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Metabolic brain connectivity after acute unilateral vestibulopathy: Longitudinal analysis and single subject classification in the rat

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Zusammenfassung

Ziel

Die Analyse der metabolischen Konnektivität des Gehirns basiert auf [^{18}F]-Fluordesoxyglucose (FDG) Positronen-Emissions-Tomographie (PET). Die Ziele dieser Arbeit waren einerseits die Anwendung von Konnektivitätsanalysen auf einen präklinischen PET-Datensatz zur akuten unilateralen Vestibulopathie (AUV) und andererseits die Untersuchung der bildgestützten Klassifikation auf Basis von Konnektivitätsinformationen.

Material und Methodik

Der untersuchte präklinische AUV-Datensatz bestand aus 85 [^{18}F]-FDG PET Bildern von Ratten, wobei je 17 Bilder an fünf Messtagen aufgenommen wurden. Ein Messtag war vor AUV und vier Messungen wurden an den Tagen 1, 3, 7 und 15 nach AUV durchgeführt. Parallel zur Bildgebung wurden klinische Verhaltensparameter der Tiere akquiriert. Die Bilder wurden nach der Rekonstruktion registriert, normalisiert und mittels eines Hirnatlas in 57 Hirnregionen segmentiert. Anschließend wurden die mittleren normalisierten Aktivitätswerte jeder Hirnregion und jedes Tieres extrahiert und für nachfolgende Analysen gespeichert. Durch die paarweise Korrelation der Aktivitätswerte aller Hirnregionen nach Pearson wurde für jeden Messtag das gruppenbasierte Hirnkonnektivitätsmuster bestimmt. Zur Analyse wurden diese Konnektivitätsmuster quantifiziert und zur Erstellung graphtheoretischer Strukturen verwendet.

Zur Klassifikation wurden die einzelnen Messtage als individuelle Klassen betrachtet und alle enthaltenen Verbindungen mit linearen Funktionen genähert. Diese linearen Funktionen repräsentierten das Konnektivitätsmuster einer Gruppe und erlaubten den Vergleich mit den im PET bestimmten Aktivitätswerten des Einzeltieres. Mittels Abgleich der Kongruenz erfolgte die Klassifikation in die Klasse mit der höchsten Übereinstimmung.

Diskussion

Vestibuläre Kompensation nach AUV aktiviert zerebrale Anpassungsprozesse, welche zur Neustrukturierung funktioneller Netzwerke führen. Die longitudinale Quantifizierung der Konnektivitätsmuster ergab kurzfristige Änderungen nach AUV, die in ihrem Verlauf den klinischen Verhaltensparametern folgten. Außerdem zeigte die graphtheoretische Analyse einen Anstieg an Verbindungen während der vestibulären Kompensation insbesondere in zum vestibulären System gehörigen Hirnregionen. Die Analyse der Hirnkonnektivität erwies sich als geeignet, um Hirnplastizität in longitudinalen Experimenten sinnvoll abzubilden. Weiterhin wurde ein neuartiger Klassifikationsansatz auf Basis des mittels Pearsons Korrelation bestimmten Konnektivitätsmusters untersucht. Hierbei konnten höhere Klassifikationsgenauigkeiten als mit Methoden des maschinellen Lernens erreicht werden. Da neurodegenerative Erkrankungen immer häufiger als komplexe Netzwerkerkrankungen beschrieben werden, könnte diese Klassifikationstechnik möglicherweise die diagnostische Entscheidungsfindung in klinisch relevanten Krankheiten wie der Alzheimer Demenz unterstützen.

Schlussfolgerung

Die Analyse der metabolischen Hirnkonnektivität eignet sich zur Untersuchung neurologischer Fragestellungen und ergänzt die im PET gängigen Analysen im Bereich der Hirnbildgebung. Die hier beschriebenen präklinischen Ergebnisse müssen auf vergleichbaren klinischen Datensätzen bestätigt werden.

Abstract

Aim

Metabolic brain connectivity analysis is based on [^{18}F]-fluorodeoxyglucose (FDG) positron emission tomography (PET). The objectives of this thesis were to apply these methods to a preclinical dataset of acute unilateral vestibulopathy (AUV) and to investigate the suitability of brain connectivity information for classification purposes.

Material and methods

The preclinical AUV dataset under investigation comprised 85 [^{18}F]-FDG PET images from rats, specifically 17 images on five distinct measurement days. One measurement day was before AUV and four follow-up measurements were performed on days 1, 3, 7, and 15 after AUV. Additionally, clinical scoring parameters were recorded in parallel to PET imaging. After image reconstruction, images were registered, normalized, and segmented into 57 brain regions using an atlas-based method. Mean normalized activity values were extracted for every brain region in every subject and stored for further processing. Brain connectivity patterns were determined for every measurement day in a population-based approach by pairwise correlation of the activity values from all brain regions with Pearson's correlation. These connectivity patterns were quantified and used to create graph theoretical structures for analysis.

