Differential Substructure Contribution to Medial Cortex Volume in Eastern Fence Lizards

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Background

- The hippocampus is a highly plastic area of the brain; volume is often used as a measure of plasticity (Yin et al., 2011).
- Overall volume, by itself, may not represent physiological or anatomical changes that result via dendritic branching, apoptosis, or production of neurons and glial cells.
- Non-homogenous hippocampal substructures may be differentially modulated (Ulinski, 1990).
- We sought to understand the differential contributions of medial cortex substructures to the overall volume of the eastern fence lizard's (*Sceloporus undulatus*) hippocampal equivalent (medial cortex).

Methods

- Brain tissue from two studies using male eastern fence lizards were used.
  - **Study 1:** Lizards were subjected to weekly corticosterone application from 2 weeks of age until 42 weeks of age (*n* = 10).
  - **Study 2:** One dose of corticosterone or vehicle control was exogenously applied to the surface of unhatched eggs (*n* = 10).
- Between nine and ten months of age, subjects were euthanized and brains were removed.
- Specimens were sectioned at 50 um and Nissl-stained with thionin (Figure 1A).
- First, overall medial cortex volume of all individuals were contoured blind to treatment group. Unbiased stereological Cavalieri procedure optimized for this species was then used to estimate the overall volume of the medial cortices.
- Next, medial cortex substrates were outlined blind to treatment group and volumes were estimated using unbiased stereological Cavalieri procedure (Figure 1B).
  - I. **Cell layer:** Soma-dense area containing projection and inter-neurons
  - II. **Outer plexiform layer:** Soma-sparse area composed of neuronal and glial projections
  - III. **Inner plexiform layer:** Soma-sparse area distinguished by presence of migrating immature neurons.

