Neuroanatomical Changes Related to a Changing Environment in Lesser Earless Lizards

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Brain–environment interactions have been examined mostly in artificial laboratory settings with classic model species; however, studies on nonmodel species in the wild have provided a new understanding of how brain morphology is linked to the environment (e.g., Jacobs et al., 1990; Sherry et al., 1992; Day et al., 2001; Powell and Leal, 2012; Roth et al., 2012). In a rapidly changing world, the study of neuromorphological plasticity or microevolution in response to environmental shifts is essential (e.g., Roth and Pravosudov, 2009; Freas et al., 2013; Powell and Leal, 2012; Roth et al., 2012). Studies on nonmodel species in the wild have provided a new understanding of how brain morphology may respond to changing environments.

**How do changes in the environment affect the brain? More specifically, how do different physical environments shape areas of the brain that facilitate the contextual perception of animals’ surroundings?** We evaluated the relationship between rapid habitat change and the volume of the medial, lateral, and dorsal cortices, in two populations of wild male and female Lesser Earless Lizards (*Holbrookia maculata*). These populations inhabit neighboring, but physically distinct, environments in southern New Mexico: White Sands and dark soil habitats. The White Sands habitat is a geologically recent environment that formed over the last 2,000–7,000 yr. There is a stark physical contrast between White Sands and the surrounding typical Chihuahuan desert habitat that is characterized by dark soil, denser vegetation, and higher competitor and predator species abundances. We observed differences in the volumes of the medial cortex, an important neuromorphological structure for navigation, but not in those of the dorsal or lateral cortices, between lizards inhabiting the White Sands and dark soil habitats. Male and female lizards in the White Sands habitat had smaller medial cortices (relative to their overall brain size) compared to those in the dark soil environment. We also found sex differences in the volume of the medial cortex; in the dark soil habitat, males had larger medial cortices than females. In summary, we have uncovered differences in neuromoronomy between habitats and between sexes in a wild species of lizard, which may reflect neuromorphological plasticity and/or rapid evolutionary change in response to a geologically recent environmental change.

**ABSTRACT.**—How do changes in the environment affect the brain? More specifically, how do different physical environments shape areas of the brain that facilitate the contextual perception of animals’ surroundings? We evaluated the relationship between rapid habitat change and the volume of the medial, lateral, and dorsal cortices, in two populations of wild male and female Lesser Earless Lizards (*Holbrookia maculata*). These populations inhabit neighboring, but physically distinct, environments in southern New Mexico: White Sands and dark soil habitats. The White Sands habitat is a geologically recent environment that formed over the last 2,000–7,000 yr. There is a stark physical contrast between White Sands and the surrounding typical Chihuahuan desert habitat that is characterized by dark soil, denser vegetation, and higher competitor and predator species abundances. We observed differences in the volumes of the medial cortex, an important neuromorphological structure for navigation, but not in those of the dorsal or lateral cortices, between lizards inhabiting the White Sands and dark soil habitats. Male and female lizards in the White Sands habitat had smaller medial cortices (relative to their overall brain size) compared to those in the dark soil environment. We also found sex differences in the volume of the medial cortex; in the dark soil habitat, males had larger medial cortices than females. In summary, we have uncovered differences in neuromoronomy between habitats and between sexes in a wild species of lizard, which may reflect neuromorphological plasticity and/or rapid evolutionary change in response to a geologically recent environmental change.

