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Investigating the Role of the Primary Motor Cortex (M1) in Upper Limb Freezing (ULF)

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1. Introduction

Idiopathic Parkinson's disease

In his infamous *An Essay on the Shaking Palsy* (1817), James Parkinson first described the signs and symptoms of a hitherto unknown disease. Over several years, he had observed the onset and gradual worsening of a cluster of peculiar motor symptoms in six of his patients. As one of the most prominent features, he identified *tremor* and hence named the condition the *shaking* palsy. With it, he associated a cluster of characteristic impairments in gait, such as shuffling, reduced step length, and festination, and further described a flexed posture, balance problems, and a risk of falling (Parkinson, 2002). James Parkinson even described nonmotor symptoms, like constipation and rapid eye movement (REM) sleep behavioral disorder, which today are considered an integral part or even prodromal markers of the disease (Obeso et al., 2017). His thorough description was (and still is) greatly acknowledged in the medical community, and over 60 years after his death, another very famous physician, Martin Charcot, would name this condition in his honor: *Parkinson's disease* (Obeso et al., 2017).

Today – more than 200 years after its first description – idiopathic Parkinson's disease (PD) is one of the most well-known movement disorders. With its main motor manifestations, *bradykinesia*, *tremor*/*rigidity*, and *postural instability* PD is the second-most-common neurodegenerative disease worldwide and affects approximately 2–3% of the world's population over 65 years (Poewe et al., 2017). By now, the emergence of typical motor symptoms has been linked to basal ganglia (BG) circuit dysfunction and dopaminergic depletion in the striatum (Obeso et al., 2008). And the groundbreaking discovery of dopamine as a movement controlling neurotransmitter has not only earned Swedish pharmacologist Arved Carlsson the *Nobel Prize in Physiology or Medicine* (2000). It has also led to the development of the potent chemical structure *L-Dopa* and revolutionized PD treatment (Poewe et al., 2017). Additional pharmacological and technical advancements such as the introduction of Deep Brain Stimulation (DBS) further extended the therapeutic spectrum.

Today, some PD symptoms, such as bradykinesia or rigidity, can be treated effectively. But PD remains a progressive disease without cure or even disease-modifying therapeutic approaches, and some of its features appear relatively resistant to *any* treatment option currently at hand. Symptoms related to gait or balance, for example, are particularly hard to treat (Sethi & Sethi, 2008). And even if initially treatable, PD symptoms lose therapeutic responsiveness when the disease progresses as ongoing dopaminergic and non-dopaminergic cell loss limits the effectiveness of dopaminergic medication (Sethi & Sethi, 2008). Consequences may range from severe fluctuations of motor symptoms to manifest *on-off-phenomena* when medication effects wear off suddenly, cause hyperkinetic states or may even lead to the emergence of new motor phenomena, such as *freezing*.

Motor automaticity impairments and freezing

Freezing is defined as the "brief, episodic absence or marked reduction of forward progression despite the intention to move" (Nutt et al., 2011). It reflects a short period of *ineffective* movement (Vercruysse et al., 2014), often with high-frequent (2–6 Hz) lower extremity trembling in place without progression (Moore et al., 2008). Most commonly, freezing episodes occur during gait (freezing of gait (FoG)), for example, when initiating movement, turning, or at narrow doorways (Jacobs et al., 2009; Nutt et al., 2011; Weiss et al., 2019). However, PD patients are susceptible to freezing during all kinds of automatic, repetitive movements (Vandenbossche et al., 2013; Vercruysse, Spildooren, et al., 2014), such as speech (Moreau et al., 2007), swallowing (Maetzler et al., 2016), or upper limb movements like typing and toothbrushing (Fahn, 1995). Sometimes freezing behavior can be alleviated through sensory stimuli, i.e., auditory or visual input; at other times, this sensory input may even evoke freezing (Hallett, 2008).

Often, freezing emerges after a prodromal sequence of accumulating motor deficits. This *transition phase* is characterized by an irregular movement frequency, disordered temporal control and inter-limb coordination, and an incremental decrease in stride length or movement amplitude (Nieuwboer et al., 2009; Nutt et al., 2011; Stegemöller et al., 2009; Vercruysse, Spildooren, et al.,

2014) but its presentation may vary. Such heterogeneity complicates the search for the one explanation grasping all aspects of it.

Basal ganglia (BG) function in health and Parkinsonism

To investigate freezing pathology, it is important to first understand how the brain controls *regular* voluntary movement. Ultimately, motor output is generated through the primary motor cortex (M1). But other cortical areas, such as the (dorsal) premotor (PMd) and supplemental motor area (SMA), are involved to plan and prepare for, initiate and coordinate movement (Arai et al., 2012). Activity within these cortical structures, particularly M1, is regulated on subcortical level through the BG loop.

The BG are a highly interconnected network of subcortical nuclei involved in different cognitive functions, such as motor control, but also associative learning, planning, working memory, and emotion (Obeso et al., 2008). They include the neostriatum (caudate nucleus and putamen), the external and internal pallidum (GPe, GPi), the subthalamic nucleus (STN), and the substantia nigra (pars reticulata (SNr), pars compacta (SNc)) and work together in segregated loops involving thalamic and cortical areas (Galvan & Wichmann, 2008). Glutamatergic efferents from the motor cortices or thalamus are projected to the striatum and STN, which then transfer the information to the output nuclei (GPi and SNr) via two separate pathways: a direct, movement facilitating pathway (monosynaptic) and an indirect, movement suppressing pathway through the intercalated GPe and STN (Galvan & Wichmann, 2008). From there, basal ganglia output is transmitted back to the cerebral cortex via the thalamus (ventral anterior (VA) and ventrolateral nuclei (VL)) or – to a lesser extent – to other thalamic nuclei (intralaminar centromedian (CM), parafascicular (Pf)) and brainstem structures (superior colliculi, pedunculopontine nucleus (PPN), reticular formation). The striatum further receives *dopaminergic* input from the SNc to its medium spiny neurons (MSNs), which also receive those corticostriatal projections. Hence, the dopaminergic inputs to the striatum act as a *gate keeper* of corticostriatal transmission: dopamine release facilitates corticostriatal

transmission onto direct pathway-MSNs and causes reduced transmission along the direct pathway, thereby facilitating movement.

In PD, however, the loss of nigrostriatal dopamine shifts the balance between both pathways towards increases in indirect pathway activity with increased STN/GPi activity, resulting in increased inhibition of thalamocortical interactions and less activation of the cerebral cortex (Galvan & Wichmann, 2008) (see Fig. 1). This results in typical PD motor symptoms.

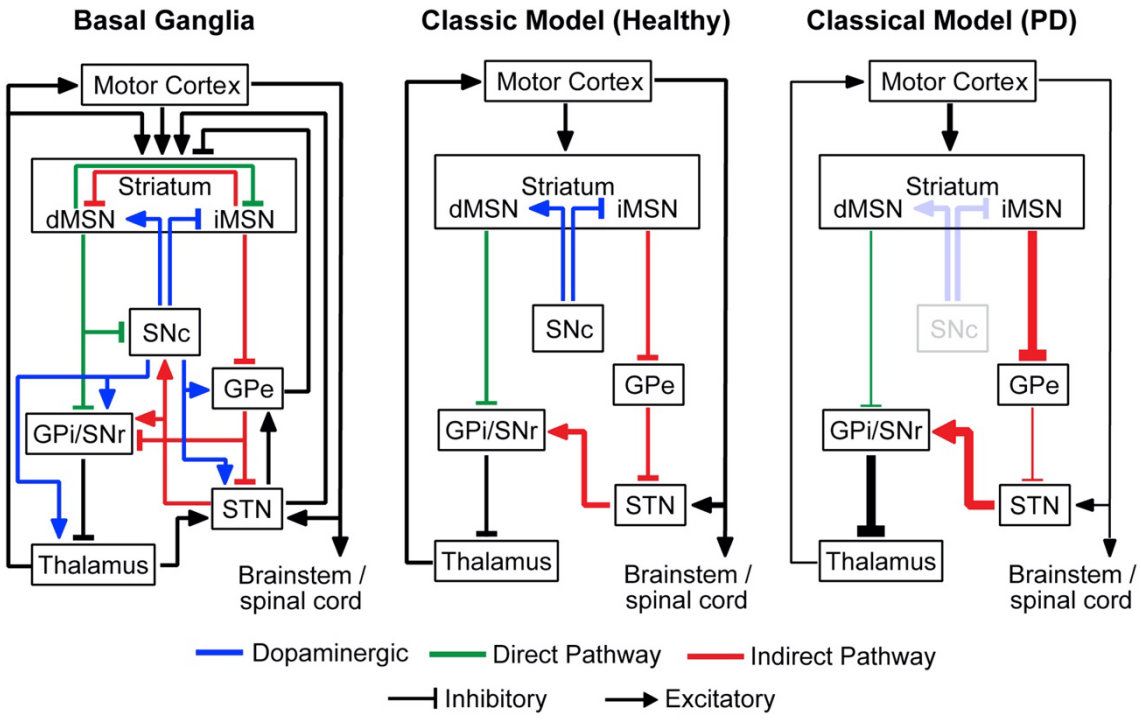

Figure 1: Basal ganglia function in health and Parkinson's disease (PD)
Left. Basal ganglia connectivity**. Center.** Simplification, highlighting the role of dopamine on direct and indirect pathway activity and motor output. In health, dopamine (blue) from the SNc to the striatum activates direct pathway (green) and inhibits indirect pathway (red) MSNs. This effect decreases GPi output, releasing inhibition on the thalamus and cortex and promoting movement. **Right.** In hypokinetic states, such as PD, the loss of SNc dopamine causes hypoactivity of the direct pathway and hyperactivity of the indirect pathway that leads to excessive GPi output. As a result, over-inhibition of the thalamus and cortex leads to a suppression of movement (figure and legend from (McGregor & Nelson, 2019), licensed from Elsevier for re-use in this thesis).

The pathophysiology of freezing

Contrary to other, more continuous gait impairing PD motor symptoms, such as bradykinesia or rigidity, freezing is a *paroxysmal* phenomenon (Nutt et al., 2011). It occurs periodically on top of the existing BG dysfunction and causes a temporary motor breakdown within an already hypokinetic state. The exact neurophysiologic pathomechanisms underlying freezing are not well understood. Typically, the execution of highly automated movements requires minimal cortical effort for healthy individuals, and, even with little to no attentional control, motor performance is very resistant to interference (Wu et al., 2015).

In PD, however, the progressive loss of dopaminergic neurons impairs connectivity, particularly frontostriatal connectivity for internal movement generation and motor automaticity (Hallett, 2008; Scholten, Klotz, et al., 2016; Vercruysse, Spildooren, et al., 2014; Wu & Hallett, 2008). Neural coding of movement also becomes less efficient, with more activation outside M1 and less connectivity within associated cortical motor areas (Wu et al., 2010). To some degree, these deficits can be compensated for through altered corticostriatal functional transmission and increased attentional control (Fling et al., 2014; Vercruysse, Spildooren, et al., 2014; Wu et al., 2015). However, the excessive reliance on the cognitive striatum to perform movements attentionally, which would otherwise be performed automatically (Redgrave et al., 2010), puts the neural system at high risk for interference (Helmich et al., 2010), and with it, the emergence of freezing (Lewis & Barker, 2009; Shine, Matar, Ward, Frank, et al., 2013).

To date, several models have been suggested to explain freezing, mainly FoG (Nieuwboer & Giladi, 2013). However, none of them covers and explains the various clinical manifestations of freezing sufficiently (Fig. 2). Lewis & Shine (2016) postulated that freezing might result from a transient increase in inhibitory BG output. Based on their updated interference model, the compensatory over-reliance on the cognitive striatum for effective movement production and the competition for limited processing resources causes transient bouts of striatal dysfunction and STN overactivity. The combination of both causes increased GPi inhibitory activity, which in turn renders the PPN, a crucial brainstem structure to

produce gait, unable to signal to the spinal cord. Such transient increase in inhibitory BG output impaires leg coordination with trembling in place and freezing (Lewis & Shine, 2016; Shine, Matar, Ward, Frank, et al., 2013). Similarly, motor breakdown in ULF probably also occurs due to a transmission failure within the BG motor circuit (Hallett, 2008). Here, signaling back to M1, the final output stage for limb movements, would be impaired and freezing would result from low cortical excitability within M1, then insufficient to signal down to the corresponding muscles to perform effective movement.

