Sectional anatomic and magnetic resonance imaging features of the head of juvenile loggerhead sea turtles (Caretta caretta)

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Objective—To compare anatomic features of cross-sectional specimens with those of MRI images of the heads of loggerhead sea turtles (Caretta caretta).

Animals—5 cadavers of juvenile female loggerhead sea turtles.

Procedures—Spin-echo T1-weighted and T2-weighted MRI scans were obtained in sagittal, transverse, and dorsal planes with a 0.2-T magnet and head coil. Head specimens were grossly dissected and photographed. Anatomic features of the MRI images were compared with those of gross anatomic sections of the heads from 4 of these turtles.

Results—In the MRI images, anatomic details of the turtles’ heads were identified by the characteristics of signal intensity of various tissues. Relevant anatomic structures were identified and labeled on the MRI images and corresponding anatomic sections.

Conclusions and Clinical Relevance—The MRI images obtained through this study provided valid information on anatomic characteristics of the head in juvenile loggerhead sea turtles and should be useful for guiding clinical evaluation of this anatomic region in this species. (Am J Vet Res 2012;73:1119–1127)
with a good understanding and knowledge of reptilian anatomic and pathophysiologic characteristics. Studies of sea turtle anatomy in which exploratory imaging was used are limited in scope. To the authors’ knowledge, no detailed MRI studies of the head of sea turtles have been reported. The head of sea turtles is particularly interesting because of the location of the CNS and important organs such as the eyes, ears, nasal cavity, glottis, trachea, and mouth. In addition, specific organs that play an integral part in the adaptation of turtles to the marine environment, such as the salt glands, are also located in the head.

Several types of head lesions in stranded loggerhead sea turtles have been described, including meningeal hemorrhages, heterophilic meningitis, encephalitis, bone fractures due to traumatic lesions of the head, and oral and esophageal lesions caused by hooks. Meningitis and encephalitis caused by parasitism by intravascular spirorchiid trematode eggs have been identified in green and loggerhead turtles. In addition, adult trematodes belonging to the genus Neosporiorchis have been found in the meninges of the brain in several loggerhead sea turtles, and multiple encephalitic intravascular or perivascular trematode eggs have been associated with granulomatous or mixed leukocyte inflammation, vasculitis, edema, axonal degeneration, and occasional necrosis. Turtles with fibropapillomatosis have multiple cutaneous fibroepithelial tumors that can develop at almost any anatomic site, including the head. Ocular fibropapillomas in sea turtles have also been described, as have pathological findings in which the only observed lesion was bilateral purulent adenitis of the salt gland. The purpose of the study reported here was to compare anatomic features of cross-sectional specimens with those of MRI images of the heads of loggerhead sea turtles and to provide anatomic details useful for veterinarians involved in sea turtle conservation.

Materials and Methods

Animals—Five juvenile loggerhead sea turtles that had been stranded in the Canary Islands (Spain) and subsequently died during hospitalization were used for this study. The turtles had been hospitalized at the Tafira Wildlife Rehabilitation Center (Las Palmas de Gran Canaria, Spain) because of severe lesions in the front and rear flippers. On the basis of SCL and sexual maturity (estimated from the appearance of their gonads), all turtles were identified as juvenile females.

Sea turtle rehabilitation at the Tafira Wildlife Rehabilitation Center was conducted with authorization of the Wildlife Department of the Canary Islands Government, in compliance with guidelines of the Tafira Wildlife Rehabilitation Center Animal Care Committee. Physical evaluation, including assessments of swimming activity, core body temperature (measured from the cloaca), food ingestion, body weight, SCL, and hydration, was performed daily in accordance with a complete clinical assessment protocol.

MRI technique—Magnetic resonance imaging of each turtle was performed immediately after death with a 0.2-T magnet and a head coil. Turtle cadavers were kept intact and were positioned with the ventrum facing downward during the MRI procedure. Magnetic resonance images were acquired with a spin-echo pulse sequence.

