Published in final edited form as:

J Neurosci Methods. 2013 May 15; 215(2): . doi:10.1016/j.jneumeth.2013.02.017.

In vivo evaluation of the effect of stimulus distribution on FIR statistical efficiency in event-related fMRI

J Martijn Jansma¹, Jacco A de Zwart², Peter van Gelderen², Jeff H Duyn², Wayne C Drevets¹, and Maura L Furey¹

¹Mood and Anxiety Disorders Program, Molecular Imaging Branch, Section on Neuroimaging in Mood and Anxiety Disorders, NIMH, NIH

²Advanced MRI/Laboratory of Functional Molecular Imaging NINDS, NIH

Abstract

Technical developments in MRI have improved signal to noise, allowing use of analysis methods such as Finite impulse response (FIR) of rapid event related functional MRI (er-fMRI). FIR is one of the most informative analysis methods as it determines onset and full shape of the hemodynamic response function (HRF) without any a-priori assumptions. FIR is however vulnerable to multicollinearity, which is directly related to the distribution of stimuli over time. Efficiency can be optimized by simplifying a design, and restricting stimuli distribution to specific sequences, while more design flexibility necessarily reduces efficiency. However, the actual effect of efficiency on fMRI results has never been tested in vivo. Thus, it is currently difficult to make an informed choice between protocol flexibility and statistical efficiency. The main goal of this study was to assign concrete fMRI signal to noise values to the abstract scale of FIR statistical efficiency. Ten subjects repeated a perception task with five random and m-sequence based protocol, with varying but, according to literature, acceptable levels of multicollinearity. Results indicated substantial differences in signal standard deviation, while the level was a function of multicollinearity. Experiment protocols varied up to 55.4% in standard deviation. Results confirm that quality of fMRI in an FIR analysis can significantly and substantially vary with statistical efficiency. Our in vivo measurements can be used to aid in making an informed decision between freedom in protocol design and statistical efficiency.

Keywords

event-related fMRI; multicollinearity; finite impulse response analysis; m-sequence

1 Introduction

Historically, the choice of an fMRI analysis method has been guided by the low signal to noise in early functional magnetic resonance imaging. Main goal was to optimize detection power, thus accepting a certain sacrifice in information and reliability by dependence on assumptions. For rapid event related MRI designs (er-fMRI), the most widely applied detection analysis is the so called 'canonical' analysis, referring to the use of a canonical hemodynamic response function (HRF) (Burock et al., 1998; Josephs et al., 1997).

Technical improvements such as the use of multi-channel receiving coil arrays and higher field strengths have since improved signal to noise. Thus, analysis methods that are optimized for information instead of detection become more appealing. These so called 'estimation' analysis methods estimate the HRF related to an event. Advantages of an estimation analyses are that they eliminate the risk of systematic bias that can be present in a detection analysis due to variation in the correctness of a HRF model. Also, an estimation analysis is better suited to test hypotheses related to differences in the onset and duration of the HRF.

A popular estimation analyses for er-fMRI method is the Finite Impulse Response (FIR) method.(Dale, 1999; Glover, 1999; Ollinger et al., 2001). A FIR employs a set of delta-pulse regressors that estimate the hemodynamic response at several time points after stimulus onset. The increase of information acquired with FIR comes with a potential loss in statistical power, compared to a detection analysis. Part of this loss is related to the fact that the HRF is modeled by more regressors then in a detection analysis. More importantly, a FIR also has increased vulnerability for dependency between regressors. Typically, er-fMRI stimulus protocols use stimuli that are randomized over time or 'jittered'. These protocols will have varying levels of statistical efficiency that are difficult to predict or control. Greater flexibility in protocol development can be achieved at the expense of statistical efficiency, as optimal efficiency introduces restrictions on protocol design flexibility. Although the theoretical framework for this is previously established (Birn et al., 2001; Buracas and Boynton, 2002; Kao et al., 2012; Kao et al., 2009; Liu and Frank, 2004; Liu et al., 2001; Wager and Nichols, 2003), there is currently no human experimental data available that has systematically quantified the effect of multicollinearity on FIR fMRI results. For a researcher, this means that it is currently difficult to make an informed choice between protocol flexibility and statistical efficiency.

