Serial resting-state fMRI functional connectivity analysis of normal rat brain development

Kajo van der Marel[1], Willem M Otte[1,2], Umesh S Rudrapatna[1], Annette van der Toorn[1], and Rick M Dijkhuizen[1]

[1]*Biomedical MR Imaging and Spectroscopy Group, Image Sciences Institute, University Medical Center Utrecht, Utrecht, Netherlands,* [2]*Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, Netherlands*

INTRODUCTION In recent years, resting-state fMRI-based functional connectivity (RSFC) analysis has emerged as a powerful and widely applied tool in the neuroimaging community for studying focal and global differences in functional brain networks between healthy and diseased subjects[1]. However, even in the course of normal brain development, profound changes in the overall organization of brain function will evidently impact measures of functional connectivity as well. Indeed, RSFC measurements have been used to advance our insight into the developmental trajectories of functional organization of healthy brains, and may help identifying patterns of delayed or disrupted development underlying, e.g., neuropsychiatric conditions[2–4]. Resting-state fMRI studies in humans of different age have revealed distinct developmental patterns of functional brain networks in the transition from childhood to adulthood[4,5]. Notably, it has been shown that the adult brain's strong *homotopic* RSFC[6,7], i.e. its significant functional connectivity between functionally homologous regions in the left and right hemisphere, is not a static phenomenon, but develops in a region- and sex-specific manner[8].

However, most human studies are limited by the cross-sectional nature of the study. Furthermore, many experimental studies are conducted in rat and mouse models of disease. Although several groups have fruitfully applied RSFC measurement to the rodent brain, its neurodevelopmental characteristics have not yet been thoroughly investigated.

The aim of the present study was therefore to characterize the developmental trajectory of functional brain organization in normally maturing rats with serial resting-state fMRI. We focused on the development of homotopic RSFC from pre-adolescence through adolescence (25-46 days of age) into adulthood (60+ days), and hypothesized that it would show region-specific trajectories.

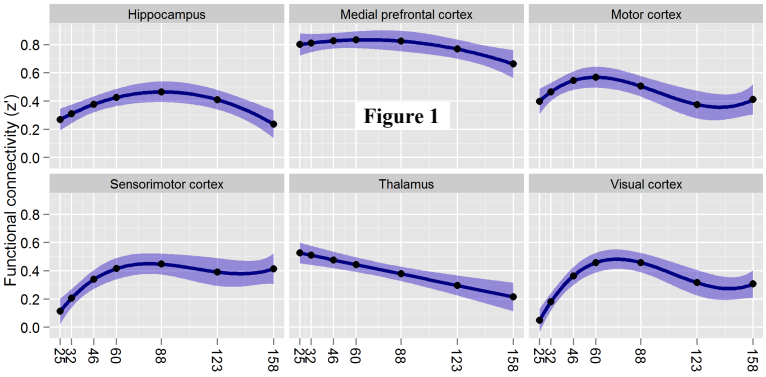

Figure 1

METHODS Serial MRI was conducted on a 4.7T horizontal bore Varian MR system with use of a 90-mm diameter Helmholtz volume coil and an inductively coupled 25-mm surface coil for signal excitation and detection, respectively. 20 male Wistar rats were scanned at post-natal days (P) 25, 32, 46, 60, 88, 123, and 158. Rats were anesthetized and mechanically ventilated with isoflurane (1.0-1.5%) in a 2:1 O_2/air mixture, and immobilized in a MR-compatible stereotactic holder. Blood oxygen saturation, heart rate and expired CO_2 were monitored during MRI, and body temperature was maintained at 37.0 ± 0.5 °C. 10 minutes of ventilation-triggered resting-state fMRI was performed using a T2*-weighted 1-shot 3D gradient-echo EPI sequence (TR/TE=32/19 ms; 12° flip angle; 64×48×32 matrix; 0.5 mm^3 voxels; 600 BOLD images). Anatomical images were obtained with a 3D gradient-echo sequence (TR/TE=6/2.5 ms; 40° flip angle; 160×100×80 matrix; FOV=40×25×20 mm^3).

RSFC preprocessing entailed motion correction, spatial smoothing, data correction with linear regression against mean signal, and band-pass filtering between 0.01 and 0.1 Hz. Anatomical images were matched to a 3D model of a rat brain atlas[9] from intra- and inter-subject registrations obtained with ANTS[10]. RSFC was calculated as the Fisher *z'*-transformed correlation coefficient between average filtered time signals. Regions-of-interest (ROIs), which included 11 cortical (prefrontal, sensorimotor, motor, visual, auditory) and 11 subcortical (striatal, amygdaloid, thalamic and hippocampal) gray matter areas, were projected from the atlas onto the functional time-series for seed-based RSFC analysis. All subsequent analyses were performed in *R* (http://www.r-project.org).

