Investigating the Earthworm (Lubricus Terrestris) as a model for ncMRI at 9.4T

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Introduction

Achieving direct neuronal current detection by MRI (ncMRI) would be an important advance for functional neuroscience. There have been conflicting reports on whether this is possible as the anticipated field modulation (~0.2%) generated by neuronal currents is at the limit of detection using current generation whole body MR systems (1,2). The aim of this project was to make measurements in a relatively simple model system, the earthworm (Lubricus Terrestris), using a high field, high sensitivity NMR probe at 9.4T to assess the feasibility of observing ncMRI with higher sensitivity in an intact organism. The main earthworm nervous system consists of three giant fibers, a median giant plus two lateral giants which act to coordinate the worm's escape response. The median giant receives sensory input from the anterior while the lateral giants receive sensory input from the posterior portion of the worm. The median giant axon is approximately 160μm in diameter while the lateral giants are about half this size.

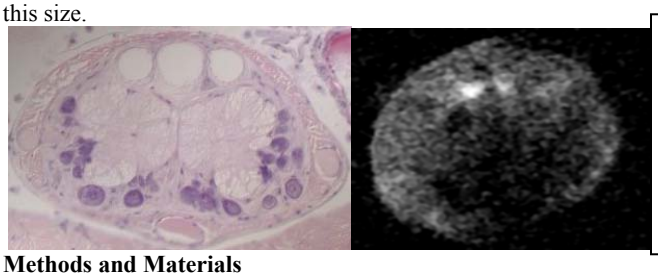

Figure 1a. Axial cross sectional image of worm (Heathcote D. et al., UWM). The three giant nerve fibres run the complete length of the animal (circular structures at top of image). Axonal currents are expected in directions both perpendicular and parallel to the main magnetic field. The components perpendicular to the field contribute to ncMRI effects. Action potentials are expected from both the medial and lateral axons with the lateral potential being delayed by about 2ms. Figure 1b. Axial FLASH image of worm at 9.4T showing three axons at the top.

Methods and Materials

Earthworms were selected with a diameter slightly less than 5mm when fully contracted, washed in water and placed in a 5mm NMR tube with moist paper to partially restrict motion and provide hydration. The stomach contents were not removed. Non-localised spectra were acquired using a 400MHz (9.4T) NMR micro-imaging/spectroscopy system (Ultrashield Plus, Bruker BioSpin, Kahrlsrue, Germany) with a broadband 5mm probe and the zg30 pulse sequence with a TR of 100ms, NEX=1 and a bandwidth of 10KHz. Manual shimming was performed prior to data acquisition. Time series of 64 or 256 spectra were acquired with 1024 complex points and analysed using Fourier analysis of the water spectral peak amplitude using Matlab. The earthworms were released unharmed into the wild on completion of the NMR acquisitions which took 15-20 minutes.

Results

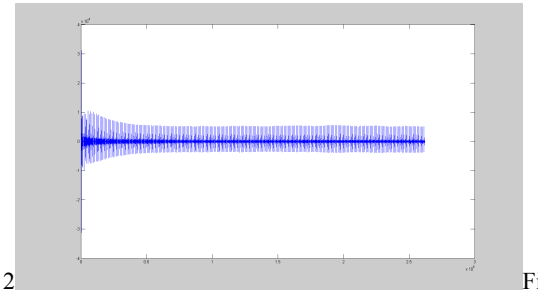

Fig. 2

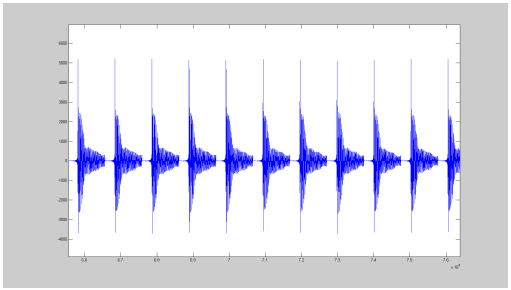

Fig. 3

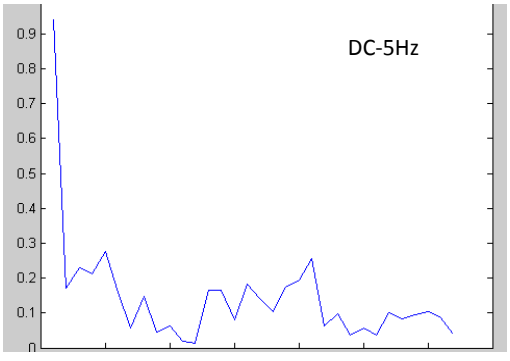

Fig. 5

Figure 2shows a time series of 64 spectra acquired from a non-anaesthetized earthworm showing high signal stability after the initial approach to equilibrium following a period of approximately 5 minutes for the worm to come to rest. Figure 3 shows a zoomed set of individual free induction decay signals from the intact functioning earthworm. Figure 4 shows the normalized Fourier transform of the peak signal time series showing no major spectral peaks from possible spontaneous axonal firing in the resting earthworm up to 5Hz.

Discussion

The earthworm has a relatively simple 'linear' nervous system which is well suited to study by NMR with large axons. Use of a high sensitivity spectroscopy probe has improved the SNR by a factor of approximately 100 times over typical experiments performed in vivo in humans at 1.5T and 3T. It has been shown that is possible to acquire stable MR data in the high resolution spectrometer from non-anaesthetized earthworms without stimulation in this initial feasibility study. The earthworm is known to be sensitive to light stimuli and, in future experiments, light from a strobe will be fed into the end of the NMR tube using a fibre optic light guide to stimulate the worm with a regular frequency to assess the possibility of measuring axonal firing with NMR.

References . [1] J.Bodurka, A. Bandettini, MRM, 2002 47:1052–1058 [2] L.S. Chow et al, MRM. 2008 60:1147-1154