Mouse fMRI : Examining the Relationship between Anesthesia, BOLD data quality and Physiology

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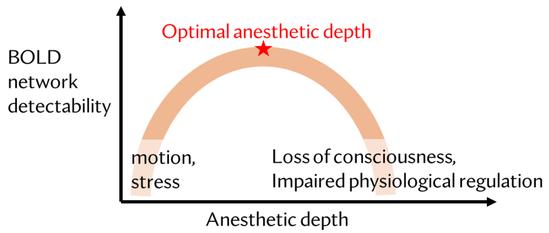
MOTIVATION

- There is growing interest in studying resting state fMRI networks in mice
- However, the use of anesthesia interferes with alertness and physiology
- Anesthetic depth that is too high or low makes it difficult to recover resting state networks
- Existing gold-standard anesthesia protocols don't account for inter-individual variability between mice, strains, and free-breathing mice [1]

Figure 1: Resting state network detectability is impaired when anesthetic depth is too light or too deep.



OBJECTIVE

Goal: Determine the optimal anesthetic and physiological conditions for a free-breathing mouse, such that rs-fMRI network detectability is improved.

Competing Hypotheses:

1) A given dose will reliably yield optimal depth– may differ across strains/sexes.
2) Individual mice will respond differently to a given dose, but their physiological parameters will indicate when they have reached optimal depth.

EXPERIMENTAL DESIGN

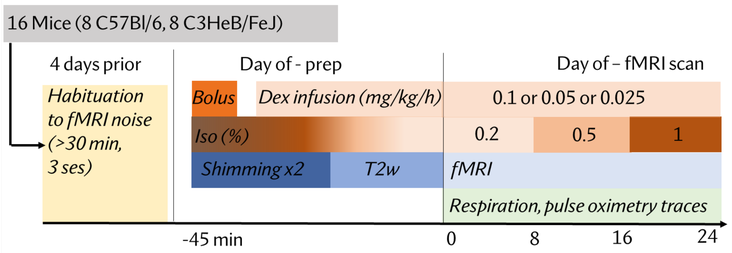


Figure 2: **Experimental design for 1 session.** *Each session was repeated 3x per mouse. Mice were habituated prior to session 1 to minimize effect of habituation across sessions. Isoflurane was induced at 3.5%, a dexmedetomidine bolus of 0.05 mg/kg was injected, then iso was reduced slowly to 0.2%. Throughout the 24 min fMRI scan, iso was varied from very low (0.2%) to standard (0.5%) to mild (1%) every 8 min [3]. Dexmedetomidine infusion was changed from low (0.025 mg/kg/h) to standard (0.05 mg/kg/h) to high (0.1 mg/kg/h) across sessions in a randomized order [3].*

METHODS - ACQUISITION

fMRI: 7T Bruker Biospec 70/30 USR, T/R CryoProbe™, GE-EPI, TR = 1 s, TE = 15 ms, matrix = 75 x 40 x 26, res = 0.25 x 0.25 x 0.5 mm, 1440 repetitions.

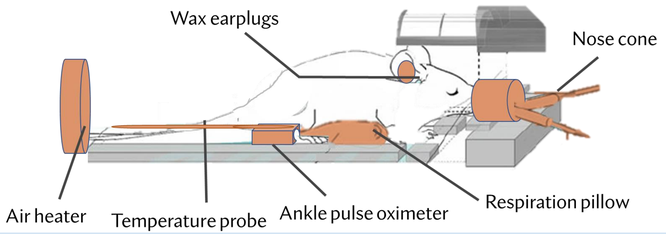


Figure 3: **The mouse setup.** *Internal temperature was maintained at 36.5°C. Physiological waveforms were recorded with the respiration pillow and pulse oximeter (SAII). Not shown: head padding was used to reduce motion. Adapted from [2].*

METHODS - ANALYSIS

fMRI processing: Commonspace registration, distortion correction, confound regression (6 motion, white matter, cerebrospinal fluid, vascular) , highpass filtering (0.01 Hz), censoring (framewise displacement, DVARS), with https://github.com/CoBrALab/RABIES

Physiology processing: Smooth, detrend, extract peaks, average within 2 min window

Analysis: Fit subject timeseries (2 min) to group somatomotor network with dual-regression. Find Dice overlap between masks of the fitted and group network = network detectability.

