masu salmon, *Oncorhynchus masou*, a releaser pheromone is secreted in the urine of ovulating females, which causes downstream males to turn and swim against the current so that they can find these receptive females (Yambem et al., 1999). As such, chemical communication can produce a very localized message, which can tell conspecifics detailed information about a given location.

Morphologies that allow for chemical communication can be quite specialized. For instance, organisms must have some way to secrete their pheromones, and in many cases, pheromones are released in the same manner as excrement, such as via urine, or even with fecal excreta (e.g., Brennan, 2010). But some organisms have special secretory glands for pheromone release, such as the femoral pores that are found on the hindlimbs of some species of lizards (e.g., Martín and López, 2000). Specialized structures are also required to detect chemical signals. The vomeronasal organ (VNO), also known as the Jacobson's organ, is a chemosensory organ separated from the nasal cavity and is found many taxa, including amphibians, mammals, and reptiles (Stoddart, 1980; Keverne, 1999). The VNO has distinct odorant receptors, and innervates a network of neurons that travel through the brain (Keverne, 1999).

Auditory communication is a modality that relies on the transfer of sound waves from sender to receiver. Organisms can produce sounds in multiple ways. For example, many birds can produce calls (single note vocalizations), and most passerine birds produce songs (elaborate vocalizations used to attract mates or defend territories), that are highly stereotyped and are often learned during a distinct developmental period (Marler and Mundinger, 1971). Song production in birds is dependent on syringeal muscles that control the syrinx, the vocal producing organ (Larsen and Goller, 2002). But, auditory communication does not have to rely on vocal performances exclusively, as external parts of the body can produce sound in some species. For example, the sage grouse, *Centrocercus urophasianus*, is a lek breeding bird species whose males use a characteristic strut with stereotyped visual and auditory components that is used to attract mates (Wiley, 1973; Gibson, 1996). The auditory part of the strut is caused by the inflation of two esophageal sacs that are lifted and then dropped at the same time that the bird's wings are moving (Wiley, 1973). Since auditory communication uses sound waves, messages can travel far and be picked up by multiple receivers. But, these messages are difficult to direct to specific receivers, and therefore can be costly, particularly if it allows a predator to hone in on where the sender is located. Auditory messages are less constrained by the environment than other modalities, such that they are not completely blocked by physical barriers like visual modalities, which require a clear line of sight. They can be modified in different environments to circumvent an obstacle, such as changing the pitch of a call in a dense forest to allow the sound waves to cut through the foliage.

Tactile communication is the fourth major type of communication and uses the sense of touch. It requires very little morphological complexity, such that organs specialized for other tasks can still be used in tactile communication, but it also has low range of transmission because information must be passed on directly (i.e., physically) from a sender to the receiver. For example, in Indian langurs, *Presbytis entellus*, touch is used to show intimacy and familiarity with another individual and to build social bonds that can be critical for survival in a group setting (Weber, 1973). In many species, tactile communication can also be manifested in the form of physical combat.

It is well understood that specific areas of the brain control certain types of sensory perception. Indeed, each communication modality uses a unique sensory network (i.e., in visual communication the visual system is used and in auditory communication the auditory system is used). For example, in macaque monkeys, lesions that cause inactivation of the lateral geniculate nucleus (LGN), a region of the brain involved in processing visual information (De Valois et al., 1965), led to a lack of behavioral responses when the monkeys were presented with visual stimuli (Schmid et al., 2010). Although there is a clear relationship between brain morphology and communication modalities, little research has investigated how variation in the cellular morphologies of these regions are associated with the degree of usage of a particular modality. To accomplish such a study, researchers would need a group of species with variable communication modalities, as well as one where the behaviors associated with communication are easily quantifiable, and tools to assess variation in brain morphology are available. Lizards make an excellent system for such a study because of their diversity in communication behaviors, particularly using visual and chemical modalities, and the availability of lizard brain atlases (e.g., Northcutt and Butler, 1974; Greenberg, 1982). Also, because the frequency of use of a modality can vary between species of lizards, studies using a phylogenetically diverse sampling can provide the opportunity to test for evolutionary relationships between the frequency of communication behaviors and development of the associated brain regions.

Lizard communication

Lizards are a diverse taxonomic group, comprised of 5907 species in 36 families (*The Reptile Database*; http://www.reptile-database.org). Most of the major variation in lizard communication modality occurs among families, although species within families often vary substantially in the frequency with which different communication modalities are used. Visual displays are particularly common in many lizard taxa. For example, head-bob and push-up displays (i.e., the raising and lowering of the head and trunk of the animal in a stereotypical fashion) are visual displays seen in many lizard families, including Iguanidae, Polychrotidae, Phrynosomatidae, and Leiocephalidae (Martins, 1993a; Ord and Stamps, 2008). Traditionally, head-bobs and push-ups are categorized as assertion or challenge displays, but head-bobs can be used to communicate disparate messages. For example, Nava et al. (2012) showed that in the fence lizard *Sceloporus undulatus*, males use relatively slow head-bobs as a sign of aggression, while relatively fast head-bobs are used in courtship displays.

Additionally, there are several groups of lizards that use a dewlap, a conspicuous throat fan extended by the bowing of the ceratobranchial cartilage (Bels, 1990), in their visual display behaviors. Although the most studied lizards that possess a dewlap are those in the speciose genus *Anolis*, (Nicholson et al., 2005), other genera are known to use the throat fan as a signal as well, including *Urosaurus* (Hover, 1985; Thompson and Moore, 1991), and *Sitana* (e.g. Patankar et al., 2013). An individual's dewlap can function in signaling information about its size, sex, and condition (Williams and Rand, 1977; Vanhooydonck et al., 2005; Cook et al. 2013). Moreover, Ord (2012) showed that in order to compensate for visual noise (i.e., foliage that blocks or distracts from the signal due to wind), some species of anoles, such as *Anolis cristatellus*, can alter how fast they perform head-bob and dewlap displays. This flexible signaling behavior allows the signal that they are intending to send to be visible through the environmental noise.

Another component of visual display common across lizards involves movements of the tail. The zebra-tailed lizard, *Callisaurus dranconoides*, constantly moves its tail, with one use of the tail curling being as an anti-predator, pursuit deterrent signal (Hasson et al., 1989). Similarly, the Mediterranean house gecko, *Hemidactylus turcicus* (Selcer, 1986) and the curly tail lizard, *Leiocephalus carinatus* (Cooper, 2001) use tail wags to attract a potential predator's attention away from their bodies, and if the tail is then attacked by the predator, the lizards can easily autotomize their tails to escape. Tail wagging in geckos can also be used as social displays (e.g., Regalado, 2003; Briggs, 2012).