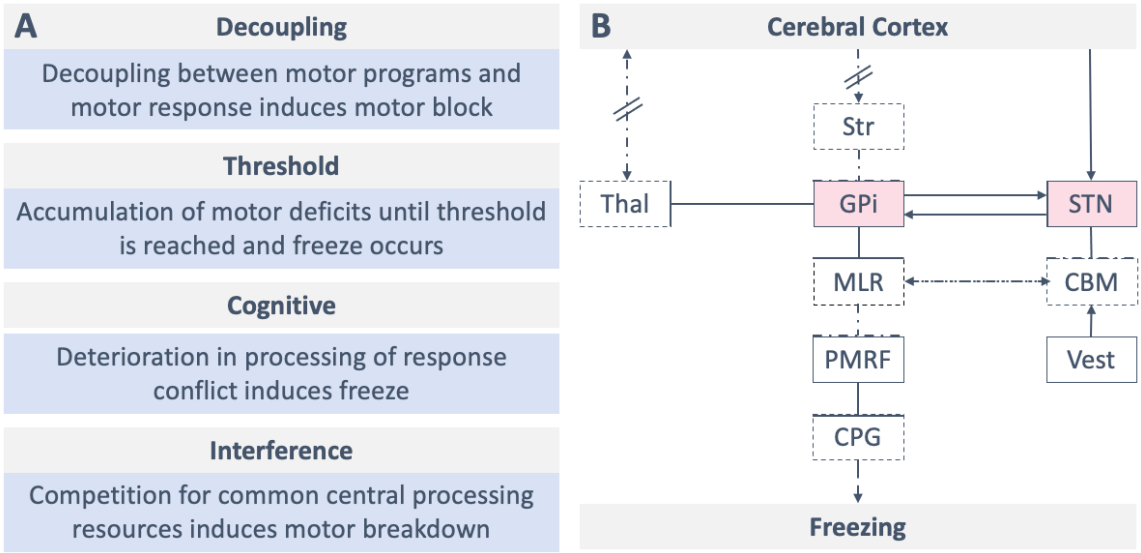

Figure 2: Theoretical models of Freezing of Gait (FoG)
A. Overview of four possible models explaining different aspects of FoG. Figure modified after Nieuwboer et al., 2009, see publication for comprehensive review. **B**. Current model as suggested by Lewis & Shine, 2016. In their model, freezing results from striatal dysfunction and subsequently increased inhibitory BG outflow. Within the BG, the GPi and SNr provide the largest inhibitory afferences to the thalamus and gait-controlling brainstem structures. Accordingly, overactivity within these nuclei will manifest as akinesia or freezing. Both, GPi and SNr receive inhibitory input from the striatum to modulate their inhibitory activity. The striatum, in turn, receives excitatory input from cortical structures, mainly frontal premotor areas (PMC, SMA). Dysfunction within the frontostriatal and corticothalamic pathways and the subsequent periodic loss of these striatal afferents would thus lead to transient unopposed motor inhibition. Typically, the probability to evoke freezing increases with dual-task performance, anxiety, irregular cueing, and perceptual obstacles. These observations may cause processing conflicts and are also implemented in the model. Dysfunctional integration of these external triggers is processed through a common pathway, the STN, which provides strong excitatory input to the GPi/SNr. In addition to transient striatal dysfunction, STN overactivity further increases inhibitory GPi capacity causing the emergence and

manifestation of 5-7 Hz oscillations between the two nuclei (STN and GPi). Ultimately, the consecutive propagation of those oscillations through the PPN/MLR to the spinal cord and lower limb musculature is believed to manifest as impaired lower leg coordination and the characteristic 5-7 Hz trembling in place during freezing. CBM = cerebellum, CPG = central pattern generators, GPi = globus pallidus internus, MLR = mesencephalic locomotor region, PMRF = pontomedullary reticular formation, PPN = peduncolopontine nucleus, STN = subthalamic nucleus, Thal = thalamus, Vest = vestibular nucleus. Arrows denote excitatory input, vertical lines (solid or broken) show inhibitory inputs.

Driving voluntary muscle activity: the cortico-muscular system

How does cortical activity translate to voluntary movement? During voluntary contraction, motor units in the corresponding muscles receive several descending volleys via the corticospinal tract and synchronize their discharge patterns at different frequencies (Brown, 2000). These frequencies do not necessarily represent the discharge rate of an individual motor unit. They instead reflect coinciding bursts of a motor unit *population* at a frequency determined by the rhythmicity of the descending activity (Brown, 2000). This descending activity must not be of cortical origin, particularly at lower frequencies (2 Hz, 5–12 Hz). But there are frequencies, for example, in the beta range (13–30 Hz), which are driven by the primary motor cortex (Brown, 2000). In general, the synchronous firing of neuronal populations leads to more substantial synaptic input to recruit motoneurons (Murthy and Fetz, 1994). However, more so than plain synchronicity, it is the *frequency* in which these neurons oscillate, determining the *functional* state of the respective cortical domain. So-called *beta oscillations* appear to play a significant role in voluntary movement (Jenkinson & Brown, 2011) and are abnormal in PD patients (Brittain & Brown, 2014). Cortical beta activity over the sensorimotor cortex indicates a relatively stable, immutable state of the motor system, maintaining the status quo (Brown, 2000). So typically, cortical beta activity is high during static motor control (Brittain & Brown, 2014) and desynchronizes just before (= Event-Related Desynchronization (ERD)) and during single, voluntary movements, then reflecting a state of increased cortical excitability (Pfurtscheller & Lopes da Silva, 1999). In short, during active motor states, such as just prior to and during motor performance, beta band amplitude decreases and movement is facilitated (= movement related beta decrease,

MRBD). On the other hand, beta amplitude increases above baseline levels after movement cessation (= post-movement beta rebound, PMBR).

We know from EEG experiments that, to produce rhythmic, repetitive movement such as gait or finger tapping, this motor cortical beta-band oscillatory activity is modulated along the movement cycle. During finger tapping, for example, a transient beta power decrease over the sensorimotor area precedes each tap to facilitate movement and is followed by a phase of resynchronization to reduce motor output (Fischer et al., 2018; Seeber et al., 2016). This alternating pattern of beta-band desynchronization (= motor facilitation) and resynchronization (= motor inhibition) allows the generation of rhythmic or cyclic motor patterns and has been shown consistently for the beta activity of the STN and M1 in repetitive upper and lower limbs movements (Androulidakis et al., 2008; Fischer et al., 2018; Seeber et al., 2016; Toledo et al., 2014). Failure of phasic beta modulation, on the other hand, leads to motor deterioration and increases susceptibility to freezing; upper limb freezing induced through a simple finger tapping paradigm, for example, has been associated with impaired beta-band modulation over the sensorimotor area (Scholten, Govindan, et al., 2016; Scholten, Klotz, et al., 2016). Recently, it has been shown that this beta band modulation failure preceded an ULF episode up to three single taps before its actual onset (*transition phase*) (Scholten et al., 2020) corroborating the observation of a gradual deterioration of motor performance prior to actual freezing.

Investigation of the motor system using Transcranial Magnetic Stimulation (TMS)

One tool to assess motor system functionality from cortex to muscle activation is TMS. In contrast to the EEG signal, which reflects current changes in dendrites of *all*, rather *widespread* pyramidal cells, TMS provides a more precise tool to measure cortical excitability directly in corticospinal neurons with similar high temporal resolution (Chen & Hallett, 1999). Here, we used TMS-evoked MEPs as indices of motor cortex excitability and probed movement amplitude-related modulation *during* successful finger tapping, in the transition phase, and during ULF.

TMS is a non-invasive, safe tool to measure *in vivo* functional and structural integrity of M1 (Burciu & Vaillancourt, 2018). A short magnetic pulse (~100µs) produces a perpendicular electric field of up to 2T (Hallett, 2007). This electrical field causes parallel, tangential current flow in the brain (Hallett, 2007) and (if applied using a focal "figure-of-8" magnetic coil) excites local cortical cells within a diameter of few millimeters (Romero et al., 2019). TMS activates these cells mostly transsynaptically through excitatory interneurons (indirect; I–waves), even though higher stimulus intensities may excite neurons directly (direct; D–waves) (Cantello, 2002). More precisely, a TMS pulse activates small *interneuron networks* and their (excitatory or inhibitory) synaptic interactions with each other (Rossini et al., 2015). This activation ultimately leads to highly synchronized depolarization of a superficial, local population of neurons, causing a series of descending corticospinal volleys (mainly I-waves) at 1.5ms intervals (Di Lazzaro & Ziemann, 2013). Applied over M1, i.e. the hand area, this cortical activation can be seen as a muscular twitch in the corresponding target muscle generating a stereotyped motor response, the motor evoked potential (MEP) (Hallett, 2007). The overall excitability of individual neurons and synaptic connections in the motor system and their local density is reflected by the stimulation threshold (Hallett, 2007). Motor threshold describes the minimal stimulus intensity needed to elicit a MEP and depends on the activity of the underlying neuronal population (e.g., rest vs. active) (Rothwell et al., 1999). When the target muscle and thus the primary motor cortex is already active, lower stimulus intensities may be required to evoke MEPs compared to rest – consequently, the active motor threshold may be lower than the resting motor threshold.

TMS-evoked MEPs are stereotyped motor responses (Di Lazzaro & Ziemann, 2013) arising after stimulation with an target muscle-specific latency of a few milliseconds. To investigate MEPs systematically during voluntary movement, MEPs are recorded over the target muscle via surface electromyography (EMG). Then, they can be detected and discriminated from the background EMG based on MEP specific shape and latency (Fig. 3). In small hand muscles, this cortico-muscular latency is, for example, between 20–40ms after a stimulus in healthy adults (Rothwell et al., 1999).

MEPs thus represent a useful indicator of integrity and excitability of the descending motor pathways, including the conductivity of interneurons, corticospinal pathways, and spinal interneurons. A characteristic feature of MEP amplitudes is their dependence on synaptic connections and their variability depending on state-dependent cortical excitability (Hallett, 2007; Siebner et al., 2009). MEP size reflects the absolute number and synchronization of motoneurons discharging in response to a TMS pulse (Cantello, 2002) and varies as a function of stimulus intensity and background muscular activity (Hallett, 2007). It is essential that it is not an increase in the number of stimulated axons but altered *responsiveness* to TMS stimulation, enlarging MEPs. This increase in MEP size is not only achieved through subthreshold increase of excitability in the *spinal* motoneuron pool but also the modulation of *cortical* excitability levels (Di Lazzaro et al., 1999). One of the most well-known examples of state-dependent cortical excitability and its effects on TMS responsiveness is the facilitation of MEPs at different levels of voluntary contraction. Typically, produced MEPs are larger when the target muscle is precontracted at the time of stimulation (Hess et al., 1987).

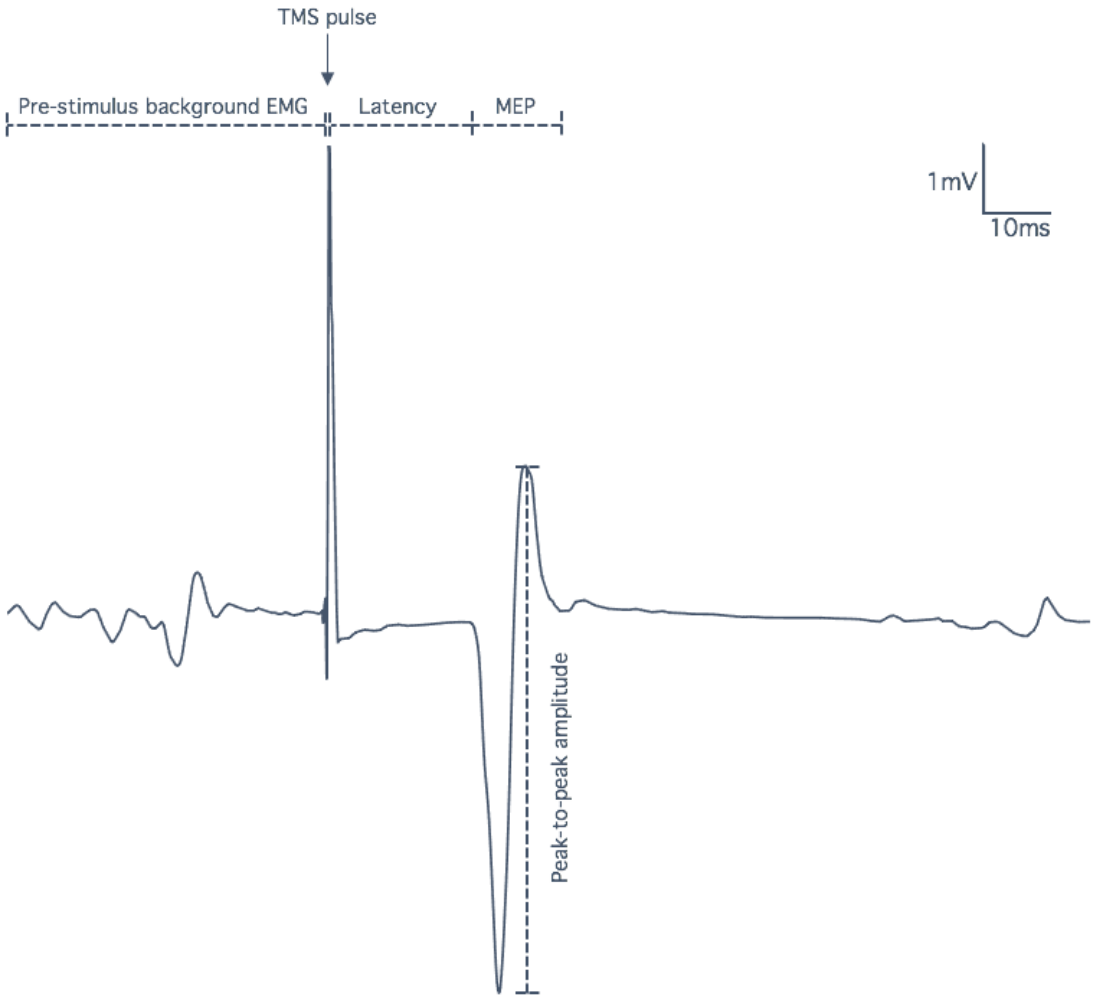

Figure 3: Example of a TMS-evoked motor evoked potential (MEP)
MEPs are stereotyped motor responses caused by single-pulse magnetic stimulation. Recorded via surface electromyography (EMG) over the right first dorsal interosseus muscle (FDI), they occur within with a target muscle-specific latency, in small hand muscles about 20-40ms after a pulse. MEPs can be detected and discriminated from the background EMG based on their specific shape and latency. In the present experiment, peak-to-peak amplitude was used as an index of cortical excitability (own data; see text for details).

Muscular activity and cortical excitability

In general, applying single-pulse TMS during active movement – for example during freezing – is difficult, as muscular activity before or at the time of stimulation may influence TMS effectivity (Hess et al., 1987; Nyi et al., 1998; Siebner et al., 2009). However, the exact relationship between the relative level of activity in distinct neuronal populations and their responsiveness to stimulation

is unclear. In their comprehensive review, (Siebner et al., 2009) strongly cautioned against the '*tempting conclusion*' that the activity of neurons automatically determines their excitability to TMS. Typically, neurons are most excitable when their membrane potential is just below threshold, but they are not yet actively discharging (so-called '*subliminal fringe*'; Denny-Brown & Sherrington, 1928 as reviewed by (Nyi et al., 1998)). High discharge rates, on the other hand, lead to reduced excitability, as previously active neurons enter a refractory period and are not excitable for some time. The complexity of the relationship between the *active* motor cortex and cortical excitability was further illustrated by Matthews (1999) (as reviewed by (Siebner et al., 2009)), who highlighted the different implications of neuronal activity on its responsiveness to synaptic input on a single-cell level. Here, the author showed that *smaller* inputs have a higher probability of increasing a stimulated *active* neuron's firing rate. In contrast, higher stimulus intensities are less effective when neuronal activity is already high, higher intensities only appear to be more effective when applied over *resting* motoneurons (Siebner et al., 2009). Siebner et al. (2009) thus concluded from this report that – if all other factors are controlled – the effect of small TMS pulses may be facilitated when the cortex is *active* and more suppressed with larger TMS pulses.

