The temporal stability of BOLD fMRI measurements in medetomidine-anesthetized rats.

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Abstract: Medetomidine anesthesia is often used for longitudinal rat fMRI experiments, but the temporal stability of BOLD readouts over long experimental times is unclear. To address the issue, we anesthetized rats for up to 6 hours, during which we monitored multiple physiological parameters and repeatedly performed task-based and resting-state fMRI. The time-period from 1.5 up to 6 hours after the start of medetomidine anesthesia was found to be suitable for stable and reproducible functional imaging experiments.

Motivation

To facilitate pre-clinical research, BOLD fMRI is being applied to experimental animals with increasing frequency. This application often necessitates the use of anesthetics, which varyingly interfere with the very generating mechanisms of the BOLD signal. In search for an agent that has minimal effects on neurovascular coupling, numerous researchers employ continuous infusion of the sedative agent medetomidine – an established practice for rat fMRI that allows for longitudinal studies (1,2). The infusion of medetomidine is often preceded by isoflurane administration, to facilitate the induction and the maintenance of anesthesia during setup. The transition between the two drugs, as well as the slow onset of medetomidine's hypnotic action create questions regarding the temporal stability of BOLD readouts, with reports being inconsistent (3,4). The goal of this study was to determine a time-window within the most widely used medetomidine protocol that allows for robust and reproducible fMRI experiments.

Materials und Methods

We used the anesthetic protocol shown in Fig. 1 to anesthetize six female adult Wistar rats for a maximum of 6 hours. Heart rate, respiratory rate, and rectal temperature were monitored throughout the anesthetic episode. With a 9.4 Tesla Bruker Biospin scanner we repeatedly acquired GE-EPI sequences every 10 minutes (TR 1.5 s, TE 15 ms, flip angle 90°, 30 consecutive coronal slices 0.5 mm thick, in-plane resolution 0.2 x 0.2 mm, 220 repetitions). The EPI runs were alternated between resting-state measurements and somatosensory fMRI with electrical forepaw stimulation (9 Hz, 3 mA, 0.3 ms pulse width).

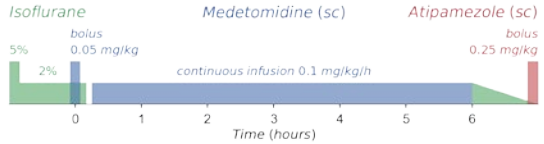

Fig. 1: The anesthetic protocol

For each electrical stimulation run, we extracted the percent signal change (ΔBOLD) from the forelimb area of the primary somatosensory cortex (S1FL) as a measure of BOLD response amplitude. For the resting-state runs, we calculated the pair-wise Pearson's correlation between multiple hand-defined regions of interest; the mean correlation across all region-pairs was taken as a measure of global functional connectivity.

Results

Physiology. The rats remained unconscious for 4-6 hours under the influence of MED infusion, with heart and respiratory rates undergoing rapid changes in the first hour, and following a slowly increasing trend afterwards (Fig. 2).

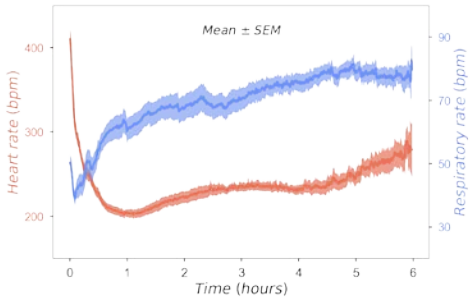

Fig. 2: Physiological parameters throughout the anesthetic episode.

Evoked BOLD responses. Electrical forepaw stimulation consistently activated contralateral somatosensory areas, including the S1FL (Fig. 3A), the secondary somatosensory cortex (S2) and the thalamus. The BOLD responses were extremely stable from 1.5 up to 6 hours after the initial medetomidine injection with a ΔBOLD of approximately 2% (1.99 +/- 0.056). Some of the earlier BOLD responses were

considerably stronger, particularly when the body temperature was low (Fig. 3B).

