Philipps Universität Marburg

Application for ethical approval of a research project from the ethics committee of the faculty of Medicine of the University of Marburg

A. Formal aspects

1. Title of the study

The networks they are a-changin' – A pilot study aimed at comparing different connectivity patterns in the auditory network during processing of speech, song and instrumental music in lefthanders without musical background and musicians.

2. Coordinator

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3. Other participants

XXXXXX

4. Multi-centre project

No.

5. Employer

None.

6. Funding status

The project isn't funded through external funds.

7. Audit contract/Investigator remuneration

There is no audit contract and no remuneration.

8. Has the present study been proposed for approval to an ethics commission before?

No.

B. Project description

9. Planned study project

9.1 Research question

The aim of the present study is to investigate differences in connectivity during processing of language, song and instrumental music in primary and secondary areas of the auditory cortex. In relation to these differences, the focus will be laid on the influence of handedness (right-handers and left-handers) and musical training (musicians and non-musicians) on inter- and intrahemispheric connectivity. In order to achieve this, combined measurements of functional magnetic resonance (fMRI) and electroencephalography (EEG) will be implemented to answer the following research questions:

1. How does a progressively growing level of musicality, which will be manipulated through the presentation of stimuli ranging from language to instrumental music, influence connectivity between different areas of the auditory cortex?
2. Is there measurable variability between connectivity models of left-handers and musicians in comparison with right-handers and non-musicians?

9.2 State of knowledge

The core areas of the auditory cortex include the primary auditory cortex (PAC), which anatomically corresponds to Heschel's gyrus (Da Costa et al., 2011), as well as the neighbouring structures in the superior temporal gyrus (STG) known as the planum temporale (PT: posterior STG) and the planum polare (PP: anterior STG) (Zatorre, 2002). In the PAC it is possible to observe a tonotopic representation of auditory stimuli with regard to their frequency spectra (Humphries, Liebenthal, & Binder, 2010), whilst the neighbouring secondary areas react to the modulation of spectrotemporal aspects of the stimuli (Norman-Haignere, Kanwisher, & McDermott, 2015). Various studies from the past years have shown distinct neural correlates of the cortical processing of language and music in the non-primary auditory cortex. Among these Rogalsky et al. (2003) were able to show in an fMRI experiment that spoken sentences activate ventro-lateral areas of the STG, whereas melodies tend to stimulate dorso-medial parts of the STG. Also in areas with overlapping activity it was possible to make distinct activation patterns between language and music visible thanks to detailed multivoxel pattern analyses (MVPA) (Norman-Haignere et al., 2015; Rogalsky et al., 2011). Furthermore, there is evidence for hemispheric lateralization effects which speak for a tendency to left lateralization of language in contrast with the processing of music, widely distributed in the right hemisphere (Josse & Tzourio-Mazoyer, 2004; Tervaniemi, 2006).
Musical training is considered to be a relevant factor of influence in the processing of language and music. Angulo-Perkins et al. (2014) were able to prove that the neural activation patterns in musicians differed from those of non-musicians in the planum polare (PP): this area was found to be bilaterally active only in musicians. Additionally, it was observed that more linguistically sensitive areas such as the planum temporale (PT) are also activated in response to musical stimuli. In relation to these findings it seems reasonable to suggest that the ability to discriminate between activation patterns in the processing of language and music in the auditory cortex of musicians might be smaller than in non-musicians and that musical stimuli in the first sample be processed in a more similar way to language.

9.3 Purpose of the study

The distinctive characteristics of language and music can be evaluated in terms of their spectrotemporal features. The processing of speech as a continuously changing sequence of broadband tones requires a high temporal resolution, whilst the slower and finer frequency variations in tonal patterns of music need a good spectral resolution (Zatorre, Belin, &