The main goal of this study was to assign concrete fMRI signal to noise values to the abstract scale of statistical efficiency. Ten healthy subjects performed a 3T fMRI experiment, with a task based on (Hariri, 2002). The task was presented with six different stimulus protocols that represented a full range of feasible efficiency levels. We calculated the within subject signal change and standard deviation of the signal change for each protocol over a selected group of active voxels.

2 Materials and methods

2.1 Subjects

Ten healthy, right-handed volunteers participated in the study. Prior to participation, all volunteers gave written informed consent, which was approved by the Intramural Review Board (IRB) of the National Institute of Mental Health at the National Institutes of Health under protocol 07-M-0021. Participants were provided with earplugs to protect their hearing from the acoustic noise generated by the MRI gradient system.

2.2 Task

Participants performed a match-to-sample task based on Hariri et al. (2002). A stimulus consisted of three female or male faces presented simultaneously in a triangle, together with a fixation cross centered within the stimulus array. Participants indicated with a button push, which of two lower faces was identical to the target face presented on top. Thirty-two trials presented a task stimulus and required a response, and thirty-one trials included only the fixation cross and did not require a response. Task stimuli were presented for 1500 ms, and were followed by a 500 ms inter trial interval, during which only the fixation cross was presented.

2.3 Measurement of multicollinearity

One measure used to identify dependency among regressors, or multicollinearity, is 'tolerance' ('TOL'). Tolerance can be described as the proportion of variance of one regressor that is not accounted for by the other regressors in the model. Tolerance can be calculated as: $[1-R^2]$, where R^2 is the determination coefficient of a multiple regression analysis with the tested regressor as a dependent factor, and all other regressors as independent factors. One advantage of TOL as a measure of efficiency is that it is standardized and can vary from zero to one, with one signifying fully independent regressors. TOL is also related to the 'variance inflation factor' ('VIF'), which equals $1/\text{TOL}$ (Montgomery and Peck, 1982). As an overall value for the level of multicollinearity for a protocol, we averaged the TOL values over all FIR regressors (average TOL or 'aTOL').

2.4 Experiment design

Protocols were kept relatively short so that subjects could readily perform six protocols during a single fMRI session. Protocol length was 63 trials (32 stimulus trials, and 31 baseline trials), adapted to the requirements of an m-sequence to have a specific number of trials ($2^n - 1$, in this study $n = 6$). The sequence ran synchronous with the scan TR (2000 ms), with one trial per TR.

Five stimulus protocols were selected from a set of 128 protocols, generated by randomly re-ordering 32 task and 31 baseline trials. Selected protocols reflected the full range of aTOL values present in the large set (respectively: 0.58, 0.66, 0.73, 0.80 and 0.90). A sixth protocol was based on a pseudo-random m-sequence. Mathematically, an m-sequence can be defined as the longest possible sequence that can be made with a (circular) shifted register, so that every step in the sequence reflects a unique state of the register, and thus should also provide a unique continuation in the sequence. In other words, there is no repeat in the pattern, until you get to the origin again. The resulting very low autocorrelation leads to a virtual absence of multicollinearity for the m-sequence based protocol, and an optimal aTOL value of 1.00 (Sutter, 1987; Sutter, 2001, Buracas and Boynton, 2002; Liu and Frank, 2004).

Previous studies have used other measurements for statistical efficiency, such as a-optimality, or d-optimality (Dale, 1999; Liu and Frank, 2004), (Wager and Nichols, 2003). Unlike the aTOL, these measures are not standardized, but the value is dependent on the characteristics of the specific design such as the number of stimuli. We also calculated these efficiency values for the six designs (values for a-optimality: 1.44, 1.24, 1.01, 1.13, 0.96, 0.88; values for d-optimality: 0.52, 0.31, 0.05, 0.13, 0.04, 0.02).

Correlation of both measures with aTOL were high ((a-optimality: -0.91, d-optimality: -0.85).

2.5 FMRI experiment

Subjects performed six runs of the same task, using the six protocols as defined above. The order of runs was varied and balanced over subjects. Subjects practiced the task prior to the scan session, until they performed well (less than 10 % incorrect responses). In order to achieve circular continuity with the fMRI signal during the actual task phase of the experiment, a requirement for optimal FIR analysis (Kellman et al., 2003), seven pre-sequence trials were added at the beginning of each run, which repeated the last seven trials of each run. These seven trials were expected to cover the effect of the delay in BOLD signal.