**RESUMEN.**—¿Cómo afectan los cambios en el ambiente al cerebro? ¿Específicamente, cómo moldean los distintos ambientes físicos las áreas del cerebro que facilitan la percepción contextual del entorno de los animales? Evaluamos la relación entre cambios repentinios en el hábitat y el volumen de las cortezas mediales, laterales, y dorsales en dos poblaciones silvestres de machos y hembras de las lagartijas Holbrookia maculata. Estas poblaciones habitan ambientes vecinos, pero físicamente disímiles en el sur de Nuevo México, EUA: las Arenas Blancas y suelos oscuros. Las Arenas Blancas son un hábitat geológicamente reciente que se formó a través de los últimos 2,000–7,000 años. Hay un contraste físico severo entre las Arenas Blancas y el entorno típico de Chihuahua, el cual se caracteriza por suelos oscuros, vegetación densa, y densidades mayores de competidores y depredadores. Observamos diferencias en el volumen de la corteza medial, una estructura neuromorfológica importante para la navegación, pero no en la dorsal o lateral, entre lagartijas que moraban en los hábitats de suelo oscuro versus las que habitaban las Arenas Blancas. Las lagartijas hembras y machos de las Arenas Blancas tenían cortezas mediales más pequeñas (relativo al tamaño cerebral total) comparadas con las que habitan en el ambiente de suelos oscuros. También encontramos diferencias en el volumen de la corteza medial entre los sexos; en el hábitat de suelos oscuros los machos tenían cortezas mediales más grandes que las hembras. Resumiendo, hemos descubierto diferencias en la neuromorofía entre hábitats y sexos en un especie silvestre de lagartija, lo cual puede indicar plasticidad neuromorfológica y/o cambio evolutivo veloz en respuesta a un cambio ambiental geológicamente reciente.
pus, is an important neuroanatomical structure for navigation (Hoogland and Vermeulen-Van der Zee, 1987; Day et al., 2001). The positive relationship, in which there is a greater cell expanse in the medial cortex of species that inhabit more spatially complex and demanding habitats, has been reported in other lizards (Powell and Leal, 2012), birds (Roth et al., 2012), bats (Safi and Dechmann, 2005), fish (Shumway, 2008), and humans (Maguire et al., 2000). Because of the dramatic differences in the habitats inhabited by Lesser Earless Lizards, we hypothesized that there would be differences in the size of the medial cortex in the White Sands and dark soil ecomorphs. The White Sands habitat has less vegetation, reduced species richness, and abundance of competitors and predators (Des Roches et al., 2011; Rosenblum and Harmon, 2011). For example, White Sands contains about half of the amount of yucca trees and shrubs as compared to the surrounding Chihuahuan desert, and three species of lizards as compared to 35 species of reptiles in the Chihuahuan desert. Therefore, we predicted that the White Sands lizards, living in a habitat with potentially lower spatial and biological complexity, would have smaller medial cortices, compared to their ancestral dark soil counterparts. We also examined adjacent brain areas, the dorsal and lateral cortices (Fig. 2) for lizards in contrasting habitats, as these areas may also be related to the ecological use of space (Day et al., 1999).

**Materials and Methods**

**Sample Collection.**—We collected 18 adult *H. maculata* specimens, 9 from the White Sands habitat (4M : 5F; White Sands National Monument, Otero County, New Mexico) and 9 from the dark soil habitat (4M : 5F; Jornada Experimental Range, Don Ana County, New Mexico and White Sands Missile Range, Lincoln County, New Mexico), during the summer of 2013. We collected lizards with the use of a common noosing technique. To obtain brain samples, we rapidly decapitated the lizards and made incisions in the skull to expose the brain. We then incubated the brains in a 4% paraformaldehyde solution for approximately 24 h. Following this fixation process, we dissected out the brains from the lizards’ skulls, and then cryoprotected them in a 30% sucrose-azide solution at 4°C, until we had returned them to the laboratory at the University of California, Berkeley. In the laboratory, the samples were frozen at −80°C until further processing the following month. To confirm that animals were adults, we examined the size of gonads (large indicated reproductively mature, versus tiny/regressed) and snout-to-vent length (SVL) (>44 mm is indicative of an adult; Rosenblum, 2008).

**Sectioning and Staining.**—We sectioned whole brains coronally into 30-μm-thick sections, on a CM3050S cryostat (Leica Microsystems, Wetzlar, Germany) at −20°C. Then we mounted every fifth section directly on silane-coated slides and stained them using a standard cresyl violet staining protocol (Day et al., 2001; Powell and Leal, 2012). Briefly, we incubated the slides, with the...
sections mounted, in 100% chloroform for 10 min and then progressively rehydrated them in 100%, 95%, 90%, and 70% ethanol for 3 min each. Then, we incubated the slides in deionized water for 5 min, after which we stained them with 0.1% cresyl violet for 10 min. The mounted sections were again dehydrated sequentially in 70%, 90%, 95%, and then 100% ethanol. Finally, we placed the mounted sections in xylene for 5 min and cover-slipped them with Krystalon mounting medium (Thermo-Fisher Scientific, Inc., Waltham, Massachusetts).