T1-weighted transverse images were acquired with the following settings: TR, 730 milliseconds; TE, 26 milliseconds; matrix, 256 × 256; and slice thickness, 3 mm. Scan time was 11 minutes and 34 seconds. T2-weighted transverse images were acquired with the following settings: TR, 3,150 milliseconds; TE, 80 milliseconds; matrix, 192 × 192; and slice thickness, 3 mm. Scan time was 9 minutes and 42 seconds. T1-weighted sagittal images were acquired with the following settings: TR, 700 milliseconds; TE, 26 milliseconds; matrix, 171 × 256; and slice thickness, 3 mm. Scan time was 11 minutes and 9 seconds. T1-weighted dorsal images were acquired with the following settings: TR, 700 milliseconds; TE, 26 milliseconds; matrix, 171 × 256; and slice thickness, 3 mm. Scan time was 11 minutes and 18 seconds. T2-weighted dorsal images were acquired with the following settings: TR, 3,000 milliseconds; TE, 80 milliseconds; matrix, 192 × 192; and slice thickness, 3 mm. Scan time was 9 minutes and 23 seconds.

Cross-sectional anatomy—After the MR images were obtained, 4 of the 5 scanned cadavers were frozen at −80°C for at least 24 hours before anatomic sectioning. Two heads were sectioned in the transverse plane, 1 head in the sagittal plane, and 1 head in the dorsal plane to approximate the MRI scans obtained. Serial parallel sections from 10 to 15 mm thick were obtained with an electric band saw. These sections were intentionally thicker than those of the MRI to maintain integrity and position of the anatomic structures in the slices.

Anatomic evaluation—Representative MR images of the head at various levels that best correlated with the sagittal, transverse, and dorsal macroscopic slices were selected. In addition, osseous anatomic preparations were used to facilitate accurate anatomic interpretation of the head. Specific terminology for sea turtles was used to describe observations.

Results

Animals—The mean ± SD SCL and body weight of the 5 turtles were 24.5 ± 2.8 cm and 6.8 ± 3.2 kg, respectively. The minimum and maximum duration these turtles spent in rehabilitation were 3 and 11 days. All turtles died because of the severity of the lesions in the flippers. No external head lesions were identified.

Representative MR images showing the reference structures for each of the transverse, sagittal, and dorsal images were selected (Figure 1). The anatomic structures were identified and labeled in the MR images and macroscopic sections, with transverse MR images presented in a rostral to caudal progression from the level of the olfactory bulb and nasopharyngeal duct to the level of the temporomandibular joint (Figures 2–9).
In the MR images, anatomic details of the head were evaluated according to the characteristics of signal of the various tissues (Table 1). Contrast between gray and white matter was poor in T1-weighted images,

Figure 1—T2-weighted MR images of the head of a cadaveric juvenile loggerhead sea turtle (Caretta caretta). Lines depict the transverse (A), sagittal (B), and dorsal (C) imaging planes. Each number (I to VI) represents the location for each MR transverse image.

Figure 2—Representative T1-weighted transverse MR image (A), T2-weighted transverse MR image (B), and anatomic section (C) at the level of the olfactory bulb and nasopharyngeal duct (level I) in a cadaveric juvenile female loggerhead sea turtle. Transverse images are oriented so that the left side of the head is to the right and dorsal is at the top. All views are rostral. 1 = Right orbit and ocular tissues. 2 = Right olfactory bulb. 3 = Right nasal bone. 4 = Interorbital septum. 5 = Frontal bone. 6 = Olfactory nerve. 7 = Left olfactory bulb. 8 = Vomer. 9 = Soft palate. 10 = Left eyelid and orbital fat. 11 = Left nasopharyngeal duct. 12 = Palatine bone. 13 = Left maxillary bone with rhamphotheca (beak). 14 = Oral cavity. 15 = Muscles of the tongue and lingual fat. 16 = Right mandible: right dentary bone with rhamphotheca (beak). 17 = Tongue. 18 = Right nasopharyngeal duct.
Figure 3—Representative T1-weighted transverse MR image (A), T2-weighted transverse MR image (B), and anatomic section (C) at the level of the cerebral hemisphere and eye (level II) in a cadaveric juvenile female loggerhead sea turtle. Transverse images are oriented so that the left side of the head is to the right and dorsal is at the top. All views are rostral. 1 = Right eye: cornea. 2 = Right eye: aqueous humor and iris. 3 = Right postorbital bone. 4 = Right eye: vitreous humor. 5 = Right salt gland. 6 = Frontal bone. 7 = Subarachnoid space. 8 = Olfactory nerve. 9 = Interorbital septum. 10 = Left eye: sclera. 11 = Left inferior eyelid and orbital fat. 12 = Left maxillary bone. 13 = Palatine bone. 14 = Oral cavity. 15 = Lingual fat. 16 = Muscles of the tongue. 17 = Tongue. 18 = Right mandible. 19 = Right groove: mandibular nerve and vessels. 20 = Soft palate. 21 = Vomer. 22 = Right cerebral hemisphere.

