A close-up of a logo

AI-generated content may be incorrect.A logo with a blue and yellow line

AI-generated content may be incorrect.

Between Head and Heart

Exploring interoception on a cortical and subcortical basis

**Master Thesis**

Master Cognitive Neuroscience Berlin (MCNB)

Department of Psychology and Education

Freie Universität Berlin

by

**Lisa Sophie Paulsen**

Mat-Nr: 5574504

**Supervisors**

Prof. Dr. Huiling Tan

University of Oxford

Prof. Dr. Felix Blankenburg

Freie Universität Berlin

Berlin, DATUM

Master’s Thesis Declaration

For the Master’s Degree Program in Cognitive Neuroscience at the Department of Education and Psychology, Freie Universität Berlin

Herewith I affirm that I wrote the work in hand autonomously and never used another source or resource as declared

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Student Location, Date

Abstract

Interoception remains an illusive connection between the brain and the body. Heart-brain coupling has been investigated mainly under the lens of arousal research but the underlying dynamics during rest continue to be poorly understood but are a nascent field. This study explores heart brain interaction on a cortical (EEG) and subcortical (STN LFP) area together with ECG during rest, while examining the effects of levodopa medication as well as the source dynamics beneath the heart-evoked potential (HEP). Neural and cardiac activity was recorded from 14 Parkinson’s disease (PD) patients after deep brain stimulation (DBS) surgery with levodopa present (MedOn) and a subset with medication absent (MedOff). Presence of levodopa indicates a higher amplitude in the HEP in both cortical and subcortical measurements. At the same time power and phase show no particular change due to medication, same as ECG features. Distinct phase coherence patterns were observed when investigating exclusively the inter-trial coherence (ITC) of the 14 subjects. With a significant ITC peak in the high delta (2-4Hz) to low theta (4-5Hz) range in the period of 100-250ms after r-peak. This pattern can be observed in both cortical and subcortical areas. This pattern coincides with the ITC analysis from Park et al. 2018 supporting their hypothesis that the HEPs underlying mechanism is based on phase-resetting. Additionally, to their theta ITC peak, our peak extended into the delta range suggesting a bottom up involvement of delta, after frontal delta has causally been implicated in attenuating HEP. That only the HEP shows a difference due to medication highlights that that levodopa modifies CNS but no difference in ECG features would suggest that levodopa’s has no impact on the ANS regulating the heartbeat.

We recorded cortical (EEG) and subcortical (STN LFP) neural activity together with ECG from 14 Parkinson’s disease patients with DBS.

and found that delta- and theta-range phase coherence underlies HEP generation. Mean ITC peaks did not correlate with power spectra, indicating that significant ITC occurs independently of power. Dopaminergic medication modulated interoceptive signaling, with clear differences in HEP but not in ECG features, diverging from previous findings (Ref). ITC patterns in EEG and STN suggest that cortico-subcortical functional alignment (CFA) may be reflected in ITC. This first study to examine heart–brain interoception across cortical and subcortical levels at rest highlights phase modulation as a key mechanism of HEP and underscores the potential of phase-based analyses for future research.

Acknowledgements

Table of Contents

[1. Introduction 7](#_Toc212466338)

[1.1. Measuring the heart-brain interaction 9](#_Toc212466339)

[1.2. Source Dynamics of the HEP 11](#_Toc212466340)

[1.3. Recordings 14](#_Toc212466341)

[1.4. Aim of the project 15](#_Toc212466342)

[2. Methods 16](#_Toc212466343)

[2.1. Patients and surgery 16](#_Toc212466344)

[2.2. Data Recording 17](#_Toc212466345)

[2.3. Study Design 18](#_Toc212466346)

[2.4. Signal preprocessing 18](#_Toc212466347)

[2.4.1. Electrocardiogram (ECG) 19](#_Toc212466348)

[2.4.2. Electroencephalography (EEG) and local field potential (LFP) 20](#_Toc212466349)

[2.5. Analysis and Statistics 21](#_Toc212466350)

[2.5.1. ECG Features Analysis 21](#_Toc212466351)

[2.5.2. HEP Analysis 22](#_Toc212466352)

[2.5.3. ITC Analysis 24](#_Toc212466353)

[2.5.4. PSI/CCC Analysis 25](#_Toc212466354)

[3. Results 29](#_Toc212466355)

[3.1. Levodopa medication shows no effect on ECG features 29](#_Toc212466356)

[3.2. Medication indicates modulation of HEP and phase coherence 30](#_Toc212466357)

[3.3. Delta and Theta phase coherence source of HEP modulation 38](#_Toc212466358)

[4. Discussion 42](#_Toc212466359)

[4.1. HEP driven by phase resetting in delta and theta 42](#_Toc212466360)

[4.2. Levodopa impact on CNS 44](#_Toc212466361)

[4.3. Phase as support mechanism for CFA circumvention 46](#_Toc212466362)

[4.4. Limitations and Outlook 47](#_Toc212466363)

[5. References 50](#_Toc212466364)

[6. Appendix 58](#_Toc212466365)

# Introduction

Parkinson’s Disease (PD)

Subthalamic Nucleus (STN)

Deep Brain Stimulation (DBS)

Local Field Potential (LFP)

Electroencephalography (EEG)

Electrocardiogram (ECG)

Inter-beat Interval (IBI)

Heartrate (HR)

Heartrate Variability (HRV)

A diagram of the brain

AI-generated content may be incorrect.Interoception is responsible for sensing, interpreting, and integrating the body's physiological conditions (e.g., hunger, thirst, pain), thus providing a moment-to-moment map of the body's internal milieu (Berntson & Khalsa, 2021; Craig, 2003). Interoception requires a complex signaling system of the afferent (bottom-up) pathways. A big focus of interoception research has been on cardiac signals as one of the most prominent interoceptive signals. Precise pathways underlying this bottom-up signaling are mostly unknown. Current Research has started to produce several possible physiological heart-brain pathways (Critchley & Harrison, 2013; Park & Blanke, 2019; Tallon-Baudry et al., 1996). The most thought of pathways starting from the heart are (i) the baroreceptors in the aortic artery travelling over the vagus nerve to the brainstem, (ii) the cardiac neurons, in the heart's walls, that signal through the vagus nerve or the spinal cord to the brainstem, and (iii) the cutaneous receptors in the skin detect cardiac changes and transfer them via the spinal cord to the brainstem. From there, they are relayed through the thalamus and terminate at the amygdala (Garfinkel & Critchley, 2016), insula (Strohman et al., 2024), primary somatosensory cortex (Kern et al., 2013) and cingulate cortex (Cambi et al., 2024; for review see Critchley & Harrison, 2013) (**Figure 1**). A connection between interoception and psychomotor processes have inferred the basal ganglia, specifically the neostriatum in a possible afferent pathway (Critchley & Harrison, 2013).

**Figure 1** Possible pathways from the heart to the brain. Cardiac neurons and Barorecptors can signal over the vagus nerve to the brainstem, and baroreceptors and cutaneous receptors can signal over the spinal cord to the brainstem. From there signals are relayed over the Thalamus onto the Amygdala, Insula, primary somatosensory cortex and the cingulate cortex. Figure credit from Park et. al., 2019

Moreover, findings in rodents suggest that cerebral blood pressure changes directly affect local neural activity. One study has seen changes in spontaneous firings after blood pressure alterations in rat slices (Kim et al., 2016). A more recent study in mice found specific baroreceptors in neural populations that open solely to the frequency of the cerebral arteries’ blood pressure (Jammal Salameh et al., 2024). Thus, indicating that there might be more and farther complex mechanisms at work for bottom-up signalling between head and heart.

## Measuring the heart-brain interaction

The increased research interest in cardiac signals has expressed itself in new behavioral and physiological measurements to help understand the intricacies of the heart-brain axis as the starting point for interoception. Behaviorally, the heartbeat counting task (Dale & Anderson, 1978; Schandry, 1981), the heartbeat discrimination task (Brener & Ring, 2016; Whitehead et al., 1977), and emotional arousal tasks (e.g. as in Gray et al., 2007; Marshall et al., 2018) have been used. Recently, the heartbeat counting task has faced repeated criticism as it utilises prior knowledge of heart rates which leads to biases and is confounded by other non-interoceptive processes (Desmedt et al., 2018; Murphy et al., 2020). A key physiological measurement for cardiac signals is heart rate variability (HRV). It reflects the variation in the interval between consecutive heartbeats (Inter-beat Interval, IBI), quantified from r-peak to r-peak measurements in an electrocardiogram (ECG) (Laborde et al., 2017). Thus, it shows the dynamic mechanism between the autonomic nervous system (ANS) and cortical interoceptive areas (Garrett et al., 2023). Findings show a positive correlation between interoceptive accuracy and higher HRV, suggesting that our ANS can modulate our interoceptive awareness (Lischke et al., 2021; Owens et al., 2018).

HRV recordings in resting state measurements?

Neurophysiologically, the main contender for quantifying interoception is the heartbeat evoked potential (HEP). The HEP is based on electrophysiological data (e.g. electroencephalography (EEG), local field potential (LFP), intracranial EEG (ECoG) or MEG), which is time-locked to the r-peaks of simultaneously measured ECG. Thus, reflecting the cortical processing of cardiac activity (Coll et al., 2021; Park & Blanke, 2019; Schandry, 1981) and more recently been connected to interoception on a broader level (Coll et al., 2021). HEP recordings are often investigated by comparing groups (Pollatos & Schandry, 2004) or using behavioral tasks (Marshall et al., 2018; Schulz et al., 2015). Resting-state recordings to investigate HEP were mainly acquired for clinical studies (Müller et al., 2015; Pang et al., 2019; Schulz et al., 2018). But especially rest recordings might be insightful when looking beyond the HEP as an ERP.

