The influence of stress on Parkinson's tremor

Jordy van Langen, BSc.

Master Thesis

Supervised by:
1. Prof. Karin Roelofs, PhD
2. Rick Helmich, MD, PhD
3. Eva Klimars, MSc.

01-07-2019

Jordy van Langen[1,2], BSc.

Master thesis (SOW-BS029)

Study program: research master Behavioural Science

Donders Institute for Brain, Cognition and Behaviour,

Centre for Cognitive Neuroimaging

Research lab: Systems Neurology (PI: Rick Helmich)

[1] Donders Institute for Brain, Cognition and Behaviour, Centre for Cognitive Neuroimaging, Radboud University, Nijmegen, The Netherlands

[2] Behavioural Science Institute, Radboud University, Nijmegen, The Netherlands

Radboud University Radboudumc

Abstract

Parkinson's disease (PD) is clinically characterized by motor slowing (bradykinesia), stiffness (rigidity) and resting tremor. From these clinical manifestations, resting tremor is the most poorly understood. Resting tremor worsens consistently during cognitively demanding tasks (such as mental arithmetic), and during psychological stress (such as time pressure or social evaluation). Exposure to these types of stress has been associated with very rapid changes in tremor amplitude, suggesting catecholaminergic involvement as opposed to corticosteroid modulations. Supportive of this idea are recent findings suggesting that the noradrenergic system is hyperactive in tremor-dominant PD and that it might play a modulating role in the pathophysiology of resting tremor. However, this has been questioned by data showing that a beta blocker (propranolol) did not inhibit the increase of tremor during a cognitive task. Alternatively, the increase of tremor during cognitive tasks may be mediated by distraction, such that a cognitive load interferes with brain systems that inhibit tremor at rest. This raises the question whether cognitive load and stress have separable effects on PD tremor. Therefore, this study examined the effect of two different tasks, with and without cognitive loading on Parkinson's tremor in eighteen tremor-dominant PD patients. We also investigated the role of the noradrenergic system in each task by measuring pupil dilation, and by correlating this with tremor power. The task with cognitive loading included cognitive co-activation by means of mental arithmetic and the task without cognitive loading was threat of electrical shock. We hypothesized that both tasks increase Parkinson's tremor as well as noradrenergic activity and explored whether noradrenergic activity mediated effects of mental arithmetic and threat-of-shock on tremor. Our findings show that both tasks resulted in an increase in Parkinson's tremor and noradrenergic activity, with stronger effects for our task with cognitive loading. However, even in a threat of shock paradigm – which is less poluted by cognitive effort or distraction – we found an increase in tremor power and pupil diameter and a (trend towards) positive correlation. This study adds to previous work by showing an effect of stress on PD tremor, independently of cognitive load. It suggests that multiple mechanisms play a role in the worsening of PD tremor during (cognitive) stress: both top-down cognitive mechanisms and bottom-up arousal systems.

Keywords: Parkinson's disease, resting tremor, noradrenergic system, cognitive stress, threat-of-shock.

1. Introduction

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder worldwide (Mhyre et al., 2012). From the existing neurodegenerative disorders, PD grows the fastest and in the previous 15 years, its prevalence has more than doubled (Feigin et al., 2017). In clinical perspective, PD is defined by motor slowing (bradykinesia), stiffness (rigidity), and resting tremor (Jankovic, 2008). From these motor symptoms, resting tremor is the most poorly understood and is characterized by rhythmic movements of one or more body parts (Nieuwhof et al., 2018). In most cases, dopaminergic medication can alleviate PD symptoms, but for resting tremor this is not always true (Helmich et al., 2012). About 75 % of PD patients have or do eventually develop a resting tremor (Tysnes & Storstein, 2017), and early patients (i.e. < 6 years from symptom onset) classify it as the second most worrisome condition (Politis et al., 2010). From a cerebral perspective, the pathological hallmark of PD is nigro-striatal dopamine depletion (Kish, Shannak, & Hornykiewicz, 1988), but the dopaminergic basis of resting tremor is unclear (Helmich et al., 2012). For instance, dopamine depletion in the striatum correlates with all motor symptoms except resting tremor (Pirker, 2003) and dopaminergic medication has a variable and sometimes no effect on resting tremor (Helmich et al., 2012).

Resting tremor consistently amplifies during acute psychological stress and during variations in cognitive and emotional states (Hemmerle, Herman, & Seroogy, 2012; Raethjen et al., 2008). Recent findings illustrate that the effect of levodopa (i.e. the most potent dopaminergic medication for Parkinson's symptoms) on tremor diminishes in a stressful compared to a neutral context (Zach et al., 2017). This implicates a critical clinical issue, since patients experience the most burden from their tremor in stressful environments, when their (dopaminergic) medication works suboptimal. Supportive of these findings is anecdotal evidence, indicating that patients often state that their tremor increases considerably under time pressure (e.g., when they are delayed for an appointment), under social evaluation (e.g., when being watched in public spaces), or when they are nervous (e.g., when giving a talk). In addition, clinical observations show that tremor increases rapidly (i.e., within seconds) after the start of simple mental arithmetic under time-pressure (e.g. counting backwards from 100 to 0 in steps of 7 during a limited time-period), which involves cognitive stress (Zach et al., 2017). While performing these mental calculations there is a consistent increase in tremor, but after its cessation, the tremor also reduces within seconds (Zach et al., 2017). This effect might be explained by neuroendocrine models suggesting that immediately subsequent to such a cognitive task, cerebral levels of catecholamines such as noradrenaline quickly build-up and briefly stay elevated, but return to their homeostasis short after stress offset (Hermans et al., 2014). In contrast to this rapid mechanism, corticosteroids have a lower temporal resolution, but preserve elevated levels for a longer time-period after stress offset (Hermans et al., 2014). This suggests that catecholaminergic mechanisms such as noradrenaline, rather than a change in peripheral hormones as adrenaline or cortisol are predominantly involved in modulating Parkinson's tremor during acute stress.

More evidence for the amplification of cerebral tremor-related activity by the noradrenergic system arises from post-mortem, animal and neuroimaging studies. For instance, post-mortem studies have shown that PD patients with a tremor-dominant phenotype have less degeneration of the locus coeruleus (LC), which is the main source of cerebral noradrenaline, compared to patients with a non-tremor dominant phenotype (Paulus, & Jellinger, 1991). Recent animal studies show that activation of the LC results in strengthened whole-brain functional connectivity (Zerbi et al., 2019) and studies in humans show that the LC has anatomical projections to all the nodes of the cerebral tremor circuit and that noradrenergic neuron degeneration is related to worsening of all PD symptoms (Delaville, De Deurwaerdère, & Benazzouz, 2011). In addition, other animal studies have reported that acute stress can produce severe motor activation (e.g. tremor) that is thought to be mediated by cerebral noradrenaline (Metz, 2007). Furthermore, nuclear imaging studies have shown that noradrenergic receptor binding in the LC is increased in PD patients compared to healthy controls (Lewis et al., 2013), and especially in tremor-dominant patients (Isaias, 2011). In addition, interventions that decreased noradrenergic hyperactivity, either pharmacologically using beta-blockers (Kissel, Tridon, & Andre, 1974; Henderson et al., 1994; Abramsky, Carmon, & Lavy, 1971) or non-pharmacologically with relaxation guided imagery (Schlesinger et al., 2009) have shown promise in diminishing resting tremor. Taken together, these findings suggest that the noradrenergic system is implicated in the pathophysiology of Parkinson's tremor, although the exact nature of its modulating effects on tremor during stress remain unclear (Hemmerle, Herman, & Seroogy, 2012).

Nevertheless, there is also evidence that has questioned the role of the noradrenergic system in tremor increases during cognitive tasks. First support for this idea comes from two groups of PD patients in a study by Marsden et al. (1967) before and after beta-blockade administration. Before beta-blockade, one group received intravenous adrenaline infusion – which activates the cerebral noradrenergic system through the vagal nerve (Tank & Lee Wong, 2015; Hermans et al., 2014) and increases tremor (Marshall & Schnieden, 1966; Constas, 1962; Barcroft, Peterson, & Schwab, 1952), while the other group performed a mental arithmetic task which involves cognitive stress (Zach et al., 2017). Before beta-blockade, both groups showed an increase in tremor, but after beta-blockade by propranolol, which is a post-synaptic beta-receptor blocker, the tremor only remained present in the mental arithmetic group and diminished in the adrenaline-infused group. This implicates that the effect of cognitive stress might not be predominantly mediated by noradrenaline and questions the association with the noradrenergic system in relation to Parkinson's tremor.

An alternative explanation for the increase in tremor during cognitive tasks is 'distraction', such that a cognitive task might interfere with cerebral mechanisms that suppress tremor. Support for this hypothesis comes from studies investigating cerebral networks for cognitive and motor systems that have a critical role in the planning and execution of movements (Aron et al., 2009). Results from these studies indicate that activation or impediments of unconscious motor processes rely on the cerebral dopamine circuit (D'ostilio & Garraux, 2012), which is depleted in PD. This idea can be linked to findings from dual-tasking studies with PD patients, in which striatal dopamine depletion is associated with impaired dual-tasking performances (Nieuwhof et al., 2017). Thus, due to a lack of dopamine availability, PD patients might become worse in performing dual-tasks such as subconsciously suppressing their tremor while performing a cognitive task.

To summarize, there is conflicting evidence that effects of cognitively demanding tasks on Parkinson's tremor are mediated by noradrenergic activity. Besides, we cannot be certain that these effects on tremor also apply for types of stress, such as fear, that have shown a more evident association with noradrenergic activity (Bitsios, Szabadi, & Bradshaw, 1996; de Voogd, Fernández, Hermans, 2016; Hodges & Spielberger, 1966; Hashemi et al., 2019; Marshall, & Schnieden, 1966; Hermans et al., 2014; Hermans et al., 2014; Constas, 1962). Therefore, in this study, we wanted to disentangle whether tremor is predominantly amplified by cognitive mechanisms during stress (such as distraction), or also by non-cognitive mechanisms (such as anxiety). We examined (1) whether two different stress tasks, one with cognitive load (mental arithmetic) and one without cognitive load (threat-of-shock), had similar or different effects on Parkinson's tremor. In addition, we investigated

(2) to what extent the noradrenergic system – by means of pupil dilation - was activated during these tasks and whether this correlated with tremor power. Compared to our mental arithmetic manipulation, in which subjects have to count backward during a limited time-period, there is more evidence relating threat-of-shock to the noradrenergic system. Threat of shock has shown to consistently produce autonomic responses such as pupil dilation (Bitsios, Szabadi, & Bradshaw, 1996; de Voogd, Fernández, Hermans, 2016), increased heart rate (Hodges & Spielberger, 1966; Hashemi et al., 2019), and increased cerebral activity in the "salience network" (Marshall, & Schnieden, 1966; Hermans et al., 2014) that is modulated by noradrenergic activity (Hermans et al., 2014; Constas, 1962). However, it is not clear if this specific type of physical fear (without an evident cognitive task) modulates Parkinson's tremor in a similar way as mental arithmetic does, since – to the best of our knowledge - this task has not been tested before in tremor-dominant PD patients.

In general, we expected that both tasks increased Parkinson's tremor as well as noradrenergic activity, but to varying extents. Specifically, we hypothesized (1) a higher tremor power for both tasks during the cognitive demanding and threat conditions (mental calculations during a limited time-period and threat of shock) compared to the non-cognitive demanding and safe conditions (rest and safe) with a greater effect for the cognitive demanding condition of our mental arithmetic task compared to the threat condition of our threat-of-shock task. Furthermore, we expected (2) more noradrenergic activity, by means of pupil dilation, for both tasks during the cognitive demanding and threat conditions (mental calculations during a limited time-period and threat of shock) compared to the non-cognitive demanding and safe conditions (rest and safe) with a greater effect for the threat condition of our threat-of-shock task compared to the cognitive demanding condition of our mental arithmetic task. Finally, we explored whether there was a correlation between tremor power and pupil diameter since the effects of our tasks on tremor power could be mediated by noradrenergic activity.

