The noradrenergic basis of Parkinson's tremor: a systems-level fMRI approach

(version 6)

PROTOCOL TITLE:

The noradrenergic basis of Parkinson's tremor: a systems-level fMRI approach.

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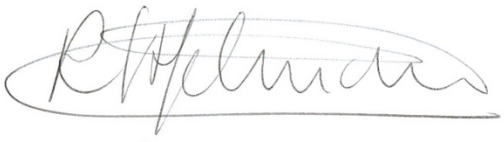
Dr. Rick C.G. Helmich *Principle investigator*		**16-4-2021**

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen
fMRI	Functional Magnetic Resonance Imaging
PD	Parkinson's disease

BOLD	Blood Oxygen Level Dependent Activity
UPDRS	Unified Parkinson's Disease Rating Scale
TRS	Tremor Rating Scale

SUMMARY

Rationale: Parkinson's disease is the second most common neurodegenerative disease worldwide. Clinically, Parkinson's disease is characterized by motor slowing (bradykinesia), stiffness (rigidity) and resting tremor. The pathological hallmark of Parkinson's disease is striatal dopamine depletion, but the dopaminergic basis of resting tremor is disputed. For instance, striatal dopamine depletion correlates with all motor symptoms except resting tremor. Furthermore, resting tremor is often resistant to dopaminergic medication. Instead, resting tremor worsens consistently during psychological stress, and recent findings suggest that the noradrenergic (stress) system is hyperactive in Parkinson's disease. Based on empirical (fMRI) data, I have recently proposed a new systems-level model of Parkinson's tremor. According to this model, tremor is initiated in the basal ganglia and amplified in the cerebello-thalamo-cortical circuit. In this study, I will use this model as the basis for understanding how the noradrenergic (stress) system amplifies Parkinson tremor.

Objective: I will test the hypothesis that the noradrenergic system amplifies tremulous activity in the cerebello-thalamo-cortical circuit. More specifically, I will test how this modulation takes place (i.e. through which brain regions and connections).

Study design: Cross-over intervention study.

Study population: Two groups of idiopathic Parkinson's disease patients with either a tremor-dominant phenotype (n=40) and with a non-tremor phenotype (n=30).

Intervention (if applicable): The intervention only involves the tremor-dominant Parkinson group. To activate the noradrenergic system, I will use a validated and controlled stress-induction task (cognitive-coactivation: alternating blocks of mental arithmetic versus rest). Furthermore, I will test whether a pharmacological intervention (propranolol 40 mg single dose) can counteract the effects of psychological stress on the tremor circuitry. Propranolol is commonly used in clinical practice to treat tremor. The control condition will be a placebo.

Main study parameters/endpoints: (1) Tremor-related activity and connectivity (quantified using concurrent EMG-fMRI); (2) Structural integrity of the locus coeruleus (quantified using neuromelanin sensitive MRI); (3) Clinical tremor severity (quantified using tremor rating scales)

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The experimental protocol will consist of clinical measurements, and performance of a simple cognitive task in the fMRI scanner. These measurements will be performed on two mornings (duration: 4-5 hours per session). Patients will arrive in a practically defined OFF state, i.e. at least 12 hours after having taken their last dopaminergic

medication. At the end of the measurement, they will resume their normal medication regime. When OFF-medication, their Parkinson symptoms may temporarily worsen, which can lead to discomfort. On one session, patients will receive a single dose of propranolol (40 mg). Propranolol is commonly used in clinical practice to treat tremor. This may sometimes lead to temporary side effects such as dizziness, bradycardia, and cold hands/feet. Patients will be extensively monitored during the measurements (blood pressure, heart rate) to avoid any health risks. In addition, our task is designed to induce psychological stress, and this may lead to some discomfort. Finally, the noise in the fMRI scanner, and lying in a small space, may lead to discomfort. If all security measures are fulfilled, then there is not risk for the patients.

Tremor is a common and debilitating symptom of Parkinson's disease. If tremor does not respond to dopaminergic treatment, then there are only few therapeutic options. Better pathophysiological insights are needed to provide a rational basis for improved treatment strategies. This study aims at better understanding the pathophysiology of Parkinson’s tremor, by focusing on the noradrenergic system. Identifying the respective neural substrates could potentially have great clinical and therapeutic implications and will also help to better understand why tremor increases dramatically during stressful circumstances. As such, this research may provide clues to target new therapies in tremor-dominant Parkinson patients.

1. INTRODUCTION AND RATIONALE

Parkinson's disease is a very common neurodegenerative disease, clinically characterized by motor slowing (bradykinesia), stiffness (rigidity), and resting tremor. Resting tremor occurs in >75% of patients, and early patients rank it as their second-most bothersome symptom[1]. The pathological hallmark of Parkinson's disease is nigro-striatal dopamine depletion[2], but the dopaminergic basis of resting tremor is disputed[3]. For instance, striatal dopamine depletion correlates with all motor symptoms except resting tremor[4], and dopaminergic medication has a variable and sometimes no effect on resting tremor[3]. On the other hand, resting tremor is consistently amplified during acute psychological stress[5,6]. Our group has recently shown that the anti-tremor effect of levodopa is reduced in a stressful vs. neutral context[7] (Fig1). This finding highlights an important clinical problem: patients suffer most from their tremor in stressful circumstances, when available (dopaminergic) therapy is least effective. It also raises interesting mechanistic questions (with potentially broad implications well beyond tremor), namely how stress can affect neural circuitries involved in normal motor function and the generation of pathological disease manifestations. Here, I will investigate the role of the noradrenergic system –which is activated during psychological stress[8,9]– in the pathophysiology of Parkinson's resting tremor.