The aforementioned visual displays are often accompanied by species-specific displays that can be used to modify the information in a display. For example, when anoles are locked in an antagonistic interaction, males take on a posture higher from their perch, they develop dark spots behind their eyes, and they develop raised nuchal and dorsal crests (Losos, 2009). Similarly, Martins (1993b) suggests that head bobs in *Sceloporus spp.* can convey different information depending on the shape (jaggedness) and number of head bobs.

Chemical communication in lizards is also quite prevalent. Many species of lizards can be seen flicking their tongues in a manner that is very much like the stereotypical behaviors of snakes. This behavior is used to bring pheromone molecules into contact with the VNO, as described above. Male desert iguanas, *Dipsosaurus dorsalis*, flick their tongues at a baseline rate of about 1.02 licks per minute to locate food, conspecifics, and nesting burrows (Krekorian, 1989). Even in the highly visual collared lizard, *Crotaphytus collaris*, individuals may use chemical cues to detect if a fecal pellet belongs to a conspecific (Wilgers and Horne, 2009).

Pheromones can send more complex messages as well. Cooper and Pèrez-Mellado (2002) showed that the lacertid lizard *Podarcis hispanica* can not only discriminate sex by using pheromones, but can also determine whether or not a female conspecific is gravid or receptive to copulation. Additionally, male and female ground skinks (*Scincella lateralis*) avoid areas where they detected pheromones of another male (Duvall et al., 1980), which could indicate that pheromones are used to define territorial boundaries.

Despite pheromones being an important method of communication for many lizards, some species use them very little, or not at all. In the green anole, *Anolis carolinensis*, Jenssen et al. (1995) reported that substrate licks occur relatively rarely (about once per hour). Consequently, Jenssen et al. (1995) concluded that pheromone use is likely not used as a means for social communication in this species, which is supported by the fact that they have a highly underdeveloped VNO (Crews and Greenberg, 1981).

Although some lizards communicate with auditory and tactile modalities, these strategies are uncommon, and so they are not a focus of this study.

Brain in communication

Compared to other vertebrate taxa, we know relatively little about the neural mechanisms that influence lizard communication. However, regions in the Social Behavior Network – the medial amygdala, lateral septum, preoptic area, anterior hypothalamus, ventromedial hypothalamus, and periaqueductal gray of the midbrain (Newman, 1999) – have homologues in most vertebrates (Goodson, 2005), and some of these have known functions in lizard reproductive behaviors, particularly in the visual displays used in courtship and aggression (reviewed in Wade, 2005). In the green anole, the preoptic area (POA), part of the social behavior network described above, has been experimentally shown to control both copulatory and antagonistic display behaviors. When the POA is bilaterally ablated, these behaviors cease to occur (Wheeler and Crews, 1978). This effect was also shown in a whiptail lizard, *Cnemidophorus inornatus*, in which individuals with lesions to the POA showed decreased courtship and copulatory behaviors (Kingston and Crews, 1994). Additionally, Greenberg et al. (1984) highlighted the role of the ventromedial hypothalamus (VMH) and the paleostriatum (PS) in courtship and challenge displays of the green anole. When the VMH of male anoles were lesioned, courtship displays were almost eliminated and challenge displays were unaffected, while individuals with lesions to the PS showed a decrease in challenge displays with courtship displays being unaffected (Greenberg et al., 1984).

Outside of the Social Behavior Network, there are other regions that are known to play some role in receiving and processing signals. For visual signals, the optic tectum (OT) (e.g., Foster and Hall, 1975), LGN (e.g., Ulinski, 1977), and nucleus rotundus (Hoogland, 1982) receive retinal projections and send these messages to other parts of the brain. Additionally, there are many motor nuclei, such as the accessory oculomotor nucleus, the trochlear nucleus, and the principal abducens nucleus that control muscles around the eye (Barbas-Henry and Lohman, 1988). For chemical signals, the VNO sends signals to the nucleus sphericus of the amygdala (NS), which is known to have chemosensory functions (Bruce, 2009). Furthermore, pheromone molecules are brought into contact with the VNO after they are picked up by the tongue, whose movement is controlled by the hypoglossal nucleus (Barnard, 1940).

Study system

In this study, I examined the neural morphology in four brain regions associated with communication using six species of lizards (Figure 1), each of which belongs to a different taxonomic family. Of these six species, two communicate primarily using visual signals: the green anole and the curly tail lizard. The green anole, *Anolis carolinensis* (Family: Dactyloidae), possesses a brightly colored dewlap that is extended and paired with pushup and head-bob displays to visually communicate to potential mates, rivals, and predators (Jenssen, 1977). The curly tail lizard, *Leiocephalus carinatus* (Family: Leiocephalidae), also communicates visually, by performing pushup and head-bob displays and by curling their tail (Evans, 1953). Two other species in this study communicate primarily using chemical signals. The spotted whiptail, *Aspidoscelis gularis* (Family: Teiidae) and little brown skink, *Scincella lateralis* (Family: Scincidae), primarily communicate using pheromones. Pheromones are secreted from femoral pores located on the hindlimbs in the spotted whiptail and from the cloaca in the little brown skink (Duvall et al., 1980; Alberts et al., 1992). The final two species in this study use both chemical and visual modalities. The Texas spiny lizard, *Sceloporus olivaceus* (Family: Phrynosomatidae), performs pushups, dorsoventral flattening (which shows off its bright blue bellies), and secretes pheromones from femoral pores on the hindlimbs (Bissinger and Simon, 1981; Roggenbuck and Jenssen, 1986). The Mediterranean house gecko, *Hemidactylus turcicus* (Family: Gekkonidae), performs tail wags, arches its back, secretes pheromones, and performs auditory clicking sounds (though auditory communication was not included in this study) (Regalado, 2003).

I investigated four brain regions associated with the visual and chemical communication modalities in these six species of lizards. I tested the hypothesis that lizards have evolved brain morphologies associated with their primary modes of communication that are more highly developed than regions associated with modes rarely used. In particular, I predicted that species that communicate using predominantly visuals cues will have larger, denser neurons in the OT, which is important in visual integration and spatial attention (Newman and Hartline, 1981), and in the LGN, which receives visual information from the retina (Ulinski, 1977). I predicted that lizard species that communicate using pheromones will have larger, more densely packed neurons in the NS, which is correlated with chemosensory function (Bruce, 2009) and is projected to almost exclusively from the accessory olfactory bulb, a projection of the VNO. Thirdly, I predicted that lizards that use both visual and chemical communication will have enlarged neuron soma size and enhanced neuron density in all three of these brain regions. I also examined neuron size and density in the preoptic area (POA). Since this region is part of the Social Behavior Network, which is important in controlling social behaviors in general, I predicted that species with higher overall rates of display (i.e., more frequent visual and chemical behaviors) will have larger, more densely packed neurons than species with lower rates of display. I used both standard and phylogenetically informed statistical methods to test whether brain morphologies have evolved convergently in species that use similar modalities.

