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Magnetic resonance imaging at 9.4 T as a tool for studying neural anatomy in non-vertebrates

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Abstract

This report describes magnetic resonance imaging (MRI) methods we have developed at 9.4 T for observing internal organs and the nervous system of an invertebrate organism, the crayfish, *Cherax destructor*. We have compared results acquired using two different pulse sequences, and have tested manganese (Mn^{2+}) as an agent to enhance contrast of neural tissues in this organism. These techniques serve as a foundation for further development of functional MRI and neural tract-tracing methods in non-vertebrate systems.

Keywords: Crustacea; MRI; Brain; Nervous system; Crayfish; Manganese

1. Introduction

Classical anatomical methods have been used for centuries to resolve the structural and functional relationships between and within organs and tissues of animals. These methods require sacrifice of the subject and preservation of the tissues by fixation, often followed by sectioning and treatment with histological stains in order to provide resolving power and contrast between tissue types. These techniques are laborious and depend on the reconstruction of serial sections in order to visualize the three-dimensional architecture of a structure. In contrast, magnetic resonance imaging (MRI) provides a window through which to view a living, functioning organism at the organ and tissue levels, without killing the subject or performing arduous reconstructions. MRI thereby allows relatively rapid imaging of specimens and the opportunity to repeatedly examine the same individual in longitudinal studies. The goal of the present study was to develop MR imaging and contrast-enhancement methods in a non-vertebrate model system, in order to lay a foundation for development of tract-tracing and functional imaging techniques that can be used for basic research in these organisms.

While MRI has become a relatively commonplace clinical approach for examining soft tissues, it has only recently been exploited in non-vertebrate animal research (Brinkley et al., 2004; Herberholz et al., 2004; Michaelis et al., 2005). Invertebrate animals present significant advantages over vertebrates for MR imaging because they do not require anesthetics, can be easily immobilized, and can stay in the magnet for many hours. This allows multiple studies, either to follow dynamic processes or to use a variety of pulse sequences, some of long duration, to elucidate different features of the animal's anatomy. Furthermore, use of a small bore, high field magnet with strong magnetic field gradients provides excellent sensitivity and resolution of anatomical structures.

We have used the Australian crayfish, *Cherax destructor*, as our model because it is a hardy creature adapted to terrestrial environments, where water may be scarce and can survive periods out of the water, unlike many crustacean species (e.g., lobsters and crabs) that are also popular neurobiological models. This is key to the animal's suitability for MRI study

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where the animal is out of the water for many hours during imaging. In addition, *C. destructor* is an excellent model for MRI studies because it will remain motionless in small, dark spaces, such as the MRI probe, for several hours and has no respiratory movements.

Introduction of the paramagnetic contrast agent Mn^{2+} into the blood system of the crayfish can enhance visualization of neural tissues. Mn^{2+} has a long history of use in MRI for enhancement of contrast in brain and other tissues in vertebrate organisms (Aoki et al., 2002; Burnett et al., 1984; Cory et al., 1987; Fornasiero et al., 1987; Geraldes et al., 1986; Leergaard et al., 2003; Lin and Koretsky, 1997; London et al., 1989; Newland et al., 1987; Pautler and Koretsky, 2002; Pautler et al., 1998; Tjälve et al., 1995, 1996; Watanabe et al., 2001; Van der Linden et al., 2002), but its use is just emerging in invertebrate organisms (Brinkley et al., 2004; Herberholz et al., 2004; Michaelis et al., 2005). Mn^{2+} is especially useful as a contrast agent in living tissues because it has an ionic radius and charge similar to calcium (Ca^{2+}), and can therefore substitute for Ca^{2+} in many biological systems (Hunter et al., 1980). It is known that Mn^{2+} is taken up by neural tissue of the related crustacean *Nephrops norvegicus* at six times the exposure concentration, in hemolymph at three times the exposure concentration, and in muscle tissue at only half the exposure concentration (Baden and Neil, 1998). Therefore, as neural tissue sequesters disproportionately more Mn^{2+} relative to other tissues, Mn^{2+} can be used as a contrast agent in MRI applications where neural tissue is the primary focus. Because *C. destructor* does not require anesthetics, there are no non-specific regions of activity as seen in studies that use light anesthesia, and there is no reduction of activity as seen in studies with heavy anesthesia (Aoki et al., 2002). Additional bonuses for contrast agent studies of the nervous system in *C. destructor* are that there is no blood brain barrier (Abbott, 1971, 1972) and the arterial vasculature is closed (Sandeman, 1967), ensuring delivery of contrast agents directly to the brain and neural tissues without artificial disruption.