2. Methods and materials

2.1 Sample size calculation

All sample size calculations were performed using Using G*power 3 (Faul, Erdfelder, Lang, & Buchner, 2007). A priori power analyses were conducted using results from a previous study in our research group (Zach et al., 2017), to estimate the sample size required to replicate the behavioral effect of our mental arithmetic task on tremor power. A minimal sample size of 12 subjects was necessary to replicate these effects at sufficient power ($1 - \beta > 0.8$) (Cohen's d of 1.05). Subsequently, a second power analysis was conducted to determine the number of subjects needed to find effects of threat of shock on tremor power. Given our relatively low shock administration frequency of 1:17, we assumed that the overall perceived stress levels would be lower than during mental arithmetic. Therefore, the effect on tremor power was also likely to be smaller, nevertheless, the effect size was still assumed to be large (Cohen's d of 0.8) since patients generally report that stress is very potent in worsening the tremor (Zach et al., 2017). Based on this effect size we calculated that a number of 15 subjects would be necessary to detect tremor amplitude changes due to threat of shock with adequate power ($1 - \beta > 0.8$). Finally, possible dropout was taken into account, leading to a total sample size of 18 tremor-dominant PD patients.

2.2 Participants

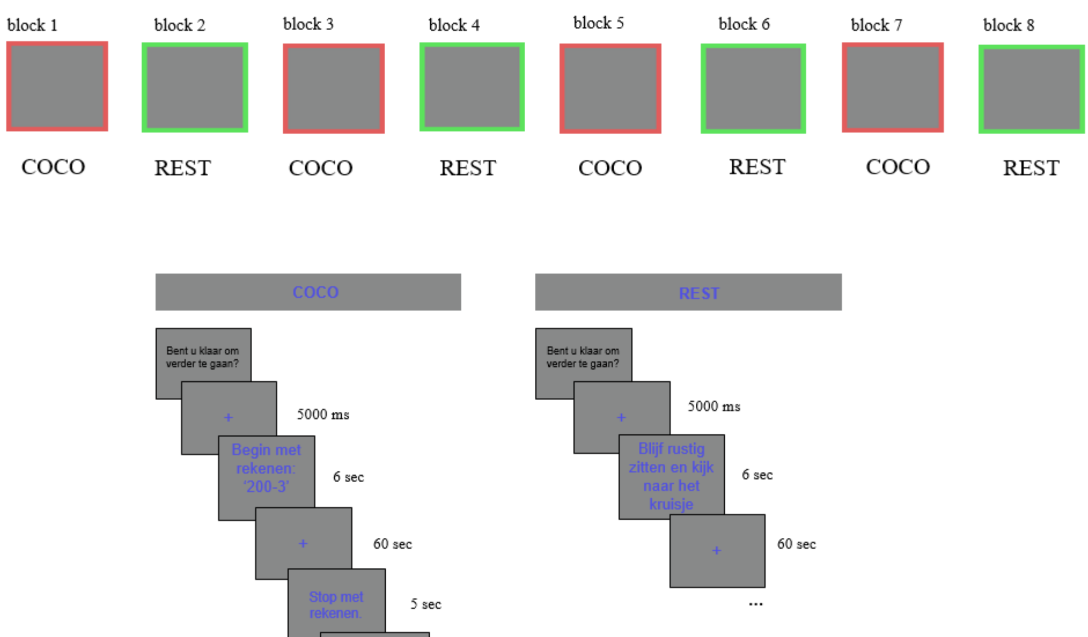
In order to be eligible to participate in this study, patients needed to meet multiple inclusion criteria. Patients were included when they had idiopathic PD according to the UK brain bank criteria (Hughes et al., 2002; Gibb & Lees, 1988), a clear resting tremor (ON or OFF medication) in at least one arm of ≥ 1 points based on item 17 of the Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (Movement Disorders Society, 2007), and when they had a mild disease phenotype since we asked them to delay one dose (i.e., the morning dose) of their medication until after the experiment. Exclusion criteria were: neuropsychiatric co-morbidity, presence of a moderate or severe head tremor, presence of severe dyskinesia's, cognitive impairment of < 26 points based on the Mini Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975), presence of a severe skin allergy, and use of a medical (dopaminergic) plaster that could not be taken off. Prior to the inclusion, all patients gave written informed consent. Patient recruitment was performed through the Parkinson outpatient clinic of the Radboud University Medical Center (Radboudumc). This assured recruiting and including clinically well-defined patients. In total, patients visited the Donders Centre for Cognitive Neuroimaging (DCCN) once. The study was approved by the local ethical review

A

board (CMO region Arnhem-Nijmegen) and was performed in accordance with the standards of the 1964 Declaration of Helsinki.

2.3 Task design

The task design is illustrated in Figure 1. Our task with cognitive load (i.e., mental arithmetic) used a within-subjects design to compare the cognitive demanding (mental calculations during a limited time-period) and non-cognitive demanding condition (rest). For our task without cognitive load (i.e., threat of shock), we used a 2 x 2 factorial within-subjects design with within-subject factors threat (threat vs. safe) and oddball (oddball vs. no-oddball). In both tasks, conditions (mental calculations vs. rest, threat vs. safe, oddball vs. no-oddball) were manipulated in a block-wise manner (see Figure 1). The order of performance for both tasks was counterbalanced between patients.



B

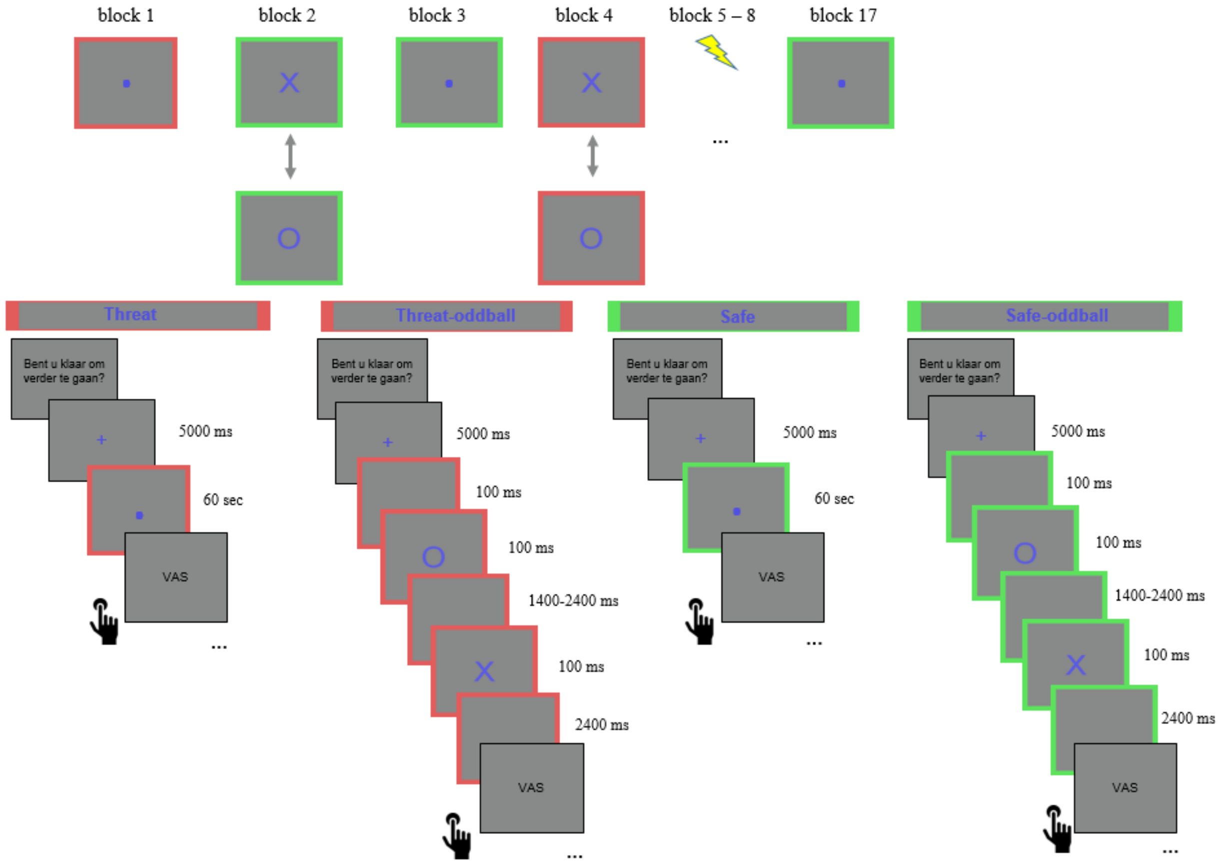


Figure 1. Overview of the task design. **A)** the mental arithmetic task consisted of 8 blocks of which block 1, 3, 5 and 7 were cognitive demanding and 2, 4, 6 and 8 were rest. **B)** the threat-of-shock task included 17 blocks of which 4 were threat, 4 were safe, 5 were threat combined with an oddball paradigm and 4 were safe combined with an oddball paradigm. Patients received only one shock during a threat-oddball block which was always pseudorandomized between the 5^{th} and 8^{th} block. The oddball paradigm included a train of standards (i.e., “O”) with an oddball (i.e, “X”) and participants were asked to count the number of oddballs during each oddball trial.

2.4 Procedure

The study procedure is presented in Figure 2. Patients were initially screened by telephone and received an information letter with accompanying insurance information before being invited for the experiment at the institute. Upon arrival, patients were installed in the experimental room in which all the sessions started at around the same time in the morning. Patients were asked to refrain from drinking coffee for ten and alcohol for 24 hours respectively to diminish potential influences on tremor. We first registered some general- disease- and tremor characteristics of the patient before continuing with a shock work-up procedure, clinical assessments, (electro)physiological registration and the experimental tasks. All patients performed both tasks between 11:00 a.m. and 01:00 p.m. The last part of the procedure consisted of a third task for another research project, which was not part of this thesis.

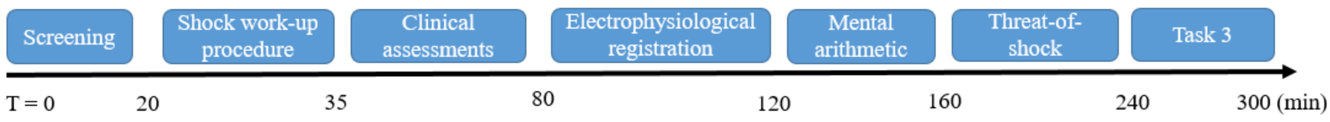


Figure 2. Overview of the study procedure. The procedure took an average of 5 hours and our expeirmental tasks were counterbalanced between each patient session.

2.4.1 Shock work-up

To determine each patient's' sensitivity to the electrical shocks, patients underwent a shock work-up procedure. To prevent carry-over effects on the threat-of-shock task later, this was performed at the beginning of the testing day. For this procedure, we used an in-house protocol based on studies that have used a threat of shock paradigm (Klumpers et al., 2010; Klumpers et al., 2010). The aim was to reach a level at which the electrical shocks felt very annoying, but did not hurt. The shock work-up procedure started after attaching the shock electrodes to the intermediate phalanges of the second and third finger of the least affected tremor hand. The shocks were transcutaneous delivered using an Innostim™ TENS 2K device (www.medimaxtech.co.uk) connected to the stimulus PC and standard Ag/AgCl electrodes that were filled with Skin Conductance Electrode Paste. After verbally explaining the instructions, the first shock was administered at the lowest intensity to explore whether the patient felt something. Thereafter, the procedure was continued starting at a minimal intensity of 16 mA and could maximally reach a final intensity of 80 mA. All electrical shocks were administered for 240-µs with a 150 Hz pulse rate. After each shock, patients had to answer '*How annoying was the electrical stimulus*', ranging from 0 = '*not annoying at all'* to 5 = '*too annoying/painful*' using a Visual Analogue Scale (VAS). When participants rated a shock, it was always checked whether this was correct to ensure that it was not an error. The shocks were clearly announced since it is important that they are very predictable (Klumpers et al., 2010). In total, the shock work-up procedure consisted of five shocks and the intensity was adjusted to reach a level that was rated as “very annoying”, meaning a rating of 4 out of the 5-point scale.