Now to investigate freezing behavior, TMS must be applied in an *active* movement paradigm which, on the one hand provokes freezing, whilst at the other hand, ensures similar levels of muscular activation throughout; also, pulses must be delivered with sufficient intensities to even produce MEPs, whilst at the same time not inducing any plasticity effects. One such experimental design combining these requirements is a simple finger-tapping task used to induce ULF (see methods section).

Common experimental approaches in FoG and ULF research

Contrary to FoG, which is notoriously difficult to evoke in laboratory settings (Nieuwboer et al., 2009), ULF can be induced through a simple finger-tapping paradigm (Nieuwboer et al., 2009; Vercruysse, Gilat, et al., 2014). Although the

production of gait is much more complex and involves much more widespread cortical activity than repetitive finger movements (Lewis & Shine, 2016; Shine, Matar, Ward, Bolitho, et al., 2013), several features of FoG and ULF are quite similar. Indeed, shared kinematic features between FoG and ULF suggest some common underlying pathophysiology (Nutt et al., 2011; Vercruysse, Spildooren, et al., 2014; Weiss et al., 2019). For example, both phenomena emerge during fast, repetitive, small-amplitude movements (Wu et al., 2015), and both frequently occur after a prodromal phase with an incremental reduction of amplitude (Fig. 4). In fact, patients experiencing FoG have a greater risk also to experience ULF at some point (Barbe et al., 2014; Nieuwboer et al., 2009; Vercruysse, Spildooren, et al., 2014).

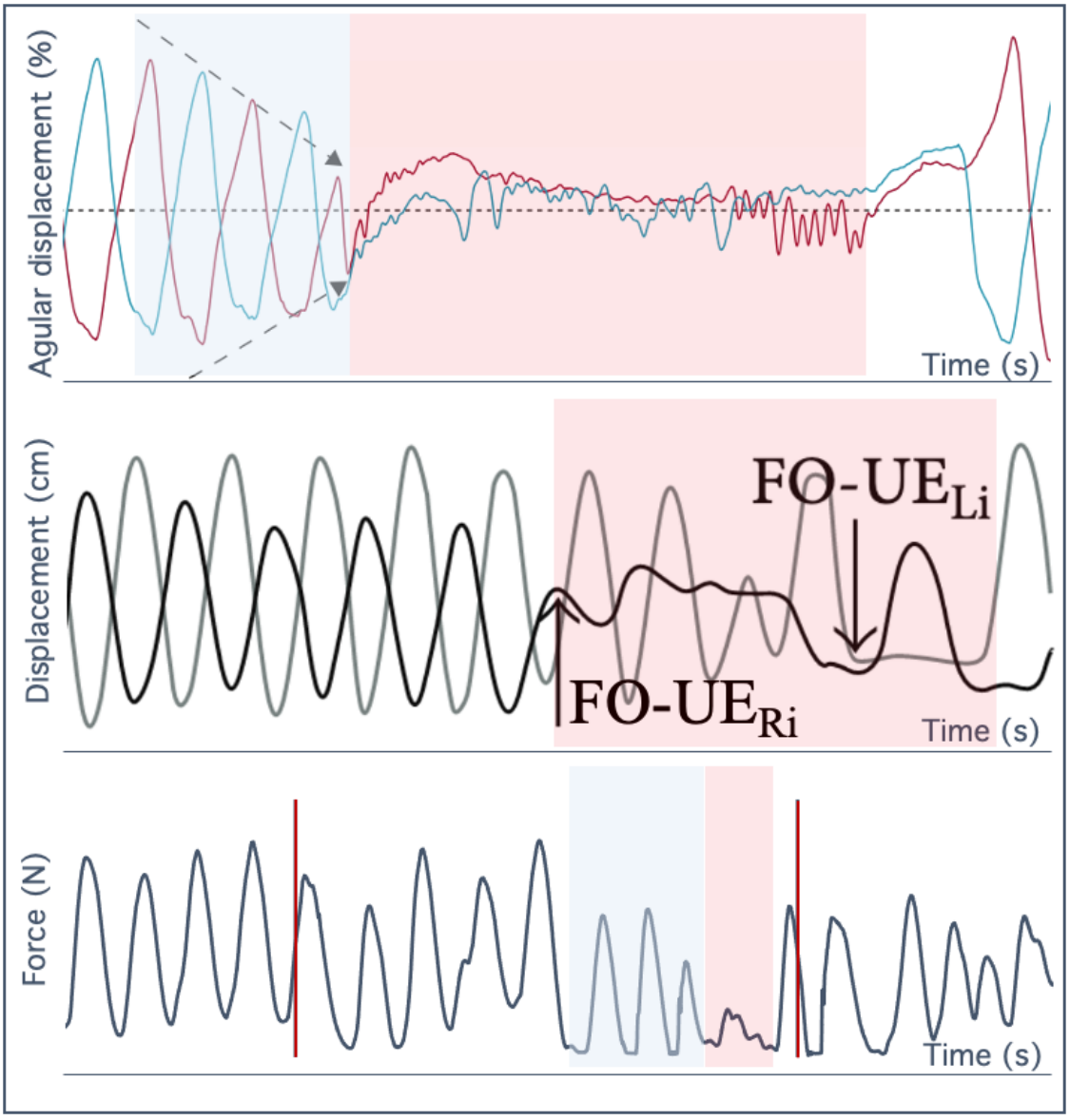

Figure 4: Illustration of different freezing phenomena
Top: Freezing episode (red) with knee trembling during gait (FoG) with transition phase of decreasing step length prior to freezing (blue); data from (Nutt et al., 2011), angular displacement (% maximum knee angle) measured with a camera. **Middle:** Freezing in a bilateral hand movement task without defined transition (black/grey lines show left/right hand); data from (Williams et al., 2013), FO-UE$_{Li/Re}$ = freezing of left/right upper extremity. **Bottom:** Own data from unilateral finger tapping task with isometric contractions. Transition phase (blue) followed by freezing (red) **Note**: Simplified axes to improve readability.

Generation of new hypotheses and present experiment

In summary, ULF ultimately represents an involuntary breakdown of effective motor output (Scholten, Govindan, et al., 2016; Scholten, Klotz, et al., 2016;

Weiss et al., 2019) and differs from voluntary motor breaks through the preserved underlying movement *intention* (movement planning, preparation). As the final output stage of the motor system, M1 plays a direct role in driving upper and lower limb muscles during cyclic movements (Petersen et al., 2001; Sidhu et al., 2012) and its functionality relies heavily on afferences from cortical and subcortical structures (Bestmann & Krakauer, 2015). In PD, however, the progressive loss of dopaminergic neurons impairs connectivity between these areas, particularly frontostriatal connectivity for internal movement generation and motor automaticity (Scholten, Klotz, et al., 2016; Wu & Hallett, 2008). Still, little is known about the excitative states of M1 during regular, repetitive movement and particularly during freezing.

From combined EEG-TMS (electroencephalography; transcranial magnetic stimulation) studies we know that beta-band desynchronization over the sensorimotor area relates to increased M1 excitability and thus greater MEP size (Ferreri et al., 2014; Hussain et al., 2019). We thus expected higher excitability (as reflected through greater MEP sizes) in the acceleration phase of tapping (= contraction) and lower excitability (smaller MEP sizes) at deceleration (= relaxation). Secondly, we hypothesized that M1 excitability is reduced during ULF compared to the acceleration phase of regular tapping. Here, we assumed that despite the preserved movement *intention* at both instances, *ineffective* motor output during freezing would be associated with reduced M1 excitability compared to *effectual* motor output during regular tapping. Third, according to previous findings from EEG studies on ULF using the same experimental task (Scholten et al., 2020), we expected that the observed modulation of cortical excitability during regular tapping would be altered in the transition phase.

2. Methods

Subjects

Eleven (n = 11) right-handed PD patients (4 females, 36.4%) participated in the study. Patients were recruited based on the following criteria: (*i*) a clinical diagnosis of akinetic-rigid PD requiring daily intake of dopaminergic medication, (*ii*) clinically verified FoG (Snijders et al., 2012), and (*iii*) a Mini Mental State Examination (MMSE) Score > 22. Conditions objecting to TMS safety guidelines, such as metallic implants, deep brain stimulators, or risk of seizure (Rossini et al., 2008), a history of other neurologic or psychiatric diseases affecting motor performance and left-handedness led to exclusion from the study. In addition to the experimental task, all patients filled in the following questionnaires: (*i*) *Beck's Depression Inventory* (BDI), (*ii*) *Edinburgh Handedness Inventory* (EDH), and (*iii*) parts I-III of the *Unified Parkinson's Disease Rating Scale* (MDS-UPDRS), consisting of two patient questionnaires on *Non-Motor Aspects of Experiences of Daily Living* (UPDRS-I, nM-EDL), and *Motor Aspects of Experiences of Daily Living* (UPDRS-II, M-EDL), as well as the clinician instructed *Motor Examination* section (UPDRS-III) in OFF medication state.

All patients provided written informed consent prior to participation. The study protocol followed the Declaration of Helsinki ("World Medical Association Declaration of Helsinki," 2013) and was approved by the local ethics committee of the University Hospital Tübingen (Baden-Württemberg, Germany; 916/2018BO1).

Preparatory measures and experimental task

The experiment was conducted in a single session after the over-night withdrawal of oral dopaminergic medication to increase the likelihood of ULF. Patients with non-oral, continuous dopamine supply (levodopa-carbidopa intestinal gel; subcutaneous apomorphine infusion) paused infusion thirty minutes before the experiment. A notable worsening of motor function assessed using the MDS-UPDRS-III OFF score) confirmed the successful dopaminergic withdrawal (Martínez-Martín et al., 2015).

Preparatory measures included (*i*) mounting of the EMG electrodes, (*ii*) identification of the individual motor hotspot, and (*iii*) determination of the individual active motor threshold (aMT) following the established procedure (Rothwell et al., 1999). All TMS pulses were then delivered at 120% aMT stimulus intensity. 2-channel EMG was recorded over the right first dorsal interosseus muscle (FDI) in belly-tendon montage (5kHz sampling rate, 0.16 Hz–1.25 kHz bandpass filter) via a 24-bit amplifier (NeurOne Tesla with Digital-Out Option, Bittium, Finland). Preparation additionally included the mounting of a TMS-compatible 64-channel EEG (EasyCap GmbH, Germany) and a 2-minute resting-state EEG recording. The obtained EEG data, however, was not part of this experiment and thus not analyzed.

Patients sat in a comfortable chair with their right arm on an armrest. The right index finger was placed on a force sensor attached to a stable metal rack to reduce motion-induced swinging. The forearm or wrist was supported with a cushion if needed. Patients were instructed to *tap as fast and accurately as possible* within a predefined force range (0–2 N) without displacing the finger (*isometric finger tapping*; Fig. 5). A computer screen in front of the chair in approximately 1m distance provided real-time visual feedback on tapping accuracy and muscular force. A 20s tapping block started with the fade-out of the word "*Pause*" in the screen center. The reappearance of the word "*Pause*" indicated a 10s break.

Overall, one session included a short individual introduction and practice followed by >10 episodes of continuous, self-paced finger tapping. After completion of the minimum ten episodes, all patients received a break of several minutes. Depending on their fatigue and cooperation level, we encouraged the patients to continue tapping in the same fashion to increase the amount of data. If a patient did not show any visible signs of freezing, we used a dual-task (verbal fluency task or arithmetic) to increase cognitive load and thus the likelihood of freezing (Scholten, Govindan, et al., 2016). All dual-tasks were performed speechless to avoid muscle artifacts in the EEG signal and assessed during the break episodes.

