Removing contrast: combining iodine-based contrast-enhanced computed tomography with serial immunostaining

Paul Heller1,2, Nikita McKnight2, Kathy Jacyniak2, Matthew Vickaryous2

1 Justus-Liebig-University Giessen, Giessen, Hessen, (Heller)
2 Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON (Heller, McKnight, Jacyniak, Vickaryous)
3 Human Health and Nutritional Sciences, College of Biological Sciences, University of Guelph, Guelph, On (Siebenaller)

INTRODUCTION

Diffusible iodine-based contrast-enhanced computed tomography (diceCT) is an increasingly common tool used to study formalin-preserved soft tissues non-invasively. This technique involves incubated specimens in iodine-based solutions. Whether specimens subjected to diceCT can then be re-used for further histological and immunostaining investigations has not been well-explored. Here we present a workflow for iodine staining and destaining, with subsequent immunostaining in a reptile, the leopard gecko (*Eubelpharis macularius).*

Figure 1. The leopard gecko (*Eublepharis macularius*). Geckos have a wide repertoire of regenerative abilities, including the tail, skin, and more recently the brain.

METHODS

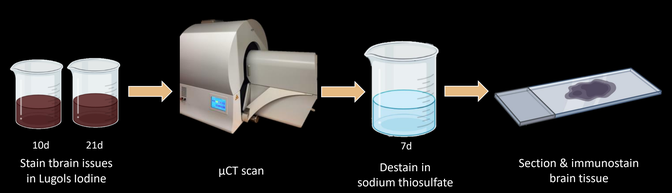

Figure 2. Methods. Two gecko heads were formalin-fixed for 24 hours, then stored in 70% ethanol. Each head was bisected , and one side was incubated in 1.25% w/v solution Lugols iodine for either 10 or 21 days. Samples were then µCT scanned, then de-stained with 1% w/v sodium thiosulfate for 7 days. Next, both treated (right side) and control (left side) tissues were processed for routine paraffin histology, sectioned at 5 µm and mounted on microscopy slides. A panel of protein markers were used to confirm immunoreactivity in each group.

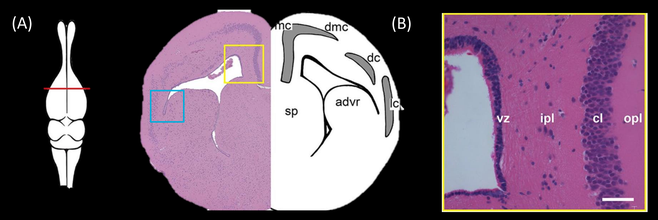

Figure 3. Anatomy of the gecko brain. (A) Transverse section through the telencephalon of the gecko brain, at the level of the medial cortex, stained with hematoxylin and eosin (red line = level of section), yellow box indicates position of (B). (B) ependymal sulcus cortex. The ventricular zone of the sulcus septomedialis is separated from the neuron-rich cellular layer of the medial cortex by a cell-sparse inner plexiform layer. The outer plexiform layer separates the cellular layer from the pial surface of the brain. Scale bar: 20 µm. advr = anterior dorsal ventricular ridge, cl = cellular layer, dc= dorsal cortex, dmc= dorsal medial cortex, ipl= inner plexiform layer, lc = lateral cortex, mc = medial cortex, opl= outer plexiform layer, sp = septum, vz = ventricular zone. Yellow and blue box indicate image positioning of figure 4. Taken from McDonald & Vickaryous, 2018.