2.6 Scans

Subjects were scanned using a GE 3T MRI scanner (GE Healthcare, Waukesha, WI, USA) equipped with an eight channel coil (scan parameters: SENSE-EPI (rate = 2), TE/TR 25/2000 ms, voxel-size: $3.5 \times 3.5 \times 3.0 \text{ mm}^3$, with 0.5 gap between slices). Subjects performed the task in runs of 90 scans (180s duration). The task was presented during scans 10-79. fMRI scans were slice-time corrected to the first slice. Scan volumes were registered to the last volume of the last run to correct for motion, using C-code based on software by Thévenaz et al. (1995) and smoothed with a Gaussian filter (FWHM: 10.5 mm).

2.7 Statistical Analysis

All analyses were performed using in-house developed software using IDL (ITTVIS, Boulder, CO, USA), unless otherwise mentioned. A multiple regression analysis was applied with 15 delta-regressors. Regressor one was identical to the stimulus distribution pattern. Regressors 2-15 were created by 'circularly' shifting the entire sequence one TR. The fifteen resulting beta-maps provide the signal change at time-points $t=0$, up to $t = 28 \text{ s}$ after stimulus presentation.

The fifteen maps were spatially normalized to MNI space using FSL software (Jenkinson et al., 2002). Subsequently, we created a mask of active voxels by selecting voxels that reached a value of $t > 3.09$ ($p < 0.001$) in each of the six protocols, in at least one of the 15 maps. We calculated the within subject average as well as the within subject standard deviation over the voxels in this mask, for each of the fifteen maps in each protocol. These fifteen values were subsequently averaged per subject and per protocol, to provide a single value for each protocol for signal change (average) as well as a noise (standard deviation).

To test the effect of statistical efficiency on fMRI results, we applied an analysis of variance over all six protocols, for both the average as well as standard deviation of the signal change (SPSS 16.0, GLM, repeated measures, linear contrast as well as contrast free for the signal change).

Additionally, we created group activity maps of three protocols covering the full range of efficiency, in order to visualize the effect of reducing within subject noise at group level, where the total noise level is a combination of the within subject and between subject noise.

3 Results

3.1 Task performance

All subjects performed the task with higher than 90 percent correct responses for all protocols. We found no effect of aTOL on task performance across the six protocols (accuracy ($F = 0.41$; $p = 0.54$), reaction time ($F = 0.63$; $p = 0.44$)).

3.2 fMRI measurements

Analysis of variance showed a significant linear contrast between the standard deviation of the signal change and aTOL ($F = 31.07$; $p < 0.001$; see figure 1a). The signal change itself did not correlate with aTOL, indicating that the six stimulus protocols did not differ in their sensitivity for estimating the amplitude and shape of the HRF ($F = 0.056$; $p = 0.48$, linear contrast; see figure 1b).

Compared to the standard deviation for the m-sequence protocol with aTOL = 1.00, the random protocol with the highest efficiency (aTOL = 0.90) showed an increase in standard deviation of 11.6 %, while the protocol with the lowest efficiency (aTOL = 0.58) showed an increase in standard deviation of 55.4 %.

The increase in variance with increased level of aTOL is further illustrated in figure 2, showing results as a function of time since stimulus onset for the protocols with the highest and lowest aTOL values. Figure 2a shows the standard deviation for each time point after stimulus onset for the protocol is higher in the protocol with the lowest tolerance (aTOL = 0.58) compared to the m-sequence protocol. Figure 2b illustrates that the average signal change over the selected voxels is comparable for all time points when comparing the m-sequence stimulus protocol and stimulus protocol with lowest tolerance (aTOL = 0.58). To illustrate the effect of signal estimation variance on group statistics, a group activation map was calculated for the m-sequence and low tolerance stimulus protocol, based on signal changes determined at 6.0 s after stimulus onset). Results, presented in figure 2c, illustrates that optimizing statistical efficiency of FIR analysis can also effect group activation patterns, as it demonstrates the more widespread activity for the m-sequence protocol than for the low tolerance protocol.

