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● *Technical Note*

PRELIMINARY ANALYSIS OF ELASMOBRANCH TISSUE USING MAGNETIC RESONANCE IMAGING

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High resolution NMR images of sixgill shark (*Hexanchus griseus*) tissue from both horizontal and sagittal orientations are presented. Structures normally radiographically undetectable are visualised here with NMR. Proton density, spin-lattice and spin-spin relaxation time maps as well as various relaxation weighted images show selective enhancement of different tissue types. Quantitative analysis of major anatomical features in the shark head is provided.

Keywords: **Magnetic resonance imaging; Proton density; T_1; T_2; Elasmobranch; *Hexanchus*; Anatomy; Cartilage; Holotypes; Taxonomy; Phylogenetics.**

INTRODUCTION

Techniques for the study of skeletal structures in cartilaginous fishes (radiographs,[1] tissue stains,[2,3] maceration[4]) are used routinely, although none produces entirely satisfactory results. Cartilages of sharks and rays (elasmobranchs) have low X-ray contrast and readily distort or become friable when heated during maceration; smaller cartilages are liable to be lost entirely. Stained, cleared whole-body preparations[3] are limited because cartilages are visualised in situ to the exclusion of muscular, vascular and neural tissues. Here we demonstrate the use of magnetic resonance imaging (MRI) to image skeletal and other tissues in the head and anterior body of the sixgill shark (*Hexanchus griseus* Bonnaterre, 1788).

SPECIMEN PREPARATION

A male six gillshark (total length 62.7 cm) in The Natural History Museum collection (reg. 1990.8.21.105) was used for this study. It had been fixed in 10% formalin and postfixed in 70% ethanol for storage.

Scanning the specimen in an ethanol jar using numerous imaging regimes yielded unsatisfactory results due to a significant residual signal intensity derived from fixative. Optimal MRI information was obtained by mounting the specimen directly in a saddle-shaped radiofrequency (RF) coil (18 cm i.d.) loosely wrapped in polyethylene to prevent alcohol loss from the specimen during the experiment.

EXPERIMENTAL

All data were acquired at a magnetic field strength of 4.7 T using a standard 33 cm, horizontal bore, Oxford superconducting magnet and a 20 mT/m gradient set controlled by a SISCO-200 (Spectroscopy Imaging Systems Corporation, Humboldt Court, Sunnyvale, CA) operating console. The saddle-shaped radiofrequency coil was tuned to an operating frequency of 200 MHz for the excitation and detection of ^{1}H signals.

A conventional spin-warp, spin-echo sequence was used to collect all high resolution data from the shark. The specimen was scanned in both horizontal (in-plane resolution 780 μm × 550 μm) and sagittal (longitudinal) planes (in-plane resolution 790 μm × 390 μm) relative to its long axis. An optimal slice thickness of 800 μm was derived from a 10 ms sinc-shaped RF pulse in the presence of a linear magnetic field gradient of 15 mT/m. Contiguous slices were obtained serially across the full width of the specimen in each plane during the imaging sequence; the sagittal scan consisted of

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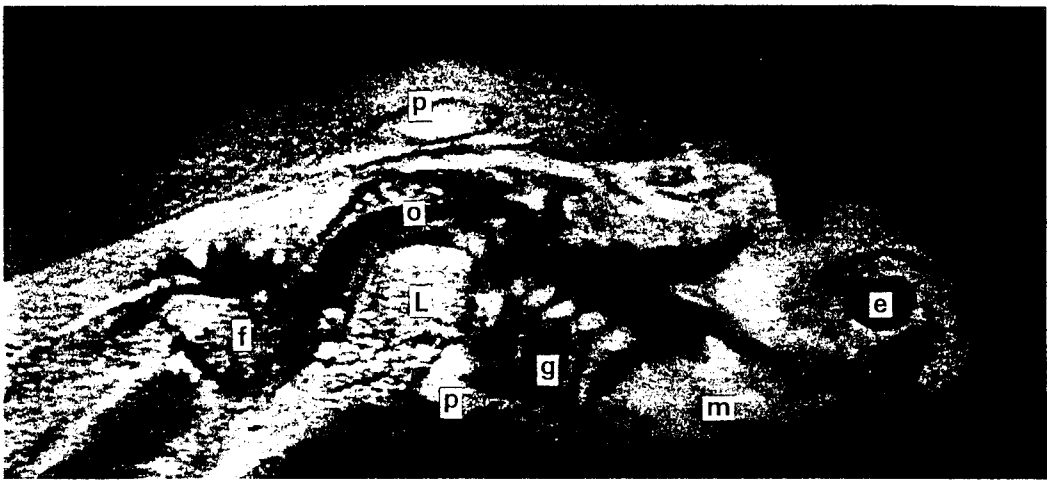

