**Starting of the „methods &execution” – part of my thesis**

# The MRI image series, both structural and functional, where obtained using a 3 Tesla Siemens Skyra – Scanner precise location and supervisor required?. Structural images in the form of 3D MPRaGEs (three-dimensional magnetization prepared rapid gradient echoes), functional images as GE – EPIs (gradient echoes – echo planar imaging (Cartesian readout)).

# The MPRaGEs where programed using a TR of 2300ms and a TE of 2.32ms with voxel dimensions of 0.938x0.938x0.9mm.

# GE-EPIs had a TR of 1800ms, a TE of 25ms and a 3.1x3.1x3.0mm voxel size. They were acquired in an interleaved fashion with 36 slices at a thickness of 3mms and a distance factor of 15%. The Phase encoding direction was anterior -> posterior.

The part of the study that I was working on had been designed as an EEG-fMRI combined examination. Thus, both data sets were acquired at the same time. For this purpose, high frequency – resistant EEG caps had to be fitted to each participant before the session within the scanner could begin. They would arrive 2 hours in advance. This seemingly over extensive timeslot was necessary, because each electrode on the cap needs to be fitted to the subjects’ skull and the impedance has to be reduced, until a setting suitable for recording is reached.

After the participants had received the safety instructions and were told about the nature and possible peculiarities of an MRI examination, they were equipped with headphones, a special visual device designed to enable the participant to look at a screen positioned at the posterior end of the bore and a two – button console (buttons were positioned on the left and right side of it.) need precise dimensions of the console

After having been introduced into the bore of the scanner, each of the participants went through the same routine running as thus:

The machine first sent out so-called dummy-pulses in order to attain the optimal recording conditions in terms of T1 saturation effects and signal decay. The entire sequence protocol that followed contained Diffusion Tensor Imaging – sequences as well as white matter nulling, designed to look at different deep - brain structures, but as none of these are relevant to my work and will be analyzed in depth at a different opportunity, I will lay my focus exclusively on the anatomic and functional sequences.

The creation of a field map is the next step when wanting to ensure high quality, precise and, most crucially, spatially conclusive recording throughout the MRI session. Basically, it establishes certain landmarks within the anatomy of interest and secures that signal originating from distinct areas is always referenced back to them and not attributed to a different origin inside the brain (especially relevant for the DTI.)

The structural sequence and three functional sequences followed the field map.

The first and third functionals were resting – state measures the analysis for which is very different, answers a different inquiry raised by the paradigm of the grand project and will be reported in a different publication.

The second functional was the task – state measure.

(The total TA was calculated at 16.35mins but was as good as never needed because participants would reach the end of the tasks in less time.)

The design of the experiment was exactly that of the Koster – tasks’ original structure (Koster, E. H., De Raedt, R., Goeleven, E., Franck, E., & Crombez, G. (2005). Mood-congruent attentional bias in dysphoria: maintained attention to and impaired disengagement from negative information. *Emotion*, *5*(4), 446.):

The task is separated into four parts: A fixation, the priming, a mask and the reaction to a target stimulus, all of which were presented in white fond on a black screen. Need precise dimensions of screen and elements in presentation (box in which the words appeared, target square etc.)

After a fixation cross was presented for 500ms, the priming used verbal, self – referencing emotional cues being either positive, negative or neutral (need! : word data base, accessible at location.) Those would appear in a box positioned either in the center on the left or the right side of the screen. Priming occurred with a duration of 1500ms, after which a 50ms blank mask was applied. Finally, a target stimulus in form of a white square was shown until the participant pressed one of the two buttons in his hands. The instructions were to press, as quickly as possible, on the side where the square appeared. This design allowed for valid as well as invalid cues depending on whether the target appeared on the same side as the prime or the opposite.

# The tool for the data analysis was MATLAB version 2013a

# The data import and preprocessing of the MRI – images were performed using the statistical parametric mapping toolbox SPM (specifically SPM12 (version 6225, downloaded from <http://www.fil.ion.ucl.ac.uk/spm/>).

# The images were imported in the form of nifti – images and then run through an SPM – batch programmed preprocessing pipeline.

# I additionally used the FSL application BET (Brain Extraction Tool, bias field & neck cleanup, robust brain center estimation, FSL, FMRIB <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>) to get rid of excessive amounts of neck and shoulder within the anatomic images. I then used the displayfunction in the SPM interface to reorient anatomic and functional images along the AC-PC line, re-centering them to their new position, with the result of a uniform and central fit before the beginning of preprocessing.