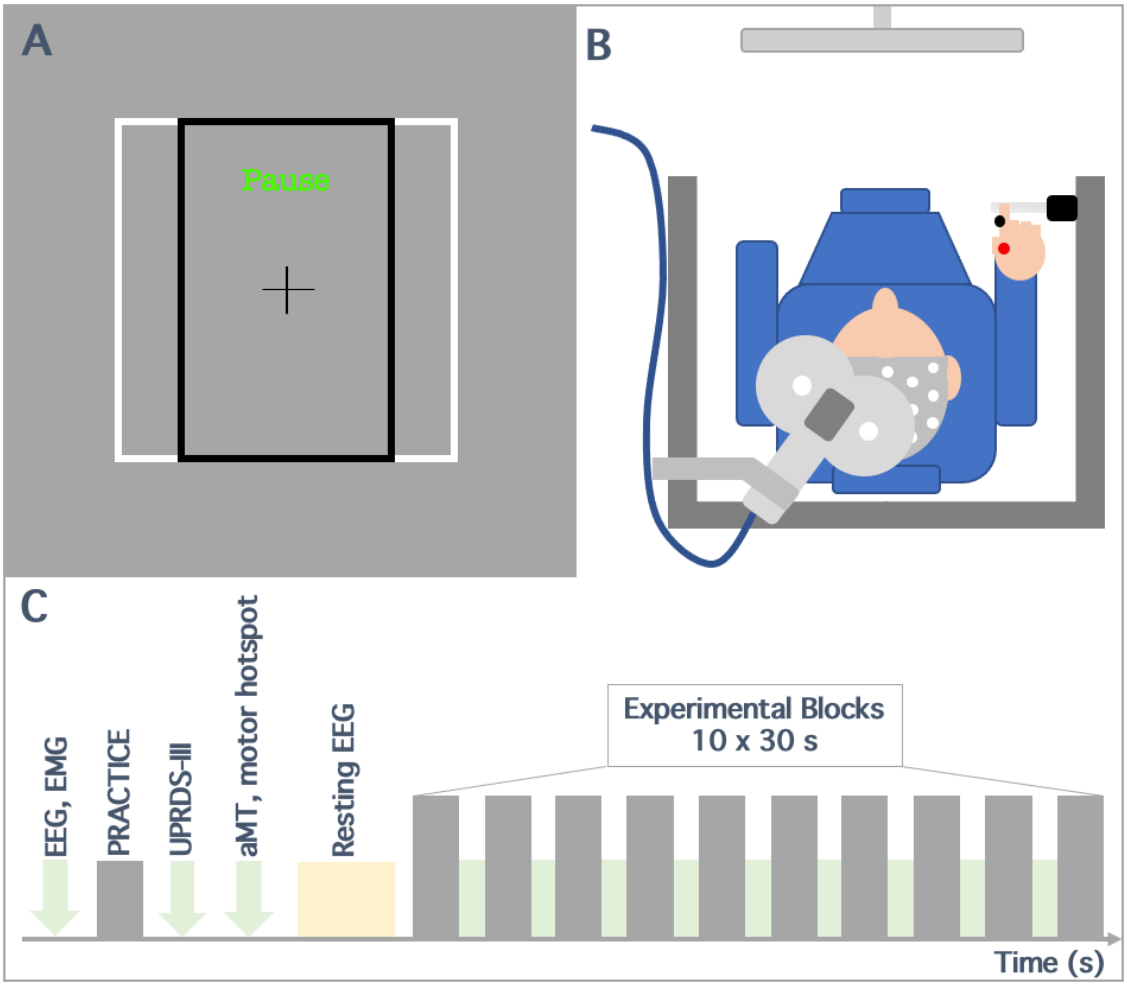

Figure 5: Illustration of the experimental setup and protocol
A Screenshot of the computer screen used to provide visual feedback on tap force. Patients were instructed to continuously increase pressure with their right index finger until the desired value of 2N and release pressure to 0N without losing touch with the force sensor. Visual feedback was provided through a black rectangle, which would extend, and collapse based on the force exerted. The alignment of the black and white square indicated a maximum of 2N, a collapsed black rectangle in the center of the screen stated no force (0N). The word "Pause" appeared during tapping breaks for a duration of 10s and instructed the patient to stop tapping and take a break. Fade-out of the word "Pause" started a new episode of 20s finger tapping. **B** Bird's eye view of the experimental setup. The force sensor and TMS coil were attached to a metal rack around the chair and then to a computer (not shown). EMG (belly-tendon montage over the right FDI) and EEG (TMS-compatible) were attached to an amplifier (not shown). **C** Illustration of the experimental protocol. First, EEG and EMG were mounted, and patients made themselves familiar with the equipment and experimental task in a short training session. A trained clinician conducted the MDS-UPDRS-III to confirm dopamine withdrawal-related worsening of motor performance. Subsequently, aMT and motor hotspot were determined (see text for more details), and a 2-minute resting EEG (yellow) was recorded (EEG data not part of this study). After that preparatory procedure, (at least) ten 20s tapping episodes (grey) with 10s breaks (green) between episodes were collected

for each patient. EEG = electroencephalography, EMG = electromyography, FDI = first dorsal interosseus muscle, MDS-UPDRS-III = Movement Disorders Society Unified Parkinson's Disease Rating Scale part III, N = Newton, TMS = transcranial magnetic stimulation).

Active motor threshold (aMT) determination and motor hotspot search

Patients pressed their right index finger on the force sensor at around 10% of maximum force (visual feedback provided) to ensure isometric FDI contraction. The minimal stimulus intensity producing MEPs of ≥200μV in at least 5/10 trials was set as aMT (Rothwell et al., 1999). TMS pulses were delivered at 120% aMT stimulus intensity. The position to produce consistent MEPs in the target muscle was identified as the motor hotspot. This cortical area was typically located 1–2cm anterior and 5–6cm lateral to the vertex on the contralateral hemisphere. The coordinates over the scalp were stored using NeuroNavigation (Localite GmbH, Germany) to allow for stable coil position and precise stimulation throughout the experiment.

Adaptive TMS triggering

TMS was applied over the left M1 using a figure-of-eight Magstim D70 remote coil (70mm winding, Magstim Ltd., UK). The coil was held over the motor hotspot in anterior-posterior orientation with the handle pointing backward and ~45° away from the midline (Ziemann et al., 1997). Single TMS pulses were triggered automatically based on a Simulink Real-Time model (R2016a, MathWorks Inc., USA). An algorithm delivered a pulse whenever the criteria were met for either ascending flank (=contraction) or descending flank (=relaxation) of the kinematic signal (see Fig. 6). The details of this approach are described elsewhere (Bergmann et al., 2019; Zrenner et al., 2018). In short, data was read, sampled down, and filtered. The signal was forward predicted by an autoregressive model (Yule-Walker, order 30), and "time zero" (="now") was determined. "Time zero" was classified as either negative-to-positive crossing (=rising flank: *ascending*) or positive-to-negative crossing (=falling flank: *descending*). The current kinematic phase was then compared to the criterion targeted (condition=ascending or

descending). Single TMS pulses were delivered if the current kinematic signal met the phase criteria of the current condition. The average stimulation delay due to real-time processing was negligible (~4.5ms). A 2.5s interstimulus interval was maintained to prevent plasticity effects due to excessive repetitive stimulation.

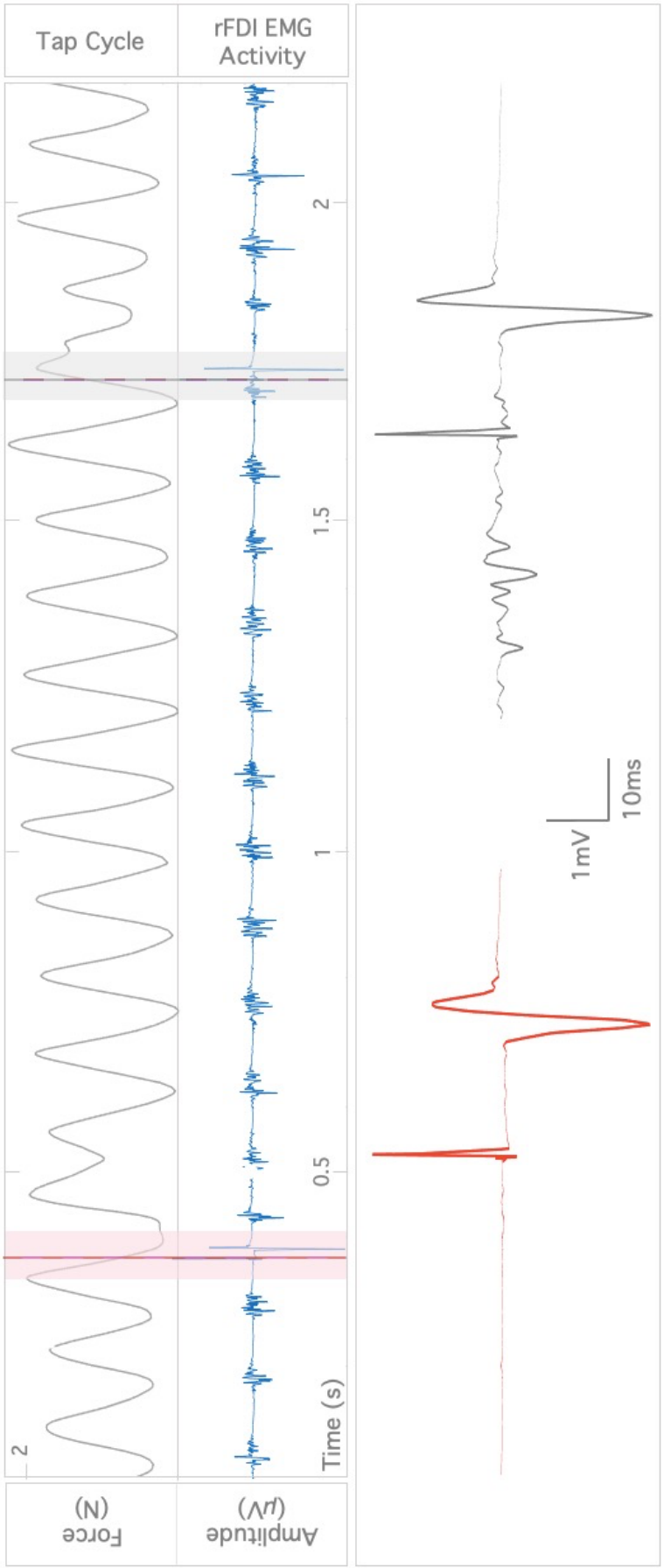
Tap Cycle
rFDI EMG Activity
Force (N)
Amplitude (μV)
Time (s)
2
0.5
1
1.5
2
1mV
10ms

Figure 6: Tap cycle with EMG and MEP discrimination (single patient)
Top. Kinematic signal and EMG activity during tapping in a single patient. **Bottom**. Magnification of the triggered MEPs and preceding EMG activity (grey). Note. The red lines indicate a single TMS pulse.

Data preprocessing and offline analysis

Detection of freezing episodes (ULF)

Prior to preprocessing, we manually searched the kinematic signal for freezing episodes. TMS is known to disrupt motor performance if applied during movement even in healthy individuals (Levit-Binnun et al., 2007). We thus carefully discriminated between true ULF and common TMS artifacts (see appendix Fig. A for examples). ULF was detected visually based on the following well-established criteria (Vercruysse et al., 2014): (*i*) irregular, hastened frequency, (*ii*) amplitude reduction >50%, (*iii*) Freezing Index (FI) > 1 (Moore et al., 2008), and (*iv*) duration ≥0.5s (see Fig. 7).

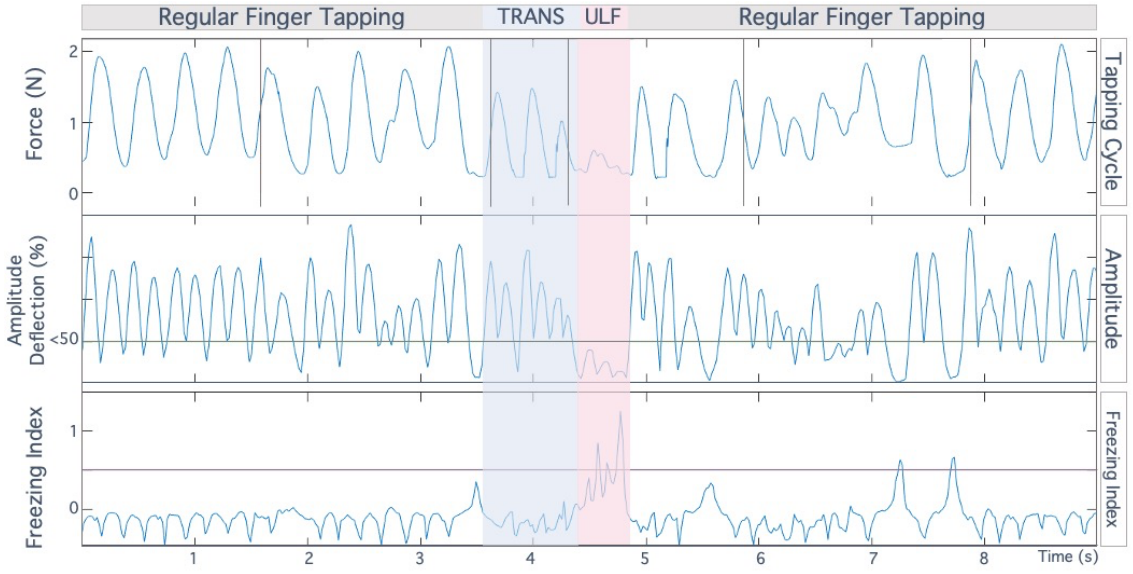

Figure 7: Tapping cycle during regular tapping, in the transition phase (blue), and during upper limb freezing (ULF, red)
Top: Kinematic (force) signal (N). **Middle**: Amplitude deflection (%). **Bottom**: Freezing index (FI). Increased, irregular tapping, amplitude deflection > 50%, a FI > 1, and a duration ≥0.5s defined ULF episodes (Vercruysse et al., 2014). MEPs elicited during ULF were classified as ULF. MEPs were classified as TRANS if they were elicited in the transition phase (≤1.2s (~3 taps) before ULF) and further divided into ascending and

descending, depending on the kinematic signal at stimulation. All remaining MEPs not assigned to either ULF or TRANS were classified as regular tapping (rT) and divided into ascending or descending based on the kinematic signal at the time of stimulation. **Note**: Grey vertical lines show examples of potential TMS trigger points within the tapping cycle to illustrate the experimental design; the actual interstimulus interval in the experiment was >2.5s. See methods for details.

Data segmentation

Preprocessing was done with FieldTrip open-source toolbox (Oostenveld et al., 2011) and customized MATLAB® scripts (Mathworks Ltd, USA, R2017a). First, we segmented the biomechanical data of each patient into individual taps. A trough-peak-trough section was considered a single, full tap if it lasted less than two seconds and the amplitude deflection from trough-to-peak and peak-to-trough was >=1 N (50%) (Scholten et al., 2020). Second, we collected the corresponding EMG signal fragments and determined the mean tapping frequencies (per patient, overall). The EMG snippets were then used to calculate the mean EMG activity per subject and the overall average (mean of subject means).

MEP extraction

The time codes of TMS pulses were collected automatically and classified based on their occurrence in the tap cycle into either *ascending* (trough-peak, 1-50% of the tap cycle) or *descending* (peak-trough, 51-100% of the tap cycle). We used a custom MATLAB script to extract the MEPs (peak-to-peak) within 20–40ms after the TMS pulse and corrected peak detection manually if necessary. EMG activity <200μV was not considered a MEP (Rothwell et al., 1999). In this case, we set the MEP value to 0 before normalization to account for “no MEP” in the following statistical analyses. MEP normalization was done separately for each tapping block due to drifts in cortical excitability between blocks within and between subjects. To do so, we determined the mean MEP size per block as an individual reference value and normalized to this value (Bergmann et al., 2019). All MEP sizes are thus presented as percent (%) change from block average.