For classification purposes, each measurement day represented a class. The group-based and class-individual connectome was transferred to a single-subject level by fitting a linear function to each connection. This enabled the evaluation of the single subject connectome by comparing the image-derived activity values to the fitted functions. Classification was performed by testing the congruence between the single-subject connectome with the class connectomes and to assign the subject to the most matching class.

Discussion

Vestibular compensation after AUV activates various adaptive cerebral processes that result in functional network rearrangement. The longitudinal quantification of the connectivity patterns demonstrated short-term changes after AUV that follow the course of the clinical scoring parameters. Furthermore, during vestibular compensation graph theoretical analysis revealed an increase in connectivity especially in brain regions associated with the vestibular system. Brain connectivity methods prove the suitability to reasonably depict short-term changes of the metabolic connectome in longitudinal experimental setups.

Moreover, classification based on Pearson's correlation-derived connective information has not been investigated so far. The described approach using linear fitting was evaluated and reached higher classification accuracies compared to machine learning methods on the same dataset. As clinically relevant neurodegenerative disorders are increasingly considered as network disorders, this classification technique could potentially support diagnostic decisions in clinically relevant diseases such as Alzheimer's disease.

Conclusion

Metabolic brain connectivity is suitable to investigate neurological questions and complements the toolkit of established cerebral image analysis in PET. The reported preclinical analysis results need to be validated on comparable clinical datasets.

Content

List of publications

Original publications

This cumulative dissertation is in accordance with the graduation regulation for natural sciences in the medical faculty of the Ludwig-Maximilians-Universität München and based on the following two publications:

Grosch, M., Lindner, M., Bartenstein, P., Brandt, T., Dieterich, M., Ziegler, S., Zwergal, A. (2021). Dynamic whole-brain metabolic connectivity during vestibular compensation in the rat. *NeuroImage*, *226*, 117588. (IF = 5.9)
https://doi.org/10.1016/j.neuroimage.2020.117588

Grosch, M., Beyer, L., Lindner, M., Kaiser, L., Ahmadi, S. A., Stockbauer, A., Bartenstein, P., Dieterich, M., Brendel, M., Zwergal, A. (2021). Metabolic connectivity-based single subject classification by multi-regional linear approximation in the rat. *NeuroImage,* 118007. (IF = 5.9)
https://doi.org/10.1016/j.neuroimage.2021.118007

Conference abstracts

The results of the investigations related to this doctoral thesis were additionally presented at national and international conferences.

Grosch, M., Kaiser, L., Ahmadi, S., Bartenstein, P., Zwergal, A., Ziegler, S. (2019). Comparison of machine learning methods for automated [18 F]-FDG PET based hydrocephalus classification. *European Journal of Nuclear Medicine and Molecular Imaging* (Vol. 46, No. SUPPL 1, pp. S757-S757)

Grosch, M., Schöberl, F., Levin, J., Bötzel, K., Dieterich, M., Zwergal, A. (2020). FV16 [18F] FDG-PET imaging of supraspinal locomotor control in Parkinson's disease. *Clinical Neurophysiology*, *131*(4), e231.

Grosch, M., Kaiser, L., Ahmadi, S. A., Bartenstein, P., Zwergal, A., Ziegler, S. (2020). Lokomotions-F-18-FDG-PET-basierte Differenzierung von Patienten mit Morbus Parkinson und gesunden Kontrollen durch maschinelles Lernen. *Nuklearmedizin, 59*(02), V41.

Further publication

Within the scope of the investigations related to this doctoral thesis, contributions to the following publication were made:

Schöberl, F., Pradhan, C., Grosch, M., Brendel, M., Jostes, F., Obermaier, K., Sowa, C., Jahn, K., Bartenstein, P., Brandt, T., Dieterich, M., Zwergal, A. (2021). Bilateral vestibulopathy causes selective deficits in recombining novel routes in real space. *Scientific reports, 11*(1), 1-16. (IF = 3.9)
https://doi.org/10.1038/s41598-021-82427-6

1. Introduction

In this chapter, the research motivation, the principles of the experimental setup, and the data acquisition procedures are presented. Furthermore, the processing of the image data is explained and the theory of metabolic brain connectivity, as it was used for the investigations within the scope of this thesis, is introduced.

1.1. Overview

Human vestibular disorders have various otological and neurological causes (Strupp et al., 2020). Preclinical animal models are suited to simulate distinct disorders in a reproducible manner and to investigate disease courses and treatment options (Straka et al., 2016). Especially the influence of comorbidities and lifestyle-dependent effects can be reduced to a minimum. Moreover, imaging modalities such as magnetic-resonance-imaging (MRI) and positron-emission-tomography (PET) are fundamental to examine cerebral processes in neurological questions (Aine, 1995). The information contained in the acquired data depends on the modality, the imaging protocol, or the pharmacokinetics of the employed radioactive tracer substance and for this reason depicts different anatomical or functional circumstances in the brain. Some of those images can be used to determine connections between individual brain regions, the so-called cerebral connectome, by analyzing the signal within those regions (Rubinov and Sporns, 2010). The connectome provides in-vivo insights into brain functioning and is appropriate to investigate neurological questions by expanding the existing toolkit of medical image analysis, symptomatic evaluations, and histology (Bullmore and Bassett, 2011).