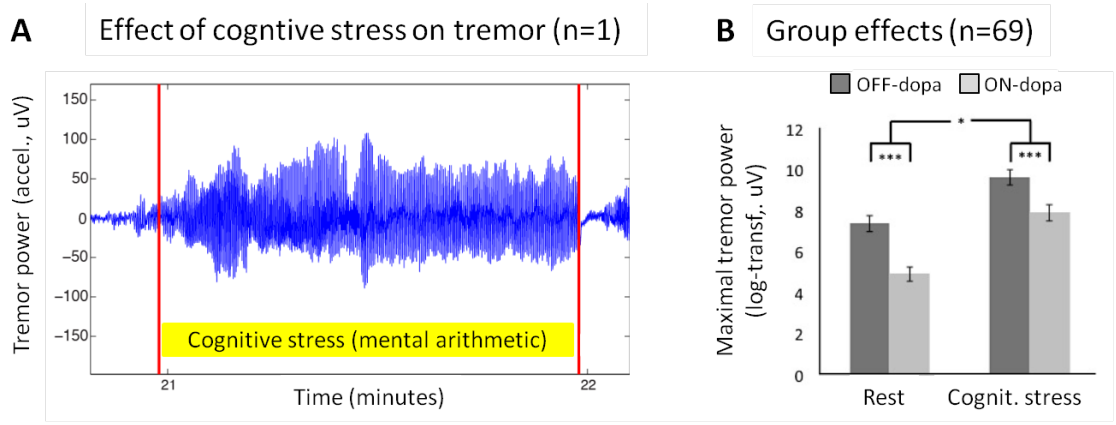

Fig1: Psychological stress immediately increases tremor (panel A) and reduces the effect of dopamine (panel B[7]).

Several pieces of evidence suggest a link between the noradrenergic system and resting tremor. For instance, post-mortem studies have shown that Parkinson patients with a tremor-dominant phenotype have *less* degeneration of the locus coeruleus (the main source of cerebral noradrenalin) than patients with a non-tremor phenotype[10]. Also, nuclear imaging has shown that noradrenalin receptor binding in the locus coeruleus is *increased* in Parkinson

patients vs. controls[11], particularly in tremor-dominant patients[12]. Furthermore, interventions that suppress noradrenergic hyperactivity, both pharmacologically (beta-blockers[13-15]) and non-pharmacologically (guided relaxation therapy[16]) can reduce resting tremor. Conversely, intravenous injection of adrenalin, which activates the cerebral noradrenergic system through the vagal nerve[8,17], increases tremor[18-20].

Despite these initial findings, the cerebral mechanisms underlying worsened tremor during acute psychological stress remain very much unclear[5]. The locus coeruleus has anatomical projections to all nodes of this circuit[21]. Animal studies have shown that an acute stressor produces motor hyperactivity, possibly mediated through direct effects of noradrenalin onto the cerebral motor circuit[22]. Another possibility is that noradrenalin interacts with the dopaminergic system. However, acute stress has been associated with increased striatal dopamine release[23,24], which would rather lead to tremor suppression than tremor worsening[25]. It is unlikely that psychological stress influences tremor through the peripheral nervous system, because the effects on tremor emerge almost immediately (Fig1A). Furthermore, adrenalin administration only increases Parkinson's tremor when injected intravenously (i.e. systemically, enabling central effects through the vagus nerve[17]), but not when injected into an artery (which distributes adrenalin to the muscle)[19]. It is also unlikely that psychological stress modulates tremor through spinal mechanisms[26,27].
To understand the pathophysiology of Parkinson's tremor, a comprehensive systems-level approach is crucial: different networks are involved, multiple nodes within the cerebello-thalamo-cortical circuit have pace-making properties[28], and network parameters (interregional connectivity) separate pathological tremor from mimicked tremor[29]. In my previous work, I have used a systems-level approach to define the cerebral mechanisms underlying Parkinson's resting tremor[3]. For this purpose, I developed a new method that directly relates cerebral activity (measured with fMRI) to fluctuations in tremor intensity (measured with concurrent EMG; Fig2)[25,30]. Using this approach, I have shown that tremor is initiated in the basal ganglia, and amplified in the cerebello-thalamo-cortical circuit[3]. More recently, I have applied network analyses (dynamic causal modelling; DCM) to this tremor circuitry. With DCM, it is possible to test how an extrinsic factor modulates cerebral activity and inter-regional connectivity. The ability to statistically test which underlying network structure best explains the fMRI data sets DCM apart from more traditional fMRI approaches. Using DCM, we have recently shown that dopamine specifically modulates the cerebello-thalamo-cortical circuit at the level of the thalamus, but only in a subset of patients with a dopamine-responsive tremor[31].

We have recently validated an experimental task that reliably modulates Parkinson's tremor (n=33; Fig 2) in a block-wise manner. This task involves alternating blocks of 60 seconds where patients (n=33) either performed mental arithmetic or observed a cross-hair. Importantly, during this task both tremor amplitude (measured with accelerometry) and pupil diameter (measured with eye tracking) rapidly increased during arithmetic blocks and decreased again during rest blocks. This time course allows a block-wise comparison of stress (arithmetic) and control (rest) conditions within one session. We have also behaviourally shown that this stress induction task (which involves cognitive effort) is more successful in modulating Parkinson's tremor (n=17) than a threat of shock task (which involves physical fear).