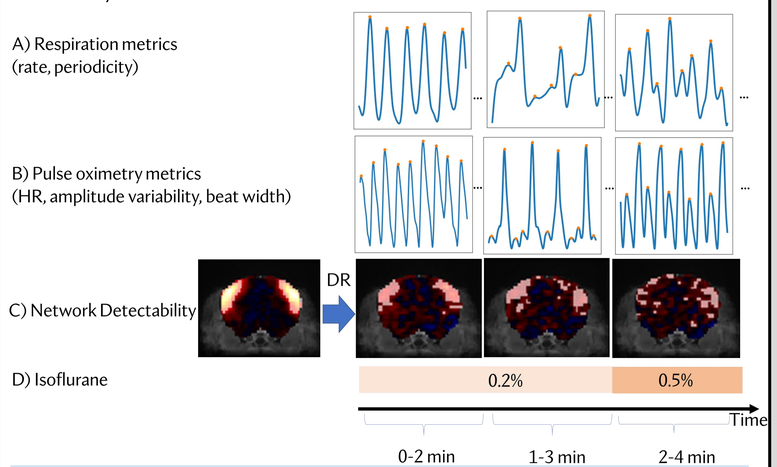


Figure 4: **Overview of metrics obtained from the analysis.** *Physiology and fMRI timeseries were analyzed in rolling windows of 2 min to obtain multiple values per subject. A) Processed respiration waveform (4 s). Orange dots are detected peaks. B) Processed pulse oximetry waveform (4 s). C) Left is the group-level somatomotor network, it is fitted to 2 min of individual-level data with dual-regression (DR). Right is the fitted network in multiple 2min windows of a single subject. Overlaid in white are masks of the top 1% voxels. D) Isoflurane levels are changing across windows.*

RESULTS: Impact of anesthesia and physiology on network detectability

The linear model and terms that were found to be significant are shown below (* : p<0.05, ** :p<0.01, ***: p<0.001).

Network detectability ~ iso *** + dex ** + resp rate * + resp periodicity *** + HR + heart amp variability + heartbeat width *** + motion *** + session order *** + sex*strain + (1|mouse)

Figure 5: **Low anesthesia is crucial for improving network detectability.** *The effect size of iso is 6 times higher than that of dex. Similar effect for both strains. See figure 8 for interpretation of Dice values.*

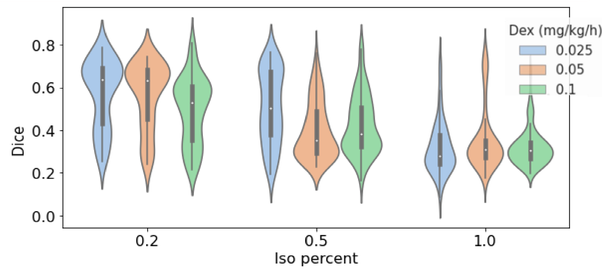


Figure 6: **High motion or aperiodic breathing are indicators of corrupt data.** *Low framewise displacement is not significantly related to network detectability, but unusually high values indicate decreased network detectability (p<0.001). A similar but inverted relationship is observed for breathing periodicity.*

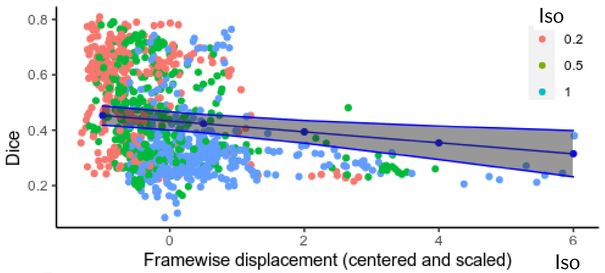


Figure 7: **Higher respiration rate and heartbeat width improve network detectability.** *Even respiration rates ~200 breaths/min can yield good Dice. However, good data can be obtained at both high and low rates.*

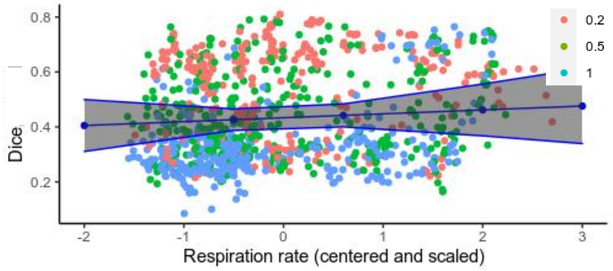
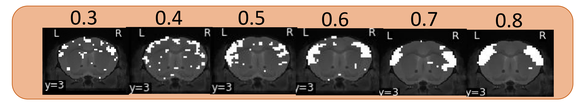


Figure 8: **Guide for interpreting Dice values.** *For a given Dice overlap, an example mask obtained by fitting the network to an individual subject is shown. A Dice of 0.6 or greater implies that the network could be well recovered.*



CONCLUSIONS

To improve the quality of rs-fMRI data acquired from free-breathing mice, researchers should focus on reducing iso and dex levels as much as possible. Anesthetic dose does not need to be tuned according to each mouse's physiology, except to avoid very aperiodic breathing or excessive motion. Hypothesis #1 is more likely than #2.

[1] Steiner, A. R., F. Rousseau-Blass, A. Schroeter. 2021. "Systematic Review: Anesthetic Protocols and Management as Confounders in Rodent Blood Oxygen Level Dependent Functional Magnetic Resonance Imaging (BOLD fMRI)—Part B: Effects of Anesthetic Agents, Doses and Timing." Animals. [2] Mueggler, T, Baltes, C., Bosshard, S., Rudin, M. 2012. "FMRI in Mice: Functional Phenotyping of Transgenic Mouse Lines Based on Hemodynamic Readouts." In Neural Metabolism in Vivo, 4:593–621. [3] Grandjean, J, Schroeter, A., Batata, I., Rudin, M. 2014. "Optimization of Anesthesia Protocol for Resting-State fMRI in Mice Based on Differential Effects of Anesthetics on Functional Connectivity Patterns." NeuroImage 102 Pt 2 (November): 838–47.