Materials and Methods

Study Species

To determine relationships between communication modalities and brain morphology, I performed behavioral observations and captured adult males from each of five lizard species (see below) in south-central Texas from May to August of 2012 and May to August 2013, and one species, the curly tail (*Leiocephalus carinatus*) in natural areas around Crooked Island Bahamas in July 2013. In Texas, I studied green anoles (*A. carolinensis*) in Palmetto State Park in Gonzales, Texas, in natural areas near San Antonio, Texas, and on the campus of Trinity University in San Antonio, Texas; house geckos (*H. turcicus*) in Palmetto State Park in Gonzales, Texas, and on the campus of Trinity University in San Antonio, Texas; spotted whiptails (*A. gularis*) and spiny lizards (*S. olivaceus*) on private properties in Bastrop, Bexar, Comal, Hays, and Travis counties in Texas. Little brown skinks (*S. lateralis*) were collected from Brazos Bend State Park in Needville, Texas, and observed at Trinity University, as described below.

Behavioral observations

I performed 10-60 min focal observations in the field on individual males for each species, except for skinks (described below), recording the occurrence of communication behaviors for each individual. Chemical communication behaviors included rubbing the hindlimbs or cloaca on the substrate (indicators that an individual is spreading femoral pore or fecal secretions), and licking the air or substrate tasting for pheromones; visual communication behaviors included dewlap extensions, push ups, dorsoventral flattening, or tail curls. In total, I collected 122.6 hours of behavioral data (Table 1).

Adult male lizards were located for observation by scanning the ground (for little brown skinks, spotted whiptails, and curly tails), trees (green anoles and spiny lizards), and buildings (Mediterranean house geckos). Because not all observed individuals could be captured, whenever possible, I identified the sex of each lizard from a distance. I visually identified the sex of green anoles, spiny lizards, and spotted whiptails by their size and secondary sex characteristics. Adult male green anoles are generally substantially larger than females, and males have a significantly larger dewlap. Male spiny lizards were distinguished by the bright blue coloration on the lateral parts of their bellies, as females of this species have white belly coloration. If a green anole or spiny lizard was captured after an observation, I examined it for enlarged post-anal scales, the presence of which further confirmed that an individual was male. Whiptails are sexually dichromatic, such that males have blue-black ventral coloration while females possess a white, unmarked belly. For curly tails, geckos, and skinks, there were no sexually dimorphic characteristics that could be consistently identified in the field prior to capture. Schoener et al. (1982) found that the snout-vent length (SVL) of male *L. carinatus punctatus*, the curly tail subspecies that inhabits Crooked Island, Bahamas, differed from female SVL by approximately 1 cm, a difference insufficient to accurately determine the sex of an individual in the field. House geckos are sexually dimorphic in head size (Johnson et al. 2005), but mean differences in head depth, length, and width between the sexes were each within 1 mm of each other. Therefore, after observations of these three species, it was necessary to capture the focal individuals to determine sex. In curly tails, I visually checked for the presence of hemipenes by eversion and in geckos, for the presence of bugles caudal to the cloacal vent, which indicated the presence of hemipenes (hemipene eversion was not performed on geckos because the tail is easily autotomized and I did not wish to stress an individual any more than necessary). Because I was unable to differentiate between male and female skinks on the basis of external characteristics, sex was determined by dissection after sacrificing the animal.

Because of the complex structural niche that little brown skinks occupy (they are primarily found under leaf litter on the ground), undisturbed skink behavior is extremely difficult to observe in the field. Therefore, I captured little brown skinks and observed their behavior in captive semi-natural conditions. These captured skinks were put into shallow plastic pools (93 cm diameter, 21 cm deep, Summer Escapes™) with leaf litter covering the bottom 1 cm of the pool. Because both male and female little brown skinks avoid areas where other male odors are sensed (Duvall et al., 1980), I wiped the pool with 100% ethanol and replaced the leaf litter between trials to ensure an individual's behavior was not influenced by the presence of a skink in a previous trial. Each skink was observed for two 60 min trials.

For the other five species in the study, 8-10 male lizards for each species (Table 1) were captured by hand or by noose after observations were complete, and their sex was confirmed as described above. I then transported lizards to Trinity University for temporary housing, following recommendations for lizard care described in Sanger et al. (2008). In brief, animals were housed in individual cages with a perch (or for geckos, a refuge), and every other day, fed 2-3 crickets coated in calcium powder. Cages were misted with water several times daily to provide drinking water and increase the humidity in the cage. The light cycle was set to 13 h light/ 11 h dark to simulate the natural summer environment.

Morphological and brain measurements

I measured snout-vent length (SVL) of each lizard to the nearest 0.5 mm using a ruler, and weighed every individual to the nearest 0.1 g using a Pesola scale. Lizards were then euthanized via rapid decapitation and brain tissues were collected immediately. Brains were flash-frozen in cold isopentane (4-methylbutane) on dry ice, and stored at -80˚C until they were sectioned. All procedures were approved by Trinity University's Animal Research Committee.

The brain of each lizard was coronally sectioned using a Leica cryostat at 20 µm in four alternate series, and sections were thaw-mounted onto SuperFrost Plus (Fisher Scientific; Hampton, NH) microscope slides. Sections were stored at -80˚C until further processing. Alternate slide series (i.e., those containing sections at 40 µm intervals) were dehydrated, cleared with xylene, and stained using thionin, which stains the Nissl bodies of golgi in neurons. Slides were coded so that species could not be identified during measurement. I measured the cross-sectional area of neuron somas (hereafter, soma size) and their density in LGN, OT, NS, and POA using a microscope at 400X magnification and the software programs FlashPoint FPG32 and ImageJ. I quantified each individual's average soma size in each region by measuring 20 arbitrarily-chosen neurons within the middle of a region on each side of the brain and averaging these 40 measurements. Density was calculated by counting the number of neurons in a 167 µm X 125 µm area, using the same sections from which the soma size measurements were taken. To see if lizards with larger brains inherently had larger neurons, I measured the length of the third ventricle, which served as a proxy for brain size.

Statistical analyses

To determine whether lizards with larger brains had larger neurons, I used linear regression analysis. There was no significant relationship between the length of the third ventricle and soma size in the LGN ($F_{1,32} = 0.87$, $R^2 = 0.036$, $p = 0.36$), OT ($F_{1,32} = 0.051$, $R^2 = 0.002$, $p = 0.82$), NS ($F_{1,29} = 2.84$, $R^2 = 0.089$, $p = 0.10$), or POA ($F_{1,29} = 2.40$, $R^2 = 0.088$, $p =$

0.13). Therefore, all analyses for these brain regions were performed using non-size corrected data. To test for differences in soma size and density between species, I used ANOVA, followed by Tukey's HSD *post hoc* tests.