MEP classification for statistical analysis

All MEPs were categorized into five conditions based on the kinematic characteristics at stimulation and their relation to ULF. MEPs were classified as TRANS if they occurred within a time frame of 1.2s (~3 taps, Scholten et al., 2020) before a freezing episode. The TRANS category was further subdivided into *ascending* (=TRANSasc) and *descending* (=TRANSdesc). MEPs produced during ULF were classified as *ULF*. No further distinction between trigger condition (ascending, descending) was made here, as increasing motor deterioration during ULF (irregular, small-amplitude kinematic signal) did not allow for further discrimination. All other MEPs were assigned to regular tapping (rT). Precisely, this condition summed up all other MEPs with the following two criteria: (*i*) not elicited during a ULF episode and – if produced before and not after ULF – (*ii*) distance to closest ULF episode >1.5s. Figure 8 schematically illustrates experimental conditions and respective numbers of MEPs assigned to them.

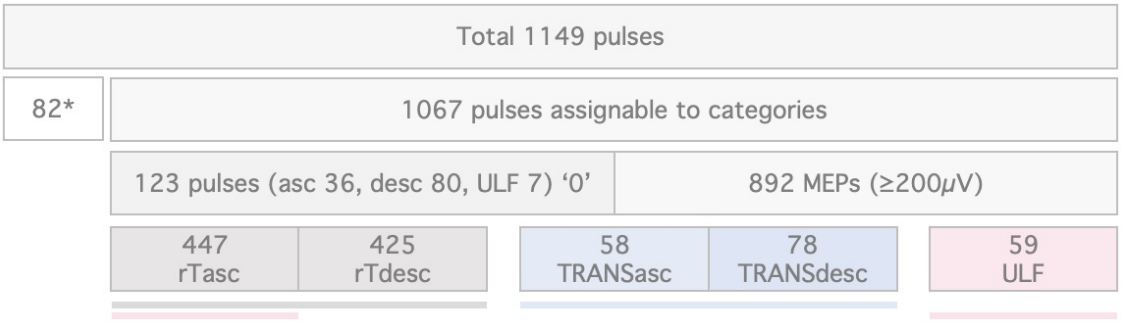

Figure 8: Schematic presentation of MEP categorization

1149 pulses were triggered in total. *For 82 triggers, the point of stimulation was attributable neither to ascending nor descending. These trials were discarded. The remaining 1067 pulses were divided into five categories (rTasc, rTdesc, TRANSasc, TRANSdesc, ULF). The respective numbers of MEPs assigned to each category can be taken from the figure. EMG activity within 20-40ms after stimulation below the threshold of 200μV was not considered an MEP. In this case, its value was set to 0 to account for no MEP in the statistical analyses. **Note**: The colored lines highlight the following statistical group comparisons.

Statistical analyses

Statistical analyses were conducted using SPSS® version 25 (IBM), Prism8 (GraphPad), and JASP; graphs and figures were made with Matlab® (Mathworks 2017a Ltd, USA), Prism 8, JASP, and Microsoft® PowerPoint. Descriptive statistics are presented as mean±SD/SEM, median with 95% confidence interval (CI), minimum, and maximum values. The dependent variable throughout all group comparisons was cortical excitability, as represented by normalized MEP amplitude size. Independent variables depended on the condition: (i) rTasc vs. rTdesc; (ii) TRANSasc vs. TRANSdesc and (iii) rTasc vs. ULF.

First, we compared MEP sizes at rTasc (contraction) and rTdesc (relaxation) during regular finger tapping. Second, we compared rTasc and ULF.
Additionally, we performed another correlation analysis using distance (= time lag between stimulation and the start of freezing) as a predictor of normalized MEP size in the 'TRANSasc' condition. Due to the non-parametric distribution of our data, we used a conservative, non-parametric statistical approach. Conducted tests included *Shapiro-Wilk tests* of normality, non-parametric *Mann-Whitney U tests*, and a non-parametric *Spearmans' Rho* correlation. All statistical tests were two-tailed; a p-value of <0.05 was considered statistically significant.

Control analysis: relationship between background EMG and MEP size

We triggered TMS pulses time-locked to the different phases of the movement cycle or freezes, ergo at different levels of background EMG activity. Thus, we expected that the MEP sizes might be influenced by this phasic EMG activation in some form (Carroll et al., 2006; Godfrey et al., 2013; Nomura et al., 2016; Sidhu et al., 2012). Contrary to conventional TMS experiments stimulating at a stable pre-stimulus baseline, FDI activation here would not be stable over time but differ with respect to the movement cycle (ascending, descending, ULF). Therefore, we controlled our MEP findings carefully for this major methodological aspect. Similar to (Nomura et al., 2016), we calculated correlation coefficients between the EMG activity 110-10ms prior to the TMS pulse and the respective MEP. Further, we calculated a linear regression analysis with experimental condition

(rTasc, rTdesc, ULF), force and background EMG as independent variables on MEP size to rule out a systematic bias by design.

3. Results

Patient characteristics

Eleven right-handed PD patients (4 females, aged 69.7 ± 9.6 years) participated in the study. All patients suffered from idiopathic PD with akinetic-rigid subtype with a mean disease duration of 11.2 ± 3.9 years. Three patients reported pronounced symptoms on the right body side, four patients experienced aggravated symptoms on the left side, and four did not observe side dominance. All patients received dopaminergic treatment routinely (oral n = 8, apomorphine pump n = 1, levodopa-carbidopa intestinal gel n=2) and experienced a noticeable worsening of PD motor symptoms after clinically supervised (overnight) withdrawal. Motor examination (MDS-UPDRS-III) in the OFF-medication state before the experiment revealed an average score of 46.25 ± 13.26, indicating moderate to severe affection of disease. FoG was recorded in all recruited patients' medical history, and except for one patient – all reported to have experienced FoG within the past month (*mean* N-FOG-Q 16.63 ± 6.1). Detailed information on the patients' clinical characteristics and test scores are presented in table 1.

Table 1: Clinical characteristics of the patient cohort

Patient	Demographics			Disease				Behavioral Measures							
	Sex	Age	Education	Onset	Duration	Side	LEDD	UPDRS III*	N-FOG-Q	M-EDL	nM-EDL	BDI	MMSE	TF	n_{ULF}
2	m	60	17	2016	4	both	729.5	55	20	20	7	16	30	2.2	40
3	f	83	9	2006	13	left	296.25	23	15	18	8	17	26	1.45	3
4	m	61	15	2012	8	left	364.5	33	0	2	6	14	26	3.51	24
5	m	66	12.5	2005	14	right	200	46	16	34	9	10	29	4.23	16
6	m	70	13	2002	17	left	819	64	22	16	10	16	30	1.54	50
7	f	78	18	2007	12	both	485	46	21	20	16	15	30	4.29	2
8	m	63	8	2009	11	right	607	43	19	12	9	8	27	5.57	26
9	f	76	17	2013	6	both	605	60	20	26	7	10	29	2.86	41
10	m	53	12.5	2004	15	right	200	37	16	18	11	4	28	1.96	16
11	f	81	13	2009	11	both	613	63	13	23	10	11	29	2.88	5
12	m	75	7.5	2009	11	left	1607.6	37	20	13	13	7	28	2.39	14
Mean	-	69.73	14.05	-	11.18	-	593.35	46.25	16.63	18.5	9	13.3	28.38	2.53	21.55
SD	-	9.57	3.3	-	3.87	-	395.05	13.26	6.1	8.2	2.91	4.27	1.57	1.29	16.35
															N=237

Note: Age, education, disease duration are displayed in years; all patients suffered from akinetic-rigid subtype. **Abbreviations**: *BDI* Beck's Depression Inventory; *LEDD* Levodopa Equivalent Daily Dose (mg; see (Schade et al., 2020) for details); (n)*M-EDL* (non) Motor Experiences of Daily Living (nM = nonmotor (UPDRS, Part I); m = motor (UPDRS, Part II)); *MMSE* Mini Mental State Examination; *N-FOG-Q* New-Freezing of Gait-Questionnaire; sex: *m* male, *f* female; *side* disease dominance (more affected side); n_{ULF} Number of Episodes of Upper Limb Freezing (ULF); *TF* mean tapping frequency over all tapping blocks per patient; *UPDRS III** Unified Parkinson's Disease Rating Scale (Motor Examination, Part III), OFF medication (table from Topka et al., 2022; reproduced with permission from Springer Nature).

Descriptive statistics

A single patient completed, on average, 15 ± 5 tapping blocks. The mean tapping frequency was 2.53 ± 1.29 Hz. All patients experienced freezing during the experiment, four patients were asked to perform dual-tasking (verbal fluency task n=3, arithmetic task due to a language barrier n=1) solely to increase the number of ULF episodes (Tab. 1). Precisely, we detected 237 episodes of ULF with an average duration of 0.83 ± 0.13 s. 109 (46%) of these ULF episodes occurred with tapping frequencies <2.5Hz and 128 (54%) when tapping with frequencies >2.5Hz. In two patients, no freezing episodes were hit by a TMS pulse. The data from those patients were thus excluded from the statistical analyses concerning ULF.

Overall, the algorithm triggered 931 single pulses (see also methods, Fig. 8). 447 pulses were triggered during the ascending slope of a finger tap (27.84 ± 9.81 % of the tap cycle (*mean* ± *SEM*)) at a median force of 1.44 N (*IQR* = 0.67). 425 pulses were applied during the descending slope (75.19 ± 8.06 % of the tap cycle) at a median force of 1.48 N (*IQR* = 0.62). An additional 136 pulses were classified as TRANS (58 ascending, 78 descending). Here, median force at the ascending slope was 1.44 N (*IQR* = 0.58) and at the descending slope 1.51 N (*IQR* = 0.56). Another 59 pulses fell into ULF (*median* force = 1.39 N, *IQR* = 0.76). A total of 105 pulses from four patients evoked no MEP (ascending (n = 31), descending (n = 67), ULF (n = 7)).

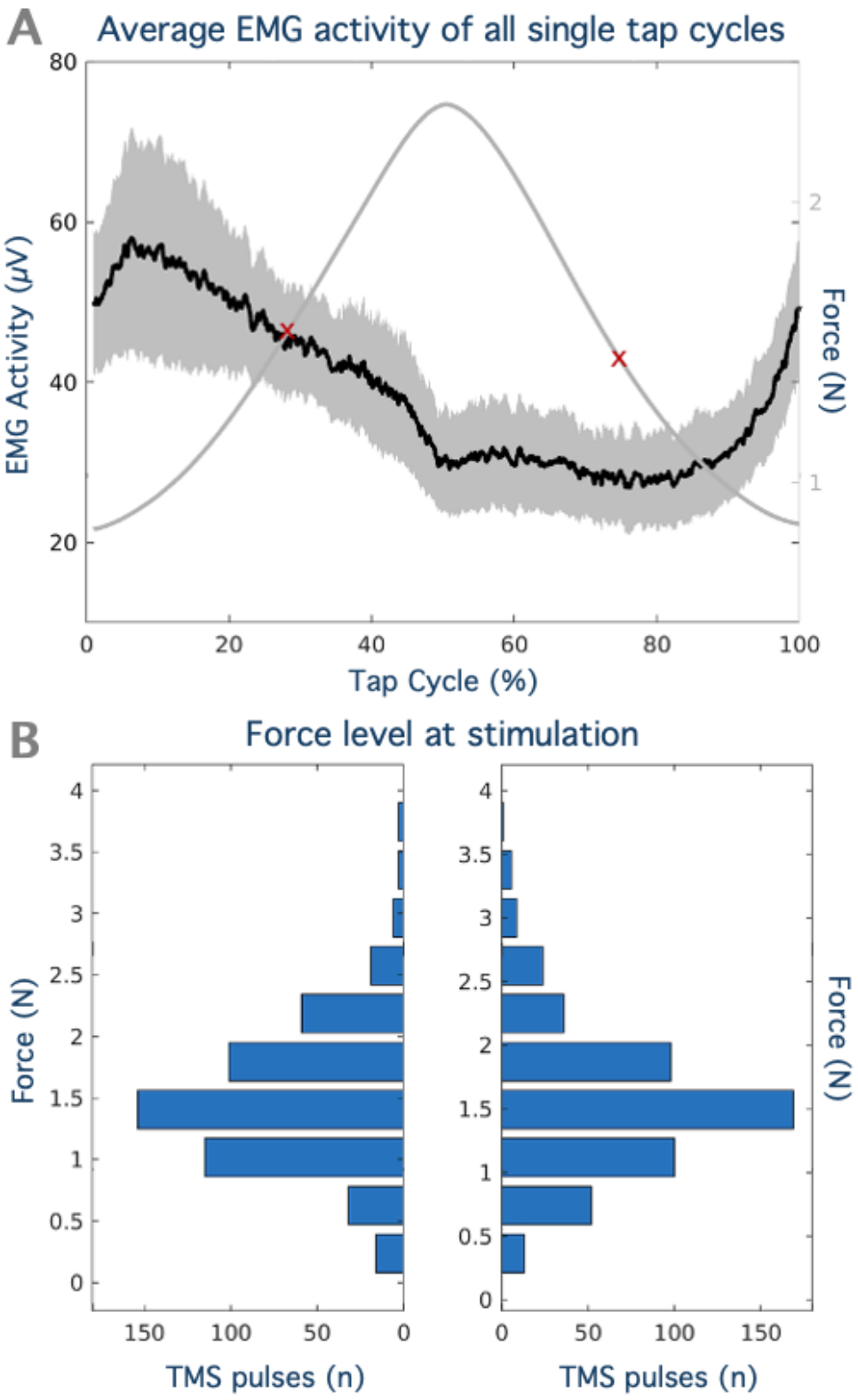

Figure 9: Background EMG activity and force levels during the tap cycle
Top: Average EMG activity (μV) of all single tap cycles (grey slope) from all patients during regular tapping. The red crosses indicate the mean times of stimulation at

ascending (left) and descending (right). **Bottom**: Distribution of single pulses (n) and their force levels (N) at stimulation. Diagram shows all pulses at ascending (left) vs. all pulses at descending (right). Figure modified from Topka et al., 2022; reproduced with permission from Springer Nature.