2.4.2 Clinical and tremor assessments

To get a subjective estimate of how sensitive patients’ tremor is in response to stressors, participants had to indicate ‘*How much does your tremor respond to stress (subjective estimate)*’, ranging from 0 = ‘*not at all’* to 100 = ‘*very much*’ on a Visual Analogue Scale (VAS). Furthermore, we performed a number of clinical tests to obtain individual disease scores that can be correlated to our outcome measures. First, we administered the Mini Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) to rule out any cognitive impairment. None of the participants had to be excluded based on this assessment. For the motor examination, we administered part III of the Movement Disorders Society - Unified Parkinson’s Disease Rating Scale (MDS-UPDRS, 2008). Since this study focusses on Parkinson’s tremor, we also assessed tremor severity using the Fahn-Tolosa-Marin Tremor Rating Scale (TRS) (Fahn, Tolosa, & Marin, 1993). The order of these assessments was always fixed and had in total an average length of 45 minutes per patient.

2.4.3 (Electro)physiological registration

Tremor was measured during all experimental tasks in a two-folded way. First, we used a Brain Products GmbH acceleration sensor MR (3 dimensions) (www.brainproducts.com) attached to the central dorsum of the most affected tremor hand. Second, we used electromyography (EMG) cables attached to the extensor digitorum communis, and flexor carpi radialis of both arms. A ground electrode was placed at the end of the ulna bone near the wrist. All EMG electrodes were filled with an Abrasive Electrolyte-Gel (Easycap GmbH) using a BD Emerald™ syringe. Before attaching the EMG electrodes, the skin was cleaned with Nuprep Skin Prep Gel (www.weaverandcompany.com) and alcohol. Pupil dilation was measured during all experimental tasks using an Eyelink 1000 plus eye-tracker with a 1000 Hz sampling rate (SR Research Ltd., www.sr-research.com/). Tremor (EMG, accelerometry) data was registered using BrainVision Recorder connected to a BrainAmp ExG amplifier (Brain Products GmbH) using a sampling rate of 5000 Hz.

2.4.4 Experimental tasks

All stimuli were presented on a BenQ 24-inch monitor with a native resolution of 1024 x 768 (4:3 ratio) and a refresh rate of 120 Hz, based on a previously performed study in our institute with a similar experimental threat of shock task (de Voogd et al., *in press*). Time accurate response registration (< 1 ms) was obtained with in-house built button pads. During all tasks, patients were seated in a comfortable chair at a fixed distance in front of the computer monitor. In order to allow the hands to hang down in an unsupported, but relaxed fashion and to move freely, small pillows were placed bilaterally under the patients' underarms reaching from the elbow to the wrist. This set-up enabled unrestricted tremulous movements of the hands. Patients gave feedback after each experimental trial by pressing buttons on the button pad with their non-dominant tremor hand. This prevented motor responses to experimental stimuli with the most affected tremor hand, which can influence tremulous activity (Raethjen et al., 2008). The researchers were present in the examination room during all experimental tasks. Before the start of each task, participants received standardized instructions on the computer screen but were verbally assisted when this was necessary. During the tasks, the lights in the experimental room were dimmed and standardized to diminish the effects of luminance on the pupil size. All tasks were programmed using Presentation® software (Version 20.0, www.neurobs.com). The task design is illustrated in Figure 1.

2.4.4.1 Task 1: Mental arithmetic

This task consisted of two conditions: (1) performing mental calculations during a limited time-period (COCO) and rest (REST). Patients read the task instructions on a computer screen and performed a short round of practice in which the two conditions were shown. Both conditions included

4 trials of 60 seconds each and were alternated in a fixed trial order. During the COCO trials, patients saw an equation at the beginning of each trial (e.g., 200-7) (RGB values: 92, 92, 226) and they were asked to count backward to 0 in silence to limit head movements. After 6 seconds the equation disappeared and a blue fixation cross (RGB values: 92, 92, 226) appeared for the rest of the trial on a gray (RGB values: 136, 137, 137) background in the center of the screen. After 60 seconds, the fixation cross was replaced by a text stating that patients had to stop calculating (RGB values 92, 92, 226). Thereafter, they had to fill in which number they had reached. As we were also interested in their levels of perceived stress during each COCO trial, they had to answer ''*How tense did you feel during the previous trial?*'' ranging from 0 = '*not at all*' to 5 = '*very much*'. On the second and final question after each COCO trial, patients had to answer ''*How difficult was the previous trial*?'' ranging from 0 = '*not difficult*', to 5 = '*very difficult*'. All patients answered these questions using a button pad. Before the REST trials, patients saw a text for 6 seconds stating that they had to sit quietly and fixate their gaze at a blue fixation cross (RGB values: 92, 92, 226). The fixation cross disappeared after 60 seconds and they were asked if they were ready to continue the task.

2.4.4.2 Task 2: Threat-of-shock with an oddball paradigm

This task was based on the design of a recently performed study in our institute (de Voogd et al., *in press*), but adapted to our study. The task consisted of four conditions: threat of receiving a shock (THREAT), no threat of receiving a shock (SAFE), threat of receiving a shock combined with an oddball paradigm (THREAT-ODDBALL), and no threat of receiving a shock combined with an oddball paradigm (SAFE-ODDBALL). The conditions THREAT, SAFE, SAFE-ODDBALL included 4 trials of 60 seconds each and the condition THREAT-ODDBALL included 5 trials of 60 seconds each, with one trial in which the shock was administered. This resulted in a total amount of 17 trials. Half of the task consisted of threat and safe conditions (THREAT & SAFE) during which the participant simply had to look at the center of the screen. These were 'threat' blocks (a red square indicating that there is a risk of receiving an electrical shock) and 'safe' blocks (a green square indicated that there is no risk of receiving an electrical shock). Each trial was followed by Visual Analogue Scales (VAS). Since we wanted to identify whether patients actually perceived stress, we asked them: ''*How tense did you feel during the previous trial?*'' ranging from 0 = '*not at all*' to 5 =

'*very much*'. Also, we wanted to identify whether our subjective threat manipulation was successful and therefore patients were asked after THREAT trials only to indicate '*'How big did you think was the chance that you would get an electrical stimulus*?'' ranging from 0 = '*very small'* to 5 = *'very* large'. With this information, we are able to compare levels of perceived stress during threat and safe trials and additionally identify if our threat manipulation was successful. The other half of the task also consisted of threat and safe blocks but combined with an oddball paradigm (THREAT-ODDBALL & SAFE-ODDBALL) (Yoshiura et al., 1999). The oddball paradigm was included in the threat of shock task to cause differences in arousal, to allow measurement of phasic pupil effects (de Voogd et al., *in press*) and to examine whether this also had an effect on Parkinson's tremor. The threat-oddball blocks consisted of a red square indicating that there is a chance of receiving an electrical shock with simultaneously a strain of oddballs (e.g. O-O-O-X-O-O-X) in the center of that square. The safe-oddball blocks consisted of a green square indicating that there was no risk of receiving an electrical shock with simultaneously a strain of oddballs (e.g. O-O-X-O-X-O-O) at the middle of the square. After each threat-oddball and safe-oddball trial, patients were asked: '*'How many oddballs did you count?''*, ranging from a minimal amount of 2 to a maximum amount of 6. Subsequently, patients also had to answer the other (two) question(s) mentioned above. The oddball strain always consisted of 30 visual stimuli (i.e., O & X) (RGB values: 92, 92, 226) with a duration of 100 ms, and an oddball frequency of 3-5 oddballs per block (i.e. X). The 'O' was the standard stimulus and the 'X' was the oddball stimulus and the letters were presented on a grey background (RGB values: 136, 137, 137). The interstimulus interval (ISI) was jittered pseudorandomly (1500-2500 ms), and every oddball was followed by a fixed interval of 2500 ms.

Previous studies with threat of shock have used different shock frequencies of for instance 1:3 trials and 1:16 trials (Klumpers et al., 2017; de Voogd et al., *in press*) for respectively investigating habituation and anticipation effects. Since we are not primarily interested in habituation effects, but more in anticipation effects, we used a relatively low shock frequency of 1:17 trials. Participants were informed that during the threat blocks they could receive an electrical shock at any time and that after receiving the first shock, the strength of the subsequent shock would be higher (Hermans et al., 2006). In fact, they received a shock only once. This resulted in 16 trials in which no shock was administered, enabling us to compare the four conditions containing 4 trials each. All trials were pseudorandomized in a block-wise trial order (i.e., 1 block contained 1 THREAT, 1 SAFE, 1 THREAT-ODDBALL, and 1 SAFE-ODDBALL trial). The electrical shock was always administered between 20 and 40 seconds in the second threat-oddball trial (i.e. between trials 5 to 8). In accordance with a previously performed study using a threat of shock manipulation (Lojowska et al., 2015), we used the instructed fear principle, meaning that we explained in advance which cues are threat (i.e., a red square) and safe (i.e., a green square). After patients read the task instructions on the monitor screen, they performed a short round of practice in which all 4 conditions were shown. No shocks were administered during the practice round. The actual task started after attaching the shock electrodes to the intermediate phalanges of the second and third finger of the least affected tremor hand. The shock was delivered in the same way as during the shock work-up.

2.5 Data reduction and statistical analysis

Offline analysis of tremor and pupil data was performed using MATLAB (MATLAB R2018b, The MathWorks, Inc.). For pre-processing and first-level analysis of EMG and accelerometry data, we used the Fieldtrip Toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011) implemented in MATLAB R2018b. First, we used a bandpass filter of 2–20 Hz to remove high frequencies of no interest and slow-frequency drifts (Zach et al., 2017). Also, we removed the linear trend from the data with the function detrend and applied a baseline correction with the function demean. Subsequently, we visually inspected the data for artifacts by means of thresholding the z-transformed value of the preprocessed data. The trial data was cut into 5-second segments to be useable for frequency analysis. After that we calculated the average tremor frequencies between a 2 and 16 Hz range using a 5 seconds Hanning taper, creating a 0.2 Hz resolution. This was then averaged across the time points and resulted in an average power spectrum across all the segments. The peak channels and peak frequency of the tremor were automatically calculated from the power spectrum. After visually inspecting the power spectra for each patient, the selected peak channel and frequency were manually corrected for some subjects in which for example the harmonic frequency was selected. Since the sensitivity of EMG electrodes can increase or decrease over time, the tremor amplitude can also be less reluctantly measured. Besides, the accelerometry power spectra showed a clear peak compared to fewer clear peaks in the EMG power spectra. Therefore, we decided to only use the accelerometry data for further analysis. Thereafter, we calculated the absolute and log-transformed mean tremor power at the peak frequency across all segments of each trial. Specifically, this resulted in averaged tremor power values for each patient across all conditions of the experimental tasks that were used for our statistical analysis. Furthermore, we calculated time-frequency-representations (TRF) between 2 and 16 Hz with a 2 s Hanning taper, resulting in a 0.5 Hz resolution. The TFR for each patient of the corresponding tremor frequency was selected, resulting in averaged regressors for each condition describing fluctuations in tremor power during the 60-s trials. These regressors were used for calculating correlation coefficients.

For pre-processing and first-level analysis of the pupil data, we used in-house software (Hermans et al., 2013; de Voogd et al., 2016a; de Voogd et al., 2016b). First, we interpolated the data by detecting and removing eye blink artifacts that were identified by pupil changes occurring too fast (< 40 ms) to represent actual pupil dilation. These artifacts were removed from the obtained signal using linear interpolation (de Voogd et al., *in press)*. On- and offset values for each trial were calculated, after which we were able to calculate the absolute mean pupil diameter values in pixels for each trial of the experimental tasks across all patients. These values were used for our statistical analysis. We also calculated a TRF for the pupil data, resulting in averaged regressors for each condition that represented variations in pupil diameter during the 60-s trials. To identify whether there was an association between noradrenergic activity and tremor power, we correlated the tremor and pupil regressors using Pearson's R correlation coefficient. Since were are correlating regressors that represent effects over a time-course, we performed a Fisher's Z-transformation on the Pearson's R correlation coefficients to account for the time-course in the data (Silver, & Dunlap, 1987). Correlations were determined in a two-fold way: (1) we calculated the correlation between each tremor power and pupil diameter regressor of each individual trial (e.g., tremor power 1st threat-oddball trial & pupil diameter 1st threat-oddball trial). Subsequently, (2) we calculated the correlation coefficient for each condition (coco, rest, threat, safe, threat-oddball, safe-oddball) per patient and for the entire sample, resulting in individual- and group level correlation values.