For each behavioral observation, I calculated the percentage of total communication displays that each individual used in visual communication, and the percentage that were used in chemical communication. I also calculated the rate of visual behavior, the rate of chemical behaviors, and the overall display rate for each individual, with each rate defined as the number of displays per minute. Percentage data were arcsine transformed to meet the assumptions of normality (Sokal and Rohlf, 1995). Each measure of individual lizard data was then averaged for each species. Using SPSS 21, I used an ANOVA with Tukey's HSD *post hoc* tests to test for differences between species in their rates of visual and chemical displays, with separate analyses for each modality. Using R, I used a series of linear regression to test for relationships between percent of visual displays and soma size and density in the LGN and the OT, as well as the percent of chemical displays and NS soma size and density. Using a molecular phylogeny of lizard taxa constructed using up to 44 nuclear genes (33,717 base pairs) from Wiens et al. (2012), pruned to include only the species in this study (Figure 2), I then calculated phylogenetic independent contrasts (Felsenstein, 1985) in R using the *ape* package (Paradis et al., 2004), and performed linear regression using contrasts for percent of visual displays against contrasts for LGN soma size and density, as well as OT soma size and density. Similarly, I performed a linear regression using the contrasts for percent of chemical displays against contrasts for NS soma size and density. Finally, I performed a linear regression using contrasts for overall display rate and POA soma size and density. All regressions using independent contrasts were forced through the origin. Because the hypotheses tested in this study were directional in nature, all regression analyses were one-tailed.

Results

Behavior

Species differed in the percentage of visual displays used ($F_{5,200} = 282.78$, $p < 0.001$), such that anoles and curly tails used visual displays more than all other species, followed by spiny lizards, then house geckos, and finally whiptails and skinks (Figure 3). They differed in the rate of visual displays ($F_{5,269} = 32.57$, $p < 0.001$; Figure 4), such that anoles had a higher rate of visual display than all other species. The six species also differed in chemical display rates ($F_{5,269} = 45.84$, $p < 0.001$; Figure 5), such that whiptails had a higher rate of chemical display than skinks, which had a higher rate of chemical display than the other four species.

Brain morphology

The soma size of the LGN ($F_{5,48} = 24.22$, $p < 0.001$), OT ($F_{5,50} = 5.97$, $p < 0.001$), NS ($F_{5,42} = 15.27$, $p < 0.001$), and POA ($F_{5,60} = 7.52$, $p < 0.001$) differed between species (Figure 6). Tukey's *post hoc* tests showed that the soma size of the LGN was largest in curly tails and spiny lizards. Curly tail LGN soma size was significantly larger than all species except for spiny lizards. Spiny lizards did not differ in LGN soma size from curly tails, whiptails, or anoles, but were larger than geckos and skinks. Whiptails did not differ in LGN soma size from spiny lizards, anoles, or geckos, but were larger than skinks. Geckos did not differ from anoles or whiptails, but were larger than skinks (Figure 6a). For the OT, geckos, spiny lizards, curly tails, and skinks had the largest soma size. Geckos had larger somas than whiptails and anoles while spiny lizards had larger somas than anoles (Figure 6a). Whiptails, spiny lizards, and skinks had the largest soma size in the NS (Figure 6b). Whiptails had larger somas than geckos, curly tails, and anoles. Spiny lizards and skinks had larger somas than curly tails and anoles. Geckos had larger somas than anoles. In the POA (Figure 6c), curly tails, geckos, whiptails, and curly tails had significantly larger soma than those in skinks and anoles.

The six species also differed in soma density in the four regions (LGN: $F_{5,38} = 10.15$, $p < 0.001$; OT: $F_{5,38} = 11.38$, $p < 0.001$; NS: $F_{5,40} = 5.23$, $p = 0.001$; and POA: $F_{5,59} = 86.71$, $p < 0.001$; Figure 7). Tukey's *post hoc* tests showed that anoles, geckos, and skinks had the densest LGN. Anoles had a denser LGN than spiny lizards, whiptails, and curly tails while geckos had a denser LGN than curly tails (Figure 7a). For the OT, whiptail somas were significantly less dense than all other species (Figure 7a). Skinks, geckos, and whiptails had a significantly denser NS than anoles (Figure 7b). Finally, skinks and geckos had the densest POA, followed by spiny lizards and curly tails, then by whiptails, and finally anoles (Figure 7c).

Brain and behavior

The soma size of the NS was positively related to the arcsine transformed percent of chemical displays (standard linear regression: $F_{1,4} = 7.06$, $R^2 = 0.64$, $p = 0.029$, regression with independent contrasts: $F_{1,3} = 3.88$, $R^2 = 0.49$, $p = 0.060$), such that species with larger soma sizes in the NS used chemical communication at a higher percentage than those with smaller soma (Figure 8). There was a significant relationship between the density of the LGN and total number of visual displays per minute (Figure 9), such that species that have a denser LGN perform higher rates of visual displays (standard linear regression: $F_{1,4} = 3.88$, $R^2 = 0.49$, $p = 0.060$, regression with independent contrasts: $F_{1,3} = 4.84$, $R^2 = 0.55$, $p = 0.046$). There were no other significant relationships between soma size or density and behavior (Table 2).

Discussion

In this study of the evolution of visual and chemical communication modalities and brain regions associated with these modalities, I found support for the general hypothesis that brain regions that are used more frequently by a given species have larger or denser neurons than in species that use the region infrequently. One brain region for each communication modality had some type of morphological relationship (i.e., either larger somas or a higher density of somas) with the modality that I hypothesized would be relevant. These results are consistent with the conclusions of Wade (2011), which reviewed the relationships between brain morphology and behavior in lizards and showed that neurons in areas that are used more frequently are larger or denser, with no study finding that neurons in a highly used region were both larger and denser.

My study has shown a significant association between visual communication and density of neurons in the LGN in a phylogenetic context. Interspecific variation in neuron density in this region predicts overall rate of visual displays for a species. Additionally, the phylogenetic relationship of these results suggests that the repeated evolution of visual communication as a primary modality is associated with the evolution of LGN morphology. Since the LGN projects from the retina (the source of visual information) to other brain regions (Aboitiz and Montiel, 2007), having more neurons packed into the region may allow for signals to be sent efficiently to multiple areas of the brain, or for signals to be sent to these regions with variable intensities (i.e., sending a weak signal with fewer neurons versus sending a strong signal with many neurons), which could result in more nuanced behavioral responses.

