Comparing rodent forepaw stimulation under two levels of Domitor anesthesia using laser doppler and fMRI at 9.4T

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Purpose

Animal anesthesia can prove challenging during fMRI studies as the anesthetic agent can inhibit cortical activity. Alpha-chloralose anesthesia is widely used in fMRI animal studies as there is minimal cortical suppression and it enables a robust BOLD response. This anesthetic, however, suffers from difficulty in controlling the level of anesthesia and its use is not compatible with survival experiments. Domitor (medetomidine hydrochloride) is a nonnarcotic sedative and analgesic that may provide some blood pressure support. Domitor is gaining wider acceptance in fMRI animal studies (reference 1,2). Our experience with rats shows that while Domitor provides some level of sedation, the rat can be easily startled and overpower the sedative effects. We have added pancuronium bromide to our anesthesia regimen to paralyze the rat, permit artificial ventilation, and prevent motion during fMRI studies. We examine the cortical responses to forepaw stimulation with two different levels of Domitor and a constant concentration of pancuronium bromide using fMRI and laser Doppler flow (LDF) to help ascertain whether higher levels of Domitor can be used safely without suppressing the functional hyperemic response.

Methods

6 Sprague Dawley rats (300 –380g) were used in the fMRI (3 rats) and LDF (3 rats) studies. The right femoral artery and vein were cannulated and used for invasive blood pressure monitoring and for continuous IV drug administration. A tracheotomy allowed for mechanical ventilation with 30% O2-70% N2. Surgery was performed under isoflurane (1.4%) vaporized into the ventilatory gas. After surgery, isoflurane was gradually reduced to zero as a continuous infusion of Domitor (0.1mg/kg/hr) and Pancurium Bromide (2mg/kg/hr) was started. A bipolar beryllium copper electrode was inserted into the second and fourth webspaces of the left forepaw. The forepaw stimulation protocol involved a current of 2mA, a duration of 3ms, and varying frequencies (3, 5, 7, 10 Hz).

Forepaw stimulation at all frequencies was repeated using a continuous infusion of Domitor (0.2mg/kg/hr) and Pancurium Bromide (2mg/kg/hr). The physiologic parameters - mean arterial blood pressure, arterial blood gases, pulse oximetry, pulse, temperature, respiratory rate, inspired / expired O2 and CO2 - were maintained under normal physiologic ranges throughout all experiments. **fMRI study**: Each stimulation sequence began with an OFF period of 40 seconds followed by three repetitions of ON for 20 seconds and OFF for 40 seconds (total scan time 3minutes 40seconds). Gradient echo scans (Single shot EPI, TE = 18.39 ms, TR = 2 sec, MTX 96 x 96, FOV = 4 cm, Number of repetitions = 110, 10 scans 1mm apart, acquisition time = 3 minutes 40 seconds) were acquired on a 9.4T Bruker MRI scanner. **LDF study:** Under isoflurane anesthesia, prior to Domitor/pancuronium bromide infusion, two 3mm x 3mm sectons of skull were thinned to translucency over the somatosensory forelimb regions (SFL1) (+0.5mm rostral, ±3.5mm lateral from bregma). At the same frequencies and Domitor/pancuronium bromide levels used in the fMRI experiments, cortical functional hyperemic response to seven stimulation trials (10s ON, 50s OFF) was bilaterally assessed through the thinned skull with LDF probes suspended above both SFL1s.

Results and Discussion

Our goal was to study the cortical hyperemic responses to forepaw stimulation at two different levels of anesthesia. The lower Domitor level (0.1mg/mg/hr) is widely used in fMRI studies today. As increased levels of anesthesia may alter cortical activity, we chose to double the Domitor dose to determine whether it would suppress the cortical response to stimulation. The electrical stimulation parameters (2mA, 3ms, 3-10 Hz) were chosen to provide a robust response without a provoking an increase in arterial blood pressure or other pain responses. At both Domitor levels and at all stimulation frequencies, a hyperemic response was observed using both fMRI (Figure 1A) and LDF (Figure 1B). This is more evident with fMRI. A higher concentration of Domitor would be ideal as it may reduce or eliminate the need for paralytics as a means to reduce motion. In this study, we have found that doubling the Domitor dose causes some diminution of cortical activity though it does not eliminate it entirely. Further studies are warranted to study cortical activity dose-response curve for this drug.

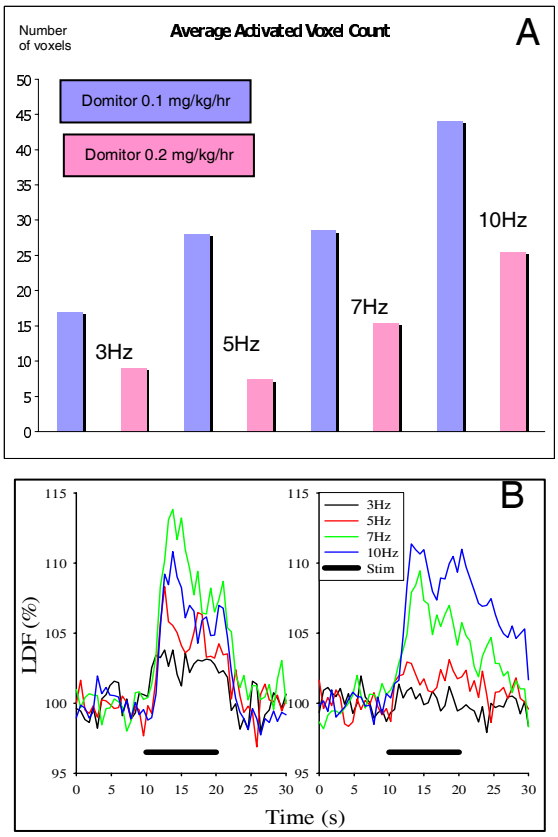

Figure 1: **A –** Shows an averaged activated voxel count in the forepaw region at four different frequencies and two levels of Domitor. An F-test ($p > 0.005$) was used as a threshold for activation. **B –** Shows the LDF % response to electrical forepaw stimulation at four different frequencies. The left graph corresponds to Domitor (0.1mg/kg/hr) while the right graph shows Domitor (0.2mg/kg/hr).

References

1) Heerschap A., *et al*, Proc. Intl.Mag.Reson.Med. 10:394, 2002
2) Weber, R. *et al*, Neuroimage. 29:1303, 2006