For statistical analysis of our mental arithmetic task, we compared the tremor power and pupil diameter for the cognitive demanding condition with the rest condition using Paired-Samples T Tests (one-tailed). Since we have a clear hypothesis about the direction of the effects, we reported one-tailed values. Statistical analysis of our threat-of-shock task was performed by comparing tremor power and pupil diameter across conditions using a 2 x 2 repeated measures Analysis of Variance (ANOVA) with factors THREAT (threat vs. safe) and ODDBALL (oddball vs. no-oddball). To test whether there was a difference in tremor power between both tasks, we performed a 2 x 2 repeated measures ANOVA with factors STRESS-HIGH (coco vs. threat) and STRESS-LOW (rest vs. safe). In addition, we calculated correlation coefficients between the tremor power and pupil diameter regressors of all conditions of both tasks using Pearson's and used Fisher's Z-transformation to account for the time characteristics of the correlation coefficients. We performed One-Sample T Tests to identify whether each condition in our tasks significantly differed from zero. In addition, we obtained a Paired Samples T Test to test whether there was a significant difference between the mental arithmetic and rest. Finally, we performed a 2 x 2 repeated measures ANOVA with factors THREAT (threat vs. safe) and ODDBALL (oddball vs. no-oddball) to test whether there was a difference between the correlations in our threat-of-shock task. Statistical analyses were performed with SPSS (IBM SPSS, Version 23.0, IMB Corp). All data was visualized in bar and line plots, obtained with respectively the plotBarScatter Toolbox (Van Nuland, 2019) and Boundedline Toolbox (Kearney, 2018) implemented in MATLAB R2018B (Mathworks, The Mathworks, Inc.).

3. Results

Eighteen idiopathic tremor-dominant PD patients were recruited for this study, but one patient was excluded due to the absence of a resting tremor during the experimental tasks and since we were not able to obtain a reliable measure of noradrenergic activity by means of eye-tracking (40% and 50% of missing data for both tasks). All data were analyzed for the remaining 17 patients (14 male, 3 female $M_{age} = 64.41$, $SD_{age} = 7.65$). 16 patients were studied OFF dopaminergic therapy, meaning at least 12 hours after their last dose of dopaminergic medication (Albanese et al., 2001). Due to side effects as a result of refraining from dopaminergic medication, 1 patient participated ON dopaminergic medication. At the beginning of the testing day, we asked each patient to indicate ''*How much does your tremor respond to stress?*'', ranging from 0 = '*not at all/never'* to 100 = *'very much/always*' on a digital sliding bar. In general, patients indicated that in daily life their tremor amplitude increases whenever they experience stress (M = 70.53 %, SD = 15.83 %). Next to our in- and exclusion criteria, this result added more confidence that we included a correct sample that matched the study purposes. Clinical patient characteristics are summarized in Table 1.

Table 1. Clinical patient characteristics (N = 17).

	Mean	Standard deviation
Age in years	64.41	7.65
Disease duration in years	4.53	3.30
UPDRS III (0 - 137)	25.88	11.92
Tremor Rating Scale A (0 - 88)	8.71	3.69
Tremor Rating Scale B (0 - 40)	4.76	4.31
Tremor Rating Scale C (0 – 28)	4.53	4.27
Mini Mental State Examination (30 - 0)	29.12	0.86
Shock intensity level (1 - 10)	7.0	1.90
Self-report tremor amplitude increase during stress (0 - 100) (%)	70.53	15.83

3.1 Visual Analogue Scale (VAS)

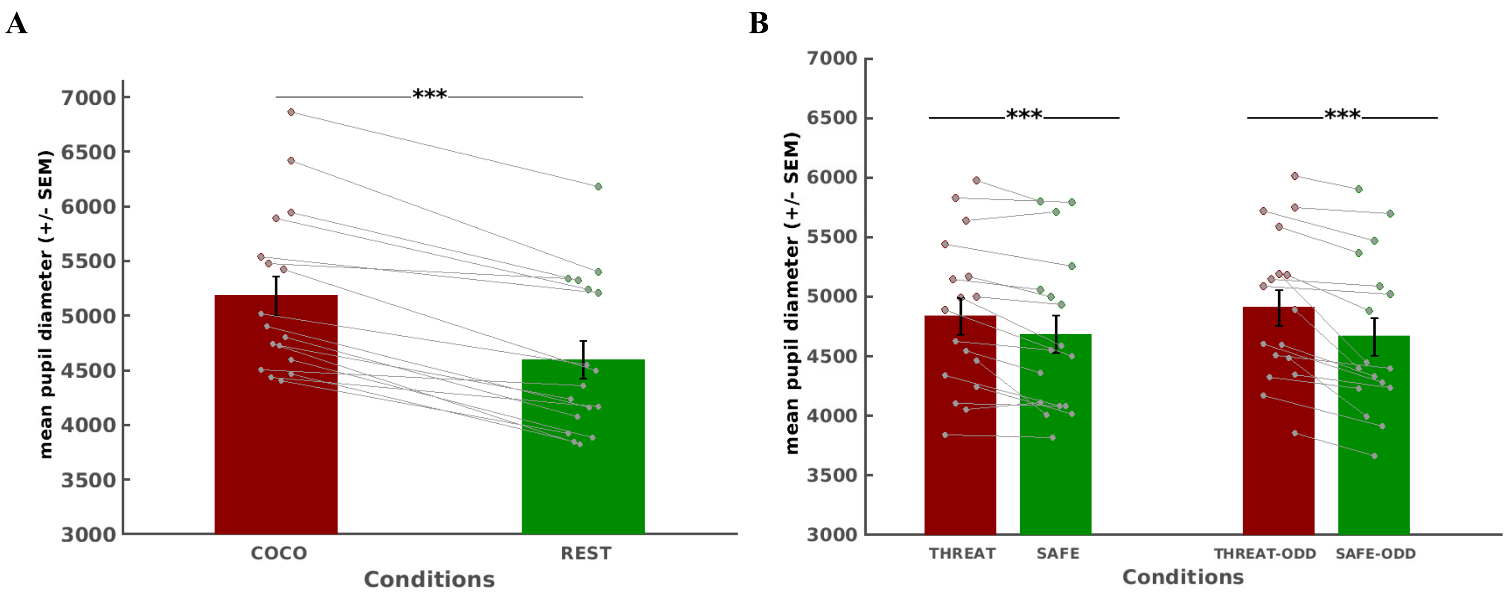
First, we explored the results of our implemented VAS during both experimental tasks. Since we wanted to identify whether patients actually perceived stress, we asked them: ''*How tense did you feel during the previous trial?*'' ranging from 0 = '*not at all*' to 5 = '*very much*'. For levels of perceived stress during our threat-of-shock task, we did find a significant main effect for threat, $F(1, 16)$ = 16.540, p < .001, with higher levels of perceived stress during the threat conditions as opposed to the safe conditions. No significant main effect for oddball, $F(1, 16)$ = 2.536 p = .131, or significant interaction effect for threat*oddball, $F(1, 16)$ = .255, p = .620 was observed. Also, we compared both tasks and found no significant difference in the levels of perceived stress between the mental arithmetic and threat conditions, $t(16) = -.797$, $p = .437$. Also, we wanted to identify whether our subjective threat manipulation was successful and therefore patients were asked after THREAT-trials only to indicate '*'How big did you think was the chance that you would get an electrical stimulus*?'' ranging from 0 = '*very small'* to 5 = *'very* large'. Between the threat conditions (threat vs. threat-oddball) we found no significant difference in the estimated chance that patients thought that they would receive an electrical shock, $t(16) = -1.186$, $p = .253$. An overview of the VAS results is listed in Table 2.

Table 2. Visual Analogue Scales (VAS) results per condition.

	Mean	Standard deviation
How tense did you feel during the previous trial? (1-5)		
Threat	2.40	1.08
Threat-oddball	2.51	0.94
Safe	1.53	0.74
Safe-oddball	1.71	0.74
Shock	2.71	1.16
Coco	2.25	0.67
How big did you think was the chance that you would get an electrical stimulus? (1-5)		
Threat	2.94	1.24
Threat-oddball	3.07	1.11
Shock	2.76	1.35

3.2 Pupil diameter

For both tasks, we detected and removed eye blink artifacts with linear interpolation ($M_{mental\ arithmetic}$ = 13.40 %, $SD_{mental\ arithmetic}$ = 10.42 %; $M_{threat\text{-}of\text{-}shock}$ = 10.36 %, $SD_{threat\text{-}of\text{-}shock}$ = 10.91 %) (de Voogd et al., *in press*). Subsequently, the pupil data were inspected and tested for normality, respectively with a Shapiro-Wilk test and Normal Q-Q plotting. Since these followed a normal distribution, we decided to use the absolute values in our analyses. In accordance with our hypothesis about noradrenergic activity, we did find a significant effect for mental arithmetic, $t(16) = 9.314$, $p < .001$ (one-tailed), with a larger pupil diameter in the cognitive demanding condition as opposed to the rest condition. A significant main effect was also observed for threat, $F(1, 16) = 26.422$, $p < .001$, with larger pupil diameter during threat conditions as opposed to safe conditions. No significant main effect was observed for oddball, $F(1, 16) = .696$, $p = .417$, but we did find a significant interaction effect for threat*oddball, $F(1, 16) = 7.409$, $p = .015$. Post-hoc Paired Samples T Tests for threat (vs. safe), $t(16) = 4.055$, $p < .001$ (one-tailed) and threat-oddball (vs. safe-oddball), $t(16) = 5.319$, $p < .001$ (one-tailed), identified that there was a main effect for threat (vs. safe), but this effect was larger in case of an oddball manipulation. For the second part of our hypothesis about noradrenergic activity, we compared both tasks and found a significant interaction effect for stress-high (mental arithmetic vs. threat)*stress-low (rest vs. safe), $F(1, 16) = 25.280$, $p < .001$, that was driven by a significant difference during the stress-high conditions but not during the stress-low conditions. We found a larger pupil diameter during the mental arithmetic condition as opposed to the threat condition, since there was a significant main effect for factor stress-high, $F(1, 16) = 124.954$, $p < .001$. No significant differences in pupil diameter were observed between the rest- and safe condition, since there was no significant main effect for factor stress-low, $F(1, 16) = 1.507$, $p = .237$. Post hoc Paired Samples T Tests for mental arithmetic (vs. threat) $t(16) = 2.994$, $p = .009$ and mental arithmetic (vs. threat-oddball) $t(16) = 2.168$, $p = .046$, confirmed that there was a main effect for mental arithmetic. Finally, we explored whether administrating an electrical shock resulted in a different in pupil size as opposed to merely the threat of receiving a shock. Here, we found a significant effect for shock administration $t(16) = 4.120$, $p < .001$ (two-tailed), with a larger pupil diameter during the shock trial as opposed to not receiving a shock. These findings do correspond to the self-reported levels of perceived stress that are depicted in Table 2. A graphical overview of the results is shown in Figure 3.



