Comparing fMRI of the rat spinal cord in the alpha-chloralose and halothane anesthetized rat during electrical forepaw stimulation

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Introduction

Anesthesia is necessary during most animal fMRI studies in order to restrict motion and minimize the stress and discomfort of the animal. General anesthesia is known to lower the basal brain activity and affect the response to stimulation. However, not all anesthetics have the same affect on neuronal activity and metabolism (1). Commonly, alpha-chloralose is used in fMRI studies because it has been found to have minimal effects on brain activity (2). While several studies have compared the effects of different types of anesthesia on functional activation in the brain, no such studies have taken place in the spinal cord. Previous fMRI studies of the rat spinal cord have used alpha-chloralose for anesthesia (3,4). Administration of an anesthetic from which rapid recovery is possible would allow flexibility in experimental design and open the door for chronic studies of spinal fMRI. The present study compares alpha-chloralose and halothane anesthetized rats during spinal fMRI and verifies the presence of neuronal activation by staining for c-fos protein, a known neuronal marker for pain (reviewed in 5).

Methods

Functional imaging of the cervical spinal cord was performed in 12 rats anesthetized with either alpha-chloralose (30 mg/mL, 80 mg/kg i.v. initially and 40 mg/kg every 90 min) (n=6) or halothane (1-2%) (n=6) using electrical stimulation (0.3 ms, 3 Hz 15 V) of the dorsal right forepaw. Blood pressure, heart rate and blood gases were monitored and maintained within the normal range. Experiments were performed using a 7 Tesla horizontal bore magnet (Magnex, U.K.) with Avance (Bruker, Germany) console. Rats were placed supine such that the bottom of the cranium was positioned at the top of the RF surface coil tuned to 300.0 MHz. A fast spin echo sequence (field of view = 3 cm, 128 x 64 matrix effective echo time of 49 - 60 ms, RARE phase encoding) was used to acquire the spinal fMRI images. Six, 2 mm thick transverse slices separated by 0.6 mm spanning from the 4th cervical (C) to the 2nd thoracic spinal cord segments were acquired. Forty time points were acquired using an asymmetric paradigm with 5 alternated periods of rest and stimulation. Data were analyzed using custom made software and functional maps of each subject were aligned to a reference image. Regions of activity were then compared with spinal cord physiology. Following functional experiments rats were transcardially perfused and the spinal cord removed for immunohistochemistry. The spinal cord segments from C3 to C8 were sectioned (20 μm thick) and incubated in primary and secondary antibodies to allow visualization of the c-fos protein by fluorescence.

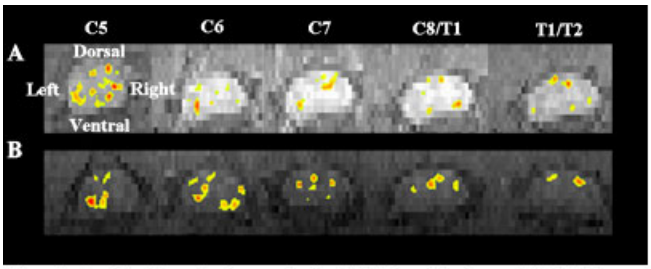

Figure 1. Combined functional maps (n=6) of A) alpha-chloralose and B) halothane anesthetized rats during electrical stimulation of the right forepaw. Red indicates areas of the most overlap followed by yellow and green.

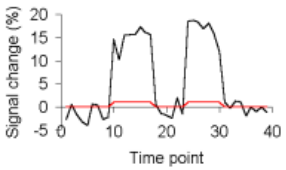

Figure 2. Average time course of the combined functional map shown in figure 1A (alpha-chloralose anesthetized rats)

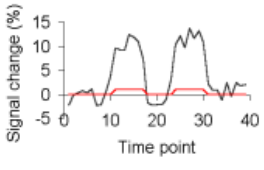

Figure 3. Average time course of the combined functional map shown in figure 1B (halothane anesthetized rats).

Results

During alpha-chloralose anesthesia (figure 1A), functional activation was greatest at C5. The combined functional map of halothane anesthetized activation (figure 1 B) is similar. In both groups, consistent functional activity was observed in the right dorsal horn and left ventral horns of the spinal cord as well as around the central canal. While location of functional activity is similar between the two experimental groups, the average percentage change in signal is less in the halothane anesthetized rats (figures 2 and 3). Immunohistochemistry was in good agreement with the fMRI results.

Discussion and Conclusions

Depth and type of anesthesia can depress central nervous system metabolic activity and reduce cerebral blood flow (6). Activity detected with fMRI is dependent upon these factors. Nonetheless, we have found that functional activity in the cervical spinal cord can be detected using both alpha-chloralose and halothane anesthesia. Signal changes were smaller in halothane anesthetized animals compared to those anesthetized with alpha-chloralose. Studies of cerebral glucose metabolism have shown lower basal levels with alpha-chloralose anesthetized rats compared to halothane anesthetized rats but comparable levels during stimulation (reviewed in 7). Alpha-chloralose is often favored because of the minimal affects it has on other physiological parameters (6). Conversely, surgical preparation of the animal in order to perform fMRI under alpha-chloralose is quite time consuming and invasive, requiring femoral artery and vein cannulations and recovery is a lengthy process making it unfeasible for chronic studies. Halothane, which is less invasive to administer, can also be used during functional imaging of the spinal cord without compromising the results. By demonstrating that halothane can be used for anesthesia during spinal fMRI studies, we have shown that in future a new range of studies including chronic experiments are feasible.

References

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