4 Discussion

The main goal of this study was to assign concrete fMRI signal to noise values to the abstract scale of statistical efficiency. This report presents measurements of the effect of stimulus protocol design and the associated statistical efficiency on fMRI signal to noise for a FIR analysis. The results show that protocol design significantly and substantially affects within subject noise in a FIR analysis, as reflected in the standard deviation in active voxels. The six tested protocols showed a difference of up to 55.4% in standard deviation over activated voxels. Thus, although a protocol may achieve a level of multicollinearity that is above what is considered acceptable in literature (typically > 0.3 TOL (Montgomery and Peck, 1982)), the effect on experimental results can be substantial, and should be considered an important factor in designing fMRI protocols. Relevance of this effect can be illustrated by the fact that the performance difference between the worst and best protocol is in the same order of magnitude as what can be achieved by increasing the field strength from 3T to 7T (Beisteiner et al.; van der Zwaag et al., 2009). It is unlikely that a researcher would apply a design with a very low efficiency if there is no need for flexibility and there are no special experimental requirements. However, if a researcher needs more flexibility, for instance due to specific stimulus timing requirements, it is possible that efficiency may indeed become much lower, even if the protocol is optimized.

Previous studies have already provided an extensive theoretical framework related to design optimization, and have provided several methods to optimize efficiency within the existing restrictions of an experiment (Dale, 1999; Kao et al., 2012; Kao et al., 2009; Liu and Frank, 2004; Liu et al., 2001; Wager and Nichols, 2003). Our results add to this body of work, in that they provide information that can be helpful in finding the right balance between flexibility and efficiency. While a protocol with high flexibility is obviously attractive, as many experimental requirements can be implemented, some requirements can substantially reduce efficiency level, even if the protocol is optimized. In that case, efficiency can be increased by reducing the requirements, for instance by limiting the number of estimated effects, or limiting the flexibility in timing of stimuli.

With the use of multi-channel coil arrays and higher field strength, an optimized FIR analysis is likely to have enough power to be applied successfully in many experimental paradigms. One important benefit will be that with FIR, it is possible to test hypotheses related to the shape of the HRF, a substantial limitation of detection analysis. FIR has already been successfully applied to test hypotheses regarding regional within-subject differences in HRF in white and grey matter (Fraser et al., 2012), and in cortical depth (Siero et al., 2011) as well as for regional differences in the visual system (de Zwart et al., 2005; Hansen et al., 2004) or auditory system (Takeichi et al., 2010). Additionally, can be used to

test hypotheses regarding an abnormal HRF as a result of a disease (see for instance Dyckman et al., 2011; Luchtmann et al., 2010 or Moessnang et al., 2011).

A second advantage of FIR analysis is that there is less risk of statistical bias and incorrect interpretation of the results due to incorrect assumptions about the HRF. This can be important for clinical fMRI, such as in presurgical planning, where the HRF may be affected by the disease. Importantly, the effect of statistical efficiency is theoretically larger in single subject analysis, such as for presurgical planning, than in a group analysis, where between subjects variation is an additional noise factor. Also, this can be valuable for complex cognitive experiments, where both the onset and length of the HRF may vary substantially between brain regions as well as between subjects, due to differences in onset and duration of underlying neuronal activity (see for instance Manoach et al., 2003, van Raalten et al., 2008, Sokol-Hessner et al., 2012).

However, estimation and detection analysis are not exclusive. A powerful analysis approach can be created by combining both. For instance, ROIs could be chosen based on a detection analysis, while hypotheses could be tested within these ROIs using a FIR analysis. Unfortunately, it is not possible to combine maximum estimation and detection efficiency in one protocol. This trade-off lies in the fact that maximum detection efficiency requires that the stimulus protocol does not include frequencies that are higher than the highest HDR spectral density, while optimal estimation efficiency does require inclusion of these higher frequencies. There is however a group of 'optimal' protocols for which it is not possible to increase efficiency in one domain without decreasing efficiency in the other domain (Birn et al., 2001; Liu and Frank, 2004; Liu et al., 2001). Several useful approaches to create optimal protocols with a specific balance between estimation and detection efficiency have been discussed by Liu et al. (2004). Importantly, the trade-off is not symmetrical: while a block protocol has high detection efficiency, it has virtually no estimation efficiency. However, a protocol with high estimation efficiency also has adequate detection efficiency, as is being demonstrated by the many er-fMRI experiments applying a detection analysis. In practice, protocols with high estimation efficiency may perform even better than expected in a detection analysis, as they also have low predictability (Liu et al., 2001). Low predictability can prevent reduction in effective detection power due to adaptation (Krekelberg et al., 2006).