Background EMG activity and background force levels

Background EMG activity was modulated along the tap cycle (Fig. 9). FDI EMG activity was slightly greater at ascending (44.28 ± 6.81 µV (*mean* ± SEM) compared to descending (28.27 µV ± 6.09; Z = –2.045, p = 0.041 (*Wilcoxon signed rank test*)). There was no significant difference between EMG activity at ascending and ULF (32.76 ± 5.1 µV; Z = –1.689, p = 0.091) or descending and ULF (Z = –1.423, p = 0.155), respectively. Also, there was no linear correlation between EMG activity and MEP sizes (Fig. 10). A linear regression analysis (R^2 = 0.0369, F(4.928) = 8.894, p = 0.004) showed that only experimental condition (rTasc vs. rTdesc (p < 0.001), rTasc vs. ULF (p = 0.0374)) predicted MEP size, not force (p = 0.883) or background EMG (p = 0.511).

We also ensured that force levels at stimulation did not differ between ascending and descending at regular tapping (U = 89944, p = 0.175) and transition (U = 2002, p = 0.254) or between ascending (rT) and ULF (U = 10178, p = 0.19). There was, however, a significant force difference between descending (rT) and ULF (U = 8891, p = 0.011).

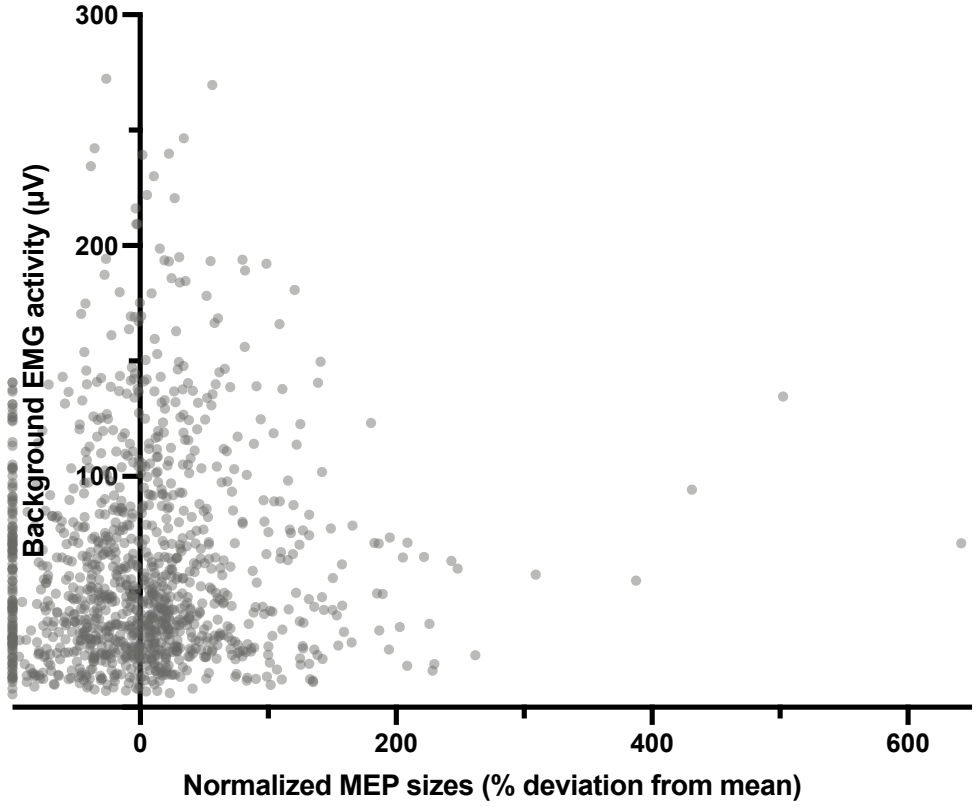

Figure 10: *Spearman's rho* non-parametric correlation analysis on EMG activity and MEP sizes
The was no linear relation between background EMG activity (µV) and normalized MEP sizes. **Note**: MEP amplitudes are presented as median percent deviation (%) from the mean. A value of -100 means no MEP >200µV was evoked.

MEP size comparisons during regular tapping, transition and ULF

We conducted three group comparisons to investigate MEP size. First, we investigated MEP size differences during the ascending and descending flank of regular tapping (= rT; rTasc vs. rTdesc). We then compared MEP size during the ascending flank (= *actual* motor output) to MEP size during ULF (= *intended* motor output) (rTasc vs. ULF). Finally, we investigated MEP sizes in the transition phase, few taps before ULF onset (TRANSasc vs. TRANSdesc). Here, we added an exploratory correlation analysis to see if MEP size during TRANSasc correlated with its distance from ULF and MEPs closer to the onset of ULF were smaller.

Table 2: Descriptive statistics for each patient and experimental condition

Patient	Regular tapping		Transition		ULF
	Asc (*n*)	Desc (*n*)	Asc (*n*)	Desc (*n*)	
2	0.24 (19)	3.41 (18)	13.11 (8)	-10.97 (11)	4.92 (14)
3	19.32 (30)	-32.67 (18)	-21.82 (1)	55.63 (1)	20.23 (2)
4	25.76 (76)	-47.25 (79)	10.78 (7)	-25.13 (16)	29.34 (7)
5	-23.10 (28)	12 (32)	-20.92 (7)	5.45 (4)	13.6 (2)
6	18.46 (38)	-19.54 (23)	-6.29 (9)	-32.71 (12)	16.75 (10)
7	1.69 (47)	8.06 (52)	-2.12 (3)	-16.68 (2)	n/a
8	-2.32 (18)	-20.44 (13)	-42.09 (2)	-62.63 (4)	-36.36 (11)
9	-0.92 (43)	10.2 (38)	0.65 (6)	2.37 (9)	-32.41 (4)
10	-35.24 (32)	-23.18 (40)	24.79 (3)	18.87 (3)	-78.36 (6)
11	5.67 (14)	1.51 (18)	10.5 (2)	-14.5 (1)	n/a
12	23.61 (102)	-38.09 (94)	22.87 (10)	-100 (15)	-100 (3)
Median	9.55 (447)	-11.19 (425)	-0.13 (58)	-20.66 (78)	-9.06 (59)

Note: All values show the % deviation from the patient mean (see methods). The numbers in brackets indicate the actual number of MEPs per patient and condition. Asc = ascending; desc = descending; n/a = not applicable since no MEP was elicited during ULF for these patients, ULF = upper limb freezing.

MEP size modulation during regular tapping

As expected, MEP sizes at the ascending and descending phase of the tap cycle differed significantly from each other. MEP amplitudes produced at ascending were significantly greater than those at descending (U = 71402, p < 0.001; Fig. 11).

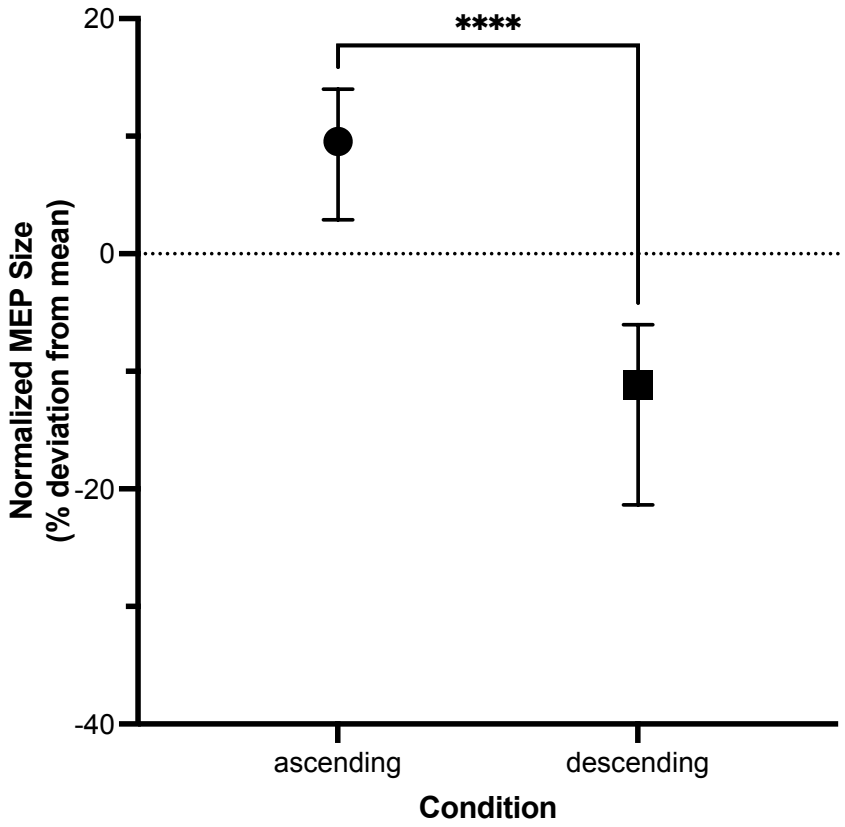

Figure 11: *Mann-Whitney U* test comparing MEP sizes at ascending to descending during regular tapping
Error bars denote 95% Confidence Interval (CI) of median; **** shows a p value < .001. **Note:** Dotted line indicates the average normalized MEP size. Figure modified from Topka et al., 2022; reproduced with permission from Springer Nature.

Reduced MEP sizes during Upper Limb Freezing (ULF)

When stimulated during ULF, the median MEP size was significantly reduced compared to ascending in regular tapping (U = 8904, p = 0.007; Fig. 12).

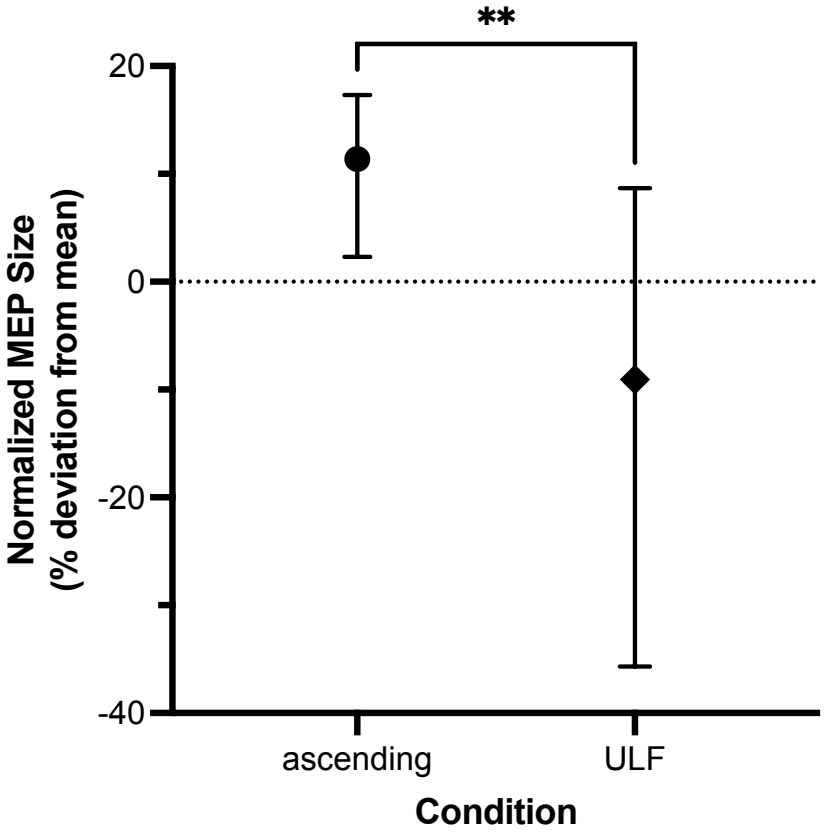

Figure 12: Mann-Whitney U test comparing MEP sizes at ascending with ULF
Error bars denote 95% Confidence Interval (CI) of median; ** shows a p value < .01. **Note:** Dotted line indicates the average normalized MEP size. Figure modified from Topka et al., 2022; reproduced with permission from Springer Nature.

Altered MEP size modulation in the transition period

When transitioning into an episode of ULF, MEP sizes at the ascending slope (TRANSasc) were still greater compared to descending (U = 1772, p < 0.03; Fig.13). However, we observed that overall MEP sizes declined during TRANS (ascending *and* descending) and did not reach the level of those evoked during regular tapping (*median* rTasc = 9.55 vs. *median* TRANSasc = -0.13 and *median* rTdesc = -11.19 vs. TRANSdesc = -20.66, descriptive observation only, no statistical test performed).