C

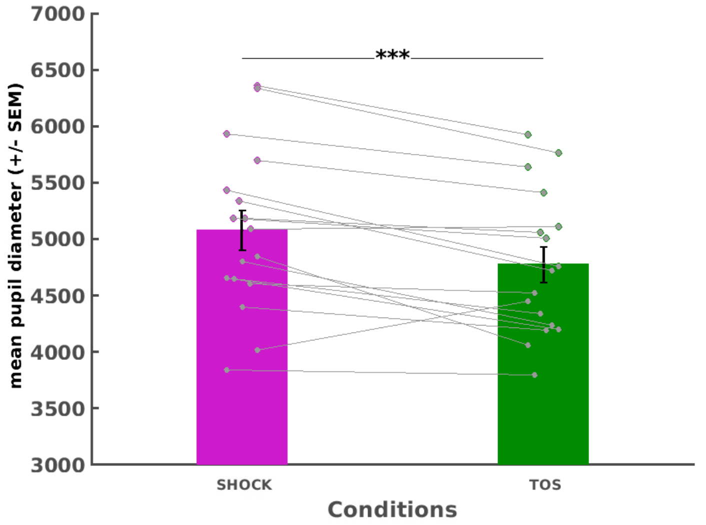
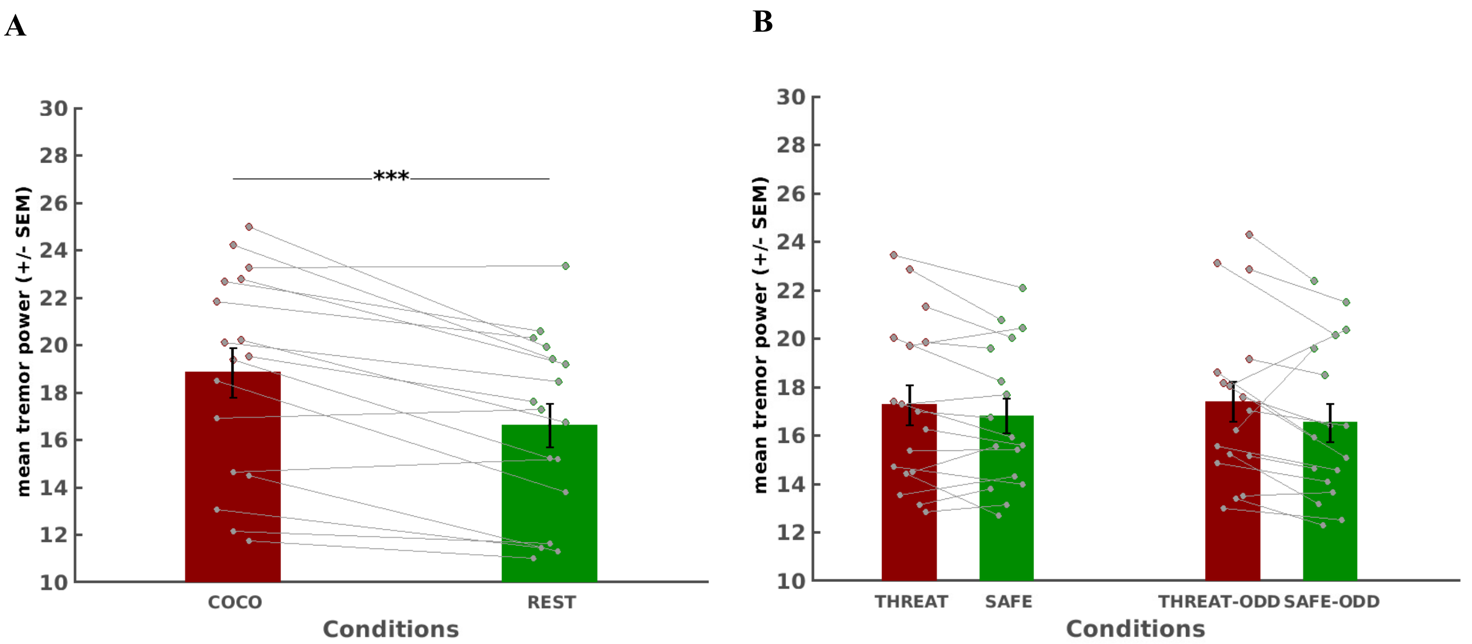


Figure 3. The effect of mental arithmetic and threat-of-shock on pupil diameter. Pupil diameter values are depicted in pixels. Panel **A** displays the individual subject and average pupil diameter values during the cognitive stress task with a significantly higher pupil diameter during mental arithmetic (coco) as opposed to rest. Panel **B** represents the individual subject and average pupil diameter values for the threat-of-shock task, with significantly higher pupil diameter values during the threat conditions. Panel **C** shows the individual subject and average pupil diameter values during the condition in which a shock was administered as opposed to the conditions in which no shock was administered. Lines between the individual data points were added to identify the trend in the data. Error bars represent the standard error of the mean.

*** indicates $p < .001$.

3.3 Tremor power

Data from both tasks were inspected and tested for normality, respectively with a Shapiro-Wilk test and Normal Q-Q plotting. Since these indicated non-normally distributed data, we performed a log-transformation on these data and used them for our analyses, in line with previous work (Zach et al., 2017; Dirkx et al., 2018). In accordance with our hypothesis about tremor power, we did find a significant effect for mental arithmetic, $t(16) = 4.985$, $p < .001$ (one-tailed), with a higher tremor power in the cognitive demanding condition as opposed to the rest condition. Also as predicted, for our threat of shock task, we observed a significant main effect for threat, $F(1, 16) = 6.519$, $p = .021$, with a higher tremor power during threat conditions as opposed to safe conditions. No significant main effect was observed for oddball, $F(1, 16) = .067$, $p = .799$, and also no significant interaction effect for threat*oddball, $F(1, 16) = .831$, $p = .376$. For the second part of our hypothesis about tremor power, we compared both tasks and found a significant interaction effect for stress-high*stress-low, $F(1, 16) = 11.652$, $p = .004$, that was driven by a significant difference during the stress-high conditions but not during the stress-low conditions. We identified a higher tremor power during cognitive stress as opposed to the threat, since there was a significant main effect for factor stress-high (mental arithmetic vs. threat), $F(1, 16) = 26.108$, $p < .001$. No differences in tremor power were observed between the rest- and safe condition, since there was no significant main effect for factor stress-low (rest vs. safe), $F(1, 16) = 1.885$, $p = .189$. Post hoc Paired Samples T Tests for mental arithmetic (vs. threat) $t(16) = 2.564$, $p = .021$ and mental arithmetic (vs. threat-oddball) $t(16) = 2.195$, $p = .043$, confirmed that there was a main effect of tremor power in the stress condition of our cognitive demanding task as opposed to the threat condition of our threat of shock task. Again, we explored whether administrating an electrical shock differed in tremor power as opposed to only the threat of receiving a shock. There we observed no significant effect for shock administration $t(16) = 1.110$, $p = .283$ (two-tailed). These findings do concur with the self-reported levels of perceived stress that are displayed in Table 2. A visual overview of the tremor power results is depicted in Figure 4.



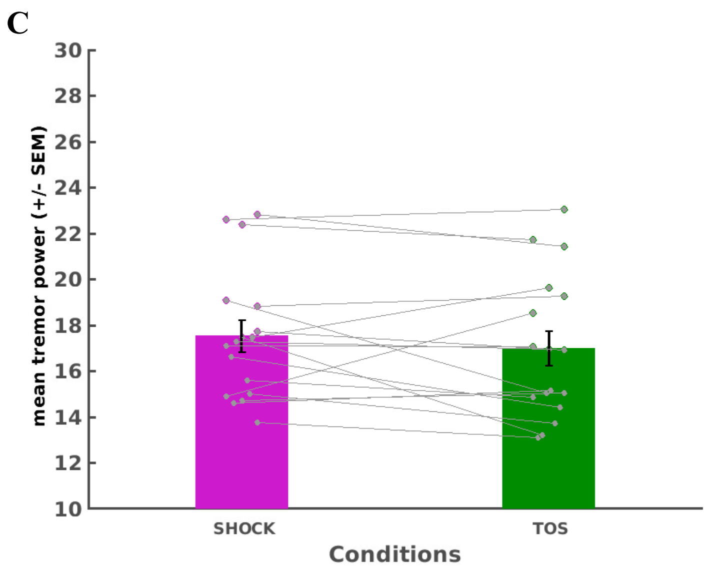


Figure 4. The effect of mental arithmetic and threat-of-shock on tremor power. Tremor power values are log-transformed. Panel **A** displays the tremor power at the individual subject and group level during the cognitive stress task with a significantly higher tremor power during mental arithmetic (coco) as opposed to rest. Panel **B** represents the tremor power at the individual subject and group level during the threat-of-shock task, with a significantly higher tremor power during the threat conditions. Panel **C** shows the individual and average tremor power during the condition in which a shock was administered as opposed to the conditions in which no shock was administered. Lines between the individual data points were added to identify the trend in the data. Error bars represent the standard error of the mean.
*** indicates $p < .001$

3.4 Correlations – tremor power and pupil diameter regressors

With respect to our exploratory hypothesis about the mediating effects on tremor power by noradrenergic activity, we performed correlations between each tremor power and pupil diameter regressor from the same condition (e.g. tremor power during safe-odd with pupil diameter during safe-odd). This enabled us to explore for example whether (1) a higher tremor power was correlated with a larger pupil diameter or (2) whether a higher tremor power was correlated with a smaller pupil diameter. With respect to our mental arithmetic task, the regressors of tremor power and pupil diameter in the stress condition showed a negative correlation, but were not statistically significant, r = -.028, p = .251. The tremor power and pupil diameter regressors in the rest condition had a positive correlation, but these were not statistically significant, r = .077, p = .066. Nevertheless, we observed a significant difference between these correlations, $t(16) = -2.501$, $p = .024$. Since we observed that the tremor power was only modulated by our threat manipulation, but not by our oddball manipulation, we calculated the average correlations for threat and safe to identify whether these were significantly different from zero. With respect to our threat-of-shock task, the regressors of tremor power and pupil diameter during threat showed a positive correlation but were not statistically significant, $r = .071$, $p = .112$. The regressors of tremor power and pupil diameter during safe showed a significant positive correlation, $r = .102$, $p = .009$, Finally, we compared correlations between these conditions but observed no significant differences for threat, $F(1, 16) = .608$, $p = .447$, oddball $F(1, 16) = .095$, $p = .751$, and threat*oddball $F(1, 16) = .348$, $p = .564$. A visual representation of the regressors and correlations are presented in respectively Figure 5 and Figure 6.

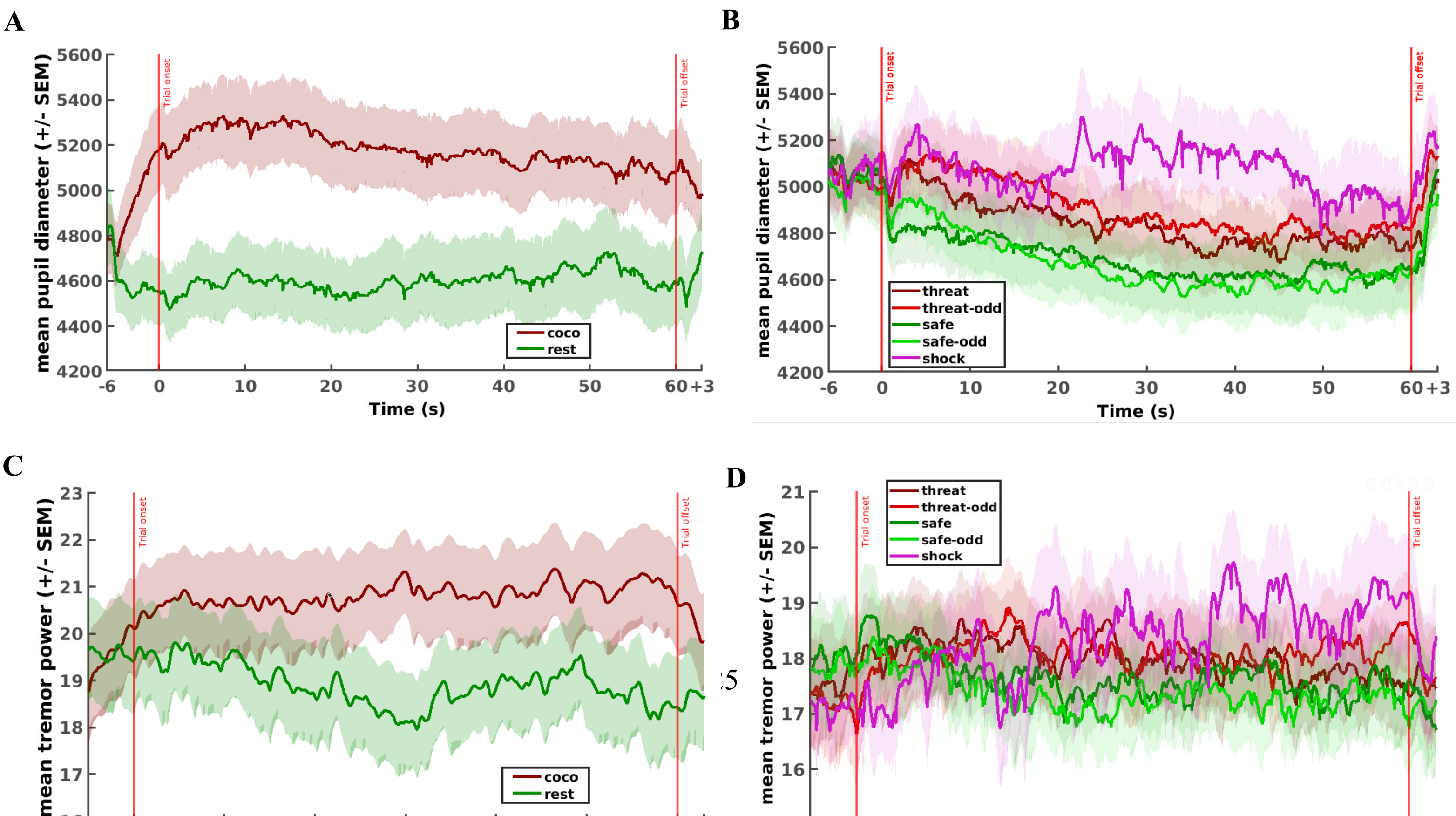


Figure 5. The effects of cognitive stress and threat-of-shock displayed during the 60 second trials. Panel **A** and **B** display the mean pupil diameter during respectively mental arithmetic (coco) and threat-of-shock. Panel **C** and **D** represent the mean tremor power during respectively mental arithmetic and threat-of-shock. Shading background colors represent the standard error of the mean. The legenda's indicate which line represents which condition.