IMMUNOREACTIVITY IS MAINTAINED FOLLOWING IODINE TREATMENT

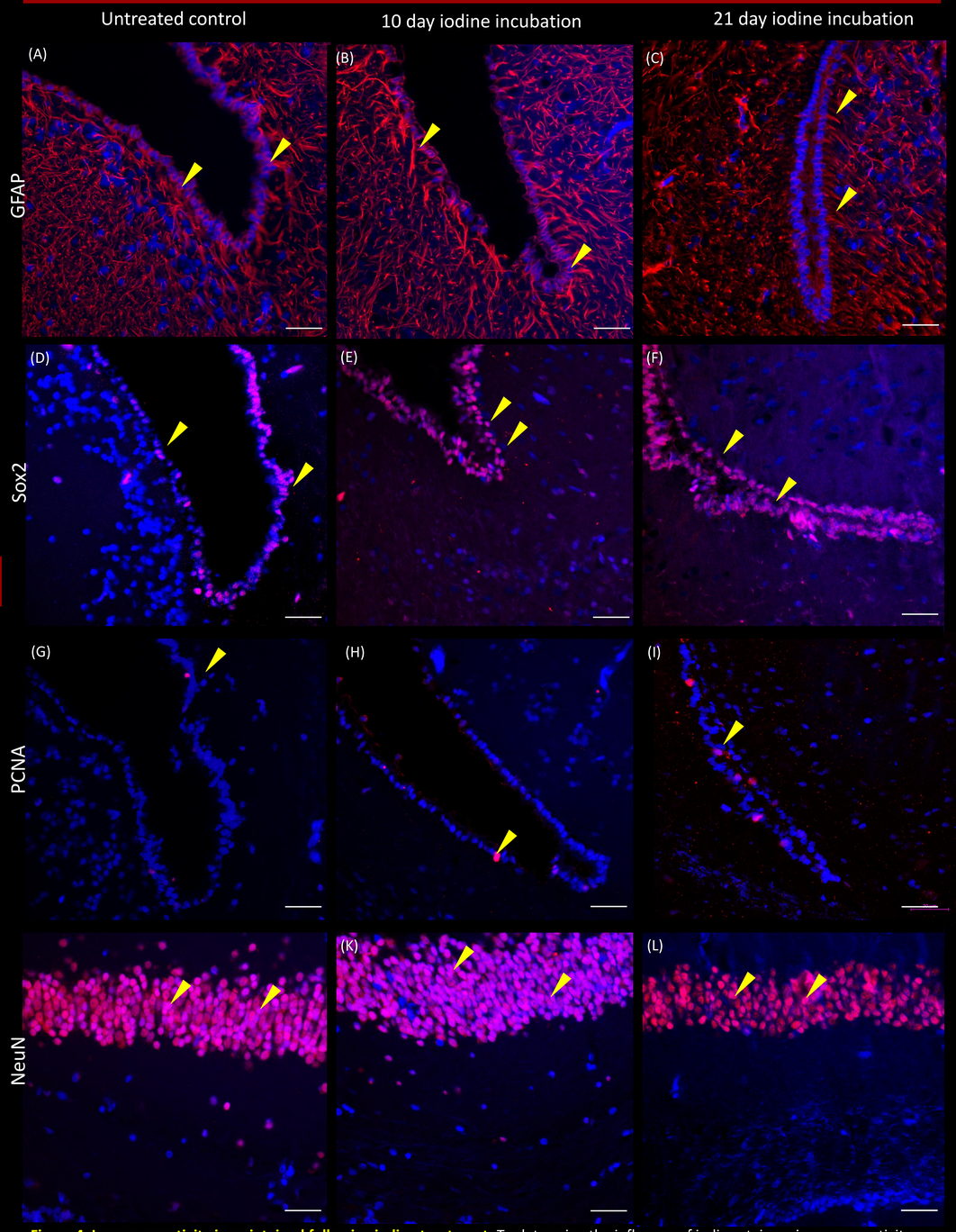

Figure 4. Immunoreactivity is maintained following iodine treatment. To determine the influence of iodine stain on immunoreactivity, gecko heads were subjected to (A,D,G,J) no iodine (B,E,H,K) 10 day or (C,F,I,L) 21 day iodine incubation, destained, and subsequently immunostained. A panel of protein markers were used in this study, including: proliferating cell nuclear antigen (PCNA; A-C), SRY-Box Transcription Factor 2 (Sox2; D,E,F), Glial fibrillary acidic protein (GFAP; G,H,I), and neuronal nuclear protein (NeuN; J,K,L). (A-F) GFAP+, Sox2+ stem cells are located lining the ventricular zone and spontaneously proliferate (G-I, PCNA+ cells indicated by arrowheads), whereas mature NeuN+ neurons reside in the cellular layer (J-L). Across all groups, immunoreactivity is maintained and does not appear to be affected by iodine treatment. Scalebar: 20µm. DAPI = 4',6-diamidino-2-phenylindole

3D RECONSTRUCTION OF THE GECKO BRAIN

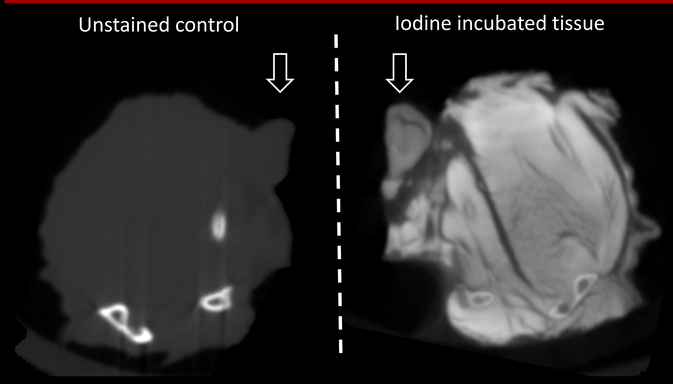

Figure 5: diceCT images of the leopard gecko brain (transverse view). µCT scans of the gecko brain and head, comparing unstained controls with 10d iodine stained tissues. Iodine provides soft tissue contrast, allowing enhanced visualization of organs and associated structures, such as the brain (arrow).

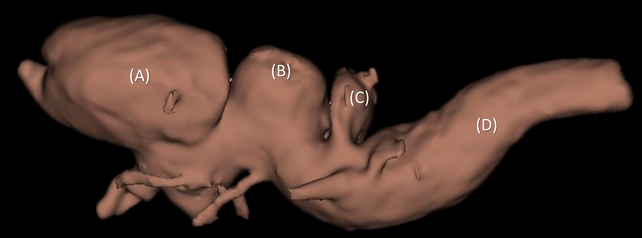

Figure 6: 3D Reconstruction of the gecko brain using 3D slicer (lateral view). Idoine-treated tissues for 10 days were imported into 3D slicer to generate a 3D reconstruction of the brain. Cerebral Hemisphere(A) Optic Lobe(B) Cerebellum(C) Medulla oblangata (D)

CONCLUSIONS

Our findings demonstrate that the immunoreactivity of reptile brain tissues can be restored following diceCT-treatment. Further, we present a methodological workflow to generate digital 3D models and details of protein localization within tissues from a single specimen.

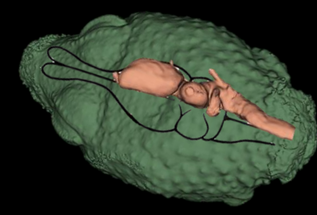

Figure 6: diceCT scanned head and brain. Future work will conduct quantitaive morphometric analyses to determine the direct effects of iodine on tissue specimens.

Boehringer Ingelheim
INNOVATION.CA CANADA FOUNDATION FOR INNOVATION | FONDATION CANADIENNE POUR L'INNOVATION
NSERC CRSNG