## Source Dynamics of the HEP

A diagram of a normal and a normal phase

AI-generated content may be incorrect.Research into the mechanisms and neural sources underlying HEP has only been picked up in recent years (Park & Blanke, 2019). One intracranial EEG study found using resting-state data that changes in HEP, in the time-frequency domain, show no time-locked changes in power but significant changes in phase coherence around 200ms after the r-peak in 4-10Hz (theta range) (Park et al., 2018). These findings, applying inter-trial coherence (ITC), led the authors to propose the hypothesis that the underlying mechanisms generating the HEP are not based on amplitude changes time-locked to the heartbeat but on a phase-resetting of the oscillations (Sauseng et al., 2007). The heartbeat resets, as the name suggests, the phase of the oscillations creating a significant phase coherence after the r-peak, which, in an event-related potential analysis, is seen as the HEP. Further competing theories have not been presented for the source dynamics of HEPs.

Figure 2 HEP Source Dynamics Theories figure is from Park et al., 2019

Delta range oscillations have been found to have connections to autonomic functions (Knyazev, 2012). Delta Power and Oscillation combined with cardiac activity showed a top-down modulation of the HEP (Patron et al., 2019). A causal connection between frontal top-down delta oscillations was shown in a recent study using tACS, where frontal delta phase synchrony attenuates the HEP, hypothesized to supress interoceptive detection in humans (Haslacher et al., 2025). Moreover, some studies could see a bi-directional coupling of frontal delta oscillations and the heartbeat during arousal tasks, finding that bottom-up cardiac activity can influence delta oscillations (Candia-Rivera et al., 2022, 2022). However, it remains unclear how delta activity in general could be attenuated by the heartbeat during rest.

Delta and theta in the source dynamics and their role

Delta increased coherence during interoception social task, delta power inhibited in the prefrontal during meditation vs controls

However, one should be aware that studies investigating HEP face a multitude of challenges. Comparisons between HEP studies are difficult due to low standardization during preprocessing, choices of HEP epochs, baseline windows and differences in the experimental designs (Park & Blanke, 2019). Further, in scalp-based recordings around the r-peak, there is a visual artefact called the Cardiac Field Artifact (CFA) (Dirlich et al., 1997; Park & Blanke, 2019). This occurs due to the strong electrical field generated by the heart itself. Computational measures have been used to remove the CFA, such as independent component analysis (ICA), subtraction method, and principal component analysis (PCA). These approaches have been found to be effective in removing prominent CFA from the HEP. However, they seem to not remove all artefactual components reliably (Park et al., 2014) and might remove important HEP components (Park & Blanke, 2019). The CFA is thought to not disturb the signal around the T-wave (Dirlich et al., 1997; Gray et al., 2007; Park et al., 2014), creating a way to use non-computational interventions. Conversely, the CFA has only a negligible effect on intracranial recordings and can be disregarded for those measurements (Park & Blanke, 2019). Although, a different artifact comes into play with intracranial recordings, the pule pressure artifact (PPA), which is based on the electrical signals of the pulse travelling through the cerebral arteries (Kern et al., 2013; Park et al., 2018). No common practice dealing with the PPA has been established since there are currently only a few studies that have investigated HEP using intra-cranial recordings. One study showed that using time-frequency analysis could be useful for removing PPA, as PPA is characterized by a low and repetitive oscillatory pattern below 2Hz (Park et al., 2018). The specific Hz range of a subject’s PPA can be calculated using their ECG heart rate values. Thus, using a high-pass filter above 2Hz, which is above a healthy humans Hz frequency of the heartbeat, is for now thought to suffice in removing the principal influences of the PPA on the HEP in intracranial recording.

Consideration of harmonics in the TFA thourgh the PPA

However, more research on the PPA and measures to extract it from the data is needed.

Dopamine and the Heart

* Vascular effects show an increase of blood pressure and heart activity
* Dopaminergic network is vast and a lot if it’s dynamics have not been explored yet
* Dopamine effect in the brain heart coupling bottom up has not been investigated this directly
* Levodopa is precursor and could create the peripheral affects , which did not cross the blood-brain barrier) which would show an increase of the cardiovascular system higher BP
* But can be administered with ecarboxylase inhibitor (like carbidopa or benserazide) which block most of the peripheral effects, decreasing the influence on the cardiovascular system.

## Recordings

The main reason for the integration of local field potentials (LFP) from deep brain stimulation (DBS) electrodes in the subthalamic nucleus (STN) and EEG is to understand the dynamics of the HEP in the cortical and subcortical areas. As mentioned above, areas in the subcortex are possibly used for relaying the signal (thalamus) and as a target region (amygdala). Although of high interest, especially recordings in subcortical regions in humans are limited to clinical purposes and clinical targets. Thus, the choice of the STN as a recording site for the subcortical measurements which is not mentioned in the possible pathways. It is clinically a highly important implantation site in Parkinson’s Disease (PD) patients for improved motor function (Bove et al., 2021; Lachenmayer et al., 2021). Taking into consideration, the new findings of a possible mechanism based on blood pressure through specific baroreceptors in neurons (Jammal Salameh et al., 2024). Based on this finding and the fact that precise pathways for the HEP are currently unknown, it can be argued that all areas in the brain, not only the ones in the possible pathways, receive cardiovascular signals. Furthermore, possible neostriatal projections in the heart-brain pathways implicate the basal ganglia in their dynamics (Critchley & Harrison, 2013). The STN being part of the basal ganglia could suggest that cardiac activity could be recorded from the STN-DBS electrodes. The experimental analysis of the subcortical data of the STN could shed some light on the dynamic influence of cardiac signals on areas outside of the possible pathways. Furthermore, the simultaneous recordings of cortical and subcortical electrodes offer the unique possibility of investigating the integration of cortical and subcortical HEP mechanisms underlying the HEP.

## Aim of the project

Following the reported literature, this thesis aims to further advance the understanding of the neural source dynamics of HEPs. The simultaneous cortical EEG and intracranial subcortical LFP recordings offer a novel opportunity for the research into HEPs. HEPs are recorded during the eyes-open resting state in both Medication Off (MedOff) and Medication On (MedOn) conditions to assess naturalistic neural processing of the heartbeat, sans the behavioral tasks and influences. Medication Off refers to the state of PD patients who have not taken their dopaminergic medication for at least six hours (SOURCES). Based on the literature, we do not expect to see HRV-related changes regarding medication but to see the HEP in both cortical and subcortical data. Furthermore, replicating the findings from Park et. al (2018), we envision that after time-frequency analysis, there are no changes in power in the data, but we can see significant phase coherence using ITC around the HEP timings in both cortical and subcortical recordings. Following that, we hypothesis that phase coherence between cortical and subcortical electrodes using cross-channel coherence (CCC), especially ipsilaterally, is significant in line with the HEP timings.

# Methods

## Patients and surgery

Fourteen PD patients (seven female) who underwent bilateral STN-DBS surgery participated in this exploratory study. At the time of the recording, their mean age was 60 years (± 1.5 years SEM), with an average disease duration of 11 years (± 1.6 years SEM). Participants were recruited from King’s College Hospital NHS Foundation Trust and St. George’s University Hospital NHS Foundation Trust, both located in London, United Kingdom. All patients gave their written and informed consent to participate in this study. The local ethics committee approved this study (St. George's University Hospital, IRAS: 46576; King’s College University Hospital, IRAS: ###). The patient’s clinical details (and location of the DBS electrodes) can be found in Table 1

For the leads the clinicians used the Medtronic 3389 (Medtronic Inc., Neurological Division, USA) with four 0.5 mm spaced contacts of 1.5 mm length with platinum‐iridium cylindrical surfaces, or the directional leads from Boston Scientific (model DB-2202, Boston Scientific, USA) or St. Jude Medical (model 6170, St. Jude Medical, now Abbott, USA), both having three segmented contacts on the middle levels. DBS implantation was guided by magnetic resonance imaging. (St. George's University Hospital).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sub. | Gender (f/m) | Age (yr) | Disease duration (yr) | Pre-OP UPDSR-III  OFF | Pre-OP UPDSR-III  ON | Pre-dominant symptoms |
| 1 | m | 57 | 11 | 41 | 16 | Rigidity |
| 2 | m | 59 | 6 | 31 | 4 | Tremor, anxiety with panic attacks |
| 3 | f | 63 | 10 | 29 | 6 | Bradykinesia |
| 4 | m | 63 | 20 | 51 | 27 | Tremor |
| 5 | f | 62 | 7 | 39 | 5 | Tremor |
| 6 | f | 63 | 10 | 29 | 8 | n/a |
| 7 | f | 64 | 14 | 31 | 17 | Tremor |
| 8 | f | 65 | 7 | 23 | 4 | Tremor |
| 9 | f | 55 | XXX | 42 | 16 | n/a |
| 10 | f | 67 | 20 | 55 | 32 | n/a |
| 11 | M | 50 | 7 | n/a | n/a | Rigidity, bradykinesia |