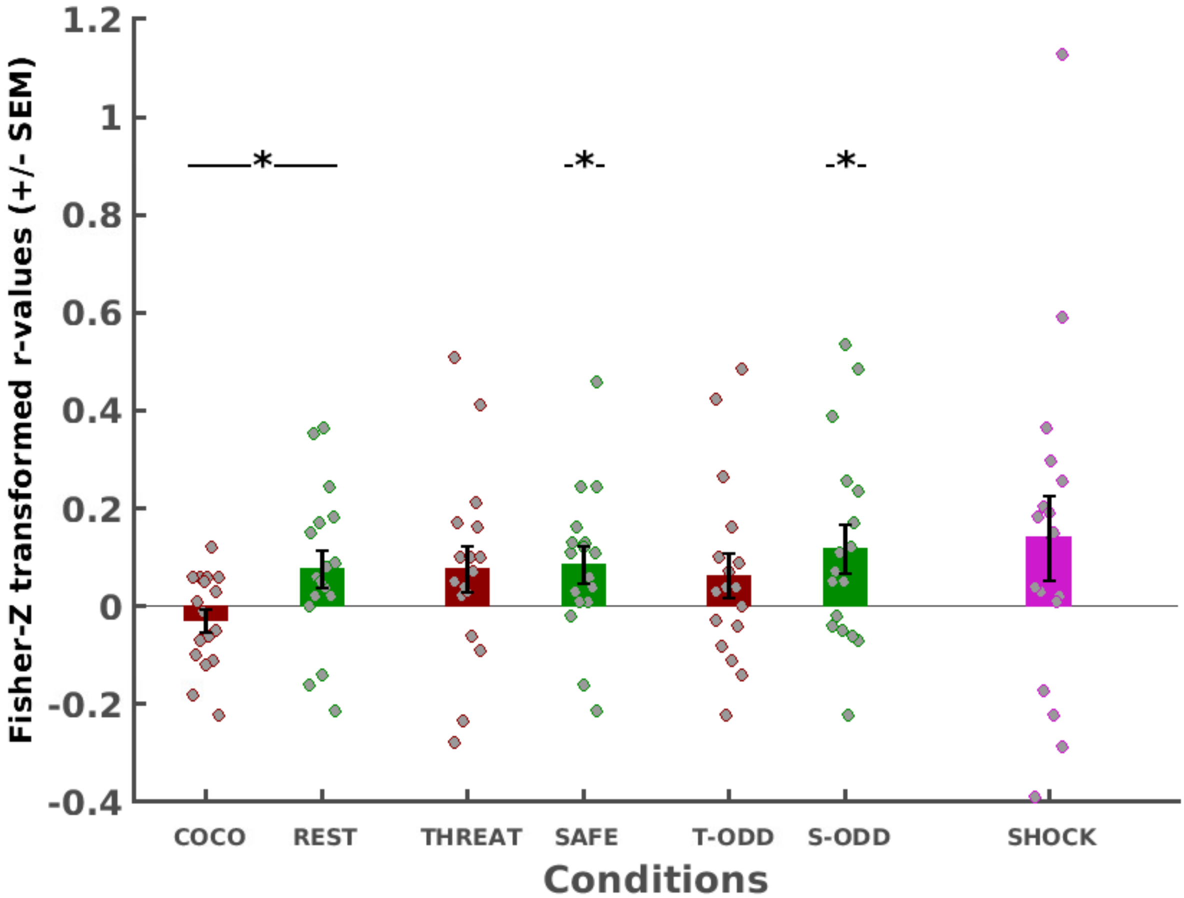


Figure 6. Fisher’s z-transformed Pearson’s R correlation coefficients between each tremor power and pupil diameter regressor (Figure 5). One-sample t-tests were performed to identify whether the correlations for the conditions (coco, rest, threat, safe) significantly differed from zero. To identify correlational between condition differences, we performed a Paired Samples T Test for our cognitive demanding task (Coco vs. Rest) and a 2 (Threat vs. Safe) x 2 (Oddball vs. No-oddball) repeated measures ANOVA for our threat-of-shock task. All correlations, but the cognitive demanding condition, showed a positive correlational trend. Data points represent the correlational values for each individual patient.
* indicates $p < .05$.

4. Discussion

In this study, we investigated the effects of two different tasks, with and without cognitive loading on Parkinson’s tremor in seventeen tremor-dominant PD patients. Furthermore, we investigated to what extent the noradrenergic system – by means of pupil dilation - was activated during these tasks and whether this correlated with tremor power. In our task with cognitive load, we tested the effects of mental arithmetic on tremor power and pupil diameter. In our task without cognitive load, we tested the effects of threat of electrical shock and an additional oddball paradigm combined with threat-of-shock on the same outcomes. We hypothesized that mental arithmetic, as well as threat-of-shock, increased Parkinson’s tremor and noradrenergic activity. Specifically, a higher tremor power was expected for both tasks during the mental arithmetic and the threat condition compared to the rest and safe condition. Since a similar cognitive task has been previously tested in PD patients with resting tremor (Zach et al., 2017), while a threat-of-shock task has never been tested before in such a sample, we expected higher tremor power for the mental arithmetic condition compared to the threat condition. We also expected more noradrenergic activity for both tasks during the mental arithmetic condition and the threat condition compared to the rest and safe condition. Due to the conflicting results of the effects of similar mental arithmetic manipulations on noradrenergic activity (Marsden, 1967; Zach et al., 2017), but consistent findings for the effects of threat-of-shock on noradrenergic activity (Bitsios, Szabadi, & Bradshaw, 1996; de Voogd, Fernández, Hermans, 2016; Hodges & Spielberger, 1966; Hashemi et al., 2019; Marshall, & Schnieden, 1966; Hermans et al., 2014; Hermans et al., 2014; Constas, 1962), we expected a larger effect on noradrenergic activity for the threat condition compared to the mental arithmetic condition. Finally, we explored whether there was a correlation between tremor power and noradrenergic activity.

In line with our hypotheses, we observed a higher tremor power and more noradrenergic activity (indexed by pupil dilation) during mental arithmetic and threat as opposed to respectively rest and safe. We also observed a higher tremor power for mental arithmetic as opposed to threat, but unexpectedly also more noradrenergic activity during mental arithmetic as opposed to threat. The latter could be explained by the fact that our mental arithmetic task is more cognitively demanding, arousing or effortful (e.g. counting backward during a limited time-period) compared to our 'cleaner' threat of shock manipulation (e.g. only counting a small number of oddballs during threat or just a fixation of gaze at a fixation-cross during threat). This can be substantiated by models that suggest that the levels of arousal that drive pupil dilation in reaction to task demands are related to the locus coeruleus-noradrenergic system (Aston-Jones, & Cohen, 2005). It could also be that other – more potent – mechanisms might play a role. For example, top-down cognitive effects could be more important compared to bottom-up noradrenergic effects, since noradrenergic mechanisms are thought to flow 'bottom-up' in order to activate systems that mediate processes of attention (Aston-Jones et al., 1996). Specifically, it has been suggested that in situations with exposure to threatening stimuli as opposed to more goal-directed endogenous stimuli, bottom-up effects are subordinate to 'top-down' processes (Jodoj, Chiang, Aston-Jones, 1998). The fact that there is a higher tremor power and more noradrenergic activity during mental arithmetic, which is more cognitively demanding as opposed to the 'cleaner' threat-of-shock task, together with the fact that that is precisely the condition where the pupil diameter does not or negatively correlate with tremor power, supports the previously mentioned distraction hypothesis in the mental arithmetic task. It could be that more cognitively demanding tasks are less related to noradrenergic activity, but with fewer available cognitive resources for tremor suppression. That could support the distraction hypothesis since there is evidence suggesting that cognitive effort predominantly relies on dopaminergic mechanisms (Shenhav et al., 2017; Westbrook & Braver, 2016). However, this should be interpreted with caution, since there are studies highlighting the complex interaction between the noradrenergic and dopaminergic system which makes it difficult to approach these as two independent processes (Xing et al., 2016). The distraction hypothesis in the mental arithmetic task can also be supported by the evolution of the tremor power and pupil diameter signals that are shown in Figure 5. There we observe that the pupil diameter slightly decreases while the tremor power increases. In the context of the 'clean' threat of shock task, where pupil dilation is not a consequence of cognitive demands or effort, but only of threat anticipation, which is more noradrenergic related (Bitsios, Szabadi, & Bradshaw, 1996; de Voogd, Fernández, Hermans, 2016; Hodges & Spielberger, 1966; Hashemi et al., 2019; (Marshall, & Schnieden, 1966; Hermans et al., 2014; Constas, 1962), there we observe a positive (trend towards) correlation between pupil diameter and tremor power.

On the one hand, our findings for mental arithmetic are consistent with previous studies reporting an increase in tremor power and noradrenergic activity (Zach et al., 2017; Marsden, 1967). Although Zach et al. (2017) observed an increase in noradrenergic activity by means of heart rate, an increase in pupil diameter – as observed in our study - is also seen as a reliable marker of noradrenergic activity (Gilzenrat et al., 2010; Murphy et al., 2011). The observed effect of threat of shock on tremor power and related noradrenergic activity is – to the best of our knowledge – a novel finding in tremor-dominant PD patients. Importantly, these effects occurred independently of cognitive load, although the magnitude of these effects was smaller than during our cognitively demanding task (mental arithmetic). This suggests that worsening of PD tremor during cognitive stress is not (only) caused by top-down cognitive mechanisms, but also by bottom-up (noradrenergic) processes involved in fear. Specifically, this may involve an activation of cerebral mechanisms that signal danger during our threat manipulation such as the salience network (Marshall, & Schnieden, 1966; Hermans et al., 2014) which is modulated by noradrenergic activity (Hermans et al., 2014; Constas, 1962). Future functional imaging studies are needed to test this hypothesis. We did not find effects of our oddball manipulation on tremor power and noradrenergic activity. The non-significant effect on noradrenergic activity is surprising, but might be explained by recent reports stating that noradrenaline has a crucial role in facilitating sustained attention (Müller & Apps, 2019) which is known to be impaired during fatigue as a consequence of a dysfunctioning noradrenergic system (Westbrook, Cools, & Braver, 2019). This can be substantiated by evidence from animal and human PD studies, indicating that cerebral noradrenaline dysfunctioning can cause motor and non-motor symptoms, such as a lack of sustained attention, to appear (Delaville, De Deurwaerdère, & Benazzouz, 2011). This suggests that during these recurring periods of counting oddballs - in which PD patients experience a certain degree of fatigue - they were probably less successful in retaining their attention. An explanation for the non-significant effect on tremor power might be explained by a lack of cognitive task demands. The previously mentioned distraction effect, suggests that a cognitive task might interfere with cerebral mechanisms that suppress tremor. However, merely counting a limited number of oddballs (i.e., 3 – 5) might not be demanding enough to interfere with cerebral mechanisms that suppress tremor. Since studies have indicated that activation or impediments of unconscious motor processes are closely related with the cerebral dopamine circuit (D'ostilio & Garraux, 2012), it might be the case that our patients were able to rely on their remaining dopamine availability and were, therefore, able to subconsciously suppress their tremor while counting the oddballs.