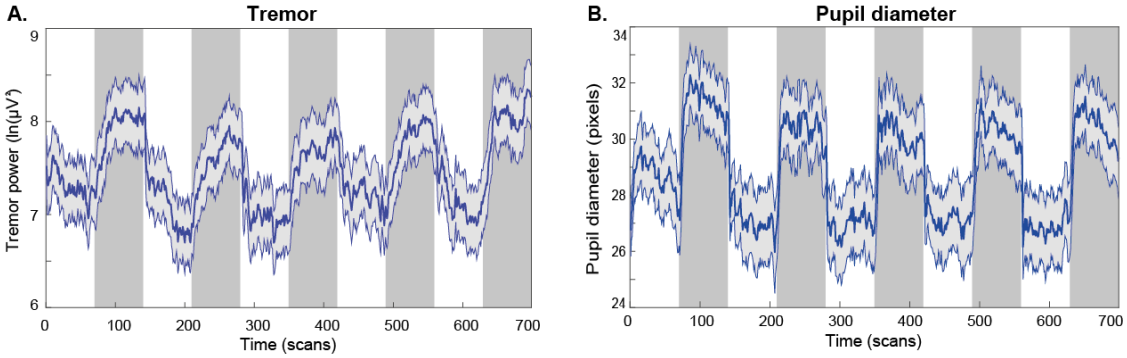

Fig 2: Effect of mental arithmetic (grey; 60 sec) and rest (white; 60 sec) on tremor amplitude (panel A) and pupil diameter (panel B) in 33 Parkinson's disease patients.

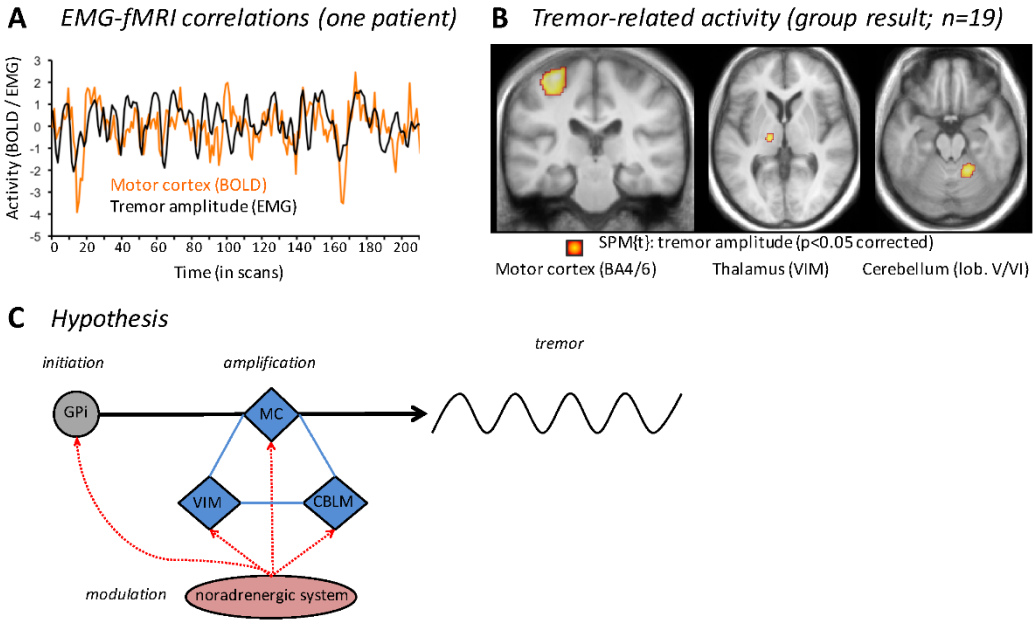

Fig 3: Method and Hypothesis

2. OBJECTIVES

In the current project, my key objective is to understand the role of the noradrenergic system in the pathophysiology of Parkinson's resting tremor. More specifically, I aim:

1. To define the cerebral mechanisms underlying stress-related amplification of Parkinson's resting tremor, and to test whether these mechanisms are sensitive to a pharmacologic intervention that suppresses noradrenergic hyperactivity (propranolol).

   Hypothesis: Based on my previous work, I expect that the noradrenergic system amplifies tremor by acting on the cerebello-thalamo-cortical circuit (Fig2C)[25]. Given the presence of functional interactions between the noradrenergic and the dopaminergic systems in the basal ganglia[9,32], I will also test the alternative hypothesis that the noradrenergic system influences tremor-related activity in the basal ganglia.

2. To test whether the structural integrity of the locus coeruleus-noradrenaline system differs between Parkinson patients with a tremor-dominant and a non-tremor phenotype.

   Hypothesis: Based on post-mortem studies, I expect reduced structural integrity of the locus coeruleus in Parkinson patients with a non-tremor phenotype [10].

3. To test whether individual differences in the structural and functional integrity of the locus coeruleus-noradrenaline system can explain individual responses of tremor to stress.