Penhune, 2002). By imagining song as including spectrotemporal characteristics of music as well as language, it is possible to develop a sequence of stimuli ranging from language to song to instrumental music and thus define them in the framework of a progressively growing degree of musicality.
To our knowledge, it's mainly the differences in activation between language and music that have been investigated to the present date, and less attention has been focused on connectivity differences between regions of the auditory cortex. The above-discussed degree of musicality, which mirrors the changes in both spectral and temporal modalities, should accordingly call forth measurable excitatory or inhibitory connectivity differences. A general consideration of connectivity patterns within and between the bilateral auditory cortices could also contribute to the discussion about the suggested lateralization effects in language and music. There are reports of specific lateralization in spectral and temporal resolution which have been associated with hemispheric dominance in the processing of music and language (Scott & McGettigan, 2013; Zatorre et al., 2002) and musical training appears to modify the related activation patterns. The present study intends to deal with the connectivity patterns which have not yet been object of investigation. At the same time, many studies in cognitive science exclude left-handed participants, since it has been shown that the processing of sensory inputs in left-handers deviate from the observed right-handed norm (e.g. Pujol, Deus, Losilla, & Capdevila, 1999). Though it is reasonable to avoid this variance in the data, the informative value coming from the inclusion of left-handers towards a better understanding of cerebral functions is nowadays acknowledged, especially as far as lateralization studies are concerned (Willems, Der Haegen, Fisher & Francks, 2014). Since this study intends to focus on lateralization effects, it seems reasonable to extend the research question to a left-handed sample.
The combined measurement of EEG and fMRI can be considered as advanced, since the neural mechanisms to be investigated are to be observed with a high temporal (EEG) and spatial resolution (fMRI). This combination is expected to provide the advantages of both methods: the information exchange between temporal and spatial aspects of neural correlates can be useful to substantiate stronger statements based on the data..
In relation to these basic research results, it is relevant to point out the possibilities for their clinical application, for example in the improvement of cochlea implants, in the therapy of hearing impairment or autism spectrum disorder. A contribution to a better understanding of psychiatric disorders is also expected..

10. Type of the research question

Basic research pilot study with healthy subjects.

11. Design

Experimental study with pseudorandomised and balanced conditions a well as temporal sequences.

12. Time plan
Planned start of the study: March 2018
Planned end of the study: February 2019

Research plan

13. Testing of hypotheses

The first hypothesis of this study is that in the processing of language, song and instrumental music varying connectivity patterns between the primary and the secondary cortex will be observed. According to the possibility to answer this first research question, the second hypothesis states that left-handers and musicians show other models of inter- and intrahemispheric connectivity in comparison with the right-handed norm group without musical training. Three participants groups will be recruited, which will differ in handedness (left- and right-handers) and musical training (musicians and non-musicians).
The stimuli chosen for the experiment are extracted from the study by Angulo-Perkins et al. (2014). In order to exclude semantic aspects as well as gender biases, sentences and song were recorded in different languages and spoken/sung by women and men in equal measure. Furthermore, the melodies used are new compositions played on various instruments, in order to exclude prior knowledge.
The regions of interest for the validation of hypotheses are the HG, the PP and the PT, since these are the core auditory areas in which unimodal cortical processing of auditory stimuli across higher and more complex areas of the cortex take place. Furthermore, the literature has widely reported findings involving these areas as related to differences in the processing of music and language (see par. 9.2). On a bilateral level there are six regions which should be considered for fMRI scanning during the stimuli-hearing task; hypothesis-based connectivity models should also be built on these regions of interest. By implementing the dynamic causal modelling (DCM) analysis tool (Friston et al., 2003), so-called winning models will be calculated, which should explain the data from the three sample groups in the best possible way. The observed patterns of connectivity will then be compared (right-handed musicians with right-handed non-musicians, left-handed non-musicians with right-handed non-musicians) and it will be interesting to investigate possible differences between left-handers or musicians and the norm group.

14. Primary endpoints

With the first research question of the study it is our aim to investigate connectivity patterns in the auditory cortices in association with this kind of stimuli (speech, song, instrumental music). The second research question, building on the first, should provide insights on intra- and interhemispheric connectivity patterns and their differences. In order to ensure consistency of results across methods and their validity, the combined implementation of EEG and fMRI is planned. As far as the spatial analysis of the regions of interest involved in the hypotheses (HG, PP, PT) is concerned, this purpose is served by the fMRI data. On the other hand, the EEG technique provides a precise definition of the temporal sequence of different stages of stimulus processing. A combined Analysis of the EEG and fMRI data allows the methods to reciprocally inform one another with the complementary aspect of the data. The common analysis of data sources should in this way lead to a better explanation of variance of the data or cover proportions of variance that haven't been considered and that are only recognisable in the combined analysis.

