Reproducibility of Resting-State fMRI Data in Rats across Three Months

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Introduction:

There has been growing interest in resting-state fMRI (rs-fMRI) using animal models, following the demonstration of intrinsic brain activities in non-human primates and rodents [1, 2]. Preclinical models have the advantage to assess the course of a disease using longitudinal paradigms, but the reproducibility of the observations (e.g. rs-fMRI signal) is critical in these studies. Since most animal fMRI scans are performed under anesthesia, individual differences in delivery of and responding to the anesthetics may lead to relatively large variations in physiology across animals and/or sessions. While the reproducibility of rs-fMRI measures in humans has been investigated extensively [3-5], its characteristics in animals are much less known. In this study, we investigate the reliability of rs-fMRI signal of rats in short (10 min), middle (2 weeks) and long (3 months) inter scan intervals.

Materials and methods:

Animals. Sixteen male Sprague-Dawley rats (initial weight of 275±25 g) were used. All animal procedures were approved by the Institutional Animal Care and Use Committee. Animals were anesthetized with a combination of 0.5-0.75% isoflurane and 0.015 mg/kg/hr dexmedetomidine hydrochloride.

MRI protocol. MRI data were acquired using a Bruker Biospin 9.4 T scanner with a quadrature surface receiver coil and birdcage volume transmit coil. Each rat was scanned on three sessions. Following the first session (S1), the second and third sessions (S2 and S3) were acquired at 2 week and 3 month intervals, respectively. Each session consisted of a high-resolution anatomical scan using a RARE sequence and two resting-state fMRI (rs-fMRI) scans, 10 min apart. The rs-fMRI scans were acquired using a gradient-echo echo-planar imaging (EPI) sequence (TR=1,000 ms, FOV=32 mm, matrix size=64*64, slice thickness=1 mm, slice number=13, volumes=520).

Image processing/analyses. Image processing was carried out using AFNI for motion correction, spatial smoothing (FWHM = 1 mm), detrending and temporally band-pass filtering (0.01 – 0.1 Hz). All 96 rs-fMRI scans (16 rats, 3 sessions, and 2 rs-fMRI scans each session) were included in a group ICA analysis (15 components) by MELODIC toolbox in FSL. A dual regression approach was used to reconstruct individual-level functional connectivity components onto each rat, session, and rs-fMRI scan. For each ICA component, a one-sample t-test was performed to obtain a robust functional connectivity map ($t > 20$) (Fig. 1).

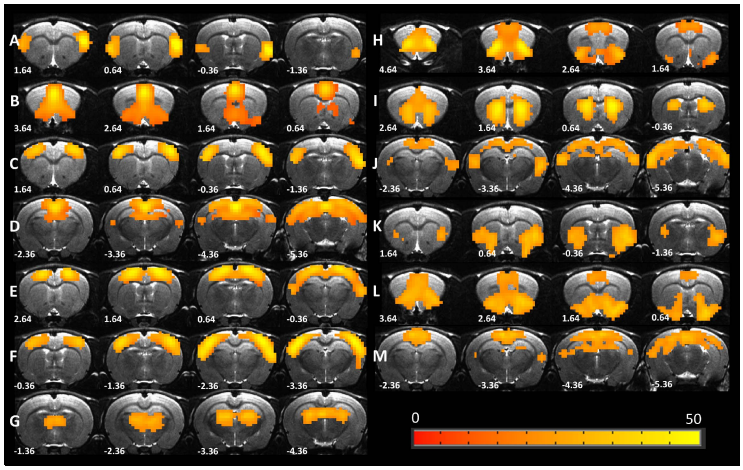

Fig.1 Functional connectivity maps resulting from the group ICA of rs-fMRI data of 16 rats. Component maps, which appear to be anatomically meaningful (13 out of 15), are shown. A) Insular cortex, B) cingulate and nucleus accumbens, C) motor cortex, D) restrosplenial/dorsal hippocampal/parietal cortex, E) somatosensory cortex (S1FL), F) barrel cortex, G) thalamus, H) prelimbic and orbital cortex, I) caudate putamen (CPu), J) visual and auditory cortex, K) ventral lateral striatum, L) cingulate/olfactory tubercle(OT), M) restrosplenial cortex. Distance to bregma (in mm) is labeled at the bottom of each slice.

Reproducibility analyses. An intraclass correlation (ICC) approach was used to investigate the reproducibility of rs-fMRI signal [6]: $ICC = (BMS - EMS) / (EMS + (k-1)*EMS)$, where k is the number of observations, BMS and EMS are between- and within-subjects mean-square errors, respectively. In this study, we computed three ICCs for each connectivity component: 1) the ICC between rs-fMRI scan 1 and 2 (ICC_1, 10 min apart); 2) the ICC between S1 and S2 (ICC_2, 2 weeks apart); and c) the ICC between S1 and S3 (ICC_3, 3 months apart). These ICCs represented the short, middle and long term reproducibility respectively.

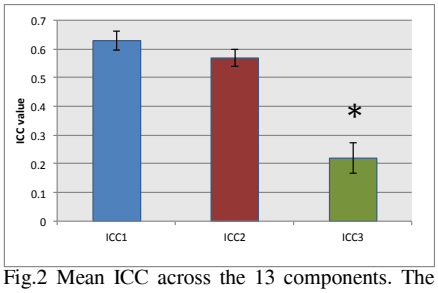

Fig.2 Mean ICC across the 13 components. The ICC_3 decreased significantly compared with ICC_1. The error bars indicate standard errors. * P<0.001

Results:

Among the 15 component maps obtained from the gICA, 13 components appeared to be anatomically meaningful (Fig. 1). These component maps included brain regions of bilateral somatosensory, motor, visual, auditory, orbital frontal, cingulate, restrosplenial and insular cortices, caudate putamen and thalamus. The ICC values for each component are listed in Table 1, in which ICC_1 and ICC_2 are generally higher than corresponding ICC_3 in each component. The mean ICC across the 13 components also showed a significant decrease in the long-term stability (ICC_3) (Fig. 2).

Tab.1 ICC values of the 13 components. The mean ICC value was calculated across components.

	A	B	C	D	E	F	G	H	I	J	K	L	M	Mean
ICC_1	0.87	0.70	0.70	0.70	0.47	0.59	0.61	0.66	0.59	0.61	0.64	0.68	0.37	0.63 SD: 0.12
ICC_2	0.58	0.72	0.55	0.62	0.72	0.66	0.40	0.61	0.49	0.44	0.63	0.54	0.44	0.57 SD: 0.11
ICC_3	0.23	-0.06	0.50	0.14	0.46	0.40	0.13	0.11	0.52	0.08	0.29	0.01	0.06	0.22 SD:0.19

Discussion and Conclusion:

Our data show that the mean ICC across brain networks during short- or mid-term scans (0.63 and 0.57 respectively) was significantly higher than that of long-term scans (0.22). This suggests that longitudinal experiments within weeks would have good reproducibility, but studies across months should be performed with caution. However, different brain networks have differential reproducibility, with the networks of motor cortices, somatosensory cortices, and caudate putamen showed good reproducibility (0.46 - 0.52) even across the long-term scans. Reliability of preclinical rs-fMRI data may also depend on anesthesia procedures, requiring additional assessments on various anesthesia protocols for longitudinal preclinical rs-fMRI experiments.

References:

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Acknowledgments:

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