Figure 4—Representative T1-weighted transverse MR image (A), T2-weighted transverse MR image (B), and anatomic section (C) at the level of the diencephalon and salt gland (level III) in a cadaveric juvenile female loggerhead sea turtle. Transverse images are oriented so that the left side of the head is to the right and dorsal is at the top. All views are rostral. 1 = Right jugal bone. 2 = Right postorbital bone. 3 = Right salt gland. 4 = Frontal bone. 5 = Diencephalon. 6 = Cerebral hemisphere (panels A and B only) and subarachnoid space. 7 = Left salt gland. 8 = Lateral pterygoideus muscle. 9 = Medial pterygoideus muscle. 10 = Pterygoid bone. 11 = Left mandible. 12 = Left groove: mandibular nerve and vessels. 13 = Laryngeal and hyoid muscles. 14 = Glottis. 15 = Nasopharynx. 16 = Right mandible. 17 = Right groove: mandibular nerve and vessels. 18 = Soft palate. 19 = Masseter muscle. 20 = Vomer.
Figure 5—Representative T1-weighted transverse MR image (A), T2-weighted transverse MR image (B), and anatomic section (C) at the level of the mesencephalon (level IV) in a cadaveric juvenile female loggerhead sea turtle. Transverse images are oriented so that the left side of the head is to the right and dorsal is at the top. All views are rostral. 1 = Right jugal bone. 2 = Right postorbital bone. 3 = Right temporal muscle. 4 = Right occipital venous sinus. 5 = Right opisthotic bone. 6 = Parietal bone. 7 = Subarachnoid space. 8 = Left occipital venous sinus. 9 = Mesencephalon: optic lobe. 10 = Lateral pterygoideus muscle. 11 = Medial pterygoideus muscle. 12 = Left masseter muscle. 13 = Left mandible. 14 = Left groove: mandibular nerve and vessels. 15 = Nasopharynx. 16 = Hyoid bone. 17 = Trachea. 18 = Neck ventral muscles. 19 = Right groove: mandibular nerve and vessels. 20 = Soft palate. 21 = Basisphenoid bone. 22 = Mesencephalon: cerebral peduncles.

Figure 6—Representative T1-weighted transverse MR image (A), T2-weighted transverse MR image (B), and anatomic section (C) at the level of the myelencephalon and cerebellum (level V) in a cadaveric juvenile female loggerhead sea turtle. Transverse images are oriented so that the left side of the head is to the right and dorsal is at the top. All views are rostral. 1 = Right squamosal bone. 2 = Right transversus cervicis muscle. 3 = Right parietal bone. 4 = Right occipital venous sinus. 5 = Right temporal muscle. 6 = Supraoccipital bone. 7 = Subarachnoid space. 8 = Cerebellum: rostral lobe. 9 = Fourth ventricle. 10 = Left exoccipital bone. 11 = Left squamosal bone. 12 = Cranial nerves and vessels. 13 = Left quadratojugal bone and auricular fat. 14 = Left groove: mandibular nerve and vessels. 15 = Depressor mandibulae muscle. 16 = Hyoid bone. 17 = Trachea. 18 = Esophagus. 19 = Neck ventral muscles. 20 = Right mandible: right articular bone. 21 = Right masseter muscle. 22 = Longus colli muscle. 23 = Basisphenoid bone. 24 = Myelencephalon.
compared with in T2-weighted images. In sagittal T1-weighted images, cerebrum and cerebellum (mostly gray matter) had intermediate signal intensity and were darker than the myelencephalon (mostly white matter), which had high signal intensity. Cerebral spinal fluid had a low signal in the subarachnoid space and inside the ventricles. In T2-weighted images, the cerebrum and cerebellum had high signal intensity, compared with that of the myelencephalon, which had intermediate signal intensity. Unlike in T1-weighted images, the CSF had high signal intensity in the subarachnoid space and into the ventricular system. No variations in anatomic characteristics or MRI signal intensity were evident; all turtles were of similar size.