15. Number of participants

In fMRI studies effect sizes aren't specified, for which reason the chosen number of participants is based on the available literature. In order to obtain significant results in connectivity differences in the auditory cortex, we strive for a sample of 20-30 individuals for each of the three participant groups. Since no directly comparable studies concerning connectivity differences in the auditory cortex have yet been published, we are going to take studies concerning activation differences in the processing of music and language using fMRI data as a reference. Among these, in the studies by Abrams (2011) and Rogalsky (2011), 20 participants were sufficient to obtain significant results. Angulo-Perkins et al. (2014) , who investigated neural activation differences between musicians and non-musicians, recruited each 28 participants for the musicians' group and 25 non-musicians. Ultimately at least 60 participants should be recruited for the project, 20 for each group, in order to be able to compare them in a significant manner. The recruitment is carried out via notices in the university facilities and the students' university mailing list (see Annex I).

16. Particularly vulnerable individuals

Minors and adults incapable of consent are not included in the study.

17. Inclusion and exclusion criteria

Inclusion criteria:

•Age between 18 and 29 years. Studies have shown that functional activation patterns measured by fMRI correlate with age of the subjects and that a BOLD signal reduction is observed in older subjects (e.g. Hesselmann et al., 2001).
• normal hearing

Exclusion criteria:

• Drugs and alcohol abuse
• diagnosed developmental language disorders, reading and writing disability
• prior history of neurologic or psychiatric disorders
• somatic illnesses, which depending on their nature and severity might interfere in the planned investigations or have an influence on the investigated parameters or endanger the participant during the experiment
• medical contraindications against the performance of an fMRI session (e.g metal parts in the body, such as implants, hearing aids, pacemakers, infusion pumps, surgical screws or disks after bone-breaking, metal splints, contraceptive coils for women etc.)
• pregnancy
• inability to observe the experimental proceedings

The inclusion and exclusion in the fMRI examination is carried out with the aid of an extra questionnaire (see Annex I), which especially points out the possible medical contraindications of fMRI sessions.

18. Statement about statistical evaluation

Data from the two chosen modalities are going to be collected, the BOLD response via fMRI and the event related potentials via EEG.

EEG-Analyse
The EEG data are going to be preprocessed and analysed using the open source MNE software package (“minimum-norm current estimates for MEG/EEG, after e.g. Gramfort et al., 2014) based on the python programming language.
Before the statistical analysis, the preprocessing will take place, in which the data will be cleaned from the gradient artefacts, which emerge via the magnetic field in the scanner, and from other physiological parameters visible in the signal, such as movement, heartbeat and breathing (cardioballistic artefacts). Likewise, the signal quality will be optimised through bandpass filtering and re-referencing. The combined implementation of EEG and MRI allows the computation of connectivity patterns between chosen cerebral areas obtained from the EEG recordings and the respective predicted sources of activation in the brain (inverse solution). In this kind of source space connectivity analysis (e.g. Barzegaran & Knyazeva, 2017) the sources of event-related potentials in EEG are calculated with the underpinning of structural MRI data, in order to verify the strength of the connections between the areas involved in a common space of voltage sources (source space).

MRT-Analyse
The preprocessing of MRI data is carried out with a preprocessing pipeline in Python. Elements from the programs AFNI, FSL, FreeSurfer, ANTs and SPM12 are used for the standard preprocessing steps of MRI data (normalisation, coregistration, etc.). Subsequently, the measured voxel activation patterns are to be represented thanks to the general linear model (GLM) in SPM12. The brain activity (the BOLD response) in the regions of interest for the research question (PP, PT, HG) are to be observed under the various stimulus-dependent conditions (speech, song, instrumental music).
In relation to the temporal differences in activation of the investigated ROIs, temporal sequences are to be extracted for the subsequent connectivity analyses. The DCM analysis tool (Friston et al., 2003) allows the generation of hypothesis-based connectivity models, which should reproduce the temporal patterning of the involved neural systems. Among the possible models included in the model space, the model that best reflects the temporal activation changes is to be determined. The extracted time series are then to be inserted into a model estimation based on bayesian statistics.
In relation to the research question a winning model for each condition (speech, song, instrumental music) will be determined. For these winning models, coupling parameters will be analysed, which should, as time constants, give insight on how fast information is transferred from one region to another. As far as these coupling parameters are concerned, it is possible to investigate further differences between participant groups (right-handed non-musicians, left-handed non-musicians, right-handed musicians) via ANOVA testing.