1.2. Objective of this thesis

The first aim of this research project was to apply the methods of brain connectivity to a preclinical dataset of acute unilateral vestibulopathy (AUV) and to evaluate the connectome in a longitudinal manner. The course of the disease was documented by [^{18}F]-fluorodeoxyglucose (FDG) PET imaging and symptomatic recording with high temporal accuracy on five measurement days (one prior to AUV and on four follow-up measurements within the first 15 days after AUV). This enabled the display of connective short-term changes arising from cerebral reorganization processes in the early phase of recovery after AUV. Clinical AUV datasets cannot provide this dense temporal sampling, as serial short-term PET imaging in patients is not possible due to regulations of radiation protection.

The second aim was to investigate classification based on PET-derived brain connectivity information. Clinically relevant disorders such as different dementia or hypokinetic syndromes are increasingly considered as complex network disorders and motivated the search for a classification approach that takes the connectome into account (Ge et al., 2020; Morbelli et al., 2013). Brain connectivity patterns change under different circumstances, for example during vestibular compensation after AUV and in Alzheimer's disease and therefore, they can potentially be used for image-based classification and to distinguish cohorts of patients accordingly (Grosch et al., 2021b; Sanabria-Diaz et al., 2013).

1.3. Acute unilateral vestibulopathy in a rat model

Preclinical animal models are suited to simulate diseases and disorders in a reproducible manner and without comorbidities influencing the course of the disease under investigation. In the Department of Nuclear Medicine at the hospital of the Ludwig-Maximilians-University Munich, laboratory animal housing, evaluation, and imaging facilities are affiliated and well established for studies of vestibular disorders (Lindner et al., 2019; Zwergal et al., 2017). All preclinical experiments reported in this thesis were conducted by experienced veterinarians with approval of the government of Upper Bavaria and in accordance with the guidelines for the use of living animals in scientific studies and the German Law for the Protection of Animals (references: ROB-55.2-2531.Vet_02-10-73, ROB-55.2-2532.Vet_02-16-93).

Unilateral vestibulopathy is a general term for vestibular disorders arising from a peripheral or central vestibular damage (Strupp and Magnusson, 2015). In the rat, a peripheral vestibular damage and the corresponding acute vestibular syndrome can be induced by chemical labyrinthectomy. Here, the left external auditory canal is exposed under anesthesia and analgesia. Through the successive application and aspiration of bupivacaine and p-arsanilic acid into the tympanic cavity, the primary sensory cells of the inner ear are irreversibly desensitized (Vignaux et al., 2012). As a result, operated rats show typical symptoms of a unilateral vestibulopathy such as nystagmus, postural deficits, and pathological locomotor behavior, which can be quantified by symptomatic scoring as described in the literature (Bergquist et al., 2008). These symptoms rapidly improve in a recovery phase, which lasts days to weeks and is driven by a cerebral adaptation process called vestibular compensation (Dutia, 2010). The symptomatic quantification is the basis to track and analyze the compensation in a longitudinal manner from a symptomatic perspective. In parallel, longitudinal metabolic PET measurements were performed to investigate potential changes in metabolism and connectome during the adaptation process. The dataset examined within the scope of this thesis was originally acquired for two studies evaluating the treatment effects of EGb761 and Betahistin after unilateral labyrinthectomy in the rat. The experimental procedures were identical in both studies. Only AUV animals with sham treatment from the control cohorts were included (Lindner et al., 2017; Lindner et al., 2019).

1.4. Positron-emission-tomography

Positron-emission-tomography (PET) is a nuclear medicine imaging procedure that relies on the beta-plus decay of radioactive atomic nuclei and that can visualize metabolism among other things in humans and animals. Hereinafter, the organism under investigation is generally named as subject.

Beta-plus decaying radioisotopes are attached to carrier molecules, yielding a so-called radiotracer, and then injected into the subject. The biomedical properties of a radiotracer and therewith its behavior within the organism depend on the used carrier molecule (Derlin et al., 2018). A popular radiotracer is [^{18}F]-fluorodeoxyglucose (FDG), which is a glucose analog containing a radioactive Fluorine-18 nucleus. The radiotracer accumulation within the subject is estimated in the PET scanner by detecting the annihilation photons emitted from electron-positron annihilations, in which the positrons originate from the decaying radioisotopes. A PET scanner is usually set up as a cylindrical detector system, consisting of multiple detector rings in which each ring is built of scintillation-based photon detector blocks optimized to detect and localize photon interactions within the detector.