Conversely, I found no relationship between communication modalities and either soma size or density in the OT. Since the OT is important in receiving retinal projections and sending information to other brain regions (Foster and Hall, 1975) and allowing individuals to orient themselves in response to visual cues (Newman and Hartline, 1981), its function may be too diverse to explain its morphology by relating it to one behavioral type, such as communication. For example, behaviors as fundamental as finding an appropriately sized perch on which to sit so that the individual does not fall off of it require the spatial integration function of the OT. Additionally, integration of visual information and subsequent orientation to external stimuli could be important for capturing moving prey or interacting with an antagonist or potential mate. Since all of these species' diets consist of live prey (although curly tails are partially herbivorous), they must be able to react to their prey item when it is moving through its environment. Territorial behavior also requires spatial orientation, as it requires lizards to keep competing males out of their territory, so they must be able to recognize the location of their territorial boundaries and where a competing conspecific is in reference to those boundaries. Furthermore, even when mates can be found using modalities other than vision (that is, in some species chemical signals can lead an individual to a potential mate), proper orientation in reference to a mate is necessary for copulation to occur. Overall, the important functions of the OT may explain why soma size or density is not a predictor of visual communication behaviors.

I have also shown that there is a significant relationship between chemical communication and the soma size of the NS in a phylogenetic context. Species that communicate with a higher proportion of chemical displays have larger somas than those with a higher proportion of visual displays, and mapping the use of chemical displays on a phylogeny suggests that this relationship has evolved multiple times. The NS is a secondary projection of the VNO (Halpern, 1987), so species that use the VNO frequently (i.e., those that use pheromones to communicate) would use the NS more. Larger neurons in the NS may be able to receive more axonal connections from regions projecting to it, so each neuron would receive more information that it then sends to other regions. Action potentials require energy (Attwell and Laughlin, 2001), which is produced by mitochondria (Kann and Kovacs, 2007). Neurons that fire more frequently may need more mitochondria to allow them a higher energy budget, leading to a larger soma size.

Finally, I found no relationship between neuron size and density of the POA and overall communication behavior. The POA is part of the Social Behavior Network (Newman, 1999) in mammals, birds, fish, and amphibians (Goodson, 2005). Since the Social Behavior Network is comprised of the same brain regions or homologues of these throughout all vertebrates, it stands to reason that its function is very important for helping an individual to survive and/or reproduce. The POA is known to play two important functions in reptiles, controlling copulation and agonistic behaviors (Wheeler and Crews, 1978). Since these behaviors are important for guarding territory and acquiring mates, it makes sense that these behaviors and their underlying mechanisms would be not necessarily show a direct relationship with communication, since that is only one aspect of social interactions.

Although the structure/function relationship of brain morphology and behavior has interested researchers for years, studies investigating this relationship within a phylogenetic context remain quite rare. Brenowitz (1997) is a unique study in that it compared brain morphology and behavior across several species. This study investigated soma size, volume, and number of neurons of brain regions related to song control in six species of birds that differ in their song repertoire size, but the data in this study were not statistically analyzed with a phylogenetic approach. To the best of my knowledge, my study is the first of its kind to look at the relationship between neural morphology and communication behaviors in a phylogenetic context. My finding that neurons in brain regions that are used more often are composed of either larger or denser neurons is one that is consistent across taxa, and thus provides support for the generality of this structure/function relationship. By using explicit evolutionary frameworks in studies of neural morphology, we will be able to more broadly understand the evolutionary relationship between brain and behavior.

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Tables

Table 1. Total hours of behavioral observation per species (Total Obs Time), average observation time per lizard (± SE), and number of brains collected per species.

Species	Total Obs Time (h)	Average Obs Time per Individual (min)	Number of Brains Collected
Green Anole	33.1	25.1 ± 7.0	10
Texas Spiny Lizard	19.6	40.6 ± 16.9	10
House Gecko	21.9	20.6 ± 9.1	10
Spotted Whiptail	18.7	26.0 ± 13.1	10
Curly Tail Lizard	20.3	35.8 ± 18.1	10
Little Brown Skink	9.0	60.0 ± 0.0	8

Table 2. Results of relationships between brain morphology and communication modalities.

Communication type	Brain morphology	F	R^2	p
Percent chemical communication	NS soma size	7.06	0.64	0.03
Visual display rate	LGN density	3.88	0.49	0.06
Visual display rate	LGN soma size	<0.01	<0.01	0.99
Percent visual display	LGN soma size	<0.01	<0.01	0.94
Percent visual display	OT soma size	3.87	0.49	0.12
Chemical display rate	NS density	0.14	0.03	0.73
Percent chemical communication	NS density	0.14	0.03	0.73
Total display rate	POA soma size	1.08	0.21	0.36

Figures

Figure 1. Six lizard species used in this study. A) *Anolis carolinensis*, green anole; B) *Sceloporus olivaceus*, Texas spiny lizard; C) *Aspidoscelis gularis*, spotted whiptail; D) *Leiocephalus carinatus*, curly tail lizard; E) *Hemidactylus turcicus*, Mediterranean house gecko; and F) *Scincella lateralis*, little brown skink. Photographs A-D and F were taken by Michele Johnson; photograph E was taken by Tom Brennan.

Figure 2. Phylogenetic relationship of species included in this study, pruned from tree in Wiens et al. (2012).

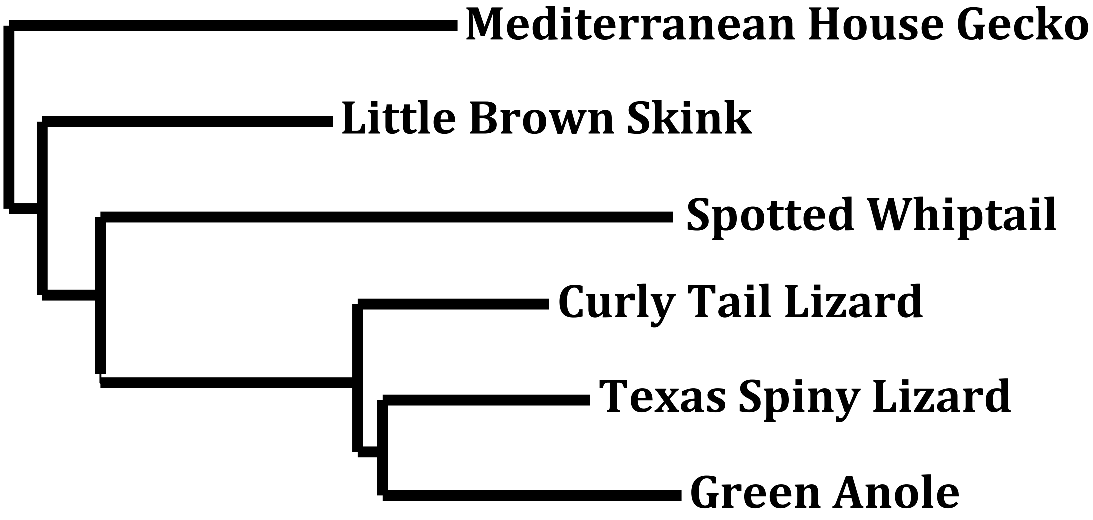


Figure 3. Communication behaviors of the six focal lizard species of this study. Curly tails and anoles use a higher percentage of visual communication than all other species, while whiptails and skinks use the highest percentage of chemical communication. Black represents visual communication, grey represents chemical communication.