**Table 1** **Patients’ clinical data**.

## Data Recording

All patients were recorded once with Levodopa medication taken and confirmed to be in effect. For 8 subjects, another recording could be done with an overnight withdrawal from Levodopa medication. The LFP recordings were done on externalised DBS electrodes around 2 to 5 days after surgery and before the implantation of the subcutaneous pulse generator. The EEG recording is split into the main data acquisition and supplemental data acquisition. For the main EEG recordings, seven electrodes were placed in frontal (F3, F4), central (C3, C4, Cz), and parietal locations (P3, P4, Pz). The main recording included 10 subjects. The remaining 4 subjects were recoded supplementarily, with differing EEG constellations, due to the different EEG channel requirements of their main studies. This current study lends itself to easy implementation as only 4 ECG electrodes are added to the setup and rest data recordings are done regardless. The increased complexity of data analysis was worth it for the additional data and subjects. The exact EEG channels can be found in Table 2.

|  |  |  |
| --- | --- | --- |
| Recording type | Subjects (N) | EEG channels |
| Main | 10 | F3, F4, C3, C4, Cz, P3, P4, Pz |
| Supplementary | 1 | C3, C4, P3, P4 |
| 1 | F3, F4, C3, C4, P3, P4 |
| 2 | Fz, Cz, Oz, Pz, C3, C4 |

**Table 2** Overview of EEG channels.

ECG was recorded using two bipolar electrodes placed horizontally and vertically along the left torso. All electrodes used a reference electrode located on the inner wrist of the patients. All signals were measured and amplified (at 2048Hz) with a TMSi Porti and its respective software (TMS International, Netherlands) on a recording laptop.

## Study Design

During the recording, the participants were seated comfortably in an armchair. For this thesis, the required data was resting data. The patients were asked to sit relaxed with eyes open for about 5 minutes. These 5-minute recordings were done with the medication present (MedOn) and, when possible, after medication withdrawal (MedOff).

## Signal preprocessing

All signal processing was done using MATLAB (v. 2024a, Mathworks, Massachusetts, USA) with custom-written scripts. All written code has been made available on the author’s GitHub (https://github.com/lipaulsen/HeadHeart). Spike2 (v. 7.2, Cambridge Electronic Design Limited) was used for the initial visual inspection. r-peak detection in the ECG Signal was done within Spike2 and manually checked. Visual cleaning was done via the exclusion of r-peak trials when major artefacts were present in the EEG and LFP data.

### Electrocardiogram (ECG)

The ECG data was cleaned by removing the DC Offset, and a two-pass 2nd order Butterworth band-pass filter (0.5Hz high pass; 30Hz low pass). The filters used were from the fieldtrip toolbox (Oostenveld et al., 2011). Afterwards, the Inter-beat Interval (IBI) and the heart rate (HR) of each patient were calculated. Using the IBI the HRV was extracted (Figure 2 B). These cardiac data features (r-peak, IBI, HR, HRV) were chosen, in this exploratory analysis, as they represent a broad field of information from the ECG signal with a solid theoretical background and complement the goals of this thesis.



B

A

**Figure 3** Preprocessing Pipeline. **(A)** The preprocessing for the EEG and LFP starts with visual artifact detection and removal in Spike2. Switching to MATLAB filtering with 50Hz Notch, High-pass at 0.1Hz and low-pass at 100Hz was done. An additional high-pass filter at 0.5Hz for EEG and 2Hz for LFP was done to take care of the PPA artifact in the LFP data. The LFP data was bipolar re-referenced and all data was down sampled to 300 and epoched time-locked to the r-peak and in the area of interest around -300ms to 600ms. All signals were baseline corrected from each epoch the values from -300ms to -100ms are subtracted. Ultimately the data was transformed into the time-frequency domain using the IIRPeak and Hilbert transform. (**B**) ECG data was visually inspected for artifact rejection and r-peak detection was automatically done in Spike two through amplitude thresholding. All detected r-peaks were manually checked. In MATLAB the DC Offset was calculated and the data was bandpass filtered at 0.5 to 30Hz. This lead to the calculation of the IBI and the HR through the ECG signal.

### Electroencephalography (EEG) and local field potential (LFP)

The EEG and LFP data were visually inspected using Spike2, and periods with a lot of visual noise were removed. In MATLAB the data was high- and low-pass filtered using a two-pass 4th order Butterworth high-pass filter at 0.5Hz and the same configuration for the low-pass filter at 100Hz (Figure 2 A).

As mentioned in the introduction, the PPA needs to be accounted for in LFP measurements. The mean heart rate over all subjects was 1.28Hz (± 0.16Hz, min 1.06Hz, max 1.65Hz). Based on this, and the fact that pulse-related oscillatory artifacts occur below 2Hz, an additional 2Hz high-pass filter was applied. Another intracranial study used a high-pass filter at 4Hz for a more conservative approach (Park et al., 2018). After consideration, the more liberal 2Hz cutoff was chosen for this data to retain the most signal information while still removing the PPA. The additional high-pass filter was not applied for the EEG data since the PPA is not present in that data. Considering the several methods for removing the CFA, both computational (ICA, PCA, subtraction) and non-computational (HEP time-window selection), the non-computational method was chosen. The CFA decreases to less than 1% during the period of the t-wave until the next r-peak compared to ECG amplitudes at the chest (Dirlich et al., 1997; Park & Blanke, 2019). Thus, the restricted time window between shortly before the t-wave to the next r-peak was chosen as the area of interest. So, this area of interest can be used to measure HEP without CFA contamination and potential signal loss through computational methods.

The EEG data and LFP data used the common average reference for re-referencing. Additionally, the LFP data was re-referenced using the bipolar re-referencing method, which is commonly used in LFP data from DBS electrodes (Li et al., 2018). Effectively this leads to one electrical signal representing for the STN per hemisphere. The filtered and re-referenced data was resampled to 300 Hz, to speed up the computation. The data was epoched around 300ms before till 600ms after the r-peak. Baseline correction was performed using 200ms of data from the 300ms to 100ms before the r-peak of each epoch. Time-frequency decomposition was performed using first an IIR Peak Filter with a Bandwidth of 2Hz and the attenuation QFac of 2Db with 148 frequency bins between 0.5 and 30Hz and a resolution of 0.2Hz. This frequency range was chosen based on previous studies and frequencies of interest of including beta frequency (13-30Hz) since working with PD data (Kern et al., 2013; Park et al., 2018). Afterwards, a Hilbert transform was applied to the filtered data using a function from the fieldtrip toolbox. The EEG spectral power and phase time series at each frequency were extracted by computing the magnitude and angle of the Hilbert-transformed signal across time, yielding time-frequency representations of power and phase dynamics.

## Analysis and Statistics

All analysis and statistical code can also be found on the author’s GitHub. The significance level for all statistical analyses was set to α = .05, if not specified otherwise.

### ECG Features Analysis

ECG data can distinguish multiple features. Features extracted here are the Heartrate (HR), in the form of beats, as r-peaks, per minute, the Inter-beat Interval (IBI), the duration of time between r-peak and r-peak, and the HRV. HRV can be calculated in multiple ways through the ECG signal. The two main approaches discern themselves between frequency-domain or time-domain calculations. Especially in recent studies solely investigating the HRV using the frequency-domain has seen wide appeal due to the ability to differentiate between low-frequency and high-frequency HRV (Fourcade et al., 2024; Malik, 1996). HRV not being the main point of analysis, the Root Mean Sum of Squared Distance (RMSSD) was chosen. It is a widespread and validated approach to HRV calculation that does not use the Fourier transform. The IBI times are squared, averaged over all values, and ultimately the square root is taken over the results.

RMSSD values are in ms and reportedly change over the lifetime so a healthy person age 30-40 years has an RMSSD HRV of 30-50ms, whereas this decreases to roughly 20-30ms in the fifties (Tegegne et al., 2020). Clinical diseases can influence the HRV of the clinical population. Taken into account in the analysis is that PD patients RMSSD HRV values are decreased compared to age-matched healthy controls (Heimrich et al., 2021).

All ECG features are compared between MedOn and MedOff condition. To inspect the difference in the features between medication a paired ttest is used. The IBI, HR and HRV values for each subject were averaged and compared between conditions. One limitation is the low number of subjects. For only 8 of the 14 patients both medication conditions datasets are available. Patients opt out of the medication withdrawal, since the increase of PD symptoms during the withdrawal period can be too uncomfortable. One of the eight patients was excluded due to Arrythmia. The patients ECG signal was extremely irregular over the entire recording, which lead to its entire exclusion. Thus, the N for the analysis here is decreased to 7.

### HEP Analysis

HEPs were computed on the EEG and LFP signals time-locked to the r-peak. r-peak detection was done using Spike2 via automatically tagging each peak exceeding the global average amplitude on a subject-by-subject basis. All automatically tagged instances were visually inspected and corrected. Epochs (−300 to 600 ms regarding the r-peak onset) presenting excessive artifacts were excluded from the analysis. After artifact rejection, each subject had 451 ± 141 epochs for each electrode. Firstly, epochs for each electrode were averaged to calculate the patients traditional HEP. Subsequently, to the traditional averaging, a hierarchical clustering approach was taken to extract waveforms. Plotting the subjects averages of the HEP it was quite apparent that the average waveforms of the HEP also show high divergence based on polarity. Hierarchical clustering can alleviate this, as it does not average but uses the pure subject-wise waveforms to create clusters over all subjects and channels. The average waveform from each channel of each subject is used. A subject and channel-wise waveforms matrix is shaped within a condition over the epoch. Hierarchical Clustering is performed using Euclidean distance and the ward algorithm. This creates a hierarchical clustering tree that takes XXX into account. MATLAB’s built-in functions were used to compute the hierarchical clustering. A table mapping the subject, channels and clusters is utilised to recover data point assignments. Averaging showed that the shifted polarity of signals lead to averaging out of useful signals. After inspection, clusters with inverse polarity were able to be flipped to correct for averaging out in this case. Hierarchical Clustering was separated into three categories (EEG, STN, ALL) based on which channels are clustered, and the two conditions (MedOn and MedOff).