As two photons originating from an electron-positron annihilation are emitted simultaneously with a photon energy of 511 keV each and in opposite directions (≈ 180°), the coincident detection of two photons contains spatial information about the annihilation site and enables the estimation of its location. The annihilation site is located along the line that connects the two detection positions in the detector modules, the line of response (LOR). From the time difference between the two detections in the modules, the annihilation position along the LOR can be restricted to a segment of the LOR by calculating the time-of-flight (TOF) difference of the photons. The TOF is related to a distance by the speed of light and the length of the corresponding segment is dependent on the timing resolution of the detector modules. During a PET scan, a multitude of annihilation events is detected and stored as raw data. Due to photon scattering and limited detector speed, energy resolution and spatial resolution, there are falsely detected coincidence photons registered in the raw data. Corrections for such errors can be incorporated in the reconstruction procedure in which the reconstruction of the raw projection data to a tomographic three-dimensional image is usually performed via iterative algorithms (e.g., maximum-likelihood expectation-maximization) (Cherry and Dahlbom, 2006; Jadvar and Parker, 2005).

PET image values represent activity concentration (Bq/ml) and depend on the administered tracer activity and the tracer biodistribution within the subject. The usage of different tracer molecules provides the opportunity to image distinct processes.

1.4.1. Tracer properties of fluorodeoxyglucose

A high metabolic glucose rate is found in the brain and in many cancers, which is why 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) became one of the clinically most used tracer molecules and FDG-PET imaging became known as metabolic imaging (Jadvar and Parker, 2005). The radiotracer [^{18}F]-FDG is taken up into the cells analogously to glucose by means of a glucose transporter. In the first step of glycolysis, [^{18}F]-FDG is phosphorylated to [^{18}F]-FDG-6-phosphate by the enzyme hexokinase. In the second step, the glycogenesis is interrupted, since for [^{18}F]-FDG the OH-group at position 2 has been replaced by a fluorine atom (Riemann, 2007). Therefore, [^{18}F]-FDG-6-phosphate accumulates within the cells. This process is called trapping and results in a relatively stable tracer concentration in the brain after an approximately 30 minutes uptake period. Thus, static image acquisition protocols are sufficient for neurological FDG-PET imaging and no pharmacokinetic modelling is required (Cherry and Dahlbom, 2006; Jadvar and Parker, 2005). It has been shown that [^{18}F]-FDG PET brain images are a measure for the regional cerebral glucose metabolism and therefore approximately represent neuronal activity (Varrone et al., 2009).

1.5. Image processing

Several uncertainties such as variable subject placement in the scanner and varying injected tracer activity are hardly avoidable in the PET scanning procedure. Therefore, project-specific image processing is necessary after image reconstruction (Figure 1a). For the investigations reported in this thesis, the imaged brains were mapped on a [^{18}F]-FDG PET template brain image using PMOD medical image analysis software (PMOD Technologies LLC, RRID: SCR_016547, v4.004) by means of translations, rotations, and scaling operations, a so-called rigid coregistration (Figure 1b). The superposition in the same spatial orientation within the template space enabled the usage of a brain atlas, which was provided with the template image and which outlined 57 brain regions anatomically determined from cryosectional images (Schiffer et al., 2006) (Figure 1c).

As PET images display quantitative information about the activity distribution within the subject, the image values depend on the initially injected amount of activity. To ensure comparability between subjects and scans, normalization procedures must be applied. Within the scope of this thesis, all images were normalized to their respective whole brain mean activity value to preserve spatial relationships in the tracer distribution and to achieve comparability.

The normalized mean activity values for each brain region and each subject were extracted by using PyRadiomics Python package and stored for subsequent analysis (Van Griethuysen et al., 2017).

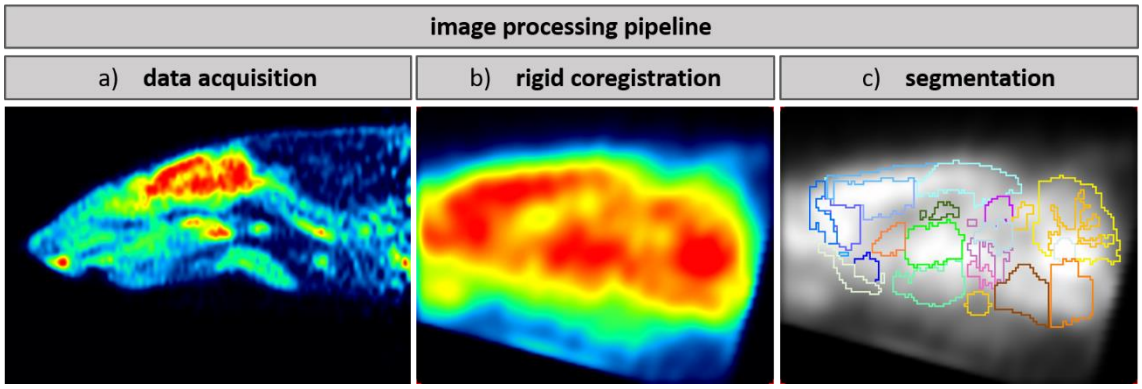

Figure 1: *Image processing pipeline. a) [^{18}F]-FDG PET image of a rat. b) Rat brain after rigid coregistration into template space. c) Atlas-based segmentation of 57 brain regions. (Figure adapted from Grosch et al., 2021b.)*