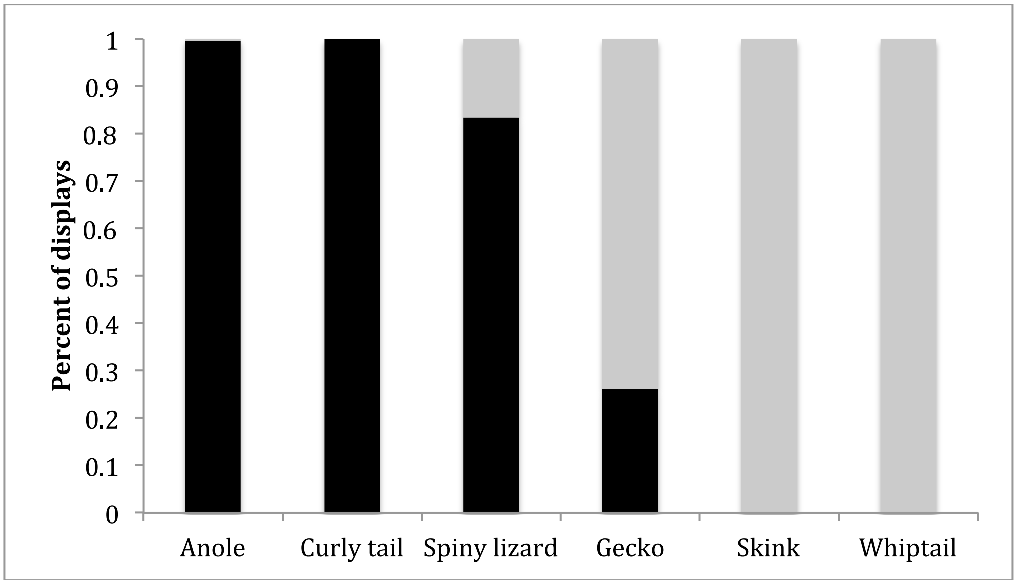


Figure 4. Average visual display rate per minute (±1 SE). Anoles perform visual displays at a higher rate than all other species.

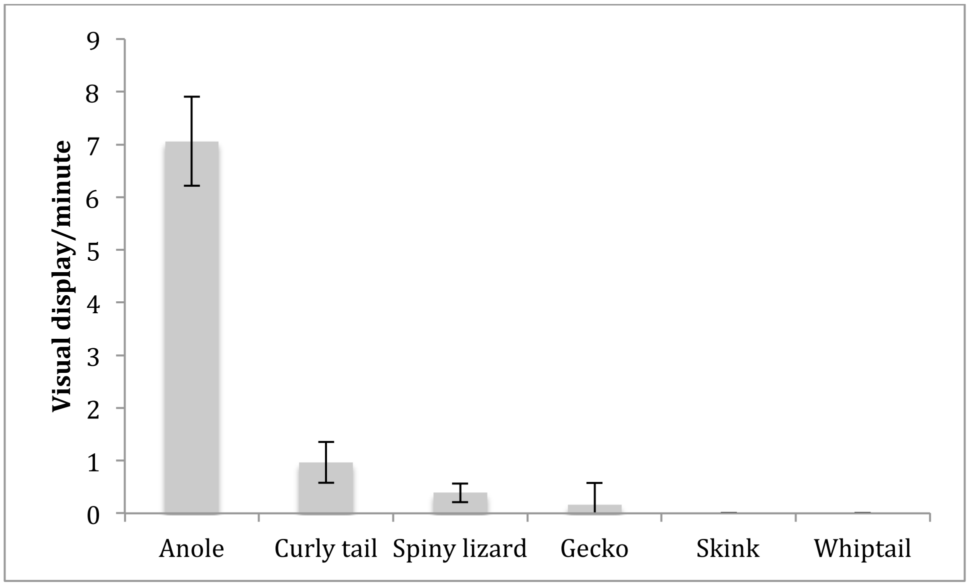


Figure 5. Average chemical display rate per minute (±1 SE). Whiptails display chemical behaviors at a higher rate than all other species, while skinks display chemical behaviors more than geckos, spiny lizards, anoles, and curly tails.

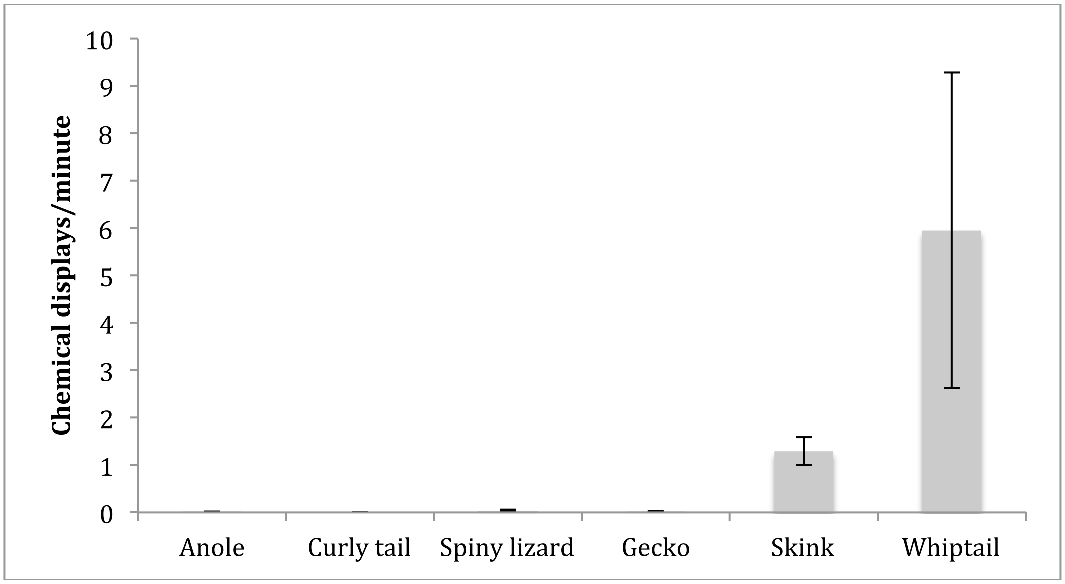


Figure 6. Average soma size (± SE) of a) the lateral geniculate nucleus and optic tectum, b) the nucleus sphericus, and c) the preoptic area.