Fig. 1. Spin-echo MR image (TR/TE: 7000/45) of *H. griseus* (sagittal section lateral to midline). Definitions: e, eye lens; f, food remains in stomach; g, gill septa; L, liver; m, Meckel's cartilage; o, oesophagus; p, pectoral girdle.

80 slices, the horizontal 55 slices. All images in both section planes were acquired with an echo time (TE) of 45 ms and a repetition time (TR) of 7 s. Eight averages were required per phase-encode step to yield images with sufficient signal-to-noise to give sub-millimetre resolution for all three axes in a total experimental time per orientation of less than 4 h.

IMAGES

Cartilage of the neurocranium, jaws, and branchial skeleton (Figs. 1 and 2) gives strong signals appearing "light" in contrast in the images obtained. Muscle tissue is "dark" in contrast. Organs of the anterior body cavity (liver, stomach) have poor differential contrast but can be distinguished by cross-sectional shape.

Separate cartilage elements of the mandibular and hyoid arches are evident in Fig. 2 [palatoquadrate (q) and hyomandibula (h), respectively]. Cartilages of the six branchial arches are evident at the bases of the gill septa (g, Fig. 1) and, although individual elements are discernable, they are difficult to identify reliably in single slices.

The spinal column (s, Fig. 2) is well contrasted against the surrounding epaxial muscle in our images. Neural tissue forming the spinal cord is distinguishable from the surrounding cartilaginous neural arches. The notochord is both externally and internally segmented with evidence, at least anteriorly, of centrum-like elaborations. Elements of the branchial skeleton (identified as pharyngobranchials due to their terminal position and orientation) lie with their medial ends adjacent to the spinal column.

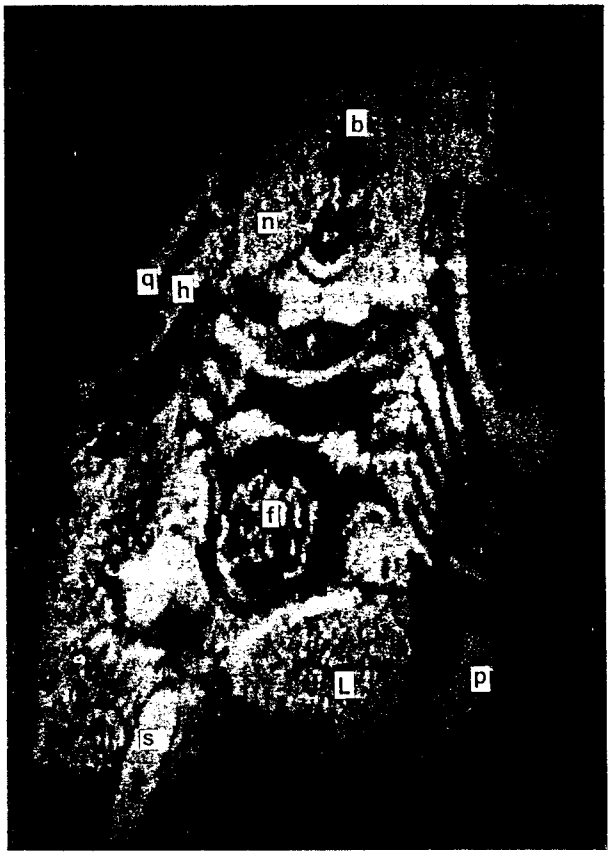

Fig. 2. Spin-echo MR image (TR/TE: 7000/45) of *H. griseus* (horizontal section dorsal to eye level). Definitions: b, brain; f, food remains in pharynx; h, hyomandibula; L, liver; n, neurocranium; p, pectoral girdle; q, palatoquadrate; s, spinal column.