Finally, our correlations did not clearly show a relationship between tremor power and noradrenergic activity. Overall, the correlations indicated a positive trend, which indicates that an increase in tremor power is associated with an increase in pupil diameter. However, only the safe conditions turned out to be significant. There was a negative correlational trend for the mental arithmetic condition. An explanation for this anti-correlational trend might be explained by a so-called 'plateau effect'. As becomes clear from panel A in Figure 5, during the first 10 seconds the pupil size increases, but slowly decreases over the rest of the time-course. Previous studies measuring pupil size during affective processing also report a similar pattern of pupil diameter with a steep increase in pupil diameter after stimulus onset which is followed by a slow decrease until stimulus offset (Partala & Surakka, 2003, Massar et al., 2019). However, we also observe this signal evolution during threat-of-shock. Therefore, this trend might be more accurately explained by the fact that more cognitively demanding tasks are less related to noradrenergic activity, as opposed to threat anticipation but more with fewer available cognitive resources for tremor suppression.

To validate whether our manipulations were successful, we implemented Visual Analogue Scales for self-reported levels of perceived stress and threat. Results indicated that during both experimental tasks, the levels of perceived stress were equal. Furthermore, our findings show that patients experienced higher levels of stress during threat as opposed to safe. Finally, we identified that our threat manipulation was successful for both of our threat conditions since patients indicated that the likelihood of receiving a shock did not differ between the two threat conditions.

In general, our findings are in line with the growing evidence that parkinsonian resting tremor is not only modulated by dopaminergic influences (i.e., the traditional view over the past century) (Zach et al., 2017), but that its pathophysiology is also modulated by other neurotransmitters such as noradrenaline, but potentially also by serotonergic mechanisms (Jankovic, 2018). Since a recent Single Photon Emission Computed Tomography (SPECT) study indicated that resting tremor severity had a lower correlation with depleted striatal dopamine transporter compared to a specific serotonergic transporter in the brain stem (Pasquini et al., 2018), the role of serotonergic mechanisms on parkinsonian resting tremor during stress could also be a target for future studies. The findings of our study suggest that noradrenaline might play a mediating role in the pathophysiology of parkinsonian resting tremor during stress, but insights into other noradrenergic-related mechanisms are needed to more extensively test and confirm this hypothesis. Our results also address a critical clinical problem, since they confirm that patients suffer from their tremor in stressful environments even when they are on their dopaminergic medication (Zach et al., 2017). Therefore, new treatment strategies that can reduce the burden of stress on PD symptoms, especially for tremor, are needed.

4.1 Limitations

One limitation is that we indirectly measured noradrenergic activity by means of pupil dilation with eye-tracking and that we did not analyze multiple (in)direct measures of noradrenergic activity (e.g., direct: locus coereuleus-noradrenergic activity with fMRI, indirect: heart rate with ECG). While both tasks showed increased pupil diameter, which correlates with cerebral activity in the LC (Murphy et al., 2014), and is a reliable marker of noradrenergic activity (Gilzenrat et al., 2010; Murphy et al., 2011), other potential mechanisms may play a role. Another issue is that there are currently no context-specific tremor-rating scales (TRS) (Elble et al., 2066). Since we did not gather clinical tremor-ratings during the conditions of our experimental tasks (coco, rest, threat, safe, threat-oddball, safe-oddball) and because simultaneous measurements of tremor with TRS and accelerometry helps in understanding the tremor changes as a result of specific manipulations (e.g., cognitive stress and threat-of-shock), it is hard to characterize the clinical relevance of our tremor results. Although our sample size is in accordance with a priori power analyses, it is still relatively small and it could be that a larger study population would strengthen our effects. For example, Zach et al. (2017) analyzed 69 patients, while we analyzed 17 tremor-dominant PD patients. Finally, it should also be mentioned that although our manipulations showed to cause the tremor effect to appear, it does not mean that our manipulations are the cause itself, only that they can cause the effect. Other studies are needed to replicate the effects of threat on resting tremor since this is the first study to report these findings. For instance, studies that take a more neurobiological perspective to investigate cerebral mechanisms underlying the tremor amplification during threat.

4.2 Conclusion

Our findings show that even in a clean threat of shock paradigm – which is less poluted by cognitive effort or distraction – there is an increase in tremor power and pupil diameter and a (trend towards) positive correlation. This study adds to previous work by showing an effect of stress on PD tremor, independently of cognitive load, although these effects were smaller in magnitude than observed during a demanding cognitive task. It suggests that multiple mechanisms play a role in the worsening of PD tremor during (cognitive) stress: both top-down cognitive mechanisms and bottom-up arousal systems. Furthermore, this is one of the first studies to take contextual factors (stressful vs. neutral) and different types of stress (cognitive vs. threat anticipation) into account and can, therefore, gain new insights about the basis of parkinsonian resting tremor. Next to PD, effects of stress on motor impairments also occur in many other neurological disorders such as Dystonia (Jankovic et al., 1991) or Tourette (Leckman, 2002) and our results might have implications that go beyond parkinsonian tremor. Our findings can gain more insight into human motor functioning, especially for tremor-dominant PD patients, as sparked by modulations in the noradrenergic system. A better understanding of resting tremor can lead to starting points for treatment. In the long-term, this study could better inform researchers and clinicians to come up with new and better-personalized strategies to reduce the burden of different types of stress on tremor in PD patients.

References

Abramsky, O., Carmon, A., & Lavy, S. (1971). Combined treatment of parkinsonian tremor with propranolol and levodopa. *Journal of the neurological sciences*, *14*(4), 491-494. https://doi.org/10.1016/0022-510X(71)90183-3

Albanese, A., Bonuccelli, U., Brefel, C., Chaudhuri, K. R., Colosimo, C., Eichhorn, T., ... & Zappia, M. (2001). Consensus statement on the role of acute dopaminergic challenge in Parkinson's disease. *Movement disorders: official journal of the Movement Disorder Society*, *16*(2), 197-201. https://doi.org/10.1002/mds.1069

Aron, A. R., Poldrack, R. A., & Wise, S. P. (2010). Cognition: Basal ganglia role. In *Encyclopedia of neuroscience* (pp. 1069-1077). Elsevier Ltd. https://doi.org/10.1016/B978-008045046-9.00410-1

Aston-Jones, G., & Cohen, J. D. (2005). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu. Rev. Neurosci.*, *28*, 403-450. https://doi.org/10.1146/annurev.neuro.28.061604.135709

Aston-Jones, G., Rajkowski, J., Kubiak, P., Valentino, R. J., & Shipley, M. T. (1996). Role of the locus coeruleus in emotional activation. In *Progress in brain research* (Vol. 107, pp. 379-402). Elsevier. https://doi.org/10.1016/S0079-6123(08)61877-4

Barcroft, H., Peterson, E., & Schwab, R. S. (1952). Action of adrenaline and noradrenaline on the tremor in Parkinson's disease. *Neurology*, *2*(5-6), 154-154. https://doi.org/10.1212/WNL.2.5-6.154

Bitsios, P., Szabadi, E., & Bradshaw, C. M. (1996). The inhibition of the pupillary light reflex by the threat of an electric shock: a potential laboratory model of human anxiety. *Journal of psychopharmacology*, *10*(4), 279-287. https://doi.org/10.1177/026988119601000404

Callister, R., Suwarno, N. O., & Seals, D. R. (1992). Sympathetic activity is influenced by task difficulty and stress perception during mental challenge in humans. *The Journal of physiology*, *454*(1), 373-387. doi: 10.1113/jphysiol.1992.sp019269

Constas, C. (1962). The effects of adrenaline, noradrenaline, and isoprenaline on parkinsonian tremor. *Journal of neurology, neurosurgery, and psychiatry*, *25*(2), 116. Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC495428/

Delaville, C., De Deurwaerdère, P., & Benazzouz, A. (2011). Noradrenaline and Parkinson's disease. *Frontiers in systems neuroscience*, *5*, 31. https://doi.org/10.3389/fnsys.2011.00031

de Voogd, L. D., Fernández, G., & Hermans, E. J. (2016). Disentangling the roles of arousal and amygdala activation in emotional declarative memory. *Social cognitive and affective neuroscience*, *11*(9), 1471-1480. https://doi.org/10.1093/scan/nsw055

de Voogd, L.D., Collin, S.H.P., Barth, M., Fernández, G., Hermans, E.J. (in press.). Phasic BOLD activity in the human locus coeruleus at different levels of tonic arousal.

Dirkx, M. F., Zach, H., Bloem, B. R., Hallett, M., & Helmich, R. C. (2018). The nature of postural tremor in Parkinson disease. *Neurology*, *90*(13), e1095-e1103. https://doi.org/10.1212/WNL.0000000000005215

D'ostilio, K., & Garraux, G. (2012). Brain mechanisms underlying automatic and unconscious control of motor action. *Frontiers in human neuroscience*, *6*, 265. https://doi.org/10.3389/fnhum.2012.00265

Elble, R. J., Pullman, S. L., Matsumoto, J. Y., Raethjen, J., Deuschl, G., & Tintner, R. (2006). Tremor amplitude is logarithmically related to 4-and 5-point tremor rating scales. *Brain*, *129*(10), 2660-2666. https://doi.org/10.1093/brain/awl190

Fahn, S., Tolosa, E., & Marín, C. (1993). Clinical rating scale for tremor. *Parkinson's disease and movement disorders*, *2*, 271-280.

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior research methods*, *39*(2), 175-191. https://doi.org/10.3758/BF03193146

Feigin, V. L., Abajobir, A. A., Abate, K. H., Abd-Allah, F., Abdulle, A. M., Abera, S. F., ... & Aichour, M. T. E. (2017). Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet Neurology, 16*(11), 877-897. https://doi.org/10.1016/S1474-4422(17)30299-5

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research*, *12*(3), 189-198. https://doi.org/10.1016/0022-3956(75)90026-6

Gibb, W. R., & Lees, A. J. (1988). The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *Journal of Neurology, Neurosurgery & Psychiatry*, *51*(6), 745-752. http://dx.doi.org/10.1136/jnnp.51.6.745

Gilzenrat, M. S., Nieuwenhuis, S., Jepma, M., & Cohen, J. D. (2010). Pupil diameter tracks changes in control state predicted by the adaptive gain theory of locus coeruleus function. *Cognitive, Affective, & Behavioral Neuroscience*, *10*(2), 252-269. doi:10.3758/CABN.10.2.252

Hashemi, M. M., Gladwin, T. E., de Valk, N. M., Zhang, W., Kaldewaij, R., van Ast, V., ... & Roelofs, K. (2019). Neural Dynamics of shooting Decisions and the switch from Freeze to Fight. *Scientific reports*, *9*(1), 4240. https://doi.org/10.1038/s41598-019-40917-8

Helmich, R. C., Hallett, M., Deuschl, G., Toni, I., & Bloem, B. R. (2012). Cerebral causes and consequences of parkinsonian resting tremor: a tale of two circuits? *Brain*, *135*(11), 3206-3226. https://doi.org/10.1093/brain/aws023

Hemmerle, A. M., Herman, J. P., & Seroogy, K. B. (2012). Stress, depression and Parkinson's disease. *Experimental neurology*, *233*(1), 79-86. https://doi.org/10.1016/j.expneurol.2011.09.035

Henderson, J. M., Yiannikas, C., Morris, J. G., Einstein, R., Jackson, D., & Byth, K. (1994). Postural tremor of Parkinson's disease. *Clinical neuropharmacology*, *17*(3), 277-285. Retrieved from: https://europepmc.org/abstract/med/9316673

Hermans, E. J., Henckens, M. J., Joëls, M., & Fernández, G. (2014). Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends in neurosciences*, *37*(6), 304-314. https://doi.org/10.1016/j.tins.2014.03.006