Statistical analysis compares the HEP group waveforms by either medication (MedOn vs. MedOff) or by location (EEG vs STN). Significance is determined using a paired t-test with FDR correction for multiple comparisons. Testing is done on the entire time epoch time window and on a time window of 100ms to 600ms after r-peak. The second time window is determined through visual inspection of all configurations, extracting the time range corresponding to the global maxima. Due to the low patient count in STN LFP studies a common practice is to use the STN hemispheres as separate patients (XXX). As this study remains exploratory and has a low number of patients the regular N and the hemispheric split is employed, to discover changes in statistical power.

### ITC Analysis

To calculate the phase coherence across single trials within one electrode, ITC was used (Tallon-Baudry et al., 1996). It describes the average of normalised instantaneous phases over single trials (Park et al., 2018)

This equation shows the implemented ITC algorithm, where is the frequency and is the time. is the number of trials and converts the phase () into a complex number on the unit circle using Euler’s formula. The resulting values for each trial can range between 0 and 1. A higher value means more coherence during the phase. ITC was calculated for both the EEG and the LFP electrodes for all subjects with the above-described epochs.

The statistical analysis was done in reference to the permutation approach from Park et al. (2018) for their ITC analysis. It uses non-parametric permutation statistics with a surrogate (Benjamini & Hochberg, 1995; Maris & Oostenveld, 2007). Surrogate r-peaks for each channel were created by randomly shifting the original r-peak timings 500ms around the event (-500ms to 500ms around the original r-peak). Thus, shifting period was chosen to keep the integrity of the original IBI and its variability and to keep within one heartbeat. Using the surrogate r-peaks, the channel data were epoched with these new times and transformed to the time-frequency domain. On the surrogate epochs, the ITC was computed as for the original data. This permutation repeated 1000 times, which led to a distribution of ITC values for each electrode that was based on chance observation. The z-scores of the distribution were calculated, and p-values for each electrode were extracted. FDR was applied to the p-values to correct for multiple comparisons. To replicate the finding of the phase-locking theory, a correlation was calculated between the ITC values and the spectral power during the same epochs. Compared to the data presented by Wang (2018), the current data set has fewer data points in total and per subject (474 derivations over 8 subjects in the original data and 108 derivations over 14 subjects). The current data was split into the different recording sites EEG (82 derivations over 14 subjects) and LFP (26 derivations over 14 subjects). The statistical approach was changed to accommodate the fewer derivations. Wang et. al. used a Pearson correlation and z-scored the data within-subject. Due to the fewer data points per subject, z-scoring the data would make the correlation unstable due to heteroscedasticity (sources). The non-parametric Spearman correlation was used to make the correlation more robust.

Following the previous investigation in the Hierarchical Clustering the ITC values were compared between MedOn and MedOff. For each channel the all MedOn and MedOff data points are tested using a paired t-test. As in the Hierarchical Clustering, the issue here remained that Med Off has fewer subjects. To keep with a within-subject design we solely utilized the subjects data that had MedOn and MedOff data. This decreased the number of subjects to 8. Beyond that, some subjects had a different configuration of EEG Channels, as explained in 2.2, therefore not all channels have the same degrees of freedom. Degrees of freedom are always reported in the results. For multiple comparison correction FDR is calculated.

### PSI/CCC Analysis

Investigating the phase coherence between two electrodes over the trials is done using the Phase Synchronization Index (PSI). In this thesis, it is also referred to as cross-channel coherence (CCC). It calculates the average of the normalised difference of phases over single trials between 2 channels.

is the PSI values for frequency and time . N is the number of trials with representing the phase angle of signal 1 of trial at the certain frequency and time. This is subtracted by the phase angle of signal 2. Where than using the Euler’s formula the complex phase difference is calculated out of. As with the ITC the PSI values range between 0 and 1 with a higher values indicating higher coherence.

Following the permutation approach from ITC the CCC analysis also uses surrogate r-peaks to create a range of surrogate epochs. Now two channels are used for this analysis at the same time and the CCC is calculated as for the original CCC data. The permutation runs 1000 times and following that Z-scores and p-values are extracted from the permutation distribution. After inspecting the original and the permutation distribution, it is unequivocal that the permutation CCC distribution differs extensively from the original CCC distribution. Normalisation approaches, like z-scoring, to bridge the gap, remained unsuccessful. Other statistical methods, which solely investigate the significant areas of the CCC of parametric tests (i.e., t-test) or non-parametric tests (i.e., Wilcoxon Signed Rank), would test against H0. This course of action does not apply in cases like PSI values, where the range is only between 0 and 1, as the null distribution of the data is not centred around 0. Alas, computational approaches circumventing this issue are out of the scope of this thesis.

Comparing the MedOn MedOff data of the CCC remained viable. As in the ITC, a paired t-test with FDR is used. The main interest remained in the phase coherence between cortical and subcortical regions. The different CCC configurations are distinguished between ipsilateral and contralateral combinations (Table 3 and Figure 3). In the EEG, a specific focus was on F3, F4, C3, C4, and Pz electrodes. These cover the motor cortex (C3, C4) and frontal regions (F3, F4), which are implicated in motor functions. These areas are susceptible to the effects of the medication in PD patients.

|  |  |  |
| --- | --- | --- |
| Directonality | Channel 1 | Channel 2 |
| Ipsilateral | STN left | F3 |
| STN left | C3 |
| STN left | Pz |
| STN right | F4 |
| STN right | C4 |
| STN right | Pz |
| Contralateral | STN left | F4 |
| STN left | C4 |
| STN right | F3 |
| STN right | C3 |

A diagram of a brain

AI-generated content may be incorrect.**Table 3** CCC channel combinations

**Figure 4** Visualisation of CCC channel combinations. The shown brain slice is a coronal cut at the basal ganglia. The rough location of the left and right hemisphere STN is indicated. As well as the hemispherical locations of the used electrodes according to the 10-20 placement of EEG electrodes. The colours of the lines indicate whether the combination is considered ipsilateral or contralateral. The graphic was done using Biorender.

# Results

## Levodopa medication shows no effect on ECG features

We tested the ECG features, IBI, HR, and HRV to test the hypothesis that parkinsonian medication has an effect on these features. These features were selected for the common use when looking into studies investigating HEPs (REFERENCE). Median IBI (Figure 4A) appears decreased in MedOn (800ms) compared to MedOff (900ms). IBI shows no significant difference between medication (p = 0.251, Cohen’s d = 0.536). Median HR (Figure 4B) increases slightly in MedOn (75bpm) with MedOff (69bpm) showing slower bpm. No significant effect was found (p = 0.338, d = 0.454). HRV Analysis (Figure 4C) shows no visual (median of both conditions = 13ms) or significant (p = 0.653, d = 0.166) difference. Single subjects show stark differences in HRV between medication, which has no effect on the group analysis.



Figure 5 Statistical Analysis of ECG Features between MedOn and MedOff. All Features are presented using a violin plot showing single data points of the IBI in both medication condition in ms. The red bar shows the median value and the dotted line connecting the conditions indicate the single subject values between conditions. (A) shows the IBI, (B) the HR and (C) the HRV data.

## Medication indicates modulation of HEP and phase coherence

We next investigated the neuronal data on medication modulation. The HEP averages were calculated for the EEG clusters of frontal (F3, F4), central (C3, C4), and parietal (P3, P4) electrodes as well as for the left (STNl) and right STN (STNr) channels. Visually all EEG clausters showed a slight increase in amplitude around 200ms after r-peak (**Figure 6A**). No visual changes could be discerned in the STN electrodes (**Figure 6B**). Based on the single channel HEPs plotted it could be seen that the HEP has a high degree of variance between of variance that get averaged out in the typical HEP calculation. Around the t-wave it can be seen that the bipolarity in amplitude. This may explain that there are no vial amplitude changes in the grand average. Especially prevalent in the case of the STN electrodes. This lead us to explore further analysis techniques to fully investigate the HEP.