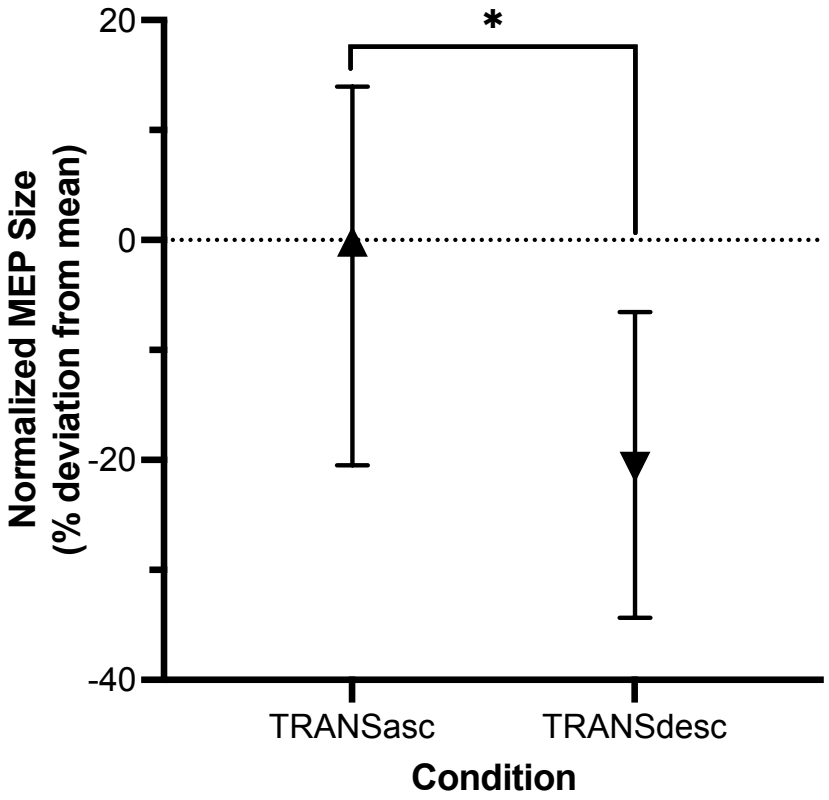

Figure 13: Mann-Whitney U test comparing MEP sizes at ascending with descending during transition
Error bars denote 95% Confidence Interval (CI) of median; * shows a p value < .05. **Note:** Dotted line indicates the average normalized MEP size. Figure modified from Topka et al., 2022; reproduced with permission from Springer Nature.

Exploratory correlation analysis

Additionally, we performed an exploratory *Spearman's rho* correlation analysis to investigate the relationship between latency between stimulation and the beginning of ULF (= distance) and the corresponding MEP produced during TRANSasc. Precisely, we used *distance* as a predictor of MEP size during TRANSasc. Statistical analysis revealed a significant relationship between both measures (r_S = 0.3994, 95% CI 0.05 – 0.66, p < .02, n = 33), potentially indicating a gradual decline of MEP amplitude in the transition phase into ULF with lower MEP amplitudes when closer to an upcoming ULF episode (Fig. 14).

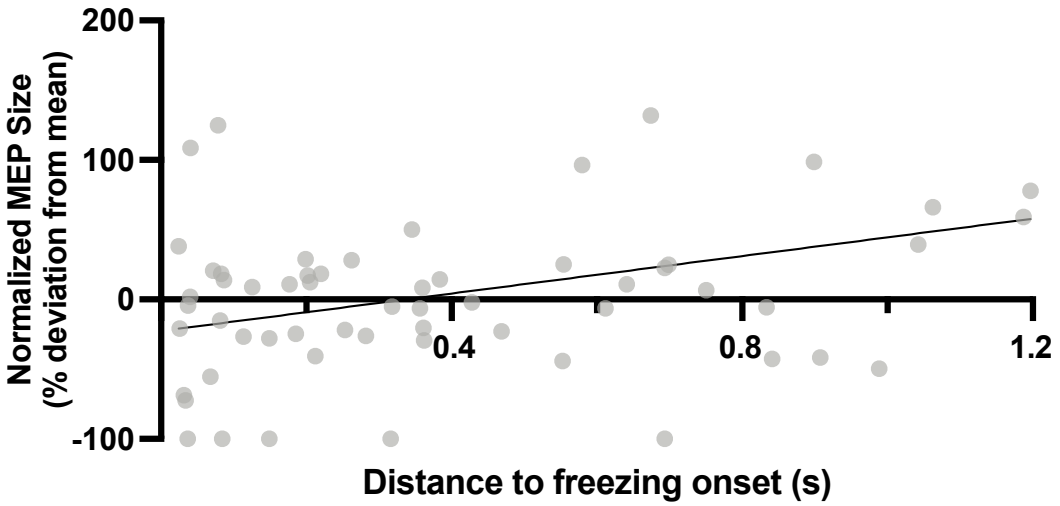

Figure 14: ***Non-parametric Spearman's rho*** **correlation between MEP sizes during the transition (TRANSasc) and their time distance (s) to the onset of freezing**
The transition period was defined as the 1.2s window ultimately before the start of freezing (~3 full taps before ULF). MEP sizes closer to the onset of a freezing episode appear to be smaller, potentially suggesting a gradual decline in cortical excitability when transitioning into freezing. **Note**: MEP amplitudes are presented as median percent deviation (%) from the mean. A value of -100 % deviation from mean MEP size means no measurable MEP.

4. Discussion

Summary of results

We used adaptive single-pulse TMS to investigate the movement phase-dependent modulation of cortical excitability during unilateral finger tapping and before respectively during upper limb freezing. When tapping regularly, cortical excitability was continuously modulated along the tap cycle: MEPs were greater with isometric contraction of the target muscle (ascending slope) and decreased with relaxation (descending slope). This modulation related to the movement cycle was maintained in the transition phase before the actual onset of freezing, but overall cortical excitability decreased (ascending and descending). The lowest levels of cortical excitability were not observed during freezing but when relaxing in the transition period.

Regular tapping: movement phase-dependent modulation of cortical excitability

Regular tapping was characterized by a movement phase-dependent, alternating excitability pattern: MEPs were greater with voluntary muscular contraction of the target muscle and decreased in amplitude when relaxing. Indeed, during single, voluntary movements, muscular precontraction has been identified as the most positive influential factor on MEP size (Hess et al., 1987), whereas reducing motor output has been related to lower MEPs (Park et al., 2016). Furthermore, when performing cyclic, repetitive motion like here, the cortical drive is reduced compared to discrete movements (Carroll et al., 2006). Such *phase*-dependent excitability fluctuations, reflecting a task-specific modulation of corticospinal responsiveness (Carroll et al., 2006; Sidhu et al., 2012), have been described consistently for both cyclic lower and upper limb movements, including finger tapping, for healthy subjects and PD patients (Carroll et al., 2006; Fischer et al., 2018; Nomura et al., 2016; Scholten et al., 2020; Seeber et al., 2016; Sidhu et al., 2012).

In PD, we know that motor cortex excitability is altered at rest (Tremblay & Tremblay, 2002) and particularly during movement (Chen et al., 2001; Pascual-

Leone et al., 1992; Valls-Sole et al., 1994) suggesting reduced modulation capacity. Compared to healthy controls, the onset of excitability enhancement in PD patients is premature (Chen et al., 2001), but the increase in muscular contraction is slower (Pascual-Leone et al., 1992). At the same time, MEP amplitudes do not grow as much with progressive increase of voluntary contraction in the target muscle (Valls-Sole et al., 1994), and the decrease of cortical excitability after movement offset (relaxation) is prolonged or delayed (Chen et al., 2001; Labyt et al., 2005; Pascual-Leone et al., 1992). Still, our results suggest that the modulation of cortical excitability during regular tapping was preserved in our PD patients to some degree, which will serve as our baseline when interpreting our findings during freezing and the transition period.

Freezing: reduction in cortical excitability

Freezing (*intended* yet *ineffective* movement) was related to decreased levels of cortical excitability compared to ascending at regular tapping (*intended* and *effective* movement). From our experiment, we can only speculate about processes outside M1 before and during freezing. Nonetheless, we believe this observation stands well in line with current pathophysiological assumptions, primarily based on fMRI findings [(decreased BOLD signal resembles decreased cortical excitability (Hamzei et al., 2002)] relating freezing to a transient functional disconnection between subcortical and cortical areas and lower motor cortical excitability (Lewis & Barker, 2009; Lewis & Shine, 2016; Shine, Matar, Ward, Frank, et al., 2013). Increasing M1 activity directly or indirectly, on the other hand, seems to facilitate motor output. Indeed, there is abundant evidence that non-invasive, excitatory cortical stimulation applied over M1 at rest or during movement improved motor performance and reduced freezing in PD (Broeder et al., 2019; Chang et al., 2016; Kim et al., 2015; Valentino et al., 2014; Yeol et al., 2014). Together, our data corroborate the previously made assumptions that upper limb freezing in a unimanual tapping task relates to low levels of cortical excitability within the contralateral M1. However, it seems necessary to emphasize that underlying cortical pathology is probably not limited to M1 and its

hypoactivity rather represents a *symptom* of incremental motor failure, the endpoint at which freezing becomes visible, rather than its *cause*.

Freezing: active inhibition vs. input problem

We cannot infer directly from our data if M1 was *actively* inhibited through basal ganglia inhibitory activity or if it instead lacked excitatory afferent input from other cortical or subcortical areas or both. But we know that M1 is a highly interconnected structure within the motor network and, apart from direct stimulation, several other cortical and subcortical processes may influence M1 activity directly or indirectly. For example, cortical excitability may increase without any intrinsic motor cortical activity at all, such as during motor imagery (Kasai et al., 1997), after motor training (Koeneke et al., 2006), after passive movement (Sasaki et al., 2017), or even through afferent sensory input (Abbruzzese & Trompetto, 2002).

We also know from repetitive TMS experiments that stimulation of M1 alone appeared not to be as effective as the combined stimulation with the ipsilateral dorsolateral prefrontal cortex (DLPFC) (Dagan et al., 2017; Yeol et al., 2014) and that stimulation at either side induced different performance enhancing mechanisms within the motor system. Sole M1 stimulation, for example, is believed to enhance striatal activity mainly through the basal ganglia motor loop, which in turn modulates inhibitory GPi activity to facilitate movement (Lewis & Barker, 2009; Lewis & Shine, 2016; Shine, Matar, Ward, Bolitho, et al., 2013). DLPFC stimulation, on the other hand, (together with M1) has been shown to increase the focal release of endogenous dopamine within the ipsilateral striatum (Strafella, 2003) while at the same time providing strong excitatory afferent input to M1 (Bestmann & Krakauer, 2015). Also, excitability and MEP sizes were lower in our experiment during rTdesc as compared to ULF, providing evidence that true motor output inhibition led to even lower levels of excitability whilst preserving motor output. Based on the available literature and our data, a lack of excitatory input causing freezing appears more likely than active inhibition.

Freezing: lowest excitability not during freezing but relaxation

Interestingly, cortical excitability was not lowest during freezing but at relaxation in the transition period. Even though we did not form a specific hypothesis and did not conduct a statistical test on this specific matter, this observation depicts a very significant finding; it indicates that freezing differs from voluntary, active inhibition at relaxation and suggests multiple mechanisms to provide the clinical picture of *motor break*. Even when tapping regularly, the average excitability level during relaxation was lower than freezing while preserving motor output, suggesting that low cortical excitability alone may not explain the emergence of freezing.

In fact, the careful balance between excitatory and inhibitory motor states requires highly adaptable, intact intra-cortical M1 activity (Floeter & Rothwell, 1999). Just before movement onset, excitatory circuits are facilitated (Hess et al., 1987; Nyi et al., 1998) and the excitability of intra-cortical inhibitory circuits declines for the selected agonist muscle (Reynolds & Ashby, 1999). Relaxation, on the other hand, though primarily achieved through spinal mechanisms (Sidhu et al., 2012), also relies on M1 activation (Buccolieri et al., 2004) and the subsequent reduction of intra-cortical inhibition to release the cortex, which then reduces motor output through spinal inhibitory neurons (Begum et al., 2005). As such, motor output inhibition does not simply reflect the cessation of muscular contraction but requires widespread cortical activation to intentionally decrease motor cortical excitability (Kato et al., 2019). This could explain why motor output was relatively unaffected at relaxation despite low excitability levels.

Freezing: no cortical hyperactivity

In our experiment, freezing was related to lower cortical excitability compared to successful movement (contraction). As we did not test MEP size during rest or included a healthy control group, this remains an anecdotal observation. However, our data provides no support that freezing may result from a *hyper*active M1, as one might suspect from its clinical presentation. Instead, the balance between cortical and subcortical activity seems to be shifted and cortical hyper- and hypoactivity due to dopaminergic denervation and compensatory

effects appear to coexist (Berardelli & Suppa, 2011). There is little evidence on the specific role of M1 during freezing in single-handed experimental setups; but in their functional magnetic resonance (fMRI) study, Vercruysse and colleagues (2014) used a bimanual finger tapping paradigm in PD patients with and without freezing and observed mostly right-lateralized increased relative fronto-cortical *activation*, including in the right M1 (Vercruysse, Spildooren, et al., 2014). In fact, no areas showed decreased activation during freezing compared to regular tapping. In their study, not the freezing episode but successful motor performance *without* freezing was associated with decreased cortical (right DLPFC and M1, left PMd) and bilaterally increased subcortical activation (dorsal putamen, pallidum, STN). The authors themselves attributed M1 hyperactivity to increased afferent sensory input due to trembling in place during freezing – and to cortical compensation processes successfully preventing a freeze (Vercruysse, Spildooren, et al., 2014). This interesting observation appears conflicting at first as we saw cortical *hypo*-excitation during freezing. However, whereas BOLD signal alterations are detectable several seconds after the observed behavior, single pulse TMS provides an almost *live read-out* of the motor system's state just at the time of stimulation (Bestmann & Krakauer, 2015) and may be more sensitive for short-lasting, transient changes in excitability. Upper limb freezing episodes are short, much shorter than gait freezing episodes (Nutt et al., 2011). A typical upper limb freeze lasts less than 5 seconds (Scholten, Govindan, et al., 2016; Vercruysse, Spildooren, et al., 2014). In our experiment, the average was even below one second, followed by few moments of recovery. Thus, it is difficult to discriminate between both activation patterns and solely attribute them to one or the other process. Indeed, some of the above described fronto-cortical areas, such as the DLPFC and PMd have also been identified as integral parts of the cortical control network, and activation within this network has previously been associated with the generation of goal-directed behavior to break or overcome a freeze (Lewis & Barker, 2009). Thus, one could speculate that those cortical activity increases were – at least to some degree – related to overcoming the freeze and did not necessarily depict the cortical state in M1 when entering the freezing episode.