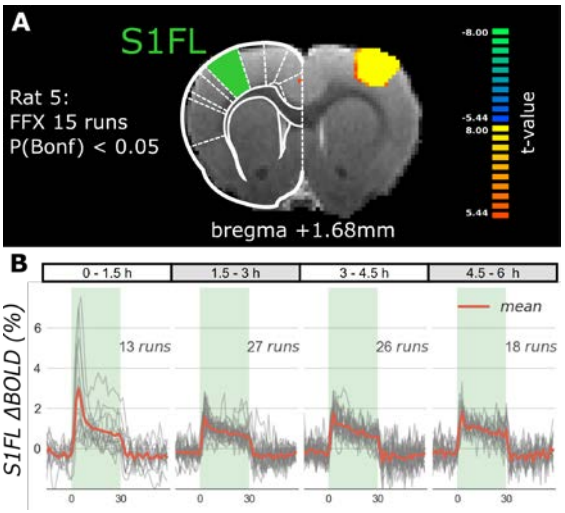

Fig. 3: The S1FL area of one example rat, functionally defined by a fixed effects analysis of all stimulation runs (A). The BOLD responses of the S1FL to electrical forepaw stimulation (shaded in green) split into four 1.5 hour time-windows (B).

Functional connectivity. Resting state global functional connectivity was stable throughout the anesthetic episode, but with some variability between individual rats (Fig. 4).

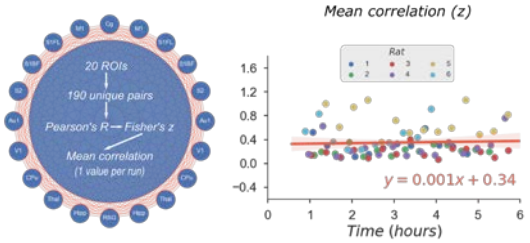

Fig. 4: Functional connectivity, taken as the mean pair-wise correlation among multiple ROIs, is stable over time.

Discussion

The anesthetic protocol based on subcutaneous continuous infusion of medetomidine is suitable for longitudinal rat fMRI experiments from 1.5 up to 6 hours after the initial medetomidine injection. Both evoked BOLD responses and resting-state functional connectivity were found to be stable within this period, with repeated measurements leading to consistent results. The initial 1.5 hours were characterized by rapid changes in physiological parameters and stronger BOLD responses, particularly in subjects with low body temperature. This might be due to slower absorption of the drug from the subcutaneous tissue at low temperatures – an effect we aim to clarify in future work.

Conclusion

Subcutaneous infusion of medetomidine is suitable for rat fMRI leading to long-lasting sedation and temporally stable results in both task-based and resting-state fMRI experiments. We propose a waiting period of 1-1.5 hours between the initial medetomidine bolus and the start of functional imaging to allow for the stabilization of all physiological parameters.

References

1. Weber R, Ramos-Cabrer P, Wiedermann D, van Camp N, Hoehn M. A fully noninvasive and robust experimental protocol for longitudinal fMRI studies in the rat. Neuroimage. 2006;29(4):1303–1310.
2. Pawela CP, Biswal BB, Hudetz AG, Schulte ML, Li R, Jones SR, Cho YR, Matloub HS, Hyde JS. A protocol for use of medetomidine anesthesia in rats for extended studies using task-induced BOLD contrast and resting-state functional connectivity. Neuroimage. 2009;46(4):1137–1147.
3. Magnuson ME, Thompson GJ, Pan W-J, Keilholz SD. Time-dependent effects of isoflurane and dexmedetomidine on functional connectivity, spectral characteristics, and spatial distribution of spontaneous BOLD fluctuations. NMR Biomed . 2014;27(3):291–303.
4. Bol CJ, Danhof M, Stanski DR, Mandema JW. Pharmacokinetic-Pharmacodynamic Characterization of the Cardiovascular, Hypnotic, EEG and Ventilatory Responses to Dexmedetomidine in the Rat. Journal of Pharmacology and Experimental Therapeutics. 1997;283(3):1051–1058.