A

B

C



The initial HEP analysis was extended to hierarchical clustering of the EEG Electrodes and the STN electrodes. A paired t-test was conducted to evaluate how medication changes (MedOn and MedOff) affect HEP. This tests whether the clustered and bipolarity corrected HEPs changes due to medication over time or amplitude. The analysis was conducted with an FDR for multiple corrections implemented after the t-test. None of the significant areas from the t-test survived multiple comparison testing. This may be due to the low number of subjects which decreases statistical power. Highlighted areas shown are before multiple comparisons. Be aware that these areas are presented as indicators of a trend in the data, rather than as statistically significant findings. It is revealed that, in the EEG electrodes shortly before the t-wave, around 200ms after the r-peak, HEP with MedOn indicate a dominant increase in amplitude compared to MedOff HEP (**Figure 7A**). Rebound of the MedOn HEP amplitude after its peak occurred around 300ms after r-peak. This appeared steeper than the MedOff rebound which happened at 400ms after r-peak. During this rebound period MedOff amplitude was higher than MedOn. The rebound trough was significantly lower than the MedOn through. Concurrently, in the STN electrodes the MedOn HEP had a dominantly higher amplitude peak compared to MedOff around 200ms after r-peak (**Figure 7B**). This peak occurred, as in the EEG electrodes, shortly before the t-wave in the ECG data. The rebound period exhibited the same pattern with the MedOn HEP shown a steep decline with a comparatively slower decline in the MedOff HEP. Subsequent analysis looked at the comparison of EEG and STN data within either MedOff (**Figure 7C**) or MedOn (**Figure 7D**). No significant amplitude changes occurred within a medication classification. EEG and STN in MedOn had a similar steep rise

Figure 6 HEP based on Averaging for EEG frontal, central and parietal regions and STN left and right. In both A+B the uppermost graphs show the grand average of the ECG amplitude over time with the black striped line indicated the r-peak. HEP graphs have the r-peak marked with a vertical line. The thick redline represents the grand average of the HEP in amplitude over time. The thin colorful lines represent the single channel HEP within that cluster. All HEPs shown here are MedOn and plotted with a Gaussian filter for smoothing of 10. (A) Figures below the ECG show the EEG channel clusters of the frontal, central and parietal electrodes. Average HEP shows only a slight increase in amplitude at the beginning of the t-wave. Single electrode HEPs show a lot of variation. (B) No visual amplitude changes in the STNl or STNr HEPs. Single channel HEPs have a high degree of variance in bipolarity.

and fall of the amplitude peak. Thus, the HEP results suggest a change through medication change.

Figure 7 Hierarchical Clustering EEG and STN MedOn vs. MedOff. Comparison between MedOn and MedOff in either EEG or STN is presented in A and B. C and D compare the EEG versus the STN Electrodes in either MedOn or MedOff. Uppermost graphs show the grand average of the ECG amplitude over time, with the black striped line indicating the r-peak. HEP graphs have the r-peak marked with a vertical line. All HEP graphs show the amplitude over time with the shading showing the Mean Standard of Error. Each graphs legend explains the colors of the lines. In A and B significant areas before Multiple Comparison are marked with a bracket on the top and an asterisk. A paired t-test evaluated statistical significance over time. All significant areas shown in the plot do not survive multiple comparison testing and are only present to show the trend in data.



C

D



A

B

\*

\*

Power data was extracted through time-frequency decomposition of the signal. Power signals were compared using a paired t-test running over time and frequency. We investigated whether medication changes induced an effect on power. As in the previous analysis, a paired t-test was used, where the significant clusters disappeared after MC. In the following statistical values and clusters are only mentioned as a means to indicate a trend in the data. Frontal EEG power (**Figure 8A**) indicated a significant increase in MedOff right around the t-wave in the alpha range (mean t(7) = 2,602, mean Cohen’s d = 0.9835). Central EEG electrodes (**Figure 8B**) showed separation of medication activation in the frequency range. Power was increased in MedOff in the beta range and in MedOn in the other lower ranges (mean t(7) = 3,136, mean d = 1,185). Indication of significance is mainly spread over the entire time axis, except for alpha range cluster which only appeared 200ms after r-peak. MedOff showed stronger power in the beta range in parietal regions (mean t(7) = 3,195, mean d = 1,208)(**Figure 8C**). Less power changes were modulated by medication in the STN electrodes. STN left showed no indication of stronger modulation, just more prevalence for higher power in MedOn (**Figure 8D**). In the right STN, MedOn increased power in a delta-range cluster shortly after r-peak until 400ms after (mean t(8) =2,792, mean d = 0,987)(**Figure 8E**).



A

B

C

D

E

Figure 8 Time Frequency Power MedOn vs. MedOff in EEG and STN. Uppermost graphs in both columns show the grand average of the ECG amplitude over time, with the black striped line indicating the r-peak. HEP graphs have the r-peak marked with a vertical line. The left column shows the different EEG regions (frontal **A**, central **B**, parietal **C**) and the right STN electrodes. Time frequency plots have the difference of MedOn-MedOff presented with the Difference in ITC values. Each graph has the mean t-value, df, mean Cohen’s d and the p-value threshold in the title.

We next investigated the medication changes (MedOn and MedOff) on the phase coherence using ITC. This was again distinguished between 3 EEG clusters (frontal, central, and parietal) and the 2 hemispheric STN channels. Paired t-test was calculated over the time and frequency levels of the ITC values. In **Figure 9** the time-frequency plots show black rimmed clusters. Those indicate significant areas before multiple comparison (MC), as none of these clusters survived that line of testing. As in the previous plot, the pre-MC significant clusters were solely used as indicators of a trend. Frontal EEG electrodes (**Figure 9A**) showed mainly a higher phase coherence in MedOff right around r-peak in high beta (21-30 Hz). This switched to a slightly higher high beta ITC value in MedOn around 550ms after r-peak. Lower frequency (delta, theta) areas showed a higher ITC in MedOn shortly before t-wave. This pattern continued over the central and parietal electrodes (**Figure 9B-C**), with higher MedOff ITC values in the beta frequency especially around or shortly after r-peak. The lower frequency ranges presented increased ITC values in the MedOn condition. Switching to LFP, the left STN channel showed a trend towards higher ITC in MedOn, contrary to the just presented EEG results. Right STN remained mainly without clear indicators of significance. Higher ITC in MedOn was present at 150ms and 550ms after r-peak in the delts/theta range, the rest remaining higher ITC MedOff values.



A

C



B



D

E

**Figure 9**: Effect of medication on ITC values in EEG and STN. Uppermost graphs in both columns show the grand average of the ECG amplitude over time, with the black striped line indicating the r-peak. time-frequency graphs have the r-peak marked with a vertical line. The left column shows the different EEG regions (frontal **A**, central **B**, parietal **C**) and the right STN electrodes. Time frequency plots have the difference of MedOn-MedOff presented with the Difference in ITC values. Each graph has the mean t-value, df, mean Cohen’s d and the p-value threshold in the title.

Furthermore, we explored the modulation of medication on ipsilateral and contralateral phase coherence between the subcortical STN and the cortical EEG electrodes. The channels investigated are on the ipsilateral side STNl-F3, STNl-C3, STNl-Pz, STNr-F4, STNr-C4, and STNr-Pz. For the contralateral connection the frontal and central hemispheric channels are switched resulting in STNl-F4, STNl-C4, STNr-F3, and STNr-C3. Significant areas after the paired t-test did not hold up to MC, but are used as an indication guide. The most prominent features, for both the frontal electrodes ipsilateral of the STN electrodes, were that the phase coherence was stronger in MedOff specifically in the low beta range (mean t(7) = -2,42, mean d = -0,915)(**Figure 10A+D**). This may be the case due to the well-known beta activity in PD patients. The removal of medication from the patients may lead to an increase in beta compared to the presence of medication to control for the PD symptoms. PD symptoms are closely related to an increase in beta range power and phase. The frontal electrodes presented randomized clusters of higher MedOn phase coherence in the lower frequency ranges. The ipsilateral central EEG electrodes exhibited diverging results compared to the parallels the frontal electrodes exuded. STNl-C3 continued with the higher low beta coherence over the entire time-axis (**Figure 10B**). Surprisingly, high beta in the later part of the cardiac cycle showed higher MedOn phase coherence. No discernible mentions could be made about the lower frequency ranges. Whereas, the right hemisphere STNr-C4 displayed a stronger prevalence for MedOn phase coherence across cortical and subcortical areas especially in the lower frequency ranges (theta and alpha) (**Figure 10E**). Ipsilateral phase interaction between the subcortical regions with Pz proved to be of more random nature, with no perceivable patterns (**Figure 10C+E**).

Figure 10 Ipsilateral phase coherence between EEG and STN electrodes Uppermost graphs in both columns show the grand average of the ECG amplitude over time, with the black striped line indicating the r-peak. Time-frequency graphs have the r-peak marked with a vertical line. The left column shows the different EEG regions (frontal **A**, central **B**, parietal **C**) and the right STN electrodes. Time frequency plots have the difference of MedOn-MedOff presented with the Difference in CCC values. Each graph has the mean t-value, df, mean Cohen’s d and the p-value threshold in the title.



A

B

C

D

E

F



B

E

Looking at the contralateral hemispheres between subcortical and cortical areas we discovered that the left STN electrode showed no detectable separation in clusters between time, frequency and medication change (**Figure 11A+B**). Right STN presented with a more separated coherence profile (**Figure 11C+D**). High beta area was mainly modulated by the absence of medication regardless of time. Contrary to this, the theta and alpha areas phase coherence seemed to be driven by the presence of medication.

Figure 11 Contralateral phase coherence between EEG and STN electrodes Uppermost graphs in both columns show the grand average of the ECG amplitude over time, with the black striped line indicating the r-peak. Time-frequency graphs have the r-peak marked with a vertical line. The left column shows the different EEG regions (frontal **A**, central **B**, parietal **C**) and the right STN electrodes. Time frequency plots have the difference of MedOn-MedOff presented with the Difference in CCC values. Each graph has the mean t-value, df, mean Cohen’s d and the p-value threshold in the title.