<u>Exposure and risks</u>

19. Statement and description of all project-related actions on the patient or experimental subject

Preparation
One day before the experiment an explanation of the procedure and of the risks of the investigation for the subject takes place. The written informed consent with regard to the

participation in the study (informed consent, see Annex I) occurs on the day of the experiment, after the participant has had time to think it over. After that, subjects are tested on their aptitude for the participation in the study. A questionnaire is conducted in order to secure that no metal objects are present in the participant's body or other disorders considered to as exclusion criteria are found (see Annex I).

Implementation

The participants are first of all requested to wear a lab coat in order to avoid marks on their clothing from the electrode gel. After that the MR-compatible EEG cap (a 32-channel system) is applied: a special gel, clinically tested to prevent irritation of the skin, is supplied to each of the points of contact of the electrodes with the skin, in order to reduce electric resistance and to optimise conduction of electrical signals on the scalp. In addition to the 31 EEG electrodes on the scalp, an extra electrode is applied on the subjects' back, which is used to record an electrocardiogram (ECG) and is also used with gel. On request of the participant, the placing of the ECG electrode can be undertaken by an investigator of the same gender.

The participant is conducted in the MRI room and is moved into the scanner by authorized staff members of the clinic. During the MRI procedure the subject lies on the examination table in a relaxed position: during the examination his/her head lies with the EEG cap on in the head coil, with soft padding on the left and on the right side, in order to prevent motion artefacts and to minimize the impact of the sound volume on the subjects. The chosen MR sequences correspond to the standard sequences recommended by the producer. Alongside the functional sequence, an anatomical T1-weighted 3D recording of the skull of about 5 minutes' duration is acquired. The following fMRI sequences last altogether approximately 30 minutes, so that the total scanner measuring time will amount to 45 minutes, including the preparation of the participant in the scanner.

The experiment will be conducted on a 3 Tesla MR scanner at the Clinic for Psychiatry and Psychotherapy in the Faculty of Medicine of the Philipps university of Marburg. The paradigm is to be presented via a projector and fMRI-compatible earphones. It is possible to monitor the experimental subjects in the MRI thanks to a camera throughout the whole experiment. Likewise, a loudspeaker system is installed allowing the investigators to enquire after the participant's conditions between the sequences.

Structural MRI

At the beginning of the experiment a localiser sequence (approx. 1 minute) is measured, followed by the structural, T1-weighted MRI measurement. These two measurements last altogether approx. 6 minutes.

Functional MRI

During the fMRI measurement a fixation cross is presented on the projector. Simultaneously, the auditory stimuli are presented via the fMRI-compatible earphones. These are 180 different stimuli consisting of 60 spoken sentences and excerpts from 60 novel songs or melodies. The sequence always includes 5 stimuli of the same category presented in a row. Considering the presentation length of 1.5 second per stimulus (for 5 stimuli in a row) and interstimulus intervals (ISI) amounting to 2.5 seconds in total, the length of a block is 10 seconds. After pseudorandomised presentation of all 180 stimuli and considering 5-6 s long breaks between blocks (for the regeneration of the BOLD signal), the total length of a run should be about 9 minutes and 30 seconds. The experiment consists of three runs. The data

from the first run are to be used for the validation of the regions of interest, whilst data for connectivity analyses will be acquired from the second and third run.
The length of the functional sequences is approx. 30 minutes including pauses between runs. In this timespan the participants have no active tasks to complete, they merely have to passively fixate the fixation cross.

Post-processing
Previously, 3 different sample groups (right-handers with and without musical training; left-handers without musical training) were recruited. For a more precise validation of the status and abilities of each participant, two questionnaires should be filled in. On the one hand a screening questionnaire investigates relevant linguistic (mothertongue/learned languages) and musical (level of musical training) aspects. On the other, another questionnaire determines the grade of handedness of experimental subjects, in order to measure their level of left-handedness (see Annex I).
Furthermore, an evaluation of the heard stimuli should test their familiarity (see annex I).
Finally, a musical hearing test (MET, after Wallentin, Nielsen, Friis-Olivarius, Vuust & Vuust, 2010; see annex II) is conducted as well as a test for the exclusion of amusia (Montreal Battery of Evaluation of Amusia, MBEA nach Peretz, Champod, & Hyde, 2003; see annex II).
For the questionnaires and tests a total length of 70 minutes should be taken into account.
Altogether (preparation, measurement, questionnaires and tests) the project will take 2 hours and 40 minutes, which mainly depends on the length of the preparation of the EEG system.