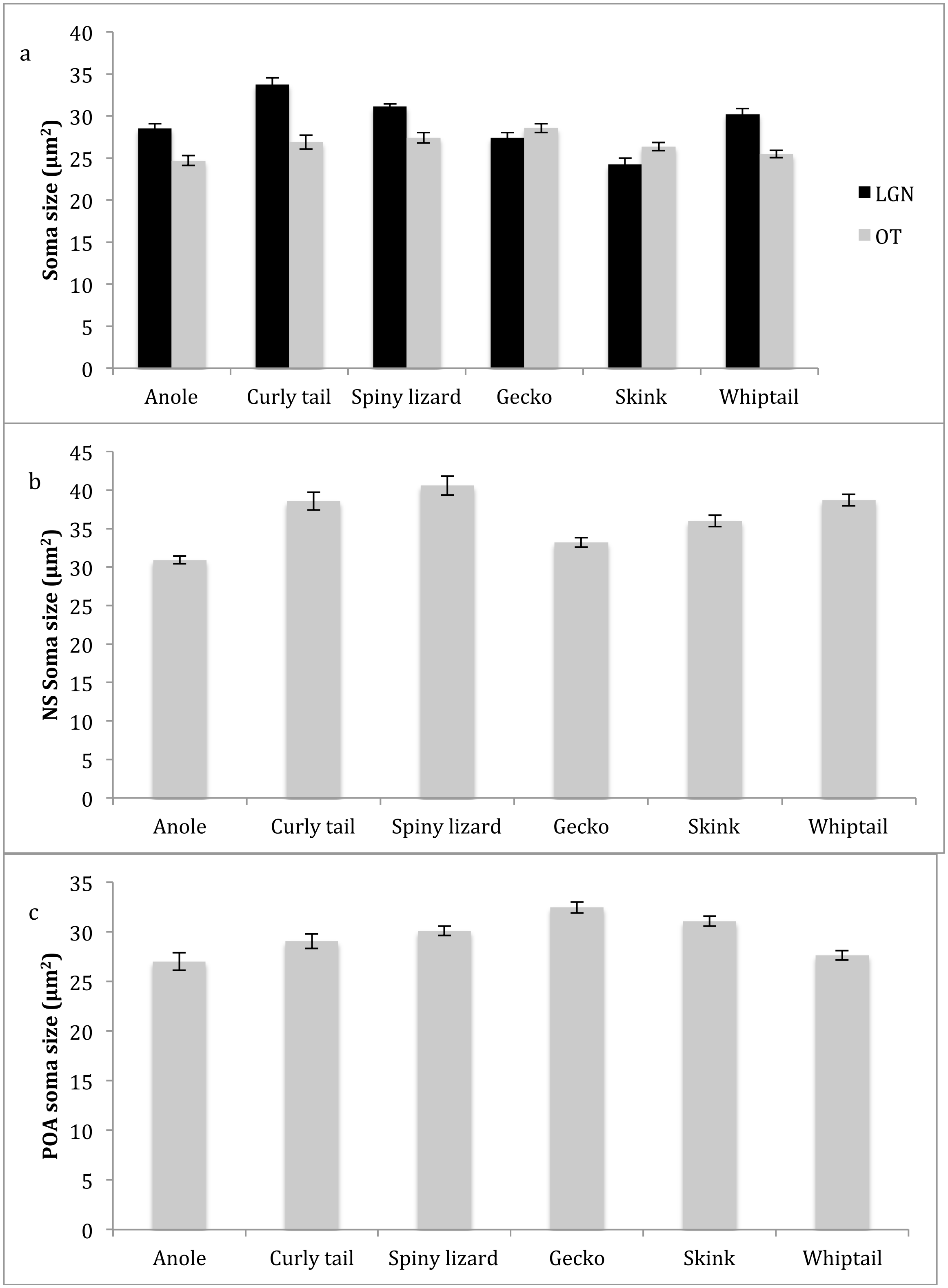


Figure 7. Average density (± SE) of a) the lateral geniculate nucleus and optic tectum, b) the nucleus sphericus, and c) the preoptic area.