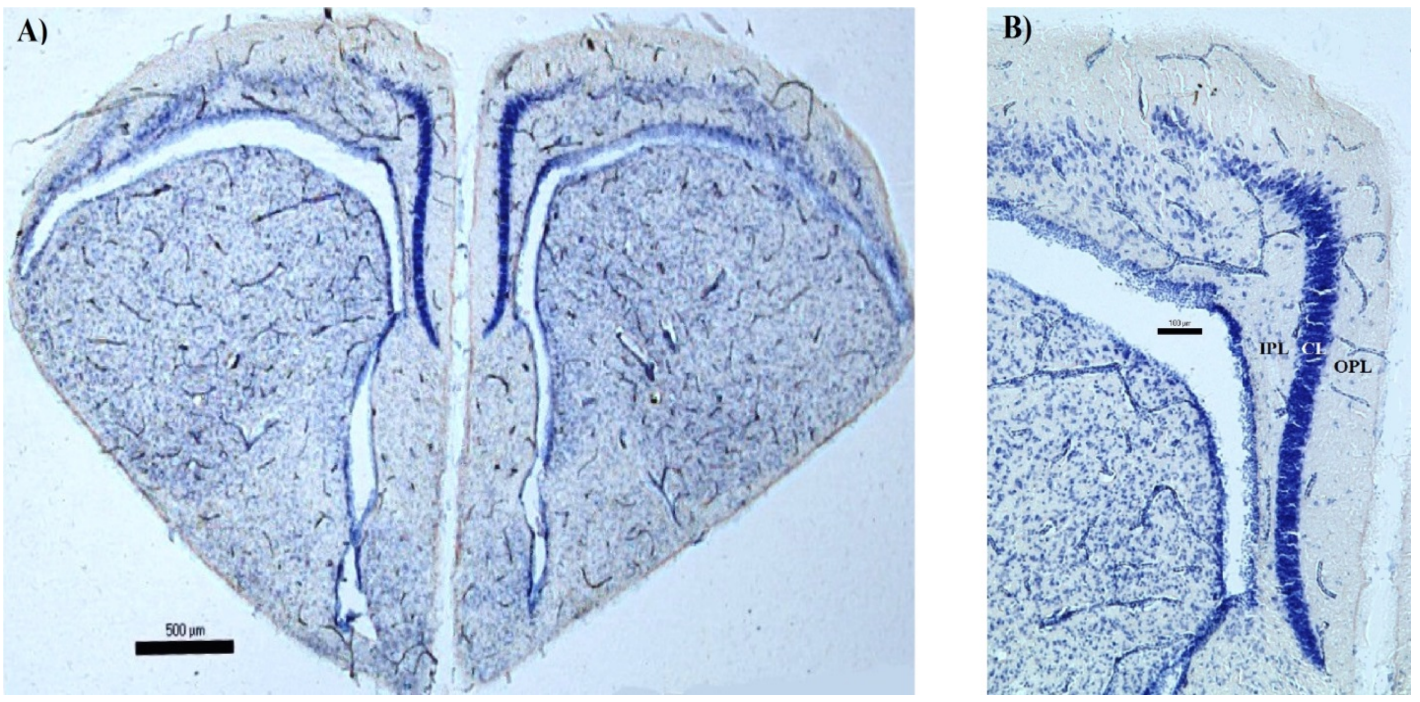

Figure 1. [a] *Nissl-stained section showing the medial cortices and remainder of the telencephalon.* [b] *Medial cortex with labeled substructures. IPL, inner plexiform layer; CL, cell layer; OPL, outer plexiform layer.*

Results

- Overall medial cortical volumes
  - **Study 1:** Experimental lizards had significantly smaller medial cortices compared to control lizards when controlling for body size, $F_{(1, 7)} = 6.18$, $p = .042$ (Table 1).
  - **Study 2**: There were no differences in medial cortex volume between experimental and control lizards when controlling for body size, $F_{(1, 7)} = 1.595$, $p = .247$ (Table 1).
- Substructure Comparisons
  - **Study 1:** Experimental lizards had significantly smaller cell layers, ($F_{(1, 9)} = 5.83$, $p = .042$) and inner plexiform layers ($F_{(1, 9)} = 9.70$, $p = .014$) than control animals. Outer plexiform layers ($F_{(1, 9)} = 2.33$, $p = .165$) did not vary between experimental and control specimens (Table 2).
  - **Study 2:** No significant differences between cell layer, inner plexiform layer, or outer plexiform layer volumes were observed (all $p \geq .332$) (Table 2).

Medial Cortex Volume	Control	Experimental	*p*
Study 1	1.54 mm³	1.21 mm³	*0.042*
Study 2	0.77 mm³	0.67 mm³	*0.247*

Table 1. *Comparisons of average overall medial cortex volume for study 1 and study 2 by experimental condition.*

Variable	Control	Experimental	*p*
*Cell Layer	.25 mm³	.20 mm³	*0.042*
*Inner Plexiform Layer	.46 mm³	.33 mm³	*0.014*
*Outer Plexiform Layer	.83 mm³	.68 mm³	0.165
**Cell Layer	.17 mm³	.15 mm³	0.440
**Inner Plexiform Layer	.24 mm³	.21 mm³	0.350
**Outer Plexiform Layer	.36 mm³	.31 mm³	0.332

Table 2. *Comparisons of medial cortex substructure volumes by study and condition. * = Study 1; ** = Study 2*

Discussion

- These results indicate that changes in inner plexiform and cell layer volumes contribute to overall fluctuations in medial cortex volume.
- Furthermore, the data suggests that null changes in medial cortex volume may not be the product of counterbalancing changes in substructure volume.
  - ie., decrease in cell layer volume masked by increases in inner plexiform layer volume.
- Cumulatively, these results demonstrate the need to analyze substructure volumes when estimating overall changes in structure volume.
- **Limitations:**
  - The sample sizes of each study (*n* = 10) were relatively small.
- **Future Research:**
  - Account for fluctuations in substructure volume when assessing volume changes in larger structures.
  - Assess other variables that contribute to cortical plasticity (e.g., new neuron production, dendritic branching).
  - Investigate potential masking effects of inverse substructure volume changes when faced with null overall volume changes.

References

Ulinski, P. (1990). The cerebral cortex of reptiles. In EG Jones, A Peters (Eds.) *Cerebral Cortex, Vol. 8A: Comparative Structure and Evolution of Cerebral Cortex Part I* (pp. 139-215). Plenum, New York.

Yin, J., Turner, G., Lin, H., Coons, S., & Shi, J. (2011). Deficits in spatial learning and memory is associated with hippocampal volume loss in aged apolipoprotein E4 mice. *Journal of Alzheimers Disease, 27*, 89-98.