**Image Capture and Analysis.**—With the use of a double-blind technique, we visualized the brain sections under an Axioimager M1 bright field microscope (Carl Zeiss Mfg., Oberkochen, Germany) and then photographed them with an Axiocam M1bright field microscope (Carl Zeiss Mfg.). Then, with the use of Photoshop CS6 (Adobe Systems, Inc., San Jose, California), we traced the area of the left side of the medial, dorsal, and lateral cortices for each brain, starting from first detection of the areas until final detection, as well as the area of the entire left hemisphere of the brain (Fig. 2). We measured these outlined areas of interest with the use of ImageJ (National Institutes of Health; 64-bit mode). To control for overall brain size, we divided the medial, dorsal, and lateral cortices in 100% chloroform for 10 min and then cover-slipped them with Krystalon mounting medium (Thermo-Fisher Scientific, Inc., Waltham, Massachusetts).

**Discussion**

We set out to determine whether differences in neuroanatomical structures were associated with habitat differences for diverging populations of wild lizards. Specifically, we examined differences in the volume of the medial, dorsal, and lateral cortices in *H. maculata* populations inhabiting two dramatically different environments: the typical dark soil habitat of the Chihuahuan Desert and the geologically recent White Sands formation. We found predictable differences in the neuroanatomy of the medial cortex between the ecomorphs and between the sexes. This finding is compelling because it suggests that structural aspects of the brain can change in response to habitat changes in wild populations of animals.

**Results**

We confirmed all animals to be adults. All animals had large gonads, indicative of sexual maturity. There were no significant differences between population SVLs (*P* = 0.168); mean ± SD SVL = 49.83 ± 5.64 mm (dark soil) and 52.83 ± 4.58 mm (White Sands).

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**Table 1.** The main effect of sex and habitat on the relative volumes of the medial, dorsal, and lateral cortices (results from the two-way ANOVAs).

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Experimental factor</th>
<th>Degrees of freedom (df)</th>
<th>Significance</th>
<th>Partial eta-squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial</td>
<td>Sex</td>
<td>1</td>
<td>0.027*</td>
<td>6.221</td>
</tr>
<tr>
<td></td>
<td>Habitat</td>
<td>1</td>
<td>0.031*</td>
<td>5.847</td>
</tr>
<tr>
<td></td>
<td>Sex * Habitat</td>
<td>3</td>
<td>0.77</td>
<td>3.690</td>
</tr>
<tr>
<td>Dorsal</td>
<td>Sex</td>
<td>1</td>
<td>0.729</td>
<td>0.126</td>
</tr>
<tr>
<td></td>
<td>Habitat</td>
<td>1</td>
<td>0.226</td>
<td>1.616</td>
</tr>
<tr>
<td></td>
<td>Sex * Habitat</td>
<td>3</td>
<td>0.746</td>
<td>0.110</td>
</tr>
<tr>
<td>Lateral</td>
<td>Sex</td>
<td>1</td>
<td>0.184</td>
<td>1.964</td>
</tr>
<tr>
<td></td>
<td>Habitat</td>
<td>1</td>
<td>0.529</td>
<td>0.418</td>
</tr>
<tr>
<td></td>
<td>Sex * Habitat</td>
<td>3</td>
<td>0.715</td>
<td>2.053</td>
</tr>
</tbody>
</table>

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**Table 2.** Analyses of the interactions between sex and habitat on the relative volumes of the medial, dorsal, and lateral cortices (results from Tukey’s HSD post hoc tests).