1.6. Metabolic brain connectivity

Generally, the brain consists of distinct brain regions that can be distinguished anatomically and/or functionally. Brain connectivity tackles the question of how these individual brain regions interact with each other and how these interactions change under different conditions or pathologies. There are different methods available with which connections can be determined (Huang et al., 2010; Wang et al., 2020; Yakushev et al., 2017). Within the scope of this thesis a population-based approach using Pearson's correlation was applied. Pearson's correlation is a mathematical description of the linear relationship between two parameters belonging to distinct elements in a set (Benesty et al., 2009).

The calculation yields two values, first, Pearson's correlation coefficient r and second, the corresponding statistical significance p. Pearson's correlation coefficient is restricted to the interval $r \in [-1, 1]$, whereby $|r| = 1$ represents a perfect linear relationship and $r = 0$ represents no linear relationship. As this test is only sensitive to linear correlations, $r = 0$ does not exclude non-linear relationships. Furthermore, absolute values between zero and one are a measure of how close to a linear relationship the two parameters are, but they provide no indication of how well linear fitting would perform.

For metabolic brain connectivity analysis, the extracted values from two brain regions were used as input parameters to calculate Pearson's correlation over a set of [^{18}F]-FDG PET-scanned individuals. A connection between two brain regions was assumed, if there was a sufficient linear relationship with satisfactory statistical significance detected, that is for example $|r| > 0.5$ and $p < 0.001$. To derive the whole-brain connectome, Pearson's correlation was calculated for all pairs of brain regions available after segmentation and the filtered results were stored in a connectivity matrix (Figure 2).

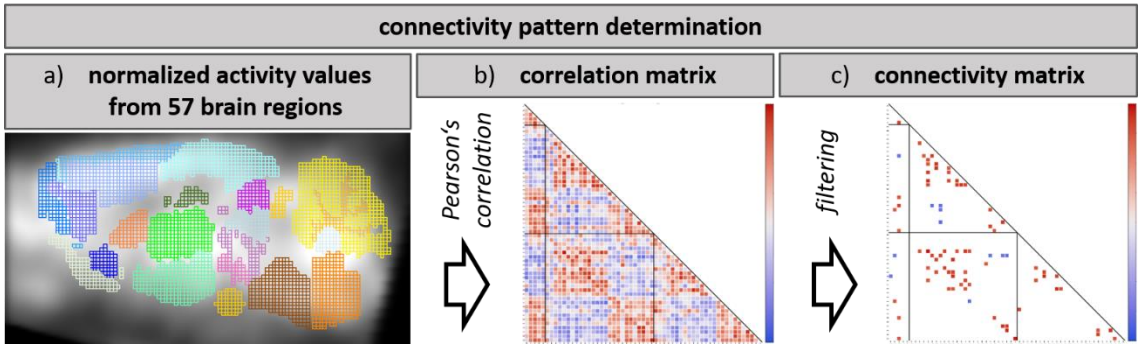

Figure 2: *Determination of connectivity matrix. a) Extraction of normalized mean activity values from 57 brain regions. b) Calculation of correlation matrix by Pearson's correlation between all pairs of brain regions. c) Filtering with correlation coefficient and statistical significance (e.g., r > 0.5 and p < 0.001). (Figure adapted from Grosch et al., 2021b.)*

1.6.1. Analyzing the connectome

The most difficult challenge was to make the derived information about the connectome manageable for interpretation. For simple quantification, the connections in the connectivity matrix were sorted according to their hemispherical affiliation and counted. This gave a first impression of hemispheric asymmetries and differences in cohorts. Another approach to quantification was provided by graph theory, where the connectome was translated into a mathematical object, a so-called graph. Basically, a graph consists of nodes and edges, whereby an edge links two nodes. Graphs have applications in many fields of our daily live as for example in modelling social networks in online platforms to suggest new contacts, or in modelling international flight traffic to determine more efficient routing (Kuyumcu and Garcia-Diaz, 2000). In the second example, the nodes represent airports and the edges flight routes between the airports. For graph theoretical analysis of the connectome, the nodes are the individual brain regions and the edges the connections determined by Pearson`s correlation, which were stored in the connectivity matrix. The implementation within the scope of this thesis was based on the NetworkX Python package (Hagberg et al., 2008). For graphs, there are several mathematical properties that can be calculated and that can be used to derive insights into the underlying dataset, for example by calculating the rich club. The rich club is a set of nodes (in this case brain regions), which are exceptionally connected by edges and therefore represent hubs within the

graph (Griffa and Van den Heuvel, 2018). From a neurological perspective, the whole brain connectivity architecture and the corresponding hub regions are of special interest when interpreting disorders or processes of brain plasticity. The methods of brain connectivity have been applied to imaging datasets covering Alzheimer`s disease amongst others and within the scope of this thesis vestibular disorders (Grosch et al., 2021b; Sanabria-Diaz et al., 2013). They provide the tools to acquire a novel view on cerebral network pathologies in neurodegenerative diseases to possibly find imaging biomarkers for early disease detection or even treatment options. Furthermore, they allow to study cerebral plasticity and functional network rearrangement in longitudinal evaluations (Grosch et al., 2021b).