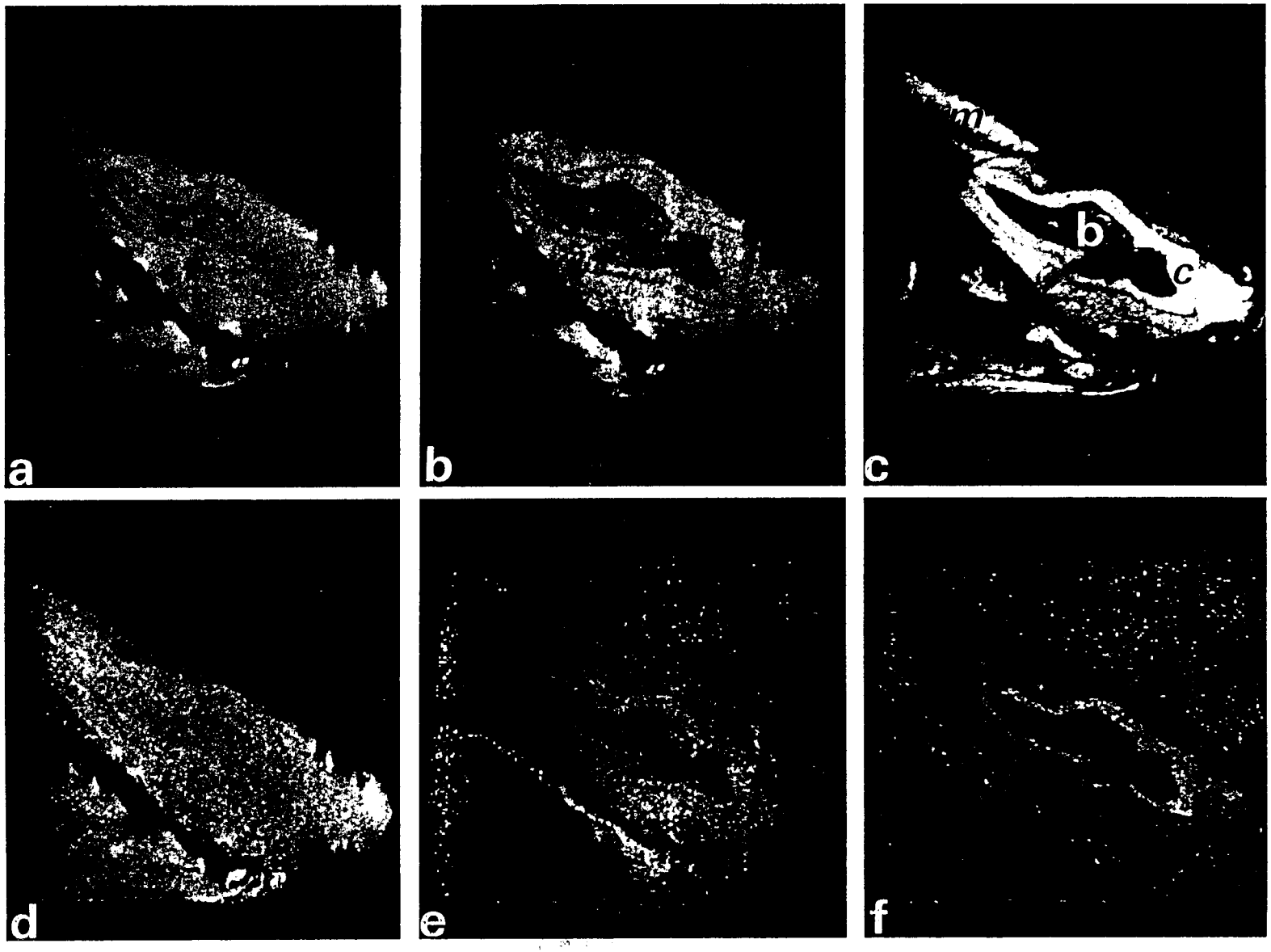

Fig. 3. Series of MR images acquired from the same sagittal section of *H. griseus* using: (a) short echo (TR/TE 6000/24); (b) long echo (TR/TE 6000/50); and (c) inversion recovery (TR/TI/TE 6000/400/24) techniques. Calculated corresponding relaxation maps are shown in (d)–(f) (proton density, spin-spin, and spin-lattice, respectively). Abbreviations in (c): b, brain; c, neurocranial cartilage; m, epaxial muscle.