   Hypothesis: I expect a positive relationship between the structural integrity of the noradrenergic system and the impact of stress on resting tremor

3. STUDY DESIGN

Interventions

I will manipulate the noradrenergic system using a 2x2 factorial design with factors PHARMACOLOGY (propranolol 40mg vs. placebo) and BEHAVIOR (stress induction vs. control) (Fig3). The two medication conditions (propranolol versus placebo) will be tested on two different days, to avoid carry-over effects. The two behavioural conditions (stress versus control) will be measured on the same day, in a block-wise manner, as shown in Fig 2. The order of the two medication conditions will be counter-balanced. Physiological markers of stress will be monitored throughout the scanning procedure (see below).

Pharmacological suppression of noradrenergic neurotransmission will be achieved using propranolol (40mg, oral dose). This is a non-selective β-adrenergic receptor-blocking agent that competes with β-adrenergic receptor stimulating agents at β1- and β2-adrenergic receptor sites. In healthy subjects, propranolol suppresses noradrenergic neurotransmission and functional connectivity within a cerebral salience network[9]. In Parkinson patients, there is mixed evidence regarding the anti-tremor effect of propranolol[13-15], probably because in most cases contextual factors (stressful vs. neutral) were not taken into account[34]. Peak plasma concentrations are reached 1 to 2 hours after administration, and remain stable for several hours[35,36]. Therefore, in line with previous studies[9], I will administer propranolol (or placebo) 105 min prior to fMRI scanning.

Behavioural noradrenergic stress induction will be achieved using a task that is well-validated task by our group, the cognitive co-activation task. This task involves alternating blocks of mental arithmetic versus rest. The dynamics of this task (rapid increases and decreases of both tremor and pupil diameter) make it possible to compare stress and rest conditions within a single fMRI session.

Measurements:

- ECG (on the first visit of the patient, prior to placebo/propranolol).
- Resting state fMRI (10 minutes, concurrent EMG recording). Subjects will be asked to keep their eyes open to continuously monitor their autonomic state. I will use an optimized multiband fMRI sequence with a high spatial resolution (voxel size=2.0 mm isotropic, to extract a reliable BOLD time course from the locus coeruleus) and a high temporal resolution (TR=1000 ms, to optimally correct for physiological noise). Connectivity measures derived from these measurements are used to test Hypotheses #1 and #3 (see *Analyses* below).

- Task-based fMRI (cognitive co-activation; 15 minutes, concurrent EMG recordings). The task will consist of five 60-second blocks during which subjects perform mental arithmetic (which will be displayed on the screen) and five 60-second blocks during which they will focus on a crosshair on the screen. For example, if 200-7 is displayed on the screen, subjects are asked to consecutively subtract 7 from each preceding number. At the end of the task, subjects will indicate how tensed they were (5-point scale).
- Autonomic responses during scanning. To validate our stress induction approach, we will record pupil diameter (using eye tracking), heart and respiration rate, blood pressure, and salivary cortisol[9]. These outcome measures are used to validate the intervention and to test Hypotheses #2 and #3.
- Neuromelanin-sensitive structural MRI scans (10 min) to localize the locus coeruleus and quantify its structural integrity[40]. This scan will only be performed once, since we do not expect an effect of task on structural brain properties. This outcome measure is used to test Hypotheses #2 and #3.
- Rating scales and questionnaires: MDS-UPDRS-III Mini Mental State Examination (MMSE), Tremor Rating Scale, Spielberger's State-Trait Anxiety Inventory, Beck's Depression Inventory, bradykinesia (computer-based peg board test[41]) and the Positive and Negative Affect Schedule (PANAS) questionnaire. These measures are used to account for sources of inter-subject variability.
- Color wheel working memory task (45 min, *outside* the scanner). This task measures the preference of individual subjects to perform a stable versus a flexible cognitive effort. This is done by asking participants to perform a visual working memory task on a trial-by-trial basis. There will be two conditions: after remembering a first set of two or four colors, participants are asked after a delay of 2 seconds to either remember a new set of two or four colors (flexibility), OR they are asked to ignore the new set of colors (distraction) and remember the previous set (stability). After this phase of the experiment (15 min + 1- min practice), participants' subjective value of cognitive effort of both conditions will be evaluated. This will be done by presenting participants with a series of choices (15 min). They will have to indicate how much they prefer to redo a block of trials of one condition to the other, or if they prefer not repeating a task at all. In order to ensure validity of the choices, participants will have to redo their selected choice (5 min). The noradrenergic system likely plays an important role in the balance between flexibility (update) and stability (ignore in the face of distraction). We expect that Parkinson patients, in whom the noradrenergic system is dysfunctional, have an

altered balance between flexibility and stability. This task is added to investigate the effect of noradrenergic dysfunction on cognition in Parkinson's disease.

Main Analyses:

Hypothesis 1: I will use EMG-fMRI to test for session-specific differences in the pattern of tremor-related activity (Fig2A)[25]. I will also use DCM to test how the locus coeruleus-noradrenaline system modulates effective connectivity within the tremor circuit, by contrasting models where behavioral and pharmacological factors modulate different connections and nodes within the tremor circuitry (Fig2C).

Hypothesis 2: I will use correlations between pupil diameter and brain activity[39] to test for session-specific differences in the locus coeruleus-noradrenaline system. Pupil diameter is correlated with cerebral activity in the locus coeruleus[39], and is regarded as a reliable marker of noradrenergic activity[42,43]. We have in-house experience with this method (collaboration with Dr Erno Hermans). Nuclear imaging (PET) is less suitable for localizing the locus coeruleus-noradrenaline system: well-validated radioligands for the cerebral noradrenaline transporter are lacking, and the limited spatial resolution of PET prevents reliable measurements in the locus coeruleus [44,45]. I will also compare the structural integrity of the locus coeruleus, assessed using neuromelanin MR-scanning[40], between patients and controls.