A

B

C

D



## Delta and Theta phase coherence source of HEP modulation

We next investigated the neural sources underlying HEP, following the example of Park et al., 2018. Our data differentiation offers a broader insight into the neural sources in subcortical regions as well. Grand-average ITC was calculated for the frontal, central and parietal EEG clusters, as well as for both hemispheres of STN electrodes. The current analysis does not depend on comparison of MedOn and MedOff, thus to broaden the statistical power solely MedOn data was used to harness the data of all 14 subjects. This exploration helped determine the frequency time phase coherence during the HEP. Over all electrodes we found that ITC significantly increases after r-peak (Figure 12 ITC across EEG and STN and correlation, using surrogate permutation statistics with a p<0.05). Strongest phase coherence in the EEG was found around 100 to 250ms after r-peak (Figure 12A-C, within the white boxes), which temporally aligns with the ECoG findings from Park et al. This period was expanded in the STN electrodes to the strongest ITC period ranging form 100 to 300ms after r-peak (Figure 12D+E). Contrary to the Park et al study we included the delta range within our analysis, with a low pass filter of 2 HZ instead of 4Hz. Including delta showed that the strongest ITC enhancement was in the delta range (2-4Hz) and the low theta range (4-5Hz). This is the case for both EEG and STN data. The main finding in Park et al was in the entire theta range of 4-7Hz, which overlaps with our findings. Add here that only the EEG electrodes show the whole freq band phase coherence around heart beat (which STN does not), could be the CFA artefact which would help with artefact removal and consideration on a dataset basis.

A

B



C

D

E

F

G



Figure 12 ITC across EEG and STN and correlation

The relationship between power and HEP has been shown multiple times in the past. We could see in the MedOn MedOff difference already that there are no time specific power changes. Purely looking at the power revealed that there is no time-locked change to the r-peak (**Figure 12A-E**). To validate our power analysis, we checked whether our power would show HEP unrelated continuous PD specific beta power increase. **Figure 8** C and D visually showed that power in beta is higher during MedOff than MedOn. This follows the common knowledge that beta power is increased in PD patients. Just extracting the beta range of our power shows that beta power remains high (**Figure 12 F+G**). Thus, our power analysis reliably showed no effect to HEP. Testing for the relationship between power and phase of the HEP, we conducted a correlation. This analysis took, inspired in Park et al., the mean ITC and mean power within the significant cluster. The specific statistical procedure and modifications based on our data is explained in 2.5.3ITC Analysis. For both EEG and STN, Spearman’s r revealed no significant correlation between ITC and power (Figure 12 F+G). All of these results replicate the findings of Park et al.. Ultimately, solidifying that phase coherence in delta and theta modulate HEP, power shows no association to HEPs and no correlational relationship between power and phase is present in HEP.

Figure 13 Power in the cardiac cycle of EEG and STN

A

E



B

C

D



F

G

# Discussion

We collected cortical and subcortical neural data from 14 patients with Parkinson’s disease and found that delta and theta range phase coherence underlie the source dynamics of HEP. Mean ITC peak data was correlated with the power spectra data of the same area and revealed no correlation, stating that significant ITC is irrespective of power. Moreover, the data indicated that the presence of dopaminergic medication in PD patients has an effect on interoceptive bottom-up signalling in the brain. Various analyses were compared between medication, showing a discernible difference in HEP. Conversely, the investigated ECG features showed no effect from medication, which also diverges from other studies in this area (Ref). Our data suggests that dopamine influences the specific HEP pattern. Finally, the ITC EEG results show a specific pattern which compared to the ITC STN proposes that CFA could be observable in ITC. Opening further research for an advancement into its legitimacy and possible methodical approaches. This is the first study investigating heart-brain interoception on a subcortical and cortical basis during rest and shows that specifically phase modulates HEP and phase analysis opens new avenues for this area of research.

## HEP driven by phase resetting in delta and theta

* Replication of Park et al successful -> support of the phase resetting hypothesis
* Additionally implicated is the delta range
  + Delta range often activated and seen in sleep, respiration but also individuals with brain damage
  + So higher delta could also be related to PD
  + tACS looking at delta in HEP have seen anti correltation
  + Delta has rough links to HEP but no real studies have investigated it yet
* High ITC area happened in out dataset directly before into the beginning of the t-wave when time locked to the r-peak
* What is the known major role of the t-wave in the cardiac cycle? Hight of aortic blood flow with ITC, peak of ejection and start of reduction of the ejection of blood into the blood stream and ventricular pressure reduces

The ITC and power analysis of the HEP in the time-frequency domain support the phase-resetting hypothesis Park et al. supposes. After replicating their analysis, we could see high phase coherence in the same time range. This is coupled with the finding that there is no correlation between ITC peak and the spectral power, which they also found. This mechanism of the source dynamics shows no power change time-locked to the r-peak in the HEP. Underlined by significant phase coherence. The evoked model (references from the Park and also a reference to a figure in the intro) would propose a time-locked change in power. A difference that is present is the frequency range implicated in the ITC peak. Park et al. see the entire theta range (4-7Hz). We can observe high delta (2-4Hz) and low theta (4-5Hz). High theta shows significant phase coherence but outside of the realm of the ITC peak. Park et al. eradicated delta signals due to high-pass filtering at 4 Hz, as a more conservative approach to circumvent PPA.

We showed that in both subcortical and cortical findings, delta phase coherence peaks between 150-300ms after r-peak and modulate the HEP during rest. Previously, heartbeat-attenuated delta findings have been observed primarily in spectral power in the frontal area during arousal tasks. Delta phase has been implicated in the top-down attenuation of the HEP. Thus, our resting data expands the topographic realm of delta involvement and further implies that the underlying patterns in creating the HEP expand beyond theta. Theta band has repeatedly been shown in vagal nerve modulation to influence the bottom-up creation of HEP. One consideration remains that delta range activity and phase can be dysfunctional in patients with brain damage (Ref). As we are using PD patients who have received DBS, which is solely considered in a medium to late progression of the disease with symptomatic damage to the basal ganglia, this is a serious consideration while interpreting our results. A study presented that ITC values of delta and theta were decreased in PD patients compared to healthy controls (Hünerli-Gündüz et al., 2023). Ultimately, our ITC findings might be lower compared to a healthy population, but as it remains significant, this indicates the strength of the ITC peak in delta and theta in underlying HEP generation via phase resetting.

We showed that the timing of the ITC peak in the cortical and subcortical areas is correlated with the timing of the t-wave peak. The ITC peak occurs shortly before the t-wave peak. Physiologically, the area shortly before the t-wave coincides with the peak of the ejection and start of the reduction of blood from the heart. For this to occur the cardiac muscles are starting to release from the contraction, causing ventricular pressure to reduce and aortic blood flow to peak.

## Levodopa impact on CNS

* Impact can be seen in the neural data especially the HEP waveforms
* No impact in the ECG
* Might suggest that levodopa regulates only in the CNS
* ANS would have impact on ECG but there no effect can be seen so ANS is not implicated by Levodopa medication
* Dopaminergic changes due to intravenous dopamine can see a rise in blood pressue (Allwood et al 1963).Rise is slow, not significant, and peaks roughly 5min after injection angoes back to baseline after roughly 15min. Oral medication might have slower effects.
* This study found that the higher the Levodopa Dose the more it can have a reduced effect on the BP (Levodopa lowers BP) <https://www.cell.com/heliyon/fulltext/S2405-8440%2823%2905084-3> but like it does not seem to do it significantly. Also we have no record of the exact levodopa dose patients were having after MedOff, could implicate that the mean dosage was on the lower end.

Our findings suggest that there is a modulation in neural signalling during the cardiac cycle through levodopa medication. ECG features show no effect from medication. Especially, HEP waveforms using the hierarchical clustering method indicated an impact on interoception processing due to the presence of medication. Further time-frequency analysis (Power, ITC, CCC) showed no clear differentiations. Contrary to our findings, other studies have seen a significant increase in HRV based on a single dose of levodopa compared to MedOff in PD patients (Meng et al., 2015). Specific medication details, such as medication type and dosage, are not available for our dataset. So further analysis based on those factors is out of the scope. To the best of our knowledge, the effects of levodopa medication on HEP have not been investigated before. Therefore, it is an interesting revelation that our data indicates that neuronal waveforms show an increase and sharpness in amplitude in the HEP. Investigating the complex mechanisms dopamine has on the brain, coupled with interoception, can help in medication adjustments. Further, it might help understand the connection between interoception and the dopamine network by uncovering how the presence of dopamine in the brain might influence bottom-up signalling from the body. Furthermore, this could be enlightening in the case of dopamine dysregulation conditions, including but not limited to PD, ADHD, schizophrenia or addiction.

## Phase as support mechanism for CFA circumvention

Our ITC findings had another revelation. In the EEG trial-based ITC, we could observe, besides the delta-theta peak, a whole frequency band spanning phase coherence right around the r-peak. This pattern was only present in the EEG electrodes and was completely missing in the LFP electrodes. A difference in the analysis of heartbeat-locked EEG and LFP data is the presence of the CFA. Which, as previously discussed, solely occurs in EEG data. One big issue in the artefact removal of the CFA is that there is no distinct and reliable measure. Previous studies have indicated time periods in which the CFA should be nonexistent in the data. But these time periods have differed greatly between studies (Ref). Other computational approaches have tried to quantify certain parts of the signal to eradicate the CFA. As we chose the non-computational approach, the phase coherence pattern right around the r-peak that is purely present in the EEG data could be a strong indicator for the CFA. This revelation comes due to the novel quality of being able to compare the ITC values of EEG to STN. In our data, we can see that the frequency band spanning significant ITC values right around r-peak are in the time period between 100ms before to 100ms after r-peak, using the baseline window of 300 to 100ms before the r-peak of each trial. This recognisable pattern cannot be seen in the power spectral analysis or in the HEP, which underlines the difficulty in pinning down specific CFA quantification. As a methodological analysis is out of the scope of this thesis, no foundational analysis can be provided. Moreover, we would like to propose this finding as a starting point for further analysis into this phenomenon, which overlaps with what the research would imply the features of CFA to be. Ultimately, this could aid the future analysis of interoceptive heart-brain research.