Optimizing statistical efficiency does introduce restrictions on the stimulus protocol. Importantly, trials cannot be omitted in the analysis without affecting the efficiency. Nor is it possible to use an optimization strategy, if trials are classified afterwards, for if a researcher is interested in activity related to specific responses.

For the m-sequence there is the additional restriction, as mentioned earlier, that the length is pre-defined by $2^n - 1$. Statistical efficiency of the m-sequence protocols is however robust for adding elements if the elements added repeat the sequence from start.

To conclude, our study shows that fMRI signal to noise in a FIR analysis is substantially affected by protocol design. The level of this effect as shown in our in vivo measurements can be used to aid in making an informed decision between freedom in protocol design and statistical efficiency.

Acknowledgments

The authors thank Peter Kellman (NHLBI, NIH) for his contributions to developing the m-sequence method for fMRI.

This research was supported by the Intramural Research Program of the National Institute of Mental Health, and National Institute of Neurological Disorders and Stroke, National Institutes of Health.

References

- Beisteiner R, Robinson S, Wurnig M, Hilbert M, Merksa K, Rath J, Hollinger I, Klinger N, Marosi C, Trattnig S, Geissler A. Clinical fMRI: evidence for a 7T benefit over 3T. *Neuroimage*. 2011; 57:1015–21. [PubMed: 21620980]
- Birn RM, Saad ZS, Bandettini PA. Spatial heterogeneity of the nonlinear dynamics in the FMRI BOLD response. *Neuroimage*. 2001; 14:817–26. [PubMed: 11554800]
- Buracas GT, Boynton GM. Efficient design of event-related fMRI experiments using M-sequences. *Neuroimage*. 2002; 16:801–13. [PubMed: 12169264]
- Burock MA, Buckner RL, Woldorff MG, Rosen BR, Dale AM. Randomized event-related experimental designs allow for extremely rapid presentation rates using functional MRI. *Neuroreport*. 1998; 9:3735–9. [PubMed: 9858388]
- Dale AM. Optimal experimental design for event-related fMRI. *Hum Brain Mapp*. 1999; 8:109–14. [PubMed: 10524601]
- de Zwart JA, Silva AC, van Gelderen P, Kellman P, Fukunaga M, Chu R, Koretsky AP, Frank JA, Duyn JH. Temporal dynamics of the BOLD fMRI impulse response. *Neuroimage*. 2005; 24:667–77. [PubMed: 15652302]
- Dyckman KA, Lee AKC, Agam Y, Vangel M, Goff DC, Barton JJS, Manoach DS. Abnormally persistent fMRI activation during antisaccades in schizophrenia: a neural correlate of perseveration? *Schizophr Res*. 2011; 132:62–8. [PubMed: 21831602]
- Fraser LM, Stevens MT, Beyea SD, D'Arcy RC. White versus gray matter: fMRI hemodynamic responses show similar characteristics, but differ in peak amplitude. *BMC neuroscience*. 2012; 13:91. [PubMed: 22852798]
- Glover GH. Deconvolution of impulse response in event-related BOLD fMRI. *Neuroimage*. 1999; 9:416–29. [PubMed: 10191170]
- Hansen KA, David SV, Gallant JL. Parametric reverse correlation reveals spatial linearity of retinotopic human V1 BOLD response. *Neuroimage*. 2004; 23:233–41. [PubMed: 15325370]
- Hariri A. The Amygdala Response to Emotional Stimuli: A Comparison of Faces and Scenes. *Neuroimage*. 2002; 17:317–23. [PubMed: 12482086]
- Josephs O, Turner R, Friston K. Event-related fMRI. *Hum Brain Mapp*. 1997; 5:243–8. [PubMed: 20408223]
- Kao L, Bulkin Y, Fineberg S, Montgomery L, Koenigsberg T. A case report: lobular carcinoma in situ in a male patient with subsequent invasive ductal carcinoma identified on screening breast MRI. *Journal of Cancer*. 2012; 3:226–30. [PubMed: 22670156]
- Kao MH, Mandal A, Lazar N, Stufken J. Multi-objective optimal experimental designs for event-related fMRI studies. *Neuroimage*. 2009; 44:849–56. [PubMed: 18948212]
- Krekelberg B, Boynton GM, van Wezel RJ. Adaptation: from single cells to BOLD signals. *Trends Neurosci*. 2006; 29:250–6. [PubMed: 16529826]
- Liu TT. Efficiency, power, and entropy in event-related fMRI with multiple trial types Part II: design of experiments. *Neuroimage*. 2004; 21:401–13. [PubMed: 14741677]
- Liu TT, Frank LR. Efficiency, power, and entropy in event-related FMRI with multiple trial types Part I: theory. *Neuroimage*. 2004; 21:387–400. [PubMed: 14741676]
- Liu TT, Frank LR, Wong EC, Buxton RB. Detection power, estimation efficiency, and predictability in event-related fMRI. *Neuroimage*. 2001; 13:759–73. [PubMed: 11305903]
- Luchtmann M, Jachau K, Tempelmann C, Bernarding J. Alcohol induced region-dependent alterations of hemodynamic response: implications for the statistical interpretation of pharmacological fMRI studies. *Exp Brain Res*. 2010; 204:1–10. [PubMed: 20502888]
- Manoach DS, Greve DN, Lindgren KA, Dale AM. Identifying regional activity associated with temporally separated components of working memory using event-related functional MRI. *Neuroimage*. 2003; 20:1670–84. [PubMed: 14642477]