Hypothesis 3: I will correlate patient-specific tremor-related patterns (#1) with noradrenergic measures (#2), and clinical tremor scores, to predict individual tremor characteristics based on the integrity of the locus coeruleus-noradrenaline system.

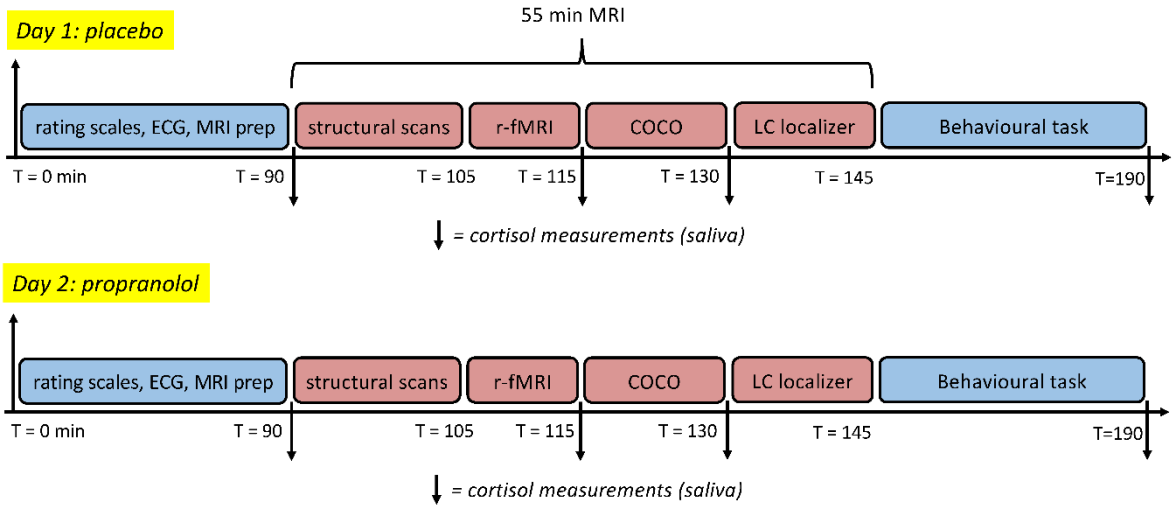

Fig4: design of the study. *Placebo and Propranolol sessions will be counter-balanced.*

4. STUDY POPULATION

4.1 Population (base)

Patients with idiopathic PD will be included through their neurologists at the Neurology department of the Radboud University Nijmegen Medical Centre, and through ParkinsonNext (see below). Nijmegen has a very large outpatient movement disorders clinic, and based on previous studies we expect no problems in recruiting enough patients for this research.

Number of subjects:
The full experiment, including testing on two different days, will be performed in 40 idiopathic Parkinson's disease patients with a tremor-dominant phenotype, defined as a resting tremor score of $\geq$2 UPDRS points for at least one arm[3,25]. This number of subjects gives reliable and replicable (validated across multiple cohorts) tremor-related activity[3,25,31]. Since my focus is to understand how different experimental factors modulate cerebral and clinical correlates of resting tremor, I will use a within-patients (cross-over) design.

In addition, I will perform a subset of measurements in 30 non-tremulous Parkinson patients. These patients will only receive structural MRI scanning, resting state fMRI, and task-based fMRI on one day, to localize structurally and functionally localize the locus coeruleus-noradrenaline system. This group will not receive any intervention (behavioral or pharmacological). I will compare the structural and functional integrity of the locus coeruleus between tremor-dominant and non-tremor Parkinson patients.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Idiopathic Parkinson's disease according to UK brain bank criteria.
- Presence of a clear resting tremor of at least one arm (UPDRS tremor-score $\geq$ 2).

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Use of beta-blockers
- Neuropsychiatric co-morbidity
- Contraindications for MRI scanning (e.g. pacemaker, implanted metal parts, deep brain stimulation, claustrophobia)
- Cardiac arrhythmias (in patient history or visible on ECG)
- contraindications for beta blockers (e.g. bradycardia, peripheral circulation disturbances, asthma or obstructive lung disease, hypotension)
- Use of medication that may interact with propranolol, e.g. other bèta-blockers, calcium antagonists, digoxine, cimetidine, hydralazine, fluvoxamine, rifampicine, barbiturates, amiodaron, flecainide, kinidine, propafenon, disopyramide, chlorpromazine, and clonidine
- Use of medication that inhibits relevant CYP enzymes that are involved in metabolizing propranolol (CYP2D6, CYP1A2, and CYP2C19): fluoxetine, paroxetine, sertraline, duloxetine, terbinafine, cinacalcet, bupropion, and ciprofloxacine
- Severe head tremor or dyskinesias
- Cognitive impairment (MMSE < 26)
- PD disease duration >10 years, severe ON/OFF medication fluctuations, or daily Levodopa-equivalent dose >1200 mg.

4.4 Sample size calculation

Formal power analyses are impossible given the highly innovative character of our approach. Our projected sample size is based on comparable fMRI experiments in PD. In these studies, a sample size of 20 patients was sufficient to find significant differences non-tremor PD patients and tremor-dominant PD patients [25,30]. In addition to tremor-dominant patients, we will also include non-tremor Parkinson patients, matched for age, gender, and non-tremor disease characteristics (duration, severity) with the tremor-dominant group. Here we aim to have two groups of at least 24 subjects (see section 8.5).