In this report, we compare images of various tissue types acquired using two different pulse sequences and test Mn^{2+}-enhancement methods for visualizing neural tissues. These studies serve as a foundation for the development of MRI methods for use in neural tract-tracing and functional MRI applications.

2. Materials and methods

2.1. Crayfish preparation

C. destructor (4–5 cm carapace length) were used in these studies. The large claws were removed at their bases so that larger animals would fit into the 30 mm inner diameter MRI probe, and also to reduce artifacts due to movement. For claw removal, the claw was squeezed until the animal autotomised the limb, so that the valve in the blood vessel to the limb closed reflexively (McVean, 1982). Claws were regenerated by the animals over the next molt period.

Mn^{2+} was injected into the pericardium of crayfish in the form of an aqueous solution of $MnCl_2$ (Sigma, St. Louis, MO), which dissociates into ions at neutral pH. The $MnCl_2$ solution was diluted with crayfish physiological saline (205 mmol l^{-1} NaCl, 5.4 mmol l^{-1} KCl, 10.2 mmol l^{-1} $CaCl_2$, 1.2 mmol l^{-1} $MgCl_2$, 2.4 mmol l^{-1} $NaHCO_3$, pH 7.4) to the desired concentration. Injections (40 μl/cm carapace length) of several concentrations of $MnCl_2$ (1, 10, 120 mM and 1 M) were tested; 120 mM resulted in images with good contrast; injection of the 1 M solution was lethal. After $MnCl_2$ injection, the animal's physical activity decreased, as evidenced by reduced tail flipping. However, there were no obvious long-term detrimental effects as individual crayfish survived as many as three sequential injections over a period of months, and survived up to 1 year after the initial injection.

2.2. MRI

Crayfish were imaged within 15 min of $MnCl_2$ administration using a Bruker Avance DRX 400 MHz NMR spectrometer with a 9.4 T vertical wide bore magnet, actively shielded gradients of 2.4 G/(cm A) with a maximum field of 96 G/cm

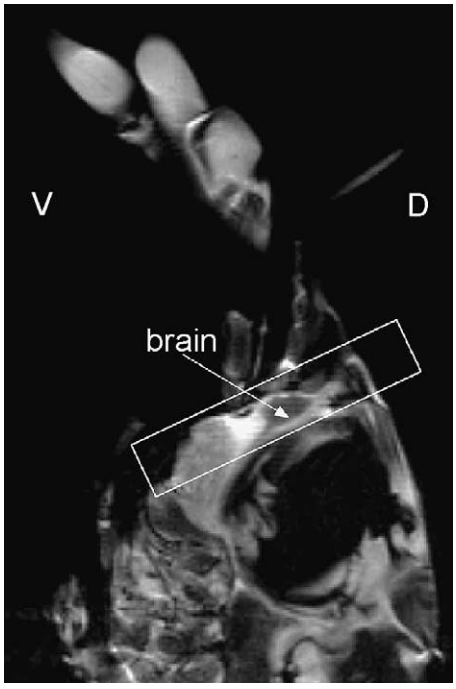

Fig. 1. Lateral view of the cephalothorax of *C. destructor* from a scout T2-weighted (RARE) image of the crayfish in one of three orthogonal planes showing orientation of slice selection for brain imaging over the area that contains the brain, eyestalks and esophageal connectives (ventral (V), dorsal (D)). A claw can be seen near the top of the image. Slices were aligned with the exoskeleton lying beneath the brain. This image was produced using RARE_BIO (FOV 4 cm, MTX 256, SLTH 1.0 mm, N_{avg} 1, TR 3112.5 ms, TE 60.8 ms, TA 1.39 min).