Physiological characterization of a robust survival rodent fMRI method

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Introduction: Anesthetics have been commonly used in animal functional MRI studies. It is well known that proper choice of anesthetics is of critical importance for the success of an fMRI experiment. The use of medetomidine, among other anesthetics, allows survival fMRI experiments in the same animals, and has gained some popularity (1-3). A recent study, using a low dose of dexmedetomidine (active isomer of medetomidine) in combination with a low dose of isoflurane, permitted fMRI data acquisition up to several hours. The rat default mode brain network has been successfully identified with this preparation (4), indicating that this protocol minimally disturbs brain network functions. However, medetomidine is known to cause peripheral vasoconstriction, respiratory suppression and bradycardia, thus independently confounding the fMRI signal. The goal of this study is to systematically characterize and to optimize physiological conditions for fMRI experiments under this anesthetic regime.

Materials and methods: A total of 17 rats were used in this study (N= 7 for fMRI; N=10 for blood gas measurement on bench). The fMRI experiment followed a protocol in (4). Briefly, rats were initially anesthetized with 2% isoflurane followed by a loading dose of dexmedetomidine (0.015 mg/kg, i.p.). Continuous subcutaneous dexmedetomidine was initiated (0.015 mg/kg/hr). Isoflurane was gradually tapered to 0.5%. Respiration rate, cardiac rate and oxygenation were non-invasively monitored (Model 1030, SA instruments). fMRI data were acquired using an electrical forepaw stimulation paradigm (3 cycles of 20 sec ON, 20 sec OFF, plus 20 sec pre-stimulus baseline), which were interleaved with 300 sec of "resting state" scans during which no stimulation was applied. Scan parameters: Bruker 9.4T scanner, TR/TE=1000/15 ms, FOV=32 mm, matrix size = 64×64. The interval between task and resting scans were about 5 min. Seed-based correlation analysis was applied to the resting state data. The bench study followed an identical protocol except that a femoral artery was catheterized for blood gas measurement (GEM Premier 3000). Blood gas data were analyzed using linear mixed-effects modeling (R package) with random slopes and intercepts for individual animals. The Kenward-Roger approach was used to estimate the degrees of freedom.

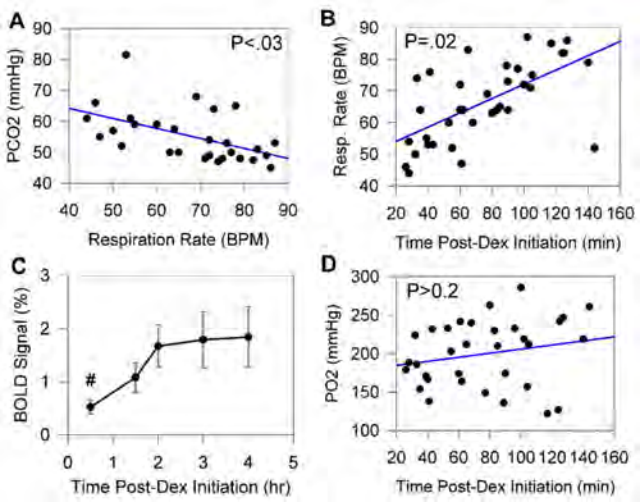

Fig. 1. Arterial PCO2 decreased as respiration rate increased over time (A, B). BOLD response to electrical forepaw stimulation increased initially, reaching a plateau 2 hrs post dexmedetomidine initiation (C). Arterial PO2 remained stable over time (D) (#, p<.01).

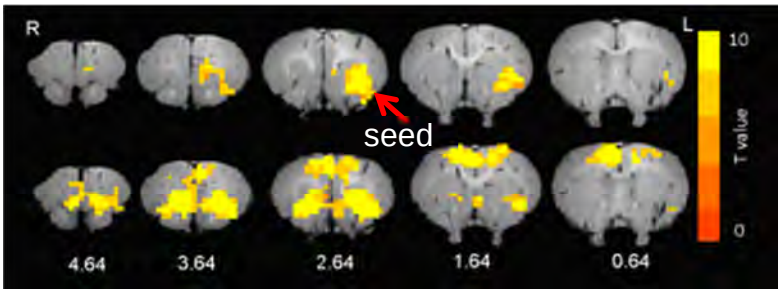

Figure 2 Comparison of t-statistical functional connectivity (fc) map with seed on the left orbital cortex (p=.001).Top panel: fc during time window I; bottom panel: fc during time window III.

Results: Figure 1A illustrates the relationship between arterial P_{CO2} and respiration rate. Respiration rate increased over time, reaching 70-85 BPM 90 min post-dexmedetomidine initiation (1B). Notably, the amplitude of fMRI BOLD response reached a plateau at this point, which was significantly higher than in the first 30-min time window (1C).There was no significant change in arterial P_{O2} over time (1D). In resting state scans, with seed in the right orbital cortex, functional connectivity patterns were unilateral surrounding the seed regions in time window I (first 30 min), but connectivity to contralateral orbital cortex, prelimbic and cingulate cortex is seen in time window III (90-150 min post dexmedetomidine initiation) (see Fig. 2), suggesting the effect of physiological conditions on resting state functional connectivity.

Discussion: These data suggest that physiological parameters reached optimal condition 90 min post dexmedetomidine initiation. Both evoked BOLD response and resting state fMRI signal were stable during this time window, consistent with the pervious report (4). Somewhat surprisingly, even with respiration rate in the range of 85 BPM, arterial P_{CO2} was about 45 mmHg, indicating that the animals were still under a slightly hypercapnic condition.

Reference: 1) Weber et al. Neuroimage 2006. 2) Zhao et al. Neuroimage 2009. 3) Pawela et al., Neuroimage 2009. 4) Lu et al. PNAS 2012.

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