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Using Magnetic Resonance Imaging (MRI) to Obtain Shapes and Sizes of Pinniped and Cetacean Brain Regions that Depend on Thyroid Hormones for Maturation

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Overview

Thyroid hormones (TH) play an integral role in neuro-development, particularly in the maturation of the corpus callosum, cerebellum, hippocampus, and inner ear (1). In rodents, persistent organic pollutants (POPs), particularly polychlorinated biphenyls (PCBs), interfere with TH signaling (2). These pollutants are widespread in the marine environment and biomagnify in marine mammals to very high levels (3). Brominated flame-retardants (BFRs) have also recently been shown to interfere with the TH system in experimental animals (4). Although at the present time BFR levels are much lower than PCBs, BFRs have been shown to biomagnify in marine mammals (5). Hence, there is concern that PCBs and BFRs with similar mechanisms of toxicity may affect neuro-development of marine mammals.

Little is known about the variability of absolute and regional brain shape and size within a population of marine mammals and among species. Magnetic resonance (MR) imaging has recently been used by other researchers to study the neuroanatomy of the fetal common dolphin, the bottlenose dolphin, the harbor porpoise, and the beluga whale (6-9). The benefit of this technique is the non-destructive and non-invasive acquisition of external and internal brain structure data, which minimizes dissection artifacts and allows more accurate determination of regional brain shapes and sizes. We are using x-ray computed tomography (CT) and MR imaging to better understand brain shape and size variability in both pinnipeds and cetaceans. Our research plan is to obtain total brain, hippocampus, cerebellum, and corpus callosum shapes and sizes (i.e. volume or area), as well as brain concentrations of PCBs and BFRs, in order to test the hypothesis that neuroanatomical alterations are seen in animals with high levels of thyroid hormone disrupting chemicals. We have devised a detailed imaging and necropsy procedure to begin to assess the neurodevelopmental health effects of thyroid hormone disrupting chemicals in marine mammals. To date, this procedure has been completed on one harbor seal (*Phoca vitulina*), two common dolphins (*Delphinus delphis*), one grey seal (*Halichoerus grypus*), and one Atlantic white-sided dolphin (*Lagenorhynchus acutus*). In this presentation, we report initial results of CT / MR imaging and necropsy of a common dolphin.

Approach

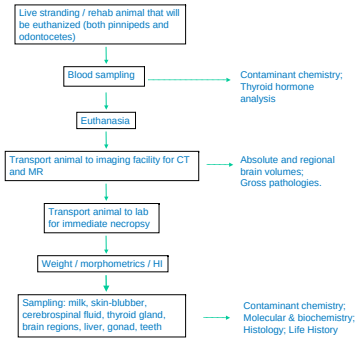


Figure 1. Imaging and necropsy procedure.

Methods

Specimen

Figure 2. Specimen CCSN04-191-Dd.

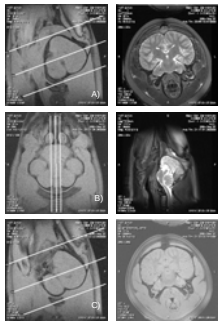
The specimen was an adult female common dolphin (CCSN04-191-Dd) that stranded alive at Squaw Island, Hyannisport, MA on September 6, 2004. The animal was moving in small circles, rolling, and listing to the right side. Because of poor health, the animal was euthanized at 12:25 by members of the Cape Cod Stranding Network, Buzzards Bay, MA. Total length and weight was 207 cm and 80 kg. At 15:00, the animal was transported to the WHOI and stored at 40°F with ice surrounding head. A letter of authorization from the National Marine Fisheries Service (NMFS) Northeast Region allowed the possession of marine mammal carcasses and parts.

CT Imaging

On 09/07/04 at 13:00 (approximately 24 hrs post-mortem), the specimen was transported to the CT facility at WHOI. Four separate scans were completed and focused on the head, whole body, lungs, and ears.

Figure 3. CT imaging of head and whole body with focus on lungs.

MR Imaging



On 09/07/04 at 17:00, the specimen was transported to the Massachusetts Eye and Ear Infirmary (MEEI) at Massachusetts General Hospital (MGH) for MRI on a Siemens Volume Zoom, 4 row detector, 1.5 Tesla machine. The MRI was completed from 19:09 - 21:42. Three different sequences were completed: 1) a Fast Spin Echo (FSE) of the whole brain that produced T2 weighted and proton density images in the axial plane; 2) a FSE of the mid-sagittal region that produced T2 weighted and proton density images in the sagittal plane; and 3) a 3D Spoiled Gradient Recalled Echo (3D-SPGR) that produced T1-weighted images of the whole brain in the axial plane.

Figure 4. MR imaging of head. A) FSE whole brain; B) FSE mid-sagittal region; C) 3D-SPGR.

Image Analysis

Computer-generated 3D models and volume estimates were completed using Amira 3.1 software (TGS Template Graphics Software, Inc.).

Whole brain. A frontal series of 64, T2-weighted images acquired during the FSE sequence was used to segment the whole brain into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). Automatic thresholding was performed to select the voxel intensity values that characterized GM, WM, and CSF. From these label fields, 3D models and volume estimates were completed. GM, WM, and CSF of the whole brain were added for a brain volume estimate. This volume estimate was converted to an estimated weight by multiplying the total brain volume by the specific gravity of brain tissue (1.036 g /cm³).