1.6.2. Other approaches to brain connectivity

There are several MRI-based methods to derive connective information. For example, by correlating the cortical thickness or the blood-oxygen-level-dependent (BOLD) signal of different brain regions, which is methodically similar to the metabolic brain connectivity method covered in this thesis. As BOLD-effect MRI is part of functional MRI (fMRI), the herewith captured connectivity is called functional connectivity (Worsley et al., 2005). Another MRI sequence is diffusion tensor imaging (DTI), where the movement (or diffusion) of hydrogen atoms is measured. By assuming that water molecules can move faster along nerve fibers, these fibers can be detected (the so-called fiber tracking). In this case, the connectome is derived from anatomical connections of nerve fibers and called anatomical connectivity (Straathof et al., 2019).

Besides the previously explained method to determine the PET-based connectome, metabolic brain connectivity has been approached with other techniques such as Sparse Inverse Covariance Estimation (SICE) and Kullback-Leibler Divergence Similarity Estimation (KLSE). In SICE, instead of calculating a connectivity matrix by Pearson's correlation, a sparse inverse covariance matrix is determined by maximum likelihood estimation. The non-zero entries in the sparse matrix represent the connections between different brain regions and are analyzed with the above-mentioned procedures (e.g., graph theory). This method requires a sample size similar to or greater than the number of investigated brain regions and was therefore not appropriate for evaluation of our dataset (Huang et al., 2010). While SICE is a population-based approach, KLSE is capable of determining connectivity patterns on a single subject level. Generally, Kullback-Leibler divergence is a statistical measure for the difference between two probability distributions (Kullback and Leibler, 1951). Here, the probability distributions are the individual voxel-values in two brain regions and if their distributions match sufficiently, those regions are assumed to be connected. The KLSE measures are evaluated between all available brain regions and, as it is the case for the other two methods, stored in a connectivity matrix that represents the connectome. Here, a special characteristic is that the connectivity matrix is not symmetric because Kullback-Leibler divergence is not symmetric. Connectivity matrices determined via Pearson's correlation are symmetric due to the mathematical correlation properties and SICE-based connectivity matrices are symmetric because covariance matrices (and their inverse) are symmetric by definition. For KLSE, this results in a directed connectome that evaluates connections between two brain regions in both directions individually. A group-wise connectome can be determined by averaging the single-subject measurements (Wang et al., 2020).

1.6.3. Connectivity-based classification

Metabolic connectivity-based classification techniques have been reported for KLSE and SICE, but classification methods relying on Pearson's correlation were missing (Huang et al., 2010; Wang et al., 2020). To fill this gap, a novel classification approach was investigated within the scope of this thesis. The fundamental idea to distinguish two (or more) classes was to determine the connectivity pattern for each class by Pearson's correlation on a group-level and to evaluate the congruence of a single subject PET-uptake pattern with the class-individual connectomes. For this project, the connectivity patterns on different measurement days from the AUV dataset were assumed as distinct classes.

To evaluate connectivity on the single subject level, all connections on the group-level were fitted with a linear function using the datapoints that were previously used to calculate Pearson's correlation (Figure 3a). Generally, a connection is detected if Pearson's correlation yields a sufficiently high correlation coefficient with sufficient statistical significance. Thus, fitting a linear function to those data points is reasonable, as Pearson's correlation coefficient is a measure for the linear relationship between two sets of parameters. Of course, the data and the fits must meet the requirements for linear regression. On the single subject level, a connection between two brain regions is determined by evaluation of the distance between the point specified by the respective uptake values and the fitted linear function from the population-based connectivity pattern (Figure 3b). This evaluation is performed for all connections in the connectivity patterns of all groups. The percentage of valid connections in a class is used as measure for the congruence between the single subject uptake pattern and the class connectome. Then, the subject is classified into to the most matching class (Grosch et al., 2021a).

Within the scope of this thesis, the above-mentioned longitudinally acquired preclinical PET dataset was used to test the classification performance. As short-term changes and high cerebral plasticity were detected during early phase vestibular compensation after AUV, each measurement day was defined as separate class. The corresponding connectivity patterns and their differences were described in detail in the first publication. Therefore, the dataset exhibited zero class-label noise, what usually cannot be achieved in clinical environments.