On March 19th 2021, data from 17 of 25 (68%) tremor-dominant patients who signed informed consent could be used for further analysis (due to 8 early drop-outs). Data from all 19 included non-tremor patients could be used for further analysis. Furthermore, ongoing data analyses from a previous study, which used the same outcome measure as done here but without a propranolol intervention, showed that 33 of 40 collected data sets contained suitable eye tracking data (83%). Accordingly, we expect that we need to include 40 tremor-

dominant patients to arrive at ±24 individuals with complete and high-quality data (40 x 68% x 83% = 23-24 subjects). Drop-out is much less for the non-tremor group, given that there is no intervention, and only one session. In sum, this means that we will measure at maximum 40 tremor-dominant and 30 non-tremor patients. To avoid more early drop-outs in the tremor-dominant group, we will take the following measures: during screening, we will prioritize patients who have also participated in the Personalized Parkinson's Project (PPP), since a recent ECG is available for these patients. Other patients will be asked whether an ECG has been made recently, if so we will give these patients priority. We will request recent ECGs and only after an MD has approved that it meets all inclusion criteria, patients will be invited to participate. Remaining patients with no ECG available are invited to come to the institute prior to the scheduled sessions, to make an ECG and make sure they can undergo the propranolol intervention. Finally, we will be stricter in selecting suitable patients during our phone screening, and we will not invite patients with a disease duration of >10 years, considerable ON/OFF fluctuations or daily levodopa-equivalent dose of >1200 mg. In addition, shortening the protocol by leaving out the threat-of-shock task will hopefully increase quality of eye-tracking data.

5. TREATMENT OF SUBJECTS

The research will be conducted OFF dopaminergic therapy. In all cases, the patients will arrive at least 12 hours after having taken their own dopaminergic medication [46]. Subjects they will take their own medication by the end of the experiment. There is a pharmacological intervention (propranolol 40 mg dispersed in water vs. cellulose dispersed in water, tested on different days) and a behavioral intervention (a task that allows for block-wise comparison of stress (arithmetic) and control (rest) conditions within one session). Thus, there are two different sessions on two different days. Peak plasma concentrations of propranolol are reached 1 to 2 hours after administration, and remain stable for several hours[35,36]. Therefore, in line with previous studies[9], I will administer propranolol (or cellulose) 105 min prior to fMRI scanning. Propranolol and cellulose will be supplied by the Radboudumc pharmacy.

In total, a patient will receive 1 tablet of Propranolol 40 mg (dispersed in water) and 1 teaspoon of cellulose (dispersed in water) on two different sessions, respectively.

5.1 Investigational product/treatment

Propranolol 40 mg tablet (dispersed in water).

5.2 Use of co-intervention (if applicable)

None.

5.3 Escape medication (if applicable)

None.

6. INVESTIGATIONAL PRODUCT

In this project, we use either propranolol 40 mg or the pharmacologically inactive cellulose dispersed in water as a placebo in order to modulate the effects induced by our stress task on tremor-related activity.

The administration of propranolol in this dose is a normal clinical treatment for treating tremor, including Parkinson's tremor[47,48]. It has been used for many decades for treating tremor, and therefore, no unexpected serious adverse events (SUSAR's) or side effects are expected.

For our study, the approved propranolol in the Netherlands is used. We have included the summary of product characteristics (SPC) as an attachment. The certificate of analysis of the supplier of cellulose has been attached as well.

6.1 Description and justification of route of administration and dosage

We use 1 tablet of propranolol 40 mg or cellulose (as placebo) dispersed in water. The 40 mg propranolol dose has clinically relevant effects on tremor[47,48] and has been shown to modulate the cerebral locus coeruleus-noradrenaline system in healthy subjects [9]. The dispersion in water makes this intervention comparable to our placebo intervention (cellulose dispersed in water). Dispersion of the propranolol tablet in water allows affordable blinding of the study subjects from treatment, as the inactive cellulose dispersed in water is indistinguishable from the dispersed tablet. We justify the feasibility of the dispersion of the tablet, with the information of the KNMP kennisbank "Oralia VTGM" (https://kennisbank.knmp.nl/article/oralia_vtgm/TradeProductOral/propranolol-tablet-10-80-mg.html), that allows dispersion of propranolol tablets in water.

6.2 Dosages, dosage modifications and method of administration

All patients will receive 40 mg of Propranolol dispersed in water on one session, and they will receive cellulose dispersed in water on one other session (separate days).

6.3 Preparation and labelling of Investigational Medicinal Product

Propranolol and placebo will be labelled according to GMP-annex 13 by the Radboudumcpharmacy.

Storage of medicinal product supplies
Medication will be stored at a pharmacy cabinet at the Donders Centre for Cognitive Neuroimaging (room 0.33; technical room nr. M291.00.055). The pharmacy cabinet consists of 6 lockable drawers. The temperature of the storage area is monitored and a temperature log maintained.

See the DCCN SOP: Study-Medication-Management_SOP_DCCN_version_1.0_newtemplate.