Statistical analysis of the developmental trajectories of homotopic RSFC was performed with multiple linear mixed-effects regressions as implemented in the *nlme* package. For each ROI, the RSFCs were regressed against linear, quadratic or cubic age predictors, with subject as random effect. Model order selection was based on minimum AIC value. Global mean RSFC over combined cortical and subcortical ROIs, respectively, were regressed against age as well, with subject and ROI as random effects.

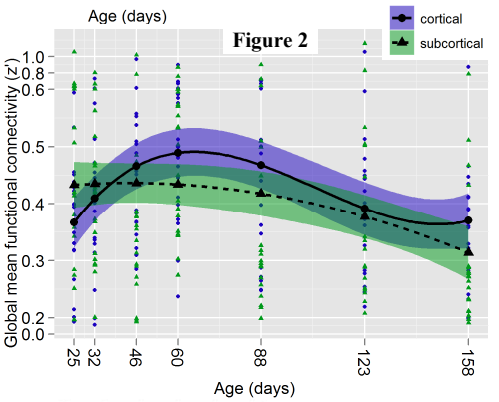

Figure 2

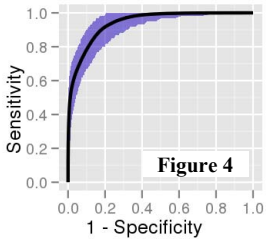

Figure 4

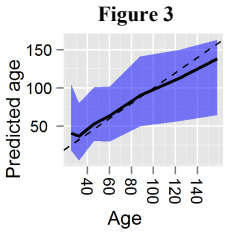

Figure 3

Predictive power of homotopic RSFC was assessed by Support Vector Machine (SVM) regression (*kernlab* package) of age against all combined RSFC values, with bootstrap estimates of prediction intervals. Secondly, a binary SVM classifier was trained to discriminate between (peri-)adolescent (P25-46) and adult (P88-158) RSFC. Bootstrap validation (*caret* package) determined sensitivity, specificity, and area under the ROC curve.

RESULTS Linear regression revealed region-specific developmental alterations in the rat brain's homotopic RSFC. For example, the fitted trajectories and 95% confidence intervals for the optimal models are plotted for 6 regions in *Figure 1*. Bilateral sensorimotor RSFC increased with age, while thalamic RSFC showed a linearly decreasing pattern. Both medial prefrontal cortex and dorsal hippocampus homotopic RSFC strengthened during adolescence, which was subsequently partially lost during early adulthood. *Figure 2* displays global mean RSFC regression overlaid on the individual mean cortical and subcortical RSFC values. The selected subcortical and cortical regions showed distinct and initially opposite developmental trajectories. Juvenile homotopic RSFC was higher in subcortical areas, but followed a consistently decreasing trajectory, whereas rapid adolescent strengthening of cortical RSFC was followed by a decline from adulthood onwards. *Figure 3* shows that the SVM-regression modeling yields reasonable estimates of subject age, based on combined homotopic RSFC values. Furthermore, as can be understood from the bootstrapped ROC curve in *Figure 4*, binary classification quite accurately distinguished adolescent from adult brains, with an area under the ROC curve of 0.94 (95% CI: 0.92-0.96).

DISCUSSION Our data show that serial RSFC measurements in 20 healthy rats enable detailed assessment of neurodevelopmental alterations in specific brain regions. As expected, RSFC trajectories differed on a region-by-region basis. Human homotopic RSFC development progresses in a similar fashion, with sensorimotor RSFC increasing in strength as opposed to higher-order processing areas[8]. Here we found that rat cortical regions that exhibited a pattern of RSFC strengthening followed by a decrease, showed peak RSFC values around the transition period from adolescence to adulthood, i.e. around P60. Furthermore, similar to RSFC's capacity to predict individual human brain maturity[3], both binary classification and regression modeling successfully exploited age-related differences in homotopic RSFC features. These findings combined suggest that normal cortical and subcortical brain maturation profiles based on RSFC may provide valuable benchmarks for identifying and characterizing neurodevelopmental delays and deficits in rodent models of neuropsychiatric disease.

References [1]Lowe *MAGMA* **23** (2010); [2]Power *Neuron* **67** (2010); [3]Dosenbach *Science* **329** (2010); [4]Fair *PNAS* **104** (2007); [5]Fair *PLoS Comp Biol* **5** (2009); [6]Stark *J Neurosci* **28** (2008); [7]Salvador *Neuroimage* **39** (2008); [8]Zuo *J Neurosci* **30** (2010); [9]Paxinos & Watson, *The rat brain* (2005); [10]Klein *Neuroimage* **46** (2009).