<table>
<thead>
<tr>
<th>Sex*Habitat</th>
<th>Medial (P)</th>
<th>Dorsal (P)</th>
<th>Lateral (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Sands female</td>
<td>0.983</td>
<td>0.902</td>
<td>0.939</td>
</tr>
<tr>
<td>(WS M)</td>
<td>0.975</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Dark soil female</td>
<td>0.983</td>
<td>0.902</td>
<td>0.939</td>
</tr>
<tr>
<td>(ds F)</td>
<td>0.975</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>White Sands male</td>
<td>0.975</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>(WS M)</td>
<td>0.975</td>
<td>1.000</td>
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<tr>
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<td>0.983</td>
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<td>0.939</td>
</tr>
<tr>
<td>(ds M)</td>
<td>0.975</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

Our models revealed that the relative volume of the medial cortex depended on sex (*F*₁,₁₇ = 6.221, *P* = 0.027) and habitat (*F*₁,₁₇ = 5.847, *P* = 0.031; Table 1). The effect size was large for both sex (*ηₚ² = 0.324) and habitat (*ηₚ² = 0.310). Specifically, dark soil males had larger medial cortices than dark soil females (*P* = 0.041), White Sands males (*P* = 0.045), and White Sands females (*P* = 0.016; Table 2; Fig. 3). The relative volume of the dorsal and lateral cortices did not depend on sex, habitat, or their interaction (Table 1; Fig. 3). Although there were no significant differences found for the lateral cortex there was a medium effect size for sex (*ηₚ² = 0.136) and the interaction of sex and habitat (*ηₚ² = 0.131) in this brain region.
One hypothesis regarding the mechanism underlying the observed neuroanatomical differences between the lizards in the different habitats is that natural selection may be acting on the medial cortex to increase fitness for the individuals living in the ancestral environment. Comparative studies in lizards, birds, and mammals have reported that the size of the homologous structure of the hippocampus is affected by selective pressures, to the degree by which the individual relies on spatial information to locate and utilize resources (e.g., Sherry et al., 1992; Powell and Leal, 2012; Roth et al., 2012). Assuming that there is a cost to maintaining neural substrates (Jerison, 1975), selection should not favor the development and maintenance of a larger than necessary brain structure. For example, if White Sands H. maculata (particularly males) have a reduced need for navigational abilities or spatial processing in their seemingly less complex environment, the size of the medial cortex may diminish over an individual’s lifespan, or through evolutionary time. Alternatively, growth in this region, resulting from postnatal neurogenesis, may occur to a lesser degree than in the ancestral habitat. In either event, the question remains as to how H. maculata actually perceive habitat complexity and whether differences observed in the medial cortex volume are heritable and have direct consequences on fitness.

In contrast to the strong patterns observed for the medial cortex, we did not find significant population or sex differences in the dorsal and lateral cortex volumes. The dorsal cortex, homologous to regions of the mammalian hippocampus, has been associated with differences in the use of space (Day, 1999; LaDage et al., 2009), whereas the lateral cortex has been found to be homologous to the mammalian olfactory cortex (Martinez-Garcia et al., 1986). Although we found no significant results for either of these brain regions, we observed a trend for males from the dark soil habitat to have larger relative lateral cortex volumes, compared to the other groups (Fig. 3, medium effect size). Further work will be necessary to understand whether the environment truly affects the dorsal and lateral cortices in this system.

In summary, we identified a volumetric difference of the medial cortex in lizards associated with a recent environmental shift. We also identified differences in this neuroanatomical structure between the sexes. In a wild population of animals, we offer support for the hypothesis that the environment—and the way that organisms are influenced by their environment—can shape neuroarchitecture. Understanding the specific neural mechanisms responsible for observed differences in medial cortex volume could be gained by incorporating additional...
metrics, such as neuron size and abundance and variation in cell connectivity (Roth et al., 2010). Understanding the ecological, evolutionary, and developmental mechanisms responsible for observed patterns also requires further work. We have not yet formally evaluated the relative contribution of adaptive genetic change, genetic drift, and developmental plasticity to the observed neuroanatomical changes, and common garden breeding experiments would be an important next step; however, our data demonstrate the importance of studying the brain’s interaction with rapidly changing environments. Our findings also emphasize the potential that sex-specific neuroanatomical patterns may be more widespread in vertebrates than currently appreciated; therefore, evaluating males and females simultaneously, something that has been rarely done in neurobiological studies, is important (Zucker and Beery, 2010; Beery and Zucker, 2011; Clayton and Collins, 2014).

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