Cerebellum. The hemispheres, mesencephalon, and the brainstem were removed from the whole brain label field. 3D models and volume estimates were completed for GM, WM, and CSF.

Corpus Callosum and Fornix. A mid-sagittal slice of a T2-weighted image acquired during the FSE sequence of mid-sagittal region was used to manually trace the corpus callosum and fornix. From this label field, the area was calculated.

Hippocampus. From the T2-weighted images acquired during the FSE sequence of the whole brain, the left and right hippocampus were manually traced. 3D models were created and volume estimates were calculated from the label field.

Necropsy and Brain Dissection

After MR imaging, the animal was transported to WHOI and stored at 40° F with ice surrounding head. Necropsy commenced on 09/09/04 at 07:58. Samples were collected for histo-pathology. Blubber, cerebrospinal fluid, thyroid, liver, and brain regions (pituitary gland, frontal cortex, corpus callosum and choroid plexus, hypothalamus, hippocampus, and cerebellum WM and GM) were collected for PCB, BFR, and halogenated phenolic analyses.

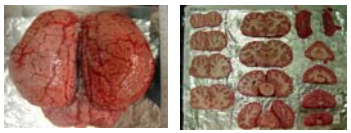


Figure 5. Total brain weight was 936.0 g. The brain was sectioned in the coronal plane 1-2 cm in thickness. Brain regions were collected using ultra-clean procedures to minimize chemical contamination.

Results and Discussion

Brain 3D Reconstructions and Size Estimates

Whole brain

The brain volume estimates were: GM = 370.94 cm³; WM = 353.60 cm³; CSF = 207.52 cm³; and total brain = 932.06 cm³. The estimated brain weight was 965.61g, while the actual brain weight was 936.00 g. The overestimation may be explained by CSF fluid loss when weighing the brain. Common dolphins ranging from 55 kg to 86 kg had brain volumes from 664cm³ to 990 cm³ (10).

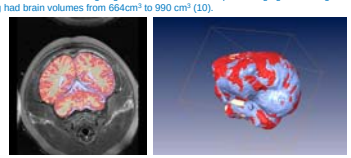


Figure 6. Label map and 3D reconstruction of brain. Red = GM; tan = WM; blue = CSF.

Cerebellum

The cerebellum volume estimates were: GM = 69.52 cm³; WM = 37.51 cm³; CSF = 57.72 cm³; and total cerebellum = 164.75 cm³. Common dolphins ranging from 55 kg to 86 kg had cerebellum volumes from 92.66 cm³ to 136.10 cm³ (10).

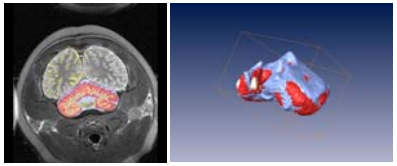
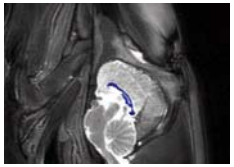


Figure 7. Label map and 3D reconstruction of cerebellum. Red = GM; tan = WM; blue = CSF.

Corpus Callosum and Fornix



The corpus callosum and fornix midsagittal area was 1.90 cm². The average midsagittal corpus callosum in humans is 6.24 cm² (11). The small corpus callosum in odontocetes is consistent with the behavioral and electrophysiological evidence for a high degree of hemispheric independence (as cited in 8).

Figure 8. Label map of mid-sagittal corpus callosum and fornix.

Hippocampus

The hippocampus volumes were: left = 0.68 cm³; right = 0.71 cm³. The average hippocampus volume in humans is 1.90 cm³ (12). These findings are consistent with earlier neuroanatomical studies in odontocetes, which found the hippocampus to be drastically decreased dorsally but well developed ventrally (13).

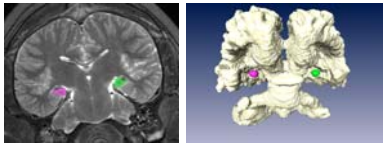


Figure 9. Label map and 3D reconstruction of the left and right hippocampus with surrounding white matter. Left = green; right = magenta.

Gross Pathology

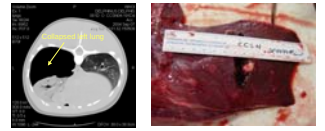


Figure 10. Collapsed left lung revealed by CT and necropsy. Cyst-like capsules present in left and right lungs.

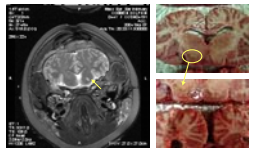


Figure 11. T2-weighted MR images suggested a necrotic region with dimensions 0.7cm x 0.4 cm located in the ventral region of the left thalamus. Internal exam revealed a yellow, necrotic region in the ventral part of the left thalamus in section #6.

Future Work

1. Increase sample size and determine the variability of absolute and regional brain shape and size within a population of marine mammals and among species.
2. Perform chemical analysis of plasma, CSF, blubber, liver, and brain regions.
3. Test the hypothesis that shape and size alterations are seen in animals with high levels of thyroid hormone disrupting chemicals.

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