- Moessnang C, Frank G, Bogdahn U, Winkler J, Greenlee MW, Klucken J. Altered activation patterns within the olfactory network in Parkinson's disease. *Cereb Cortex*. 2011; 21:1246–53. [PubMed: 21047984]
- Montgomery D, Peck E. Multicollinearity. *Introduction to linear regression analysis*. 1982:299–300.
- Ollinger JM, Shulman GL, Corbetta M. Separating processes within a trial in event-related functional MRI. *Neuroimage*. 2001; 13:210–7. [PubMed: 11133323]
- Siero JCW, Petridou N, Hoogduin H, Luijten PR, Ramsey NF. Cortical depth-dependent temporal dynamics of the BOLD response in the human brain. *J Cereb Blood Flow Metab*. 2011; 31:1999–2008. [PubMed: 21505479]
- Sokol-Hessner P, Hutcherson C, Hare T, Rangel A. Decision value computation in DLPFC and VMPFC adjusts to the available decision time. *Eur J Neurosci*. 2012; 35:1065–74. [PubMed: 22487036]
- Sutter, E. A practical nonstochastic approach to nonlinear time-domain analysis. Plenum; New York: 1987.
- Sutter EE. Imaging visual function with the multifocal m-sequence technique. *Vision Res*. 2001; 41:1241–55. [PubMed: 11322969]
- Takeichi H, Koyama S, Terao A, Takeuchi F, Toyosawa Y, Murohashi H. Comprehension of degraded speech sounds with m-sequence modulation: an fMRI study. *Neuroimage*. 2010; 49:2697–706. [PubMed: 19878726]
- van der Zwaag W, Francis S, Head K, Peters A, Gowland P, Morris P, Bowtell R. fMRI at 1.5, 3 and 7 T: characterising BOLD signal changes. *Neuroimage*. 2009; 47:1425–34. [PubMed: 19446641]
- van Raalten TR, Ramsey NF, Duyn J, Jansma JM. Practice induces function-specific changes in brain activity. *PloS ONE*. 2008; 3:e3270. [PubMed: 18827897]
- Wager TD, Nichols TE. Optimization of experimental design in fMRI: a general framework using a genetic algorithm. *Neuroimage*. 2003; 18:293–309. [PubMed: 12595184]

Figure 1. within subject results of the fMRI experiments

a. Standard deviation of signal change, averaged over all time points, as function of tolerance (aTOL). This value showed a significant linear contrast with aTOL.

b. Measured activation-related signal change, averaged over all time points, as a function of tolerance (aTOL). No significant linear contrast with aTOL was found.

Figure 2.

a. Standard deviation of the hemodynamic response signal change observed in the fMRI measurements for the m-sequence protocol (blue) and the random protocol (red), averaged over active voxels per subject and over subjects.

b. The measured hemodynamic response function, observed in the fMRI measurements for the m-sequence protocol (aTOL = 1.00, blue) and a random protocol with high multicollinearity (aTOL = 0.58, red), averaged over active voxels per subject and over subjects. Results illustrate that there is no significant effect of multicollinearity on the average signal.

c. Group activation, calculated for the signal at time point $t = 6.0$ for protocols with a high (1), medium (2) and low (3) tolerance. Group statistics illustrate the effect of within subject variance in signal estimation on group statistics, indicating higher detection sensitivity with higher tolerance.