Functional connectivity in the rat at 11.7 T: Impact of physiological noise in resting state fMRI

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ABSTRACT

Resting state functional MRI (rs-fMRI) of the brain has the potential to elicit networks of functional connectivity and to reveal changes thereof in animal models of neurological disorders. In the present study, we investigate the contribution of physiological noise and its impact on assessment of functional connectivity in rs-fMRI of medetomidine sedated, spontaneously breathing rats at ultrahigh field of 11.7 Tesla. We employed gradient echo planar imaging (EPI) with repetition times of 3 s and used simultaneous recordings of physiological parameters. A model of linear regression was applied to quantify the amount of BOLD fMRI signal fluctuations attributable to physiological sources.

Our results indicate that physiological noise – mainly originating from the respiratory cycle -dominates the rs-fMRI time course in the form of spatially complex correlation patterns. As a consequence, these physiological fluctuations introduce severe artifacts into seed-based correlation maps and lead to misinterpretation of corresponding connectivity measures. We demonstrate that a scheme of motion correction and linear regression can significantly reduce physiological noise in the rs-fMRI time course, remove artifacts, and hence improve the reproducibility of functional connectivity assessment.

In conclusion, physiological noise can severely compromise functional connectivity MRI (fcMRI) of the rodent at high fields and must be carefully considered in design and interpretation of future studies. Motion correction should be considered the primary strategy for reduction of apparent motion related to respiratory fluctuations. Combined with subsequent regression of physiological confounders, this strategy has proven successful in reducing physiological noise and related artifacts affecting functional connectivity analysis. The proposed new and rigorous protocol now opens the potential of fcMRI to elicit the role of brain connectivity in pathological processes without concerns of confounding contributions from physiological noise.

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Introduction

Functional magnetic resonance imaging (fMRI) of the brain's "resting state" (rs-fMRI) has gained considerable interest during the last 15 years after its first application to probe functional connectivity (Biswal et al., 1995). More recently, it has found its way into animal research (Lu et al., 2007; Pawela et al., 2008, 2009a; Zhao et al., 2008) and has since evolved into a unique tool to investigate functional connectivity in animal models of various pathologies, for example recently demonstrated in stroke (van Meer et al., 2010). In this context, functional connectivity MRI (fcMRI) could complement studies of fMRI and extend the scope to brain networks that are not accessible through sensory stimulation. In addition, fMRI as well as

fcMRI can profit from the ultrahigh fields available in animal MRI regarding the gain in signal-to-noise, sensitivity and resolution.

The terms *rs-fMRI* and *fcMRI* are often used interchangeably, although having a slightly different meaning. To avoid confusions, we will use the term *rs-fMRI* when emphasizing the conditions under which data was acquired; *fcMRI* is used in a more general context focusing on the application as a tool to reveal connectivity networks.

As assessment of functional connectivity relies on spontaneous rs-fMRI signal fluctuations, it is important to know all contributions to these fluctuations. Apart from inherent thermal noise and hardware imperfections (Weisskoff, 1996), the rs-fMRI signal time course is affected by physiological noise that scales linearly with the signal intensity (Kruger and Glover, 2001). As a consequence, the significance of physiological noise increases with field strength and can be the dominating source of temporal noise in human fMRI at high field (Triantafyllou et al., 2005). Although physiological noise comprises spontaneous fluctuations of potentially neuronal origin, there are various interfering sources of non-neuronal fluctuations, most prominently related to the cardio-respiratory cycle (Glover et al., 2000). Contributions of these sources are substantial and have

recently been quantified for fMRI in the human brain at 7.0 Tesla (Bianciardi et al., 2009). Particularly the respiration related signal fluctuations are known to increase with field strength and to depend on the geometric dimensions (Raj et al., 2000). In translation to high field fMRI and fcMRI of the rodent, a significant and potentially confounding contribution of physiological noise can thus be expected. Therefore, the sensitivity to detect changes of functional connectivity in pathological processes could be compromised in the presence of physiological noise. Moreover, the applicability of temporal filtering to remove physiological fluctuations is limited, because the high respiratory and cardiac rates are usually undersampled.

With this motivation we conducted BOLD rs-fMRI experiments in healthy, spontaneously breathing rats under Medetomidine sedation at ultrahigh field of 11.7 Tesla with simultaneous physiological recordings in order to identify and quantify the contribution of non-neuronal sources of physiological noise to the rs-fMRI signal. We applied a scheme of motion correction and subsequent linear regression with the physiological parameters to correct for these contributions. Finally we compared the original and corrected data regarding functional connectivity analysis to assess the impact of physiological noise on connectivity measures.

Material & methods

Experimental protocol

Animal experimental protocol

MRI experiments were performed on spontaneously breathing, male Wistar rats (n = 22, 300–350 g) using Medetomidine sedation with slight modifications to a previously described protocol (Weber et al., 2006). All experiments were conducted in accordance with the German Laws for Animal Protection and were approved by the local animal care committee and regional governmental body (Bezirksregierung Köln).

