Statistical pitfalls in pharmacological MRI

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Introduction

fMRI is increasingly used to monitor the modulating effects of pharmacological agents on human brain function [1]. Often a generalized linear model approach [2] (e.g. SPM, http://www.fil.ion.ucl.ac.uk/spm/) is applied to analyze fMRI data. Within this toolbox, a canonical form of the hemodynamic response function (HRF) is used as a standard regressor to analyze the signal course of the measured data. Despite potential effects of the examined drugs on the mechanisms of neurovascular coupling, most pharmacological MRI (phMRI) studies are analyzed under the premise of an unchanged hemodynamic response function (HRF). This assumption may lead to false negative activation changes when the HRF underlies considerable alterations induced by the employed drugs. Here we demonstrate this statistical pitfall on an exemplary phMRI study and show how this assumption may lead to incorrect conclusions if HRF changes are neglected in statistical analysis methods.

Methods

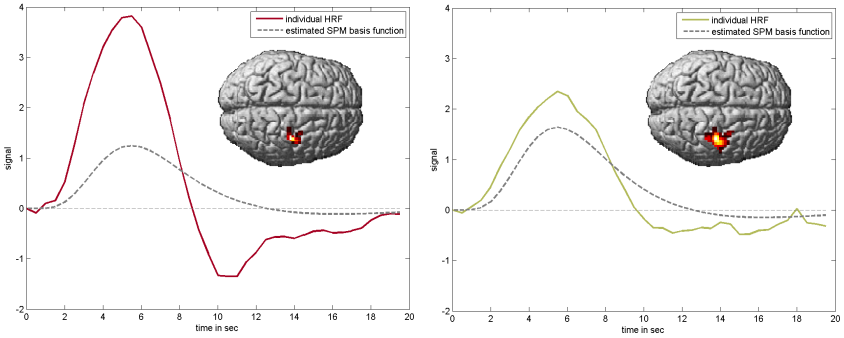
Fourteen healthy right-handed volunteers (8 m, 6 f, mean 23.2±1.9 years) were examined before and after drinking of an ethanol/orange-juice mix (mean blood alcohol concentration [BAC] 0.82±0.07 ‰). A minimum BAC of 0.7‰ had to be reached to be included into the statistical analysis. The time course of the HRF was measured with a repetition time of 500 ms over 20 s. Subjects watched a flickering checkerboard for 1 s, and had to perform a single finger-tap simultaneously. SPM5 was used to determine activation changes in the brain. Furthermore, the corresponding averaged signal-courses of each region were determined for each individual subject. Subsequently, the individual HRFs were fitted with a triple gamma-variate function using the Levenberg-Marquardt algorithm. The fit with an analytic function yielded an increased time resolution and a subsequent optimized estimation of the parameters amplitude, time to peak (TTP), full width at half maximum (FWHM) and area under the curve (AUC) of the positive BOLD response. The mentioned parameters were tested for ethanol caused changes using a paired t-test (SPSS, Version 15.0, http://www.spss.com/).

Results and discussion

RMC, LMC, SMA as well as VC exhibited an increased BOLD signal against the rest condition in both the pre- and post-ethanol conditions. No significant change ($p<0.05$) was observed in the baseline level after ethanol ingestion. Although the individual HRFs varied considerably, ingestion of ethanol changed the shape of observed HRF notably. On average, amplitude significantly decreased in each activated area with a mean reduction between 14.1% and 34.1%. The most evident decrease was detected in the SMA. The FWHM showed a significant increase ($p<0.05$) in the RMC (0.39±0.44 s), the LMC (0.38±0.55 s), and the VC (0.43±0.58 s), whereas this effect was not observed in the SMA. The post-stimulus undershoot shifted towards positive values. To estimate whether the reduced amplitude was balanced by an increased FWHM, the area under the curve (AUC) of the positive overshoot was determined. While the AUC remained unchanged in the RMC, LMC, and VC ($p<0.05$), a significant decrease of 29.9% ($p<0.036$) was observed in the SMA under alcoholic conditions.

The figures show exemplary pre- and post-ethanol curves of a volunteer of the presented study whereas a standard SPM analysis (inlets depicting the results of an event-related analysis using the canonical SPM-built-in HRF) had revealed a false-positive activation increase after ethanol ingestion. A possible explanation for the paradox result that the activation seems to be increased while the BOLD amplitude obviously decreased may be provided by the reduced undershoot in the post-ethanol condition: the overshoot now influences more strongly the fit to the canonical HRF as indicated by the smaller distance between measured and fitted curve in fig. 1b as compared to fig. 1a. This in turn results in a higher beta value that may be erroneously interpreted as an increased activation.

Our findings suggest that whenever statistical methods utilize assumptions about the HRF (like General Linear Models do), and simultaneously experimental conditions may alter the HRF time-course erroneous interpretations may result. The results of our experiments also imply that in studies with sparse HRF-sampling the BOLD signal should be inspected (especially in pharmacological fMRI studies where changes in neurovascular coupling are to be expected).



(1a) sober ethanol (1b)

The curves show raw data and estimated HRF of the RMC averaged

Solid line: averaged HRF, broken line: SPM-based estimation to the HRF using the basis function multiplied with the average beta value across the ROI.

[1] Honey, G. and Bullmore, E., Human pharmacological MRI, Trends in Pharmacological Sciences, 25, 366-374, 2004
[2] Friston et al., Analysis of fMRI time-series revised, Neuroimage, 2, 45-53, 1995