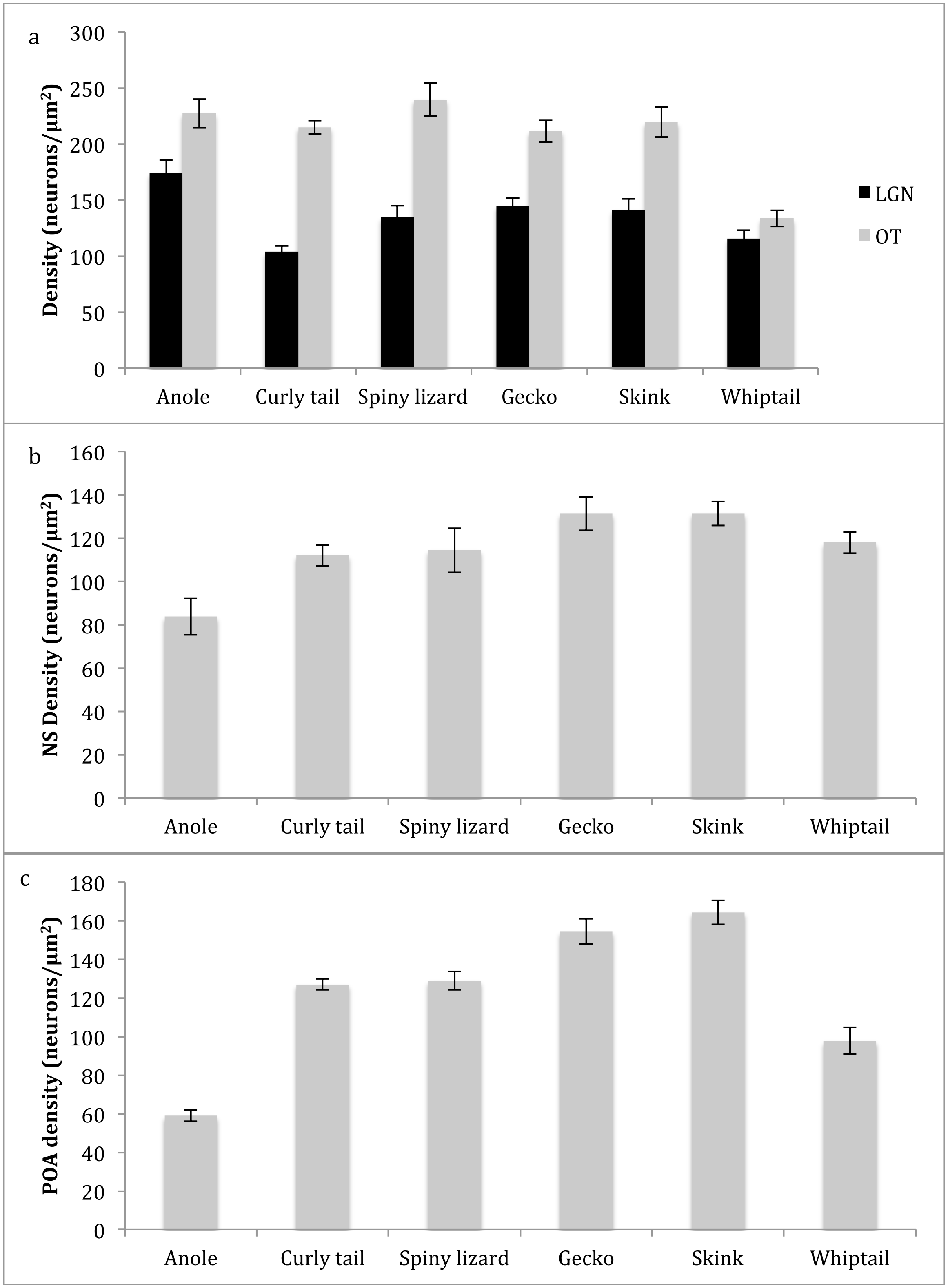
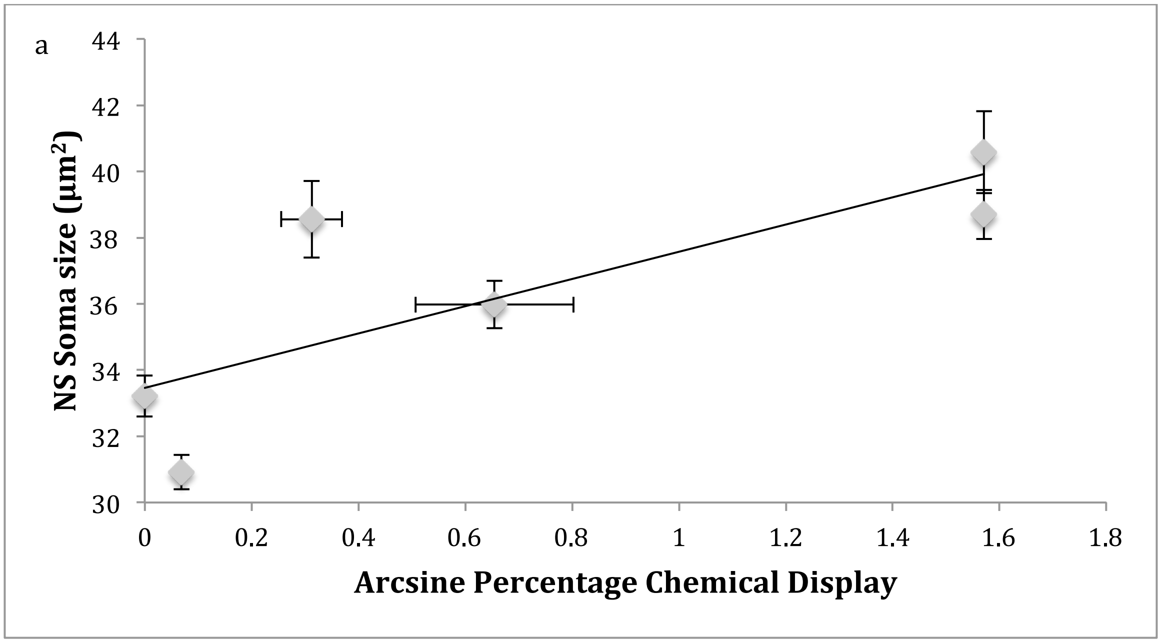


Figure 8. a) Average soma size (± SE) of the nucleus sphericus for each species plotted against the arcsine transformed percent chemical display (± SE). b) Contrasts of NS soma size plotted against the contrasts of the arcsine transformed percent chemical display, with regression line forced through the origin. Contrast data were calculated using phylogeny in Figure 2 and positivized for graphical visualization.



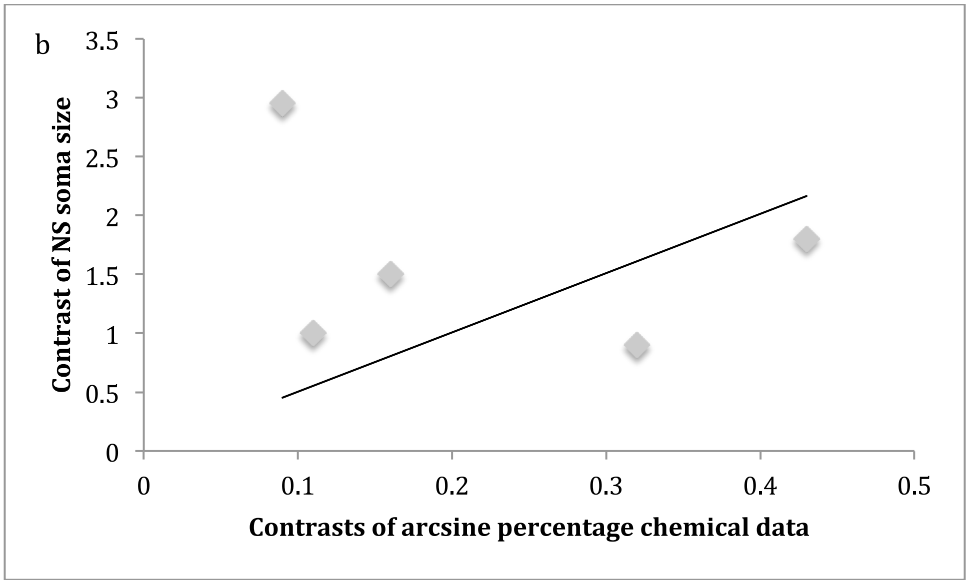


Figure 9. a) Average density (±SE) of the lateral geniculate nucleus for each species plotted against the rate of visual display (±SE). b) Contrasts of LGN soma density plotted against the arcsine transformed percent chemical display, with regression line forced through the origin. Contrast data were calculated using the phylogeny in Figure 2 and positivized for graphical visualization.