6.4 Drug accountability

We will keep an accountability log at the Donders Centre for Cognitive Neuroimaging both for Propranolol and for the placebo (cellulose). We have attached an example drug accountability log to this study protocol.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

Tremor-related activity and connectivity (quantified using concurrent EMG-fMRI) as a function of behaviourally induced stress and the pharmacological intervention (Propranolol).

8.1.2 Secondary study parameters/endpoints (if applicable)

Structural integrity of the locus coeruleus (quantified using neuromelanin sensitive MRI).

8.1.3 Other study parameters (if applicable)

The response of non-tremor PD symptoms (such as bradykinesia and rigidity), as well as age and gender, will be used as covariates in the analyses, despite careful matching of the two groups on these parameters. We will also quantify the effect of the intervention (Propranolol) on clinical tremor severity (rating scales).

8.2 Randomisation, blinding and treatment allocation

Not applicable.

8.3 Study procedures

There are no invasive procedures in this study. The procedures that all subjects will undergo are also listed under STUDY DESIGN.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.5 Replacement of individual subjects after withdrawal

Our goal is to investigate two groups of 24 patients (tremor-dominant vs. non-tremor Parkinson's disease). We count on 6 dropouts per group based on bad eye tracking data, and up to 10 additional dropouts in the tremor-dominant group due to our strict inclusion criteria (ECG and measurement of blood pressure). We will therefore include at maximum 40 tremor-dominant and 30 non-tremor patients.

8.6 Follow-up of subjects withdrawn from treatment

Not applicable.

9. SAFETY REPORTING

9.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / the experimental intervention]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that at any dose:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgement, the event may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse events. SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse event. This is for a preliminary report with another 8 days for completion of the report.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable.

9.3 Annual safety report

Not applicable.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5 Data Safety Monitoring Board (DSMB)

Not applicable.

10. STATISTICAL ANALYSIS

The data will be presented quantitatively.

10.1 Primary study parameter(s)

Functional MRI data will be pre-processed and analyzed with SPM12 (Statistical parametric mapping). As outlined above (STUDY DESIGN), we will test for tremor-related cerebral activity and functional connectivity as a function of TASK (stress vs. control) and PHARMACOLOGY (Propranolol vs. placebo). This gives rise to four activation images per subject (one for each condition), which will be entered into a 2 x 2 repeated measures ANOVA in SPM12. We will test for effects in pre-defined regions of interest, i.e. brain areas showing where we previously observed tremor-related activity (the basal ganglia, the brain stem, and the cerebello-thalamo-cortical circuit[3,25]).

10.2 Secondary study parameter(s)

- Locus coeruleus structural integrity. We quantify structural integrity of the locus coeruleus by comparing signal intensity (on the neuromelanin-sensitive MRI scan) between tremor-dominant and non-tremor Parkinson patients in an independent sample t-test, as done before [49]. Locus coeruleus signal intensity will be normalized with respect to the surrounding brain stem intensity.
- Clinical scores. Clinical tremor severity will be compared between the two sessions using paired t-tests. Non-tremor clinical scores will be compared between the two groups (tremor-dominant vs non-tremor) using t-tests.

10.3 Other study parameters

Not applicable.

10.4 Interim analysis (if applicable)

Not applicable.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study under this protocol involving human subjects will be conducted in the respect of the following codes:

- The "Declaration of Helsinki 2007-2008" and its subsequent amendments
- The Medical Research Involving Human Subjects Act (WMO)
- The applicable laws and regulatory requirements governing the conduct of biomedical research projects involving human subjects.
- The DCCN Standard Operating Procedures

11.2 Recruitment and consent

It is the responsibility of the investigator to obtain informed consent from each subject participating in the study, after explanation of the aims, methods, benefits and potential hazards of the study. The consent must be obtained before any study-specific procedures are performed where subject will have unlimited time to consider their decision. Only subjects who are able to give legal consent will be entered into the study. It must be made completely and unambiguously clear to each subject that they are free to refuse to participate in the study, or that they can withdraw their consent at any time and for any reason, without incurring any penalty or withholding of treatment on the part of the investigator. Signed informed consent must be kept on file by the investigator, and documented in the applicable site file.

Adequate patient recruitment is ascertained by the Parkinson Centre Nijmegen (ParC), which has been recognized since 2005 as Centre of Excellence by the National Parkinson Foundation. Up to 500 new patients with parkinsonism are carefully evaluated each year within the day care centre of ParC, thus assuring adequate recruitment of precisely defined patients. If needed, additional recruitment of patients can take place through our excellent collaboration within the national ParkinsonNet / ParkinsonNext, an award-winning health care concept that consists of a series of now 64 regional networks around general hospitals. ParkinsonNext is a website hosted by ParkinsonNet where patients can register online and indicate whether they are interested in participating in (particular types of) scientific research. The movement disorders neurologists (Dr. B.R. Bloem, Dr. R. Esselink, Dr. B. Post, Dr. M. Timmer, Dr. Van de Warrenburg, Dr. P. Praamstra, Dr. R. Helmich) or the movement disorders fellow will identify patients eligible to participate to

the study (see inclusion and exclusion criteria above). In a first step, the neurologist will briefly provide the patient with some information about the nature of the research and ask whether he/she would be interested in learning more. If a patient has already explicitly indicated his/her willingness to participate in this particular research on ParkinsonNext, then this step will be omitted. If the patient agrees, Mrs. Anouk van der Heide, the executive researcher, or the research-assistant will send him/her more information. The patient then has to send back a coupon indicating whether he/she is willing to apply to the study. If so, Mrs. Anouk van der Heide or the research assistant will contact him/her and explain further the study before practical measurements can be carried out. Each subject will receive as much time as he/she thinks necessary to consider his/her participation.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable.