Data used for this work was acquired in two studies (n = 5 / n = 17) that also involved BOLD fMRI using electrical forepaw stimulation. In both studies, animals were initially anaesthetized using Isoflurane in a mixture of N_2O (70%) and oxygen (30%). Animals were placed in a plastic cradle in prone position. The head was carefully fixed using a bitebar and earbars.

After positioning, anaesthesia was maintained at ~1.5% Isoflurane for adjustments and additional imaging experiments. After completion of these, a bolus of 0.5 ml Medetomidine solution (Domitor®, Pfizer; 1 ml/kg bodyweight added to 10 ml of saline solution) was injected subcutaneously. Onset of the agent's effect was observed by a decrease of respiratory and cardiac rates after which Isoflurane was slowly discontinued within the next 5-10 minutes. 15 to 20 minutes after the bolus, continuous infusion of Medetomidine solution was started at 1 ml/h and N_2O was replaced by N_2. After completion of the imaging session, Atipamezol (Antisedan®, Pfizer; 1 ml/kg bodyweight) was injected subcutaneously together with ~2 ml of saline to reverse the sedative effect and substitute for fluid loss during the experiment.

Physiological monitoring & recording

To observe physiological parameters during MRI experiments, an MR compatible monitoring system (Small Animal Instruments Inc., NY, USA) was used. Via breakout module the system was connected to a custom-made data acquisition system based on DASYLab (measX, Mönchengladbach, Germany) that allowed continuous recording of physiological parameters and, moreover, received the MRI trigger channels.

The monitoring system was operated using a fiber optic temperature probe, respiration pad and fiber optic pulse oxymeter to monitor body temperature (T), oxygen saturation (sO_2), respiratory and cardiac rates (rBPM / cBPM) as well as their corresponding wave-

forms. The acquisition system logged the slow parameters (T, sO_2, rBPM, cBPM) every 10 s over the whole session while MRI triggers, respiratory and cardiac waves were recorded during rs-fMRI data acquisition with a temporal resolution of 1 kHz.

To maintain body temperature at 37 °C during the imaging session, a feedback-controlled water circulation system (medres, Cologne, Germany) was used to supply the base of the cradle and an additional heating pad on the back of the animal.

MR imaging protocol

Experiments were conducted on a 117/16 BioSpec system (Bruker BioSpin, Ettlingen, Germany) with Avance II hardware and a BGA9s gradient system with maximum strength 750 mT/m and a minimum ramp time of 125 μs. Transmission was achieved with a quadrature volume resonator (inner diameter 72 mm) and a standard rat brain quadrature surface coil ($\sim 30 \times 30$ mm^2) was used for signal reception (Bruker BioSpin, Ettlingen, Germany). MRI experiments were executed with ParaVision 5 software.

Adjustments. Animals were positioned in the bore to align the forelimb region of the primary somatosensory cortex (S1fl) to the magnet isocenter, determined by its distance caudal to the rhinal fissure (~5.4 mm).

To optimize field homogeneity, an implementation of MAPSHIM in ParaVision 5 was used for shimming. After acquisition of a fieldmap, a local shim was performed on a $8.0 \times 6.5 \times 6.0$ mm^3 voxel containing the cortical region of interest.

EPI imaging parameters. For acquisition of the actual rs-fMRI scans, gradient echo planar imaging (gEPI) was performed using a 96×96 imaging matrix with an in-plane resolution of 300×300 μm^2 at a bandwidth of 250 kHz. The k-space center was sampled at an echo time (TE) of 17.5 ms with an asymmetric echo at 25% echo position. Five consecutive slices of 1.2 mm thickness were acquired and a separate trigger pulse was played out for each. Each scan consisted of 100 volumes and five additional dummy scans at the start. The repetition time (TR) was chosen to TR = 3000 ms (n = 5) and TR = 2840 ms (n = 17), respectively. In both cases, a volume delay of 2500 ms was used, meaning that the five slices were acquired within ~500 ms. Within each imaging session, one (n = 17) or two (n = 5) of these rs-fMRI scans were conducted and used for analysis.

To enable determination of the raw noise present in the EPI time series, a separate scan with five frames at 0° flip angle was acquired in each session (Triantafyllou et al., 2005) containing noise only.

Data analysis

Unless noted otherwise, all image based processing was performed with ImageJ (Version 1.42q; National Institutes of Health, Bethesda, USA; *http://rsbweb.nih.gov/ij*) using custom-made plug-ins and macros that utilize the Apache Commons Maths Library (Version 2.1; The Apache Software Foundation; *http://www.apache.org*). Results of ROI analyses were handled and further processed using Excel (Microsoft Corporation, Redmond, USA). Processing of physiological monitoring data was performed using IDL (ITT Visual Information Solutions, Boulder, USA).

Prior to any further processing, native ParaVision EPI datasets were converted to 32-bit NIFTI format (Neuroimaging Informatics Technology Initiative; *http://nifti.nimh.nih.gov*). Image intensity was mapped back to the original raw data range to ensure comparability of scans within a session and with respect to the noise determination. To make the datasets suitable for motion correction and brain extraction using FSL tools (FMRIB Software Library; *http://www.fmrib.ox.ac.uk/fsl*), voxel size was scaled up in the NIFTI header by a factor of 10.