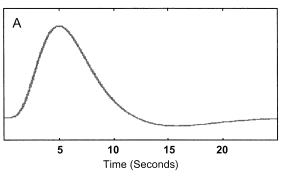
# The pipeline used for this purpose included the following steps: Realign & Unwarp, Slice Timing (correction), Corregister: Estimate, Segment, Normalize: Write, Smooth and again Normalize:Write.

# The first two steps adapted the functional images to the specific parameters (TR, TE, number of slices etc.) of our data collection. The third step corregistered the anatomic images and the unwarped mean functional image. Segmentation created three substance – maps from the corregistered images, something that is very useful to be used as mask in succeeding analysis, as it can be applied in order to focus eclusively on “the right signal” meaning cortical and subcortical “activation” (change of oxygen-bound hemoglobin over time) and not, for instance, activity of adjoining musculature which is irrelevant to a cfMRI paradigm. Normalization writes the nifti – images to a normalized template of a brain (anatomic references taken from the brain atlas created by the Montreal Neurological Institute), a step used in the attempt to create a global approach to shape, size, connection and transition of brain regions when it comes to Brain sciences. Though this procedure comes with a cost, the loss or distortion of brain asynchronies typical for human neural architecture (e.g. elongated temporal-occipital pole of the right hemisphere), it is a necessary one, if the results of unconnected studies are supposed to be interpretable in the same fashion. Smoothing is a step I am very skeptical to use as this runs a three-dimensional Kernel function over the signal in each voxel, normalizing it in a certain fashion. Nonetheless, suppressing noise and effects of residual differences in function and anatomy is essential for a clean inter - subject averaging. I have therefore decided to insert it into my preprocessing.

After the preprocessing was completed, I turned towards the construction of my first level analysis.

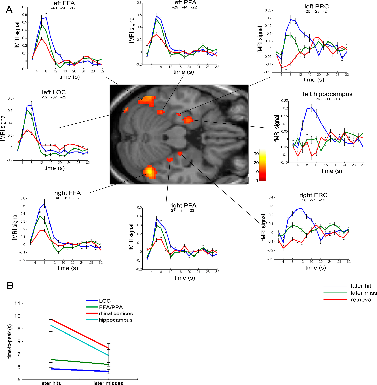
The general linear model (GLM) is a standard approach for setting all the relevant vectors into an interactive matrix. As this is a good preparation for setting up a Dynamic Causal Model on second level analysis (and also standard in SPM12), I chose it for within - subject examination. The usual architecture of a GLM includes one vector for each event (in our case 3 emotions x congruency (2) x side (2) = 12 events -> 12 vectors), a series of vectors to remove slow drift and similar artefacts, vectors to account for outsiders (abnormally high and/or sudden changes in amplitude or frequency) and movement as well as a constant vector which is a basic requirement for the matrix algebra used by the statistic parametric mapping toolbox.

The preprocessed data, still essentially a collection of sums and products from systematically arranged and re-arranged numbers, is then convolved with a basis function, thereby resulting in the typical HRF function for every voxel, whose interactions, (in-) congruencies, (a-) synchronies, quantities, means, variances, change-rates and correlations can then be analyzed statistically. There are different kinds of basis functions depending on the nature and quantity of assumptions made about the physiology underlying hemodynamic blood flow and blood oxygenation level adaptation. The standard is the so-called canonical HRF, a double gamma function with its’ characteristic steep increase, peak after approximately 6 seconds, fall and posterior undershoot until returning back to baseline about 16-18 seconds after the response has been triggered.



(Image taken from Lindquist, M. A. (2008). The statistical analysis of fMRI data. *Statistical Science*, *23*(4), 439-464.)

I decided to use a so – called temporal basis set to convolve my imaging data with. It is a linear combination of the standard HRF function + time derivative + dispersion derivative, allowing for more variation in shape and timing and thus being able to account for different HRFs in different areas of the brain as well as across different conditions, a flexibility shown to be of significant importance.

 (A demonstration of such a difference shown here in a visual presentation task by Summerfield, C., Greene, M., Wager, T., Egner, T., Hirsch, J., & Mangels, J. (2006). Neocortical connectivity during episodic memory formation. *PLoS Biol*, *4*(5), e128.

Information about the mathematical approach and arguments for different modelling techniques is available from Lindquist, M. A., Loh, J. M., Atlas, L. Y., & Wager, T. D. (2009). Modeling the hemodynamic response function in fMRI: efficiency, bias and mis-modeling. *Neuroimage*, *45*(1), S187-S198..)