11.4 Benefits and risks assessment, group relatedness

This research involves capacitated adults. This research has no direct benefit for the participants. The scientific benefit of this study is to achieve a better understanding of the pathophysiology of a severe symptom (Parkinson's tremor). The outcomes of this study may give rise to future new treatments for Parkinson's tremor.

The burdens of this study are relatively small: there are no invasive procedures, and the time asked from each participant is two day-parts of 4 hours.

During the experiment, the subjects will be exposed to a magnetic field. This is not dangerous and poses no risk as long as no metal objects are located on the subject, as these can be heated or magnetized. Therefore, all subjects will be informed in advance and checked for the presence of metal in or on the body. See also the brochure of the Donders Centre for Cognitive Neuroimaging. Because the subjects will have to spend approximately 1 hour (two times) in the MRI scanner, it is important that none of them has the fear of tight spaces. As mentioned before, claustrophobia will be an exclusion criterion in this study.

Considering the extensive exclusion criteria, the screening procedure, and constant monitoring of the subjects we do not expect any side effects. However, some discomfort is expected from several sources:

- Resurgence of the parkinsonian symptoms following withdrawal of anti-parkinsonian medication.
- The noise and the relative confined space of the MRI scanner.
- The length of the experimental procedure, which will however be kept as short as possible.
- Potential and short-lasting side effects (such as nausea, orthostatic hypotension) due to a single dose of Propranolol (40 mg). If they occur, these side effects are fully reversible. There is no risk for SUSAR's, given the extensive and long clinical experience that is available with treating tremor-dominant Parkinson patients with Propranolol.

11.5 Compensation for injury

Please see the "standard Verzekeringstekst_info_brochure_NED" brochure for details about the Insurance.

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

The Donders Centre for Cognitive Neuroimaging has taken out insurance for the subjects in this scientific research. This insurance covers losses caused by death or injury resulting from participation in this scientific research, which reveals itself during the participation of the subject in the scientific research or within four years thereafter. The personal injury is deemed to have revealed itself at the time it is reported to the insurer.
In the event of a claim, you may contact the insurer directly.

<u>The insurer is:</u>
Onderlinge Waarborgmaatschappij Centramed B.A.
P.O. Box 191

2270 AD Voorburg, The Netherlands
Tel.: +31 70 3017070
Email: Schade@centramed.nl

The insurance provides a maximum cover of € 650,000 per subject and € 5,000,000 for the entire research, and € 7,500,000 per annum for all examinations of the same client.

The above amounts are included in the "Besluit verplichte verzekering bij medisch-wetenschappelijk onderzoek met mensen". Information on this "besluit" can be found at the website of the Central Committee Clinical Research involving Humans: www.ccmo.nl.

11.6 Incentives (if applicable)

All subjects will receive compensation for travel expenses.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

The investigator will ensure that the subject's anonymity will be maintained. On all documents subjects will be identified only by an identification code – not by their names or other assigned number. The investigator will keep a separate Subject Identification Code List which matches identifying codes with the subject's names. Documents will be maintained by the investigator in strict confidence. Subject anonymity has to maintain upon all archiving steps. After cessation of the whole study (finalized and signed research report) the investigator will store the investigator file and copies of the forms to fulfil his responsibility to maintain adequate human study records for at least 2 years after the final publication or longer if required by the applicable legislation. Handling of personal data will comply with the Dutch Personal Data Protection Act.

Subjects will be given a (study specific) unique identification number, The executive researcher and (if applicable) the assigned team members only will have the key to the code giving access to personal data. Personal data will be kept separately from the experimental data acquired.

12.2 Monitoring and Quality Assurance

This study will be monitored according to the local guidelines at the Donders Centre for Cognitive Neuroimaging (by Mrs. Sabine Kooijman, research coordinator).

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed

the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 End of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the investigator will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

Not applicable.

13. STRUCTURED RISK ANALYSIS

Based on the revised version of the guideline."Quality Assurance of Research in humans 2.0" by the Dutch federation of academic medical centers, NFU) a risk assessment is in place (table 1).

Table 1 The risk classification depends on the probability of adverse effects, the extent of damage (seriousness) and the vulnerability of the participants (source: NFU, Kwaliteitsborging Mensgebonden onderzoek, 2.0).

Extent of additional risk/ Degree of damage	**Minimal damage**	**Moderate damage**	**Serious damage**
Minimal chance	Negligible risk	Negligible risk	Moderate risk
Moderate chance	Negligible risk	Moderate risk	High risk
High chance	Moderate risk	High risk	High risk

Recommendations

Given the negligible risk of the MRI procedure, and the low risk of minor and short-lasting side effects when prescribing Propranolol 40 mg to Parkinson patients, we classify this research as a negligible risk research ("verwaarloosbaar risico"), according to the NFU guidelines; Kwaliteitsborging mensgebonden onderzoek 2.0; Nederlandse Federatie van Universitair Medische Centra.

Monitoring of studies is regarded as an essential part of research in humans ensuring safe and sound scientific research. The level of monitoring is attuned to the degree of the risk involved, indicating ***minimal*** *on site monitoring*. A qualified monitor assesses all studies once a year/ or once during the data acquisition phase. Outcomes will be documented and reported to the sponsor.

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