## Limitations and Outlook

* Small sample. Higher power would be great and it’s such an easy data set to aquire with other recordings
* Consistency of electrodes and conditions, when considering this as a proper study. This study as it was exploratory used all kinds of data available but a more consistent set of electrodes, conditions and subjects would be extremely helpful in getting reliable results.
* The high amount of artifacts in this line of research, where for this specific kind of artifacts not a lot of research has been done so current practices are either under or overcorrecting. Which would severely influence the results making the statistical reliability low. A consistent and agreed upon preprocessing procedure for general handleing of ECG, EEG and other recording methods. This has been already suggested and in parts attempted by park blanke 2019 but an updated approach and as more research is focused on interoception would be needed.
* High variability of data due to heart conditions especially in clinical research with previous conditions. Length of IBI can differ a lot, this was taken into account and checked her ebut clinical research inthat area is more limited than healthy younger subjects that have a more regular dataset pattern.

The study design follows exploration along the data. This can harbour many advantages for curiosity but conversely give limitations. Conceptually the requirements for data acquisition is low, with rest recordings of 5min and 2 extra ECG electrodes added onto an already used EEG amplifier. It can very easily be implemented into other EEG studies. A pitfall here is the different combinations of EEG electrodes. Some electrodes might only have an N of 1 or 2, so a more consistent and stable set of electrode parameters would alleviate analysis issues. Moreover, this study only had a subset of the patients in both conditions. For further studies, we would aim to have a reliable condition split. Following this, the study’s main draw back for most statistical tests was its low sample size, which inadvertently decreased statistical power. Thus, all the comparisons for medication are not statistically significant after multiple comparison. Investigating the influence of medication on neural responses of interception with a high sample size would greatly improve statistics.

As discussed previously investigating time-locked heart and brain data leads to artefact management in the cortical region with CFA and in the subcortical region with the PPA. Currently there is no common ground for a procedure to deal with either artefact in the different modalities of EEG and LFP. Ultimately, leading researchers to possibly under- or overcorrect during artefact removal, influencing results. This lack of shared pre-processing practice has been noted before. Park and Blanke have suggested a pre-processing procedure in their 2019 paper. Only few papers reported to have known or done this procedure. For future research, an updated approach taking current developments into account would be admirable.

Finally, clinical research with an older population with previous conditions can also include heart conditions apart from the PD diagnosis. In our study patients were included based on the DBS surgery and PD diagnosis, other diagnosis of the heart were not regarded. Controlling for this, previous to recordings for heart conditions or heart medication taken by the patients could help exclusion factors, in the case of strong arrythmias, and take medication and their influence on the heart brain coupling into consideration. Further, the influence of levodopa medication on the cardiovascular system has been presented. The exact medication and dosage after MedOff were not recorded for each participant. This limits the interpretation of the medication influence. Henceforth, it would be suggested to record dopaminergic medication to control for the ecarboxylase inhibitor sometimes found in levodopa medication which inhibit cardiovascular influence.

Heart conditions and dopamine medication changing vascular system by increasing blood pressure

We don’t know which medication the subjects got to control for the peripheral effect. Future research should take care of medication to control for plausible cardiovascular difference based on pharmaceutical intervention of dopamine outside of blood-brain barrier.

# References

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bove, F., Mulas, D., Cavallieri, F., Castrioto, A., Chabardès, S., Meoni, S., Schmitt, E., Bichon, A., Di Stasio, E., Kistner, A., Pélissier, P., Chevrier, E., Seigneuret, E., Krack, P., Fraix, V., & Moro, E. (2021). Long-term Outcomes (15 Years) After Subthalamic Nucleus Deep Brain Stimulation in Patients With Parkinson Disease. *Neurology*, *97*(3). https://doi.org/10.1212/WNL.0000000000012246

Brener, J., & Ring, C. (2016). Towards a psychophysics of interoceptive processes: The measurement of heartbeat detection. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1708), 20160015. https://doi.org/10.1098/rstb.2016.0015

Cambi, S., Solcà, M., Micali, N., & Berchio, C. (2024). Cardiac interoception in Anorexia Nervosa: A resting‐state heartbeat‐evoked potential study. *European Eating Disorders Review*, *32*(3), 417–430. https://doi.org/10.1002/erv.3049

Candia-Rivera, D., Catrambone, V., Thayer, J. F., Gentili, C., & Valenza, G. (2022). Cardiac sympathetic-vagal activity initiates a functional brain–body response to emotional arousal. *Proceedings of the National Academy of Sciences*, *119*(21), e2119599119. https://doi.org/10.1073/pnas.2119599119

Coll, M.-P., Hobson, H., Bird, G., & Murphy, J. (2021). Systematic review and meta-analysis of the relationship between the heartbeat-evoked potential and interoception. *Neuroscience & Biobehavioral Reviews*, *122*, 190–200. https://doi.org/10.1016/j.neubiorev.2020.12.012

Critchley, H. D., & Harrison, N. A. (2013). Visceral Influences on Brain and Behavior. *Neuron*, *77*(4), 624–638. https://doi.org/10.1016/j.neuron.2013.02.008

Dale, A., & Anderson, D. (1978). Information Variables in Voluntary Control and Classical Conditioning of Heart Rate: Field Dependence and Heart-Rate Perception. *Perceptual and Motor Skills*, *47*(1), 79–85. https://doi.org/10.2466/pms.1978.47.1.79

Desmedt, O., Luminet, O., & Corneille, O. (2018). The heartbeat counting task largely involves non-interoceptive processes: Evidence from both the original and an adapted counting task. *Biological Psychology*, *138*, 185–188. https://doi.org/10.1016/j.biopsycho.2018.09.004

Dirlich, G., Vogl, L., Plaschke, M., & Strian, F. (1997). Cardiac field effects on the EEG. *Electroencephalography and Clinical Neurophysiology*, *102*(4), 307–315. https://doi.org/10.1016/S0013-4694(96)96506-2

Fourcade, A., Klotzsche, F., Hofmann, S. M., Mariola, A., Nikulin, V. V., Villringer, A., & Gaebler, M. (2024). Linking brain–heart interactions to emotional arousal in immersive virtual reality. *Psychophysiology*, *61*(12), e14696. https://doi.org/10.1111/psyp.14696

Garfinkel, S. N., & Critchley, H. D. (2016). Threat and the Body: How the Heart Supports Fear Processing. *Trends in Cognitive Sciences*, *20*(1), 34–46. https://doi.org/10.1016/j.tics.2015.10.005

Garrett, L., Trümbach, D., Spielmann, N., Wurst, W., Fuchs, H., Gailus-Durner, V., Hrabě De Angelis, M., & Hölter, S. M. (2023). A rationale for considering heart/brain axis control in neuropsychiatric disease. *Mammalian Genome*, *34*(2), 331–350. https://doi.org/10.1007/s00335-022-09974-9

Gray, M. A., Taggart, P., Sutton, P. M., Groves, D., Holdright, D. R., Bradbury, D., Brull, D., & Critchley, H. D. (2007). A cortical potential reflecting cardiac function. *Proceedings of the National Academy of Sciences*, *104*(16), 6818–6823. https://doi.org/10.1073/pnas.0609509104

Haslacher, D., Reber, P., Cavallo, A., Rosenthal, A., Pangratz, E., Beck, A., Romanczuk-Seiferth, N., Nikulin, V., Villringer, A., & Soekadar, S. R. (2025a). Heartbeat perception is causally linked to frontal delta oscillations. *Communications Biology*, *8*(1), 1466. https://doi.org/10.1038/s42003-025-08933-9

Haslacher, D., Reber, P., Cavallo, A., Rosenthal, A., Pangratz, E., Beck, A., Romanczuk-Seiferth, N., Nikulin, V., Villringer, A., & Soekadar, S. R. (2025b). Heartbeat perception is causally linked to frontal delta oscillations. *Communications Biology*, *8*(1), 1466. https://doi.org/10.1038/s42003-025-08933-9

Heimrich, K. G., Lehmann, T., Schlattmann, P., & Prell, T. (2021). Heart Rate Variability Analyses in Parkinson’s Disease: A Systematic Review and Meta-Analysis. *Brain Sciences*, *11*(8), 959. https://doi.org/10.3390/brainsci11080959

Hünerli-Gündüz, D., Özbek İşbitiren, Y., Uzunlar, H., Çavuşoğlu, B., Çolakoğlu, B. D., Ada, E., Güntekin, B., & Yener, G. G. (2023). Reduced power and phase-locking values were accompanied by thalamus, putamen, and hippocampus atrophy in Parkinson’s disease with mild cognitive impairment: An event-related oscillation study. *Neurobiology of Aging*, *121*, 88–106. https://doi.org/10.1016/j.neurobiolaging.2022.10.001