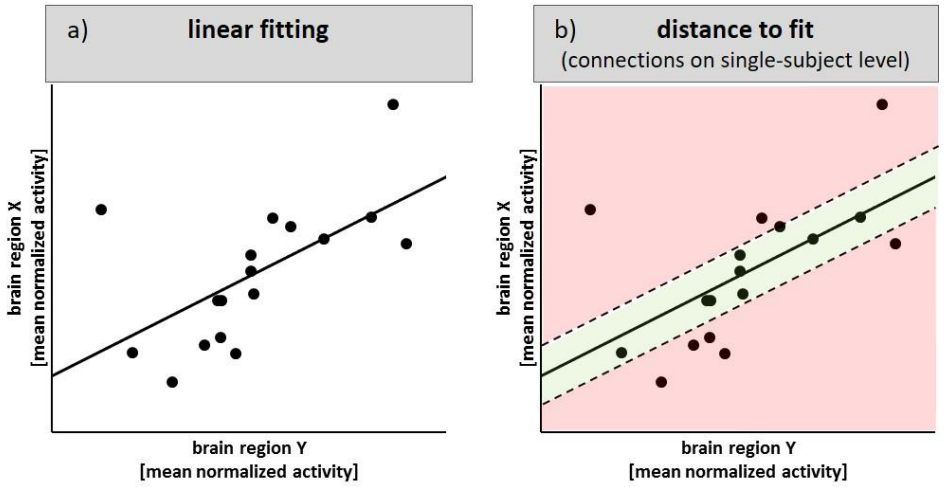

Figure 3: *Single-subject classification approach. a) Fitting a linear function to the data points used for Pearson's correlation. b) Single-subject evaluation for every connection enables classification. (Figure adapted from Grosch et al., 2021a.)*

2. Project report

When this doctoral project started in October 2018, the first goal was to achieve the necessary skills to process and analyze PET-imaging data properly. While doing so, a machine learning (ML) pipeline was set up and tested in cooperation with colleagues from the Department of Nuclear Medicine and the German Center for Vertigo and Balance Disorders (DSGZ). This pipeline was used for preliminary studies on neurodegenerative diseases as well as for benchmarking purposes in the second publication. At that time, first brain connectivity analyses were performed on preclinical PET-imaging data from the DSGZ and the main goal of this thesis consolidated. The corresponding self-written Python code yielded interesting results and was developed further to incorporate state-of-the-art brain connectivity analysis tools as inspired by the literature (Sanabria-Diaz et al., 2013). This resulted in the first publication about short-term changes after unilateral labyrinthectomy in the rat. In parallel to writing the manuscript, the idea of using the connectivity information for classification emerged and evolved in various internal discussions. After submission of the first publication, the code was extended to cover the novel classification idea and corresponding testing procedures. The results were promising and therefore evaluated more in depth to improve and finalize the method. This resulted in the second publication about using the Pearson's correlation-based connectome for classification purposes.

All coauthors gave their written consent that the two relevant publications are used for this cumulative dissertation and confirmed that neither of them is part of another doctoral thesis. Their signatures were provided to the doctoral office of the medical faculty of the LMU Munich.

2.1. Contribution to first publication: Brain connectivity analysis after unilateral vestibulopathy

First, I collected and organized the dataset from two previous studies. This included particularly the unprocessed PET-images directly after reconstruction and the clinical scoring data from symptomatic evaluations performed by the veterinarians. Then, I registered the images in PMOD medical image analysis software (PMOD Technologies LLC, RRID: SCR_016547, v4.004) and segmented the brains into 57 brain regions with W.Schiffer's rat brain atlas (Schiffer et al., 2006). Subsequently, the brains were normalized to their whole brain mean and the normalized activity values extracted for following brain connectivity analysis. Normalization and extraction were both performed with self-written Python scripts incorporating SITK and PyRadiomics Python packages, respectively (Lowekamp et al., 2013; Van Griethuysen et al., 2017).

Starting from scratch, I wrote the complete brain connectivity analysis code by myself, incorporating established Python packages such as Numpy, Pandas, SciPy, and NetworkX (Hagberg et al., 2008; Harris et al., 2020; McKinney, 2010; Virtanen et al., 2020). Therefore, I searched for methodic inspiration in the literature and implemented ideas from different publications in a single analysis class (Griffa and Van den Heuvel, 2018; Sanabria-Diaz et al., 2013). The development was iterative in cooperation with medical experts, as they provided me the neurological background to specifically tackle distinct questions. In retrospect, the main challenge was to reduce the dimensionality of the huge amount of information contained in the brain connectivity analysis to a manageable level and to develop reasonable ways to display the results.

For interpretation purposes of the connectivity results, I wrote MATLAB scripts to set up voxel-wise analysis of the images in statistical parametric mapping software (SPM; Wellcome Department of Cognitive Neurology, Great Britain). Furthermore, I wrote a Python script that performed and evaluated an ANOVA with Bonferroni correction for multiple testing on the clinical scoring data of the rats.

Using the results from the aforementioned analysis, the manuscript was drafted. Here, I wrote the first draft particularly of the materials and methods, the results, and the discussion sections. The discussion is based on an interdisciplinary interpretation of the connectome within a clinical and methodic context. The manuscript was corrected and finalized in cooperation of physicians, veterinarians, and physicists, especially with support of my doctoral supervisor Prof. Dr. Sibylle Ziegler and my medical supervisor PD Dr. Andreas Zwergal.

