Functional imaging of the cervical spinal cord of the halothane and alpha-chloralose anaesthetized rat during noxious and innocuous thermal stimulation of the forepaw.

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Introduction: Previous animal studies investigating functional imaging of the spinal cord (spinal fMRI) have examined patterns of functional activity during noxious electrical and chemical stimulation[1-3] and have also compared the effects of different anaesthetics[4]. The first objective of this study is to observe patterns of functional activity during thermal stimulation at noxious (48 °C) and innocuous (40 °C) temperatures. Noxious and innocuous thermal afferent fibres terminate closely within the spinal cord (lamina I and lamina II respectively) therefore similar activity maps are anticipated. The second objective is to compare two types of anaesthesia (α -chloralose and halothane) and examine differences in the activity maps and signal change during thermal stimulation. Alpha-chloralose has traditionally been used for animal fMRI however it is suitable only for acute studies. This study shows that fMRI activity in the rat spinal cord can be detected under halothane anaesthesia.

Materials and Methods: The cervical spinal cords of 24 rats were imaged in a 7T Bruker system (Magnex, U.K.) with Avance (Bruker, Germany) console. Twelve animals were anaesthetized with α-chloralose (30 mg/ml, 80 mg/kg i.v. initially and 40 mg/kg every 90 min) and 12 with halothane (1-2%). Animals were placed supine with the cervical spinal cord centred on a quadrature coil tuned and matched at 300 MHz. A fast spin echo sequence (FOV = 3 cm, 128 x 64 matrix, TE_{eff} = 86 ms) was used to acquire the images. Six, 2 mm thick transverse slices centred on the 2nd to 7th cervical (C) vertebrae (~C3-C8 spinal cord segments) were acquired. Functional images were acquired during 2 periods of thermal stimulation of the right forepaw with either 40°C (n=6) or 48°C (n=6) alternated with 3 periods of rest. Data were analyzed by correlation to the paradigm using custom made software written in MatLab.

Results: During noxious stimulation in the α -chloralose anaesthetized group functional activity was greatest at the C6 vertebra(C7 spinal cord segment) (Figure 1A). Lesser amounts of functional activity were observed above and below this slice. During innocuous thermal stimulation functional activity was also greatest at the C6 and C7 vertebrae (Figure 1B). Consistent activity was observed in the right dorsal horn at the level of the C3 and C4 vertebrae. In the halothane anaesthetized rats, during noxious stimulation, functional activity was observed in the right dorsal horns or near the central canals in all slices (Figure 1C). During innocuous thermal stimulation the greatest number of active pixels were observed at the C5 and C6 vertebrae. The peak signal changes were higher in both anaesthetic groups during noxious stimulation (Figures 2 A, C) compared to that obtained during innocuous stimulation (Figure 2B, D). The average time courses of the halothane animals were slightly higher than those of the α -chloralose anaesthetized animals (Figure 2).

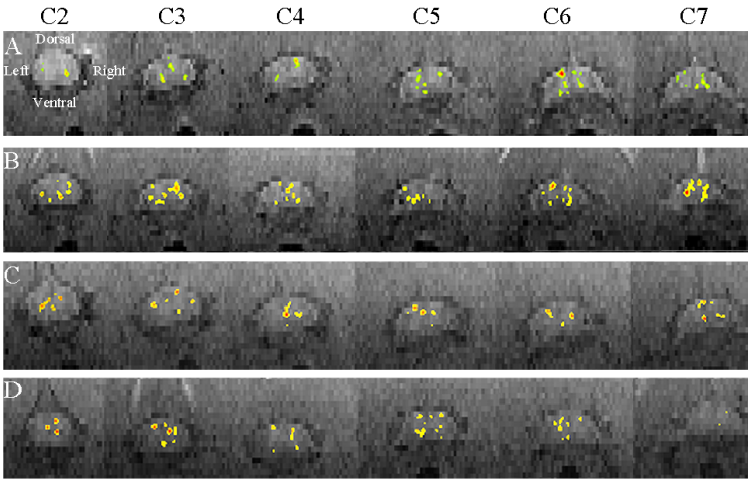

Figure 1. Combined activation maps of α -chloralose animals during 48°C (A) and 40°C (B). Halothane anaesthetized animals are shown in during 48°C (C) and 40°C (D). Labels indicate the vertebral level of each slice.

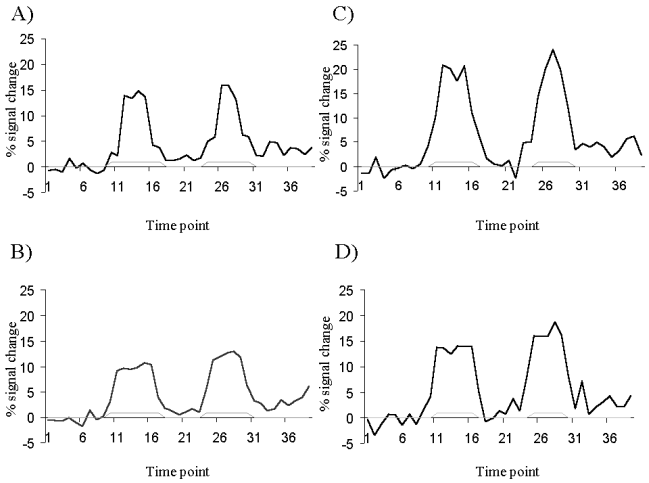

Figure 2. Average time courses of the combined activation maps shown above with α -chloralose during A) 48°C and B) 40°C, and halothane during C) 48°C and D) 40°C, thermal stimulation.

Discussion: The rat forepaw is innervated by afferent fibres terminating in spinal cord segments C5 to C8[5]. The C5 spinal cord segment aligns approximately with the C4 vertebra and the C8 spinal cord segment approximately aligns with the C6/C7 disc. Therefore activity was expected to occur mainly between C4 and C6 vertebrae. The axons of thermal heat afferent neurons terminate mainly in lamina II, but some also terminate in lamina I of the spinal cord. They become silenced at noxious temperatures (e.g. 48°C). Noxious heat information is carried by Aδ and C fibres which terminate mainly in lamina I[6]. The terminations in the dorsal horn between these two modalities are similar. Therefore, the functional activity maps are expected to show similar patterns of activity. Active pixels were observed in the right dorsal horn at the level of the C4 vertebra in all four groups. Three of the four groups demonstrated activity in the left ventral horn at the level of the C5 vertebra. Active pixels were observed bilaterally in the dorsal horns at the level of the C6 vertebra in all of the groups. However, activity in the ventral horn was less consistent in the halothane anaesthetized animals at the same level. It is known that anaesthesia acts on the spinal cord in order to prevent movement[7]. Less consistent ventral horn activity could reflect the suppression of activity by halothane. Peak percentage signal changes were greater in the animals stimulated with 48°C. In a previous human spinal fMRI study, higher percentage signal changes were observed with noxious cold stimulation than with innocuous cold[8].

Conclusions: Spinal fMRI detected activity in expected physiologically relevant areas during noxious and innocuous stimulation. Greater signal changes were observed during more intense stimulation in both anaesthetic groups. Fewer active pixels were observed in combined functional maps of halothane compared to α -chloralose anaesthetized animals. This may reflect the extent of neuronal activity taking place under halothane anaesthesia. Therefore, we have demonstrated that fMRI of the spinal cord can be performed in halothane anaesthetized animals, enabling chronic animal spinal fMRI studies.

References:

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