Further, low resolution images of 2.0 mm thickness were acquired in the sagittal plane from the same specimen (Fig. 3) to ascertain the relaxation characteristics of the different tissue types identified at high resolution. Conventional spin-warp, spin-echo images with a long repetition time (TR) of 6000 ms at two different echo times (TE) of 24 and 50 ms were acquired (Figs. 3a and 3b). These data sets provided both proton density (Fig. 3d) and spin-spin (Fig. 3e) relaxation information. Combination of the short echo time data with a further study acquired using the inversion recovery sequence, (inversion time, $T_1 = 400$ ms) with the same TE and TR of 24 and 6000 ms, respectively (Fig. 3c), provided a spin-lattice relaxation map (Fig. 3f).

From these data it was found that the spin concentration was evenly distributed throughout the head (Fig. 3d) indicating good specimen fixation. Spin-lattice relaxation times of three main tissue types varied dramatically (e.g., when quoted in terms of mean value ± SEM: brain, 1050 ± 20 ms; cartilage, 2124 ± 20 ms; muscle 767 ± 16 ms), whereas the spin-spin relaxation times for the same tissue types showed less variation (brain, 43 ± 1 ms; cartilage, 52 ± 1 ms; muscle 36 ± 1 ms).

DISCUSSION

The results presented here show that MRI is applicable to the noninvasive study of internal structure in preserved sharks and that cartilage is particularly well visualised with T_1-weighted acquisition strategies. MRI may also be effective in the study of other fish groups with extensive skeletal chondrification (e.g., holocephalans and chondrosteans).

Relaxation time maps provide the necessary information for sequence optimisation and tissue characterisation, especially at the onset of an imaging protocol. It is clearly important to note that relaxation characteristics quantified here are dependent on fixation, which may vary between specimens, and the field strengths used.[5]

Lack of calcification of the notochord in *H. griseus* combined with relatively poor structural differentiation of centra in this species[6] contributes to resolution difficulties with standard radiological techniques. At best, only portions of the spinal column can be visualised and counts of total vertebral numbers from radiographs in *H. griseus* have not been made successfully.[1,7] Study of the structure of the *H. griseus* precaudal spinal column using the protocols described, has shown for the first time the anatomy in situ of the notochord and neural arch cartilages; high resolution MRI offers the potential for providing complete vertebral counts without resorting to dissection in this species and other hexanchoid and squaloid species where radiography has proved ineffective.

Three dimensional (3D) reconstruction provides a method of maximising the information content of two dimensional serial slices. Coloured, shaded 3D images of isolated vertebral centra of the blue shark (*Prionace glauca*) have been made from resin-embedded and sliced material.[8] Techniques for 3D reconstruction are applicable to MRI and computerised tomography (CT) derived data[9]; 3D reconstructions from serial CT scans have been used to show the in situ structure of the cranial skeleton of the coelacanth (*Latimeria chalumnae*),[10,11] a primitive bony fish. Preliminary work currently in progress suggests that morphological study of elasmobranchs from NMR images could be greatly enhanced by application of 3D reconstruction techniques, particularly to cranial cartilages and muscles.

This study demonstrates the potential of MRI for the study of preserved fishes in, for example, museum collections. It will in addition prove to be of particular value for the study of species represented by single specimens (holotypes) which cannot be dissected. MRI will also aid in determining the quality of fixation of specimens, monitoring fixation state during long-term storage, and identifying appropriate fixation protocols.

An important source of anatomical data for elasmobranch taxonomic study is derived from time-consuming and invasive dissections; our results show that MRI represents a potentially revolutionary advance in speeding up data collection, providing multiple observations on the same specimen and obviating the need for destruction of specimens inherent in conventional dissection. For the first time the internal anatomy of holotypes can be successfully explored. These results have important implications for the anatomical study of elasmobranchs and advancing understanding of their phylogenetic relationships and functional morphology through recognition of novel structural features.

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