Discussion

Magnetic resonance imaging is a valuable tool for evaluating organs located in the head. However, images cannot be accurately interpreted without thorough knowledge of the tomographic or planimetric anatomy of the subject species. In the present study, the head of loggerhead sea turtles was evaluated because this species is commonly evaluated in captivity and in the wild for various purposes.

Exploration of anatomic structures within the heads of loggerhead sea turtles and clinical evaluation of soft tissues is laborious because of the turtles’ anatomic complexity. This complexity typically makes it difficult to diagnose morphological changes through physical examination and conventional radiographic evaluations. Contemporary image-based diagnostic techniques such as CT and MRI make it possible to obtain views of body sections from various tomographic planes, providing images with good anatomic resolution, high contrast between various structures, and excellent tissue differentiation.10,15

Magnetic resonance imaging allows good discrimination between the cephalic bones and soft tissues, in comparison with other conventional image-based techniques, because of a higher contrast resolution of the anatomic structures. The technique is useful for understanding the morphology and positions of various soft tissues.10,15 Another important advantage is that MRI can be used to obtain images via various anatomic planes without repositioning of subjects. Likewise, no harmful effects of MRI, such as those attributable to the ionizing radiation associated with conventional radiography and CT, have been described. On the other hand, more time is required for data analysis when MRI is used rather than another imaging technique.15 Magnetic resonance imaging is also expensive, compared with other imaging techniques, but its use in wildlife is justified in endangered species because of the amount of diagnostic information obtained with little risk to the animals evaluated.15 Another disadvantage to MRI is that sedation can be necessary when imaging the head and flippers because their movement may produce artifacts on the images.9 When T1-weighted images are used, the technique can also be used in the diagnosis of some bone diseases,28 although for other bone diseases, CT may be preferable for operators lacking experience with MRI.11

The parameters (ie, TR, TE, section thickness, interslice spacing, and scan time) used in the present...
study to obtain MR images in sagittal, transverse, and dorsal spatial planes can be used as an initial valid reference for exploratory evaluation of the head of juvenile loggerhead sea turtles. Excellent discrimination of soft and mineralized tissues was evident in the images obtained in our study. Because cadavers were used, images with IV contrast medium (gadolinium) could not be obtained. In the future, it would be important to perform new studies of the head in live sea turtles by the administration of gadolinium and also to establish...
Turtles are diagnostic for species identification. Lateral (maxillary, palatine, and pterygoid) structures of loggerhead sea turtles are better differentiated by use of MRI. The jaw muscles of sea turtles are mostly located inside the skull, occupying deep positions. Head muscles appear homogenously gray with intermediate CT density but appeared light gray in T1-weighted MR images and dark gray in T2-weighted MR images.

The brain of sea turtles is longitudinally arranged along the midline of the skull, and the major components are telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon. The lateral ventricles of the cerebral hemispheres, the third ventricle and cerebral aqueduct, and the fourth ventricle of the cerebellum and medulla form the encephalic ventricular system. The contrast between gray and white matter in the present study was higher in T2-weighted images, compared with T1-weighted images or CT images. Cerebrospinal fluid could be seen in the subarachnoid space and appeared dark gray (low signal intensity) on the T1-weighted MR images and white on the T2-weighted MR images (high signal intensity); however, on CT, CSF appears dark gray to black (low CT density).