Security and side effects
EEG and MRI are both measuring methods that are routinely used in the clinical context. There hasn't been any proof of side effects to the present day. No risks associated with these techniques are known, as long as the exclusion criteria are respected (application of a MRI-compatible EEG system, no examination of subjects with metal implants in MRI etc.). The MRI-compatible EEG system is an authorized product of the Brain Products GmbH company (BrainAmp MR, for further details see http://www.brainproducts.com/productdetails.php?id=5), which conforms to all up-to-date security standards and was extensively tested on Siemens scanners such as the one used in the university of Marburg. For this reason combined measurements are assumed to represent no greater risk for the participants.

20. Termination criteria

Participants can drop out of the study or interrupt each part of the experiment (including the MRI measurement) at any time and without giving explanations. The participants are told that an interruption of the experiment won't have any negative consequences. During the MR investigation the participants have the possibility to terminate the experiment by pressing an emergency button at any time. Additionally, when possible (e.g. during breaks between measurements) the participants will be asked how they are feeling, in order to prevent incidents.

21. Documentation

The documentation of all collected data takes place electronically and is pseudorandomised, so that data can only be reconducted to the subject thanks to a key list separately stored by the investigator. More precise information about the pseudorandomisation process and of further use of the data is explained in point “D. Data protection” of this statement.

22. Number of competing studies in the same clinic/department on the same patient groups

No patients are included in the present study.

23. Recruitment

Duration of the recruitment: March 2018 - June 2018
Place of recruitment: university facilities in Marburg
Recruitment procedure: E-mail to students and university staff mailing lists of the Philipps university of Marburg, notices in the PUM (see annex I).

24. Expense allowance for patients/participants

Participants can receive course credits or €10 per hour. Therefore participants receive altogether €25 or 2.5 hours in course credits. Furthermore, participants can additionally acquire a CD with their structural MR data.

C. Clarification and consent

25. Information sheet, informed consent

Participants are informed in detail about the reasons of the study and the risks connected to participation through a written information sheet (see annex I). Each participant is required to give his/her written consent by signing the informed consent form. The information sheet explains that the present study is only research-oriented and thus no neuroradiological analysis of the MR data nor a clinical analysis of the EEG data in a clinical or diagnostic framework is conducted. However, it is explained that it is possible that anomalies are discovered in the MR images and in the EEG data, which may potentially have clinical relevance (“incidental finding”). It is made clear that in case there were clues for an incidental finding, the investigator will personally inform the participant and recommend a neuroradiological diagnosis.

26. Information: responsible person

The clarification about the study is held by the investigator. He/she signs the informed consent form after the signature of the participants. The informing investigator will ensure that the content of the information sheet has been understood completely by the participants. The original signed version of the informed consent form is kept by the investigator, whilst the participant receives a copy.

D. Data protection

27. Data collection

Data are pseudonymised through the assignment of a participant key, thanks to which no attribution of identity of the participants is possible without the key list. In order to allow open and reproducible research according to the guidelines for good scientific practice, the data should be made available to other research groups in neuroscience after the end of the project on the established open science platforms openfmri (https://openfmri.org/) and github (https://github.com/). Additionally, the so called defacing is foreseen, in which faces and thus recognition features of the participants are cancelled from the fMRI data. In such a way the attribution of identity to the single participants through recognition of face or teeth is not possible. Participants receive further informational material commonly used in open research (Open Brain Consent Documentation, see annex I) and can decide for themselves, if they agree to this use of their data or not. If they don't agree, they are not excluded from the study and this represents no disadvantage for the participants.

28. Data storage and transmission

All data saved in the computer are only identifiable via the key list, to ensure that the identity of the participants stays pseudonoymised. To generate the key code, participants are asked to write down a random 6-digit sequence including number 0 to 9 and the beginning and end letters of their hometown in capital letters (e.g. 340692 MN). The connection between collected data and the identity of participants can be made thanks to the key list. The latter is stored separately from the data and is deleted two years after the end of the project. Participants are informed in writing that the data are saved and analysed in a computer and that confidential treatment is guaranteed according to the law.

29. Information from the treating physician (release from oath of secrecy)

None.

30. Signature

Marburg, the ____

Date, signature of the project leader XXXXXX

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