Jammal Salameh, L., Bitzenhofer, S. H., Hanganu-Opatz, I. L., Dutschmann, M., & Egger, V. (2024). Blood pressure pulsations modulate central neuronal activity via mechanosensitive ion channels. *Science*, *383*(6682), eadk8511. https://doi.org/10.1126/science.adk8511

Kern, M., Aertsen, A., Schulze-Bonhage, A., & Ball, T. (2013). Heart cycle-related effects on event-related potentials, spectral power changes, and connectivity patterns in the human ECoG. *NeuroImage*, *81*, 178–190. https://doi.org/10.1016/j.neuroimage.2013.05.042

Kim, K. J., Ramiro Diaz, J., Iddings, J. A., & Filosa, J. A. (2016). Vasculo-Neuronal Coupling: Retrograde Vascular Communication to Brain Neurons. *The Journal of Neuroscience*, *36*(50), 12624–12639. https://doi.org/10.1523/JNEUROSCI.1300-16.2016

Knyazev, G. G. (2012). EEG delta oscillations as a correlate of basic homeostatic and motivational processes. *Neuroscience & Biobehavioral Reviews*, *36*(1), 677–695. https://doi.org/10.1016/j.neubiorev.2011.10.002

Laborde, S., Mosley, E., & Thayer, J. F. (2017). Heart Rate Variability and Cardiac Vagal Tone in Psychophysiological Research – Recommendations for Experiment Planning, Data Analysis, and Data Reporting. *Frontiers in Psychology*, *08*. https://doi.org/10.3389/fpsyg.2017.00213

Lachenmayer, M. L., Mürset, M., Antih, N., Debove, I., Muellner, J., Bompart, M., Schlaeppi, J.-A., Nowacki, A., You, H., Michelis, J. P., Dransart, A., Pollo, C., Deuschl, G., & Krack, P. (2021). Subthalamic and pallidal deep brain stimulation for Parkinson’s disease—Meta-analysis of outcomes. *Npj Parkinson’s Disease*, *7*(1), 77. https://doi.org/10.1038/s41531-021-00223-5

Li, G., Jiang, S., Paraskevopoulou, S. E., Wang, M., Xu, Y., Wu, Z., Chen, L., Zhang, D., & Schalk, G. (2018). Optimal referencing for stereo-electroencephalographic (SEEG) recordings. *NeuroImage*, *183*, 327–335. https://doi.org/10.1016/j.neuroimage.2018.08.020

Lischke, A., Pahnke, R., Mau-Moeller, A., & Weippert, M. (2021). Heart Rate Variability Modulates Interoceptive Accuracy. *Frontiers in Neuroscience*, *14*, 612445. https://doi.org/10.3389/fnins.2020.612445

Malik, M. (1996). Heart Rate Variability: Standards of Measurement, Physiological Interpretation, and Clinical Use: Task Force of The European Society of Cardiology and the North American Society for Pacing and Electrophysiology. *Annals of Noninvasive Electrocardiology*, *1*(2), 151–181. https://doi.org/10.1111/j.1542-474X.1996.tb00275.x

Maris, E., & Oostenveld, R. (2007). Nonparametric statistical testing of EEG- and MEG-dataଝ,ଝଝ. *Journal of Neuroscience Methods*.

Marshall, A. C., Gentsch, A., Schröder, L., & Schütz-Bosbach, S. (2018). Cardiac interoceptive learning is modulated by emotional valence perceived from facial expressions. *Social Cognitive and Affective Neuroscience*, *13*(7), 677–686. https://doi.org/10.1093/scan/nsy042

Meng, L., Dunckley, E., & Xu, X. (2015). Effects of a single dose levodopa on heart rate variability in Parkinson’s disease. *Zhonghua Yi Xue Za Zhi*, *95*(7), 493–495.

Müller, L. E., Schulz, A., Andermann, M., Gäbel, A., Gescher, D. M., Spohn, A., Herpertz, S. C., & Bertsch, K. (2015). Cortical Representation of Afferent Bodily Signals in Borderline Personality Disorder: Neural Correlates and Relationship to Emotional Dysregulation. *JAMA Psychiatry*, *72*(11), 1077. https://doi.org/10.1001/jamapsychiatry.2015.1252

Murphy, J., Brewer, R., Plans, D., Khalsa, S. S., & Catmur, C. (2020). Testing the independence of self-reported interoceptive accuracy and attention. *Quarterly Journal of Experimental Psychology*, *73*(1), 115–133. https://doi.org/10.1177/1747021819879826

Oostenveld, R., Fries, P., Maris, E., & Schoffelen, J.-M. (2011). FieldTrip: Open Source Software for Advanced Analysis of MEG, EEG, and Invasive Electrophysiological Data. *Computational Intelligence and Neuroscience*, *2011*(1), 156869. https://doi.org/10.1155/2011/156869

Owens, A. P., Friston, K. J., Low, D. A., Mathias, C. J., & Critchley, H. D. (2018). Investigating the relationship between cardiac interoception and autonomic cardiac control using a predictive coding framework. *Autonomic Neuroscience*, *210*, 65–71. https://doi.org/10.1016/j.autneu.2018.01.001

Pang, J., Tang, X., Li, H., Hu, Q., Cui, H., Zhang, L., Li, W., Zhu, Z., Wang, J., & Li, C. (2019). Altered Interoceptive Processing in Generalized Anxiety Disorder—A Heartbeat-Evoked Potential Research. *Frontiers in Psychiatry*, *10*, 616. https://doi.org/10.3389/fpsyt.2019.00616

Park, H.-D., & Blanke, O. (2019). Heartbeat-evoked cortical responses: Underlying mechanisms, functional roles, and methodological considerations. *NeuroImage*, *197*, 502–511. https://doi.org/10.1016/j.neuroimage.2019.04.081

Park, H.-D., Blanke, O., Bernasconi, F., & Salomon, R. (2018). Neural Sources and Underlying Mechanisms of Neural Responses to Heartbeats, and their Role in Bodily Self-consciousness: An Intracranial EEG Study | Cerebral Cortex | Oxford Academic. *Cerebral Cortex*, *28*, 2351–2364. https://doi.org/10.1093/cercor/bhx136

Park, H.-D., Correia, S., Ducorps, A., & Tallon-Baudry, C. (2014). Spontaneous fluctuations in neural responses to heartbeats predict visual detection. *Nature Neuroscience*, *17*(4), 612–618. https://doi.org/10.1038/nn.3671

Patron, E., Mennella, R., Messerotti Benvenuti, S., & Thayer, J. F. (2019). The frontal cortex is a heart-brake: Reduction in delta oscillations is associated with heart rate deceleration. *NeuroImage*, *188*, 403–410. https://doi.org/10.1016/j.neuroimage.2018.12.035

Pollatos, O., & Schandry, R. (2004). Accuracy of heartbeat perception is reflected in the amplitude of the heartbeat-evoked brain potential. *Psychophysiology*, *41*(3), 476–482. https://doi.org/10.1111/1469-8986.2004.00170.x

Sauseng, P., Klimesch, W., Gruber, W. R., Hanslmayr, S., Freunberger, R., & Doppelmayr, M. (2007). Are event-related potential components generated by phase resetting of brain oscillations? A critical discussion. *Neuroscience*, *146*(4), 1435–1444. https://doi.org/10.1016/j.neuroscience.2007.03.014

Schandry, R. (1981). Heart Beat Perception and Emotional Experience. *Psychophysiology*, *18*(4), 483–488. https://doi.org/10.1111/j.1469-8986.1981.tb02486.x

Schulz, A., Ferreira De Sá, D. S., Dierolf, A. M., Lutz, A., Van Dyck, Z., Vögele, C., & Schächinger, H. (2015). Short‐term food deprivation increases amplitudes of heartbeat‐evoked potentials. *Psychophysiology*, *52*(5), 695–703. https://doi.org/10.1111/psyp.12388

Schulz, A., Stammet, P., Dierolf, A. M., Vögele, C., Beyenburg, S., Werer, C., & Devaux, Y. (2018). Late heartbeat-evoked potentials are associated with survival after cardiac arrest. *Resuscitation*, *126*, 7–13. https://doi.org/10.1016/j.resuscitation.2018.02.009

Strohman, A., Isaac, G., Payne, B., Verdonk, C., Khalsa, S. S., & Legon, W. (2024). Low-intensity focused ultrasound to the insula differentially modulates the heartbeat-evoked potential: A proof-of-concept study. *Clinical Neurophysiology*, *167*, 267–281. https://doi.org/10.1016/j.clinph.2024.09.006

Tallon-Baudry, C., Bertrand, O., Delpuech, C., & Pernier, J. (1996). Stimulus Specificity of Phase-Locked and Non-Phase-Locked 40 Hz Visual Responses in Human. *The Journal of Neuroscience*, *16*(13), 4240–4249. https://doi.org/10.1523/JNEUROSCI.16-13-04240.1996

Tegegne, B. S., Man, T., Van Roon, A. M., Snieder, H., & Riese, H. (2020). Reference values of heart rate variability from 10-second resting electrocardiograms: The Lifelines Cohort Study. *European Journal of Preventive Cardiology*, *27*(19), 2191–2194. https://doi.org/10.1177/2047487319872567

Whitehead, W. E., Drescher, V. M., Heiman, P., & Blackwell, B. (1977). Relation of heart rate control to heartbeat perception. *Biofeedback and Self-Regulation*, *2*(4), 371–392. https://doi.org/10.1007/BF00998623

# Appendix