Eyes appear homogeneously gray with intermediate CT density, whereas they appeared dark gray (low signal intensity) in T1-weighted MR images and with high signal intensity in T2-weighted MR images. Salt glands, which are responsible for removal of excess salt from the body, are the largest glands in the head and are found dorsal and medial to the eyes. Salt glands appear homogeneously gray with intermediate CT density, whereas they appeared light gray in T1-weighted MR images and dark gray in T2-weighted MR images, representing possibly the best differentiation achievable with T2-weighted MRI. The lumen of air-filled structures of the respiratory (nasal cavity, glottis, and trachea), digestive (oral cavity and esophagus), and sensory (ear) systems appear black in CT and MR images, although associated mucosal surfaces are better differentiated by use of MRI.

Preliminary MRI evaluations of sea turtles (data not shown) confirmed that cadavers could be used to provide accurate anatomic characteristics. The use of non-decapitated specimens in the present study prevented the presence of air in the subarachnoid space. However, the absence of blood flow in cadavers must be taken into account when applying cadaveric findings to live turtles.

Table 1—Relative signal intensity of the tissues of the head of cadaveric juvenile female loggerhead sea turtles.

<table>
<thead>
<tr>
<th>Anatomic structure</th>
<th>T1-weighted MR images</th>
<th>T2-weighted MR images</th>
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<tbody>
<tr>
<td>Cortical bone and subcondral bone</td>
<td>Signal void</td>
<td>Signal void</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Intermediate</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Temporomandibular joint (fibrocartilaginous joint disk)</td>
<td>High</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Muscles</td>
<td>Intermediate</td>
<td>Low</td>
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<tr>
<td>Vessels</td>
<td>Intermediate</td>
<td>Low</td>
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<tr>
<td>CNS</td>
<td>High</td>
<td>Intermediate</td>
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<tr>
<td>White matter</td>
<td>Low</td>
<td>High</td>
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<tr>
<td>Gray matter</td>
<td>Low</td>
<td>Intermediate</td>
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<tr>
<td>CSF</td>
<td>Low</td>
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<tr>
<td>Eye</td>
<td>Low</td>
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<tr>
<td>Lens</td>
<td>Low</td>
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<tr>
<td>Aqueous humor</td>
<td>Low</td>
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<tr>
<td>Vitreous humor</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Salt gland</td>
<td>Intermediate</td>
<td>Low</td>
</tr>
<tr>
<td>Air-filled structures</td>
<td>Signal void</td>
<td>Signal void</td>
</tr>
<tr>
<td>Fat</td>
<td>High</td>
<td>Intermediate</td>
</tr>
</tbody>
</table>

MRI appearance of cortical and subchondral bone was white (signal void) and the bone marrow was also gray (intermediate signal intensity) in T2-weighted MR images.

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Computed tomography is an excellent method for the detailed assessment of cephalic bone structures in juvenile loggerhead sea turtles, and the present study showed that MRI was useful for visualization of bone, soft tissues, and fluids. The spin-echo T1 sequence could be used to provide scans to identify anatomic structures, and the primary use for T1-weighted images could be to provide a precontrast baseline representation and to identify certain specific tissue changes (ie, hemorrhage). On the other hand, the spin-echo T2 sequence was useful for anatomic study and is recommended for identifying pathological changes. Magnetic resonance images obtained in the sagittal plane, as opposed to the transverse plane, allowed better evaluation of the topographic anatomic structures in the median plane, which were fundamentally the cranial cavity and the CNS as well as the associated and topographically related structures. The images yielded in the present study should be useful as initial reference material to aid clinical studies of loggerhead sea turtles.

References

1. Pritchard PCH. Evolution, phylogeny and current status. In: Esaoe, Genoa, Italy.
2. Radiodiagnostic Service of Veterinary Hospital Los Tarahales, Las Palmas de Gran Canaria, Spain.

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