Hermans, E. J., Van Marle, H. J., Ossewaarde, L., Henckens, M. J., Qin, S., Van Kesteren, M. T., ... & Fernández, G. (2011). Stress-related noradrenergic activity prompts large-scale neural network reconfiguration. *Science*, *334*(6059), 1151-1153. doi: 10.1126/science.1209603

Hodges, W. F., & Spielberger, C. D. (1966). The effects of threat of shock on heart rate for subjects who differ in manifest anxiety and fear of shock. *Psychophysiology*, *2*(4), 287-294. https://doi.org/10.1111/j.1469-8986.1966.tb02656.x

Hughes, A. J., Daniel, S. E., Ben-Shlomo, Y., & Lees, A. J. (2002). The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. *Brain*, *125*(4), 861-870. https://doi.org/10.1093/brain/awf080

IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

Isaias, I. U., Marotta, G., Pezzoli, G., Sabri, O., Schwarz, J., Crenna, P., ... & Cavallari, P. (2011). Enhanced catecholamine transporter binding in the locus coeruleus of patients with early Parkinson disease. *BMC neurology*, *11*(1), 88. https://doi.org/10.1186/1471-2377-11-88

Jankovic, J. (2008). Parkinson’s disease: clinical features and diagnosis. *Journal of Neurology, Neurosurgery & Psychiatry*, *79*(4), 368-376. doi:10.1136/jnnp.2007.131045

Jankovic, J., Leder, S., Warner, D., & Schwartz, K. (1991). Cervical dystonia: clinical findings and associated movement disorders. *Neurology*, *41*(7), 1088-1088. https://doi.org/10.1212/WNL.41.7.1088

Jankovic, J. (2018). Reader response: The nature of postural tremor in Parkinson disease. *Neurology*, *91*(15), 723-723. https://doi.org/10.1212/WNL.0000000000006312

Jodoj, E., Chiang, C., & Aston-Jones, G. (1998). Potent excitatory influence of prefrontal cortex activity on noradrenergic locus coeruleus neurons. *Neuroscience*, *83*(1), 63-79. https://doi.org/10.1016/S0306-4522(97)00372-2

Kearney, K. (2018). Boundedline Toolbox version 1.4.0.0. Retrieved from: https://github.com/kakearney/boundedline-pkg

Kish, S. J., Shannak, K., & Hornykiewicz, O. (1988). Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. *New England Journal of Medicine*, *318*(14), 876-880. doi: 10.1056/NEJM198804073181402

Kissel, P., Tridon, P., & Andre, J. M. (1974). Levodopa-propranolol therapy in parkinsonian tremor. *The Lancet*, *303*(7854), 403-404. doi: 10.1016/S0140-6736(74)93165-1

Klumpers, F., Raemaekers, M. A., Ruigrok, A. N., Hermans, E. J., Kenemans, J. L., & Baas, J. M. (2010). Prefrontal mechanisms of fear reduction after threat offset. *Biological psychiatry*, *68*(11), 1031-1038. https://doi.org/10.1016/j.biopsych.2010.09.006

Klumpers, F., van Gerven, J. M., Prinssen, E. P. M., Niklson Roche, I., Roesch, F., Riedel, W. J., ... & Baas, J. M. P. (2010). Method development studies for repeatedly measuring anxiolytic drug effects in healthy humans. *Journal of Psychopharmacology*, *24*(5), 657-666. https://doi.org/10.1177/0269881109103115

Klumpers, F., Kroes, M. C., Baas, J. M., & Fernández, G. (2017). How human amygdala and bed nucleus of the stria terminalis may drive distinct defensive responses. *Journal of neuroscience*, *37*(40), 9645-9656. https://doi.org/10.1523/JNEUROSCI.3830-16.2017

Leckman, J. F. (2002). Tourette's syndrome. *The Lancet*, *360*(9345), 1577-1586. https://doi.org/10.1016/S0140-6736(02)11526-1

Lewis, S. J., Pavese, N., Rivero-Bosch, M., Eggert, K., Oertel, W., Mathias, C. J., ... & Gerhard, A. (2012). Brain monoamine systems in multiple system atrophy: a positron emission tomography study. *Neurobiology of disease*, *46*(1), 130-136. https://doi.org/10.1016/j.nbd.2011.12.053

Marsden, C. D., & Owen, D. A. L. (1967). Mechanisms underlying emotional variation in parkinsonian tremor. *Neurology*, *17*(7), 711-711. https://doi.org/10.1212/WNL.17.7.711

Marshall, J., & Schnieden, H. (1966). Effect of adrenaline, noradrenaline, atropine, and nicotine on some types of human tremor. *Journal of neurology, neurosurgery, and psychiatry*, *29*(3), 214. Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC496021/

Massar, S. A., Lim, J., Sasmita, K., & Chee, M. W. (2019). Sleep deprivation increases the costs of attentional effort: performance, preference and pupil size. *Neuropsychologia*, *123*, 169-177. https://doi.org/10.1016/j.neuropsychologia.2018.03.032

MathWorks, Inc. (2018). MATLAB : the language of technical computing : computation, visualization, programming. Natwick : MathWorks, Inc.

Metz, G. A. (2007). Stress as a modulator of motor system function and pathology. *Reviews in the Neurosciences*, *18*(3-4), 209-222. https://doi.org/10.1515/REVNEURO.2007.18.3-4.209

Mhyre, T. R., Boyd, J. T., & Hamill, R. W., & Maguire-Zeiss, K.A. (2012). Parkinson's disease. In J. Robin Harris (Ed.), *Protein Aggregation and Fibrillogenesis in Cerebral and Systemic Amyloid Disease*, (pp. 389-456). doi: 10.1007/978-94-007-5416-4_16

Müller, T., & Apps, M. A. (2019). Motivational fatigue: A neurocognitive framework for the impact of effortful exertion on subsequent motivation. *Neuropsychologia*, *123*, 141-151. https://doi.org/10.1016/j.neuropsychologia.2018.04.030

Murphy, P. R., O'connell, R. G., O'sullivan, M., Robertson, I. H., & Balsters, J. H. (2014). Pupil diameter covaries with BOLD activity in human locus coeruleus. *Human brain mapping*, *35*(8), 4140-4154. https://doi.org/10.1002/hbm.22466

Murphy, P. R., Robertson, I. H., Balsters, J. H., & O'connell, R. G. (2011). Pupillometry and P3 index the locus coeruleus–noradrenergic arousal function in humans. *Psychophysiology*, *48*(11), 1532-1543. https://doi.org/10.1111/j.1469-8986.2011.01226.x

Nieuwhof, F., Panyakaew, P., van de Warrenburg, B. P., Gallea, C., & Helmich, R. C. (2018). The patchy tremor landscape: recent advances in pathophysiology. *Current Opinion in Neurology*. doi: 10.1097/WCO.0000000000000582

Nieuwhof, F., Bloem, B. R., Reelick, M. F., Aarts, E., Maidan, I., Mirelman, A., ... & Helmich, R. C. (2017). Impaired dual tasking in Parkinson's disease is associated with reduced focusing of cortico-striatal activity. *Brain*, *140*(5), 1384-1398. https://doi.org/10.1093/brain/awx042

Nuland, A. (2019). plotBarScatter Toolbox version 2019. Retrieved from: https://github.com/annemonelies/MatlabGraphs

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. 10.1155/2011/156869

Partala, T., & Surakka, V. (2003). Pupil size variation as an indication of affective processing. *International journal of human-computer studies*, *59*(1-2), 185-198. https://doi.org/10.1016/S1071-5819(03)00017-X

Pasquini, J., Ceravolo, R., Qamhawi, Z., Lee, J. Y., Deuschl, G., Brooks, D. J., ... & Pavese, N. (2018). Progression of tremor in early stages of Parkinson's disease: a clinical and neuroimaging study. *Brain*, *141*(3), 811-821. https://doi.org/10.1093/brain/awx376

Paulus, W., & Jellinger, K. (1991). The neuropathologic basis of different clinical subgroups of Parkinson's disease. *Journal of Neuropathology & Experimental Neurology*, *50*(6), 743-755. https://doi.org/10.1097/00005072-199111000-00006

Pirker, W. (2003). Correlation of dopamine transporter imaging with parkinsonian motor handicap: how close is it? *Movement disorders: official journal of the Movement Disorder Society*, *18*(S7), S43-S51. https://doi.org/10.1002/mds.10579

Politis, M., Wu, K., Molloy, S., G Bain, P., Chaudhuri, K., & Piccini, P. (2010). Parkinson's disease symptoms: the patient's perspective. *Movement Disorders*, *25*(11), 1646-1651. https://doi.org/10.1002/mds.23135

Pruessner, J. C., Dedovic, K., Khalili-Mahani, N., Engert, V., Pruessner, M., Buss, C., ... & Lupien, S. (2008). Deactivation of the limbic system during acute psychosocial stress: evidence from positron emission tomography and functional magnetic resonance imaging studies. *Biological psychiatry*, *63*(2), 234-240. https://doi.org/10.1016/j.biopsych.2007.04.041

Raethjen, J., Austermann, K., Witt, K., Zeuner, K. E., Papengut, F., & Deuschl, G. (2008). Provocation of Parkinsonian tremor. *Movement disorders: official journal of the Movement Disorder Society*, *23*(7), 1019-1023. https://doi.org/10.1002/mds.22014

Schlesinger, I., Benyakov, O., Erikh, I., Suraiya, S., & Schiller, Y. (2009). Parkinson's disease tremor is diminished with relaxation guided imagery. *Movement disorders: official journal of the Movement Disorder Society*, *24*(14), 2059-2062. https://doi.org/10.1002/mds.22671

Shenhav, A., Musslick, S., Lieder, F., Kool, W., Griffiths, T. L., Cohen, J. D., & Botvinick, M. M. (2017). Toward a rational and mechanistic account of mental effort. *Annual review of neuroscience*, *40*, 99-124. https://doi.org/10.1146/annurev-neuro-072116-031526

Silver, N. C., & Dunlap, W. P. (1987). Averaging correlation coefficients: Should Fisher’s z transformation be used? *Journal of Applied Psychology, 72*(1), 146-148. http://dx.doi.org/10.1037/0021-9010.72.1.146

Tank, A. W., & Lee Wong, D. (2015). Peripheral and central effects of circulating catecholamines. *Compr Physiol*, *5*(1), 1-15. doi: 10.1002/cphy.c140007

Tysnes, O. B., & Storstein, A. (2017). Epidemiology of Parkinson’s disease. *Journal of Neural Transmission*, *124*(8), 901-905. https://doi.org/10.1007/s00702-017-1686-y

Westbrook, A., Cools, R., & Braver, T.S. (2019). Editorial. *Neuropsychologia, 123*, 1-4. https://doi.org/10.1016/j.neuropsychologia.2019.01.010

Westbrook, A., & Braver, T. S. (2016). Dopamine does double duty in motivating cognitive effort. *Neuron*, *89*(4), 695-710. https://doi.org/10.1016/j.neuron.2015.12.029

Xing, B., Li, Y. C., & Gao, W. J. (2016). Norepinephrine versus dopamine and their interaction in modulating synaptic function in the prefrontal cortex. *Brain research*, *1641*, 217-233. https://doi.org/10.1016/j.brainres.2016.01.005

Yoshiura, T., Zhong, J., Shibata, D. K., Kwok, W. E., Shrier, D. A., & Numaguchi, Y. (1999). Functional MRI study of auditory and visual oddball tasks. *Neuroreport*, *10*(8), 1683-1688. doi: 10.1097/00001756-199906030-00011

Zach, H., Dirkx, M. F., Pasman, J. W., Bloem, B. R., & Helmich, R. C. (2017). Cognitive stress reduces the effect of levodopa on Parkinson's resting tremor. *CNS neuroscience & therapeutics*, *23*(3), 209-215. https://doi.org/10.1111/cns.12670

Zerbi, V., Floriou-Servou, A., Markicevic, M., Vermeiren, Y., Sturman, O., Privitera, M., ... & Wenderoth, N. (2019). Rapid Reconfiguration of the Functional Connectome after Chemogenetic Locus Coeruleus Activation. *Available at SSRN 3334983*. https://doi.org/10.1016/j.neuron.2019.05.034