For publication, I was corresponding author and submitted the draft to NeuroImage. During the peer-review process, I coordinated the handling of the reviewer questions and collected the answers for timely resubmission.

2.2. Contribution to second publication: Image classification by linear approximation of the connectome

The idea of this novel classification approach emerged in discussions with physicians from the Department of Nuclear Medicine. They wondered whether brain connectivity information could expediently support clinical diagnostic decision making in neurodegenerative diseases. After brainstorming this idea, I started developing classification procedures based on the fundamental functions of the connectivity analysis class created for the first publication. The main challenge was to transfer a population-based connectome by Pearson's correlation to a single subject level. Therefore, I implemented a pipeline that derived brain connectivity patterns for different classes and approximated the connections class-wise by fitting linear functions. Then, those functions represented the brain connectivity patterns of the individual classes and allowed for single subject evaluations. The estimation of congruence between a single subject PET uptake pattern and the class connectomes as depicted by linear functions enabled me to classify with a higher accuracy than state-of-the-art machine learning methods (support vector machine, random forest classifier). Classification performance was measured as accuracy and area under the receiver operating characteristic curve at which the latter calculation was imported from Scikit-learn Python package (Pedregosa et al., 2011).

As basis for these investigations, the used dataset was already organized and processed for the first publication as described above. To counter the limited sample size, I improved the statistical significance of my investigations by implementing a leave-one-out cross validation procedure that fitted linear functions according to the connectome derived from n-1 subjects in each class and subsequently classified the left-out subjects by pattern congruence estimation (n = number of subjects in a class). This process was repeated n-times and is a commonly used cross-validation procedure in machine learning applications. Furthermore, I implemented easy to use functionality to test the classification performance for different combinations of connectivity and classification parameters, because the report of classification performance with respect to those parameters is the essential part of the publication.

Following the data analysis, I wrote the first draft of the manuscript and organized the internal review process with my coauthors. Therefore, I collected suggestions, implemented corrections, and scheduled frequent meetings to discuss the updates. The parts concerning the neurological motivation and the clinical relevance of the described method were mainly written by my coauthors with medical background. After finalizing and submitting the manuscript, I coordinated two rounds of revisions. For me, this meant particularly implementing code functionality to answer reviewer questions, reproducing the results, updating the manuscript, and writing of the response letters. Furthermore, I operated again as corresponding author during the publication process (proofing, organizational issues).

3. Conclusion and Outlook

This thesis aimed to apply the methods of metabolic brain connectivity to vestibular disorders. Particularly, a rat model of acute unilateral vestibulopathy was investigated in a longitudinal manner and a novel connectivity-based classification approach was introduced. Novel insights into the cerebral plasticity during early-phase recovery after peripheral vestibular damages were acquired and reported.

Currently, metabolic brain connectivity methods are applied to a clinical dataset of acute unilateral vestibulopathy to study the human cerebral connectome during vestibular compensation. As mentioned in the introduction, there are other approaches to metabolic brain connectivity and metabolic brain connectivity-based classification available and it would be interesting to examine these methods on datasets covering vestibular disorders. Furthermore, the classification performance of our method needs to be evaluated on different clinical datasets to estimate its general applicability in clinical environments. All mentioned connectivity-based classification methods should be compared to each other as the proposed method has only been tested on a vestibular disorder dataset and the other methods so far only have been tested on Alzheimer's disease datasets. A comparison of the results could potentially provide deeper insights into the biological meaning depicted with the individual methods.

To expand the field of brain connectivity in PET imaging, different tracers could be investigated with respect to their applicability for brain connectivity analysis. There are tracers available that depict cerebral synaptic density, and which potentially are suited for brain connectivity analysis. Nevertheless, a proper interpretation would be necessary that takes biomedical properties of the tracer and methodic requirements likewise into account.

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5. Original publications

5.1. First publication: Dynamic whole-brain metabolic connectivity during vestibular compensation in the rat

The following document was the first publication used for this thesis.

Grosch, M., Lindner, M., Bartenstein, P., Brandt, T., Dieterich, M., Ziegler, S., Zwergal, A., 2021b. Dynamic whole-brain metabolic connectivity during vestibular compensation in the rat. Neuroimage 226, 117588

DOI: 10.1016/j.neuroimage.2020.117588

5.2. Second publication: Metabolic connectivity-based single subject classification by multi-regional linear approximation in the rat

The following document was the second publication used for this thesis.

Grosch, M., Beyer, L., Lindner, M., Kaiser, L., Ahmadi, S.A., Stockbauer, A., Bartenstein, P., Dieterich, M., Brendel, M., Zwergal, A., Ziegler, S., 2021a. Metabolic connectivity-based single subject classification by multi-regional linear approximation in the rat. Neuroimage, 118007

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