Freezing: higher MEP size variability reflecting motor system instability

Interestingly, MEP size variability during freezing was much greater than at relaxation or contraction during the transition (observation, see Appx. E). This is remarkable insofar as it is the process of relaxation and the stepwise reduction of muscular contraction known to increase response variability (Park et al., 2016). Intuitively, one would attribute this increase in response variance largely to sample size differences between experimental conditions. However, the number of events was roughly comparable for all subgroups (TRANSasc 59, TRANSdesc 78, ULF 58 events), and it is possible that factors other than sample size also contributed to this observation. In fact, high variability during freezing could indicate a high level of motor system instability during the freezing episode. Indeed, hitting the short freezing episodes was challenging, and we had no control over *when* the TMS pulse was triggered within a freezing episode. Thus, there were very likely different compensatory mechanisms involved during these episodes, increasing excitability to re-establish intended motor output. Some freezes may have been hit closer to breaking the freeze instead of its onset when we believe that excitability was lower. Higher response variability could thus reflect underlying processes either of the motor cortex resetting itself actively or other cortical compensatory mechanisms that increase M1 excitability to break or overcome the freeze (Shine, Matar, Ward, Frank, et al., 2013).

Transition: overall reduction in cortical excitability

Transitioning into freezing was reflected by overall decreased excitability compared with regular tapping at both contraction and relaxation, while phasic modulation was still intact. This finding somewhat resembles decreased fronto-cortical activation seen in PD patients when trying to maintain successful tapping performance with increasing motor demands (Vercruysse, Spildooren, et al., 2014). Similarly, in our experiment, the beginning motor failure became first visible not during freezing but in the transition phase, when overall cortical excitability declined. In general, the emergence of irregular, hastened movement during tapping – but not freezing – has been suggested to result from a reduced

M1 recovery capacity between taps (rebound synchronization) and the subsequent overlapping of neuronal activity associated with the forthcoming movement (Stegemöller et al., 2016). Indeed, speeding up during tapping impaired performance and increased the number of freezing episodes (Heremans et al., 2019). Interestingly, in their experiment, the probability of ULF did not correlate with disease severity but was inversely correlated with cognitive performance in a standardized screening test (Montréal Cognitive Assessment, MoCA)(Heremans et al., 2019). Patients with lower scores were more likely to freeze, suggesting that available cognitive processing capacity represented a valuable resource to prevent or alleviate freezing. Indeed, freezing has been suggested to result from paroxysmal decoupling of basal ganglia and frontal cognitive control network, rendering insufficient afferent input to M1 to produce effective movement (Shine, Matar, Ward, Frank, et al., 2013). Although somewhat speculative, our observation of overall lower levels of cortical excitability in the transition phase could have reflected the increasing failure of this cognitive control network and the associated accumulation of motor deficits. In either case, our data show that a worsening of motor performance correlated with measurable alterations in cortical excitability and that these observations preceded the actual onset of ULF.

Correlation analysis: anecdotal evidence for a relationship between MEP size and time to freeze onset

Lastly, we found anecdotal evidence that – during the transition – the distance to the onset of a freeze correlated with MEP size: the closer to the next freeze an MEP was triggered, the smaller its amplitude. This analysis, however, was done with exploratory character and will not be interpreted to the full extent. Indeed, it seems plausible that MEPs shortly before a freezing episode are smaller, particularly when entering a freeze has been associated with low levels of excitability. However, a sample size of 58 data points from 11 patients only certainly does not provide substantial evidence to challenge this view and rather serves hypothesis generating purposes.

Methodological considerations

No effect of force

Muscular pre-contraction is the most well-known factor during single movements to increase and stabilize MEP amplitudes (Hess et al., 1987), and significant force differences between both contraction and relaxation would have seriously complicated data interpretation. We ensured that force levels did not differ much between both conditions through our experimental design. Hence, we believe that the above-presented results cannot be attributed to simple force effects but reflect underlying connectivity processes.

Dealing with background EMG levels

Contrary to conventional TMS experiments stimulating at a stable pre-stimulus EMG baseline, stimulation in our experiment occurred at constantly changing background EMG levels. Background EMG activity was higher at contraction than relaxation or freezing. This phasic EMG modulation was expected as TMS pulses were triggered time-locked to the different phases of the tap cycle. Even though the reduction of motor output is ultimately achieved through inhibitory activity at the spinal level (Sidhu et al., 2012), it is well-established that EMG activity at the time of stimulation influences the size of cortically evoked MEPs in some form (Carroll et al., 2006; Godfrey et al., 2013; Nomura et al., 2016). MEPs most likely do not superimpose on background EMG, but it is not clear how exactly this EMG activity interacts with TMS responsiveness in a way that would allow predicting and mathematically correct for the magnitude of MEP facilitation. Generally, EMG level at stimulation and MEP response are not linearly related, and a large share of variance cannot be predicted through the intensity of EMG activity before stimulation. Some authors identified EMG activity 100–50ms preceding a TMS pulse as one predictor of MEP size but reported high inter-individual differences (Hasegawa et al., 2001) or poor predictive power (R^2=15%; Mitchell et al., 2007). Still, it is common practice to account for the magnitude of pre-stimulus background EMG when calculating MEP amplitudes, particularly when stimulating *during* cyclic movement instead of static force or rest. Even though the evidence is limited, this is mainly done through the calculation of ratios

between MEP sizes at two defined times with different background EMG levels, i.e., the ratio between a defined window of pre-stimulus EMG (Sidhu et al., 2012) or maximum voluntary contraction (MVC) EMG and the EMG activity at the respective experimental condition (Carroll et al., 2006; Godfrey et al., 2013)). However, these approaches were used on healthy probands, not PD patients known for their altered responsiveness to TMS at rest and during muscular contraction (Lefaucheur, 2005; Valls-Sole et al., 1994). Instead, we followed the proposed method by Nomura and co-authors (Nomura et al., 2016) and showed that MEP sizes and the mean level of EMG background activity 110-10ms before stimulation were not linearly related. Although this approach does not entirely exclude potential confounding, it rules out a systematic, linear influence of EMG activity on MEP size. This was supported through an additional regression analysis supporting the influence of experimental condition while at the same time showing no effects of force and background EMG on MEP sizes.

Sample size

Neurophysiological experiments are often based on limited sample sizes and are known for their high inter- but also intrapersonal variability and unpredictability (Rossini et al., 2015), and our experiment is no exception to that. For example, we recorded 237 individual freezing episodes from eleven patients and triggered more than 1000 single TMS pulses – yet only 59 of those hit a freezing episode. This is a reasonable amount, considering that one of our goals was to explore the feasibility of using single-pulse TMS during repetitive movement to investigate upper limb freezing. In that sense, our data show that it is possible to characterize the current state of the motor system during movement and freezing with a phase-locked single-pulse TMS paradigm. However, small sample sizes have lower statistical power, and the generalizability, reliability, and reproducibility of experimental results are somewhat limited. Still, we believe our data provide valuable insight into M1 physiology in and around upper limb freezing.

Open questions: does cortical hypoactivity cause freezing?

Our data suggest that episodes of upper limb freezing may relate to motor cortical hypoactivity. It remains unclear however, *when* during freezing hypoactivity occurs – and, if it truly causes it. We observed a decline in cortical activity during the transition phase prior to freezing and inferred that excitability levels ultimately reach levels below some kind of threshold to cause freezing. We argued that the underlying movement intention could explain why cortical activity was lowest at relaxation during the transition phase to explain why motor output was still intact then. In the end, however, there is one major limitation to this argument: we had no control over *when* during an episode of freezing the TMS pulse would hit. This means our data do not reflect cortical activity levels at the *onset* but rather a mean of cortical activity levels during all freezing episodes. It is possible that some pulses occurred when M1 was already in a recovery phase trying to overcome the freeze. During that time, a switch from automatic to attentive motor control through activation of the PMd, a central hub of the cognitive control network, is believed to bypass deficient fronto-striatal pathways to restore motor output (Zang et al., 2022) and with it cortical activity. This would have caused a bias towards slightly higher excitability levels and counteract somewhat the interpretation that this observation is mainly driven by movement intention. Indeed, we saw higher MEP size variability during freezing compared to relaxation at regular tapping or transition. Due to the low sample size, however, we did not perform a statistical test and this evidence remains anecdotal.

Open questions: where do we go from here?

We tested our patients after sufficient dopaminergic withdrawal to increase the number of freezing episodes. This was necessary but might have confounded our results. There is plenty of evidence from TMS studies on the parkinsonian M1 showing that some of the functional abnormalities in PD, i.e. reduced short-interval intracortical inhibition (SICI) or shorter cortical silence periods (CSP) resulting in higher cortical excitability at rest or during voluntary contraction, respectively, can be restored through the administration of L-dopa (Berardelli & Suppa, 2011; Chen et al., 2008). As stated in the introduction, freezing is not very

responsive to dopaminergic treatment. So, if freezing pathology differs from typical PD-related basal ganglia dysfunction, it would be interesting to replicate our experiment in an on-state to see if and how cortical excitability levels change. With the goal of treatment in mind, future research directed to the transition phase when – according to our experiment – excitability levels are already changing but motor output is still intact, may help to identify parameters predicting either a freeze or successful prevention freezing.

5. Conclusion

We showed with our experiment that phase-locked single-pulse TMS is a feasible tool to investigate M1 excitability during regular finger tapping and upper limb freezing in PD patients. We provide insight that freezing relates to M1 hypoactivity as reflected by low cortical excitability comparable to the relaxation phase of regular movement. The lowest excitability levels were not observed during freezing but few taps before in the transition period when motor output was still intact, suggesting that M1 hypoactivity alone may not cause freezing. Instead, the overall excitability decrease in the transition phase indicates that freezing ultimately emerges with the loss of excitatory input from premotor and other frontal areas.

Overall, our data suggest that freezing reflects an episodic event of ineffective motor output that differs in its physiology from rest or ongoing repetitive movement. We identified M1 as a crucial structure in the pathophysiology of upper limb freezing, representing a potential target for adaptive brain stimulation approaches. Further, we found that M1 excitability was reduced even before motor breakdown and before freezing was detectable in the kinematic signal. We hope to use this knowledge to identify parameters to predict upcoming freezing episodes to improve the future of freezing treatment in PD patients.

Zusammenfassung

Die vorliegende Studie untersucht den Zusammenhang zwischen der Erregbarkeit des primären Motorkortex und verschiedener Bewegungszustände während regelmäßiger Fingerbewegungen (reguläres Fingertapping Kontraktion/Relaxation (rTasc/desc) bzw. vor/während Freezing der oberen Extremitäten (TRANSasc/TRANSdesc, ULF).

In einem isometrischen Fingertapping-Paradigma wurde mittels transkranieller Magnetstimulation (Einzelpulse) zu zwei fest definierten Zeitpunkten im Bewegungszyklus (zu 25 (= aufsteigend) bzw. 75% (=absteigend) eines Tapzyklus) anhand der Größe des evozierten motorischen Potentials (MEP) die aktuelle Erregbarkeit des Motorkortex bestimmt. Dabei konnte gezeigt werden, dass die kortikale Erregbarkeit entlang des Bewegungszyklus gleichförmig mit zunehmender isometrischer Kontraktion anstieg bzw. während der Relaxation abnahm. Während des Freezing, also wenn keine suffiziente Bewegung produziert werden konnte, zeigte sich die Erregbarkeit im Vergleich zur erfolgreichen Kontraktion im regulären Tapping reduziert. Die sogenannte Transitionsphase, also jene prodromale Phase, in der kinematisch erste Veränderungen des regulären Bewegungsablaufes kurz vor einer Freezing-Episode auftreten (z.B. Abnahme von Frequenz und Amplitude), die Bewegung jedoch noch intakt ist, war durch eine allgemeine Abnahme der Erregbarkeit gekennzeichnet. Interessanterweise fanden sich die absolut niedrigsten Werte kortikaler Erregbarkeit nicht während des Freezing selbst, sondern während der Transition (bei Relaxation) – trotz zu dieser Phase noch erhaltenem motorischen Output.

Unsere Ergebnisse legen daher nahe, dass der Zusammenbruch des Bewegungsablaufes wie wir ihn während Freezing-Episoden sehen, sich auf physiologischer Ebene unterscheidet von dem, was wir beobachten, wenn das Bewegungsausmaß willkürlich reduziert wird (Relaxation). Möglicherweise geschieht dies auch deshalb, weil erregende dopaminerge, fronto-striatale Afferenzen im Rahmen der Parkinson-Erkrankung fehlen und nur in einem gewissen Ausmaß durch andere Kortexareale ersetzt werden können.

Unsere Ergebnisse untermauern daher die Beteiligung des primären Motorkortex und dessen die Bedeutung seines aktuellen Erregungszustands während regulärer Fingerbewegungen und insbesondere in der Entstehung von Freezing der oberen Extremität. Wir hoffen mit diesen Erkenntnissen dazu beizutragen, zugrundeliegende Pathomechanismen besser zu verstehen und Parameter abzuleiten, die anhand des physiologischen oder kinematischen Signals drohende Freezing-Episoden vorhersagen können und möglicherweise ein therapeutisches Fenster öffnen für neue hirnstimulierende Ansätze.

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7. Erklärung zum Eigenanteil der Dissertationsschrift

Die Arbeit wurde in der Klinik für Neurologie, Abteilung Neurodegeneration unter Betreuung von **Prof. Dr. med. Daniel Weiss** durchgeführt.

Die Konzeption der Studie erfolgte in Zusammenarbeit mit **Prof. Daniel Weiss** und **Prof. Dr. med. Ulf Ziemann, sowie Dr. med. Christoph Zrenner, Dr. rer. nat. Paolo Belardinelli, Dr. rer. nat. Marlieke Schneider** und mich.

Sämtliche Versuche wurden (nach Einarbeitung durch Labormitglieder **Dr. med. Christoph Zrenner** und **Dr. rer. nat. Paolo Belardinelli**) von mir zusammen mit **Dr. rer. nat. Marlieke Schneider** durchgeführt.

Die statistische Auswertung erfolgte durch mich, **Prof. Dr. med. Daniel Weiß** und **Dr. rer. nat. Marlieke** Schneider.

Ich versichere, das Manuskript selbständig (unter Anleitung durch **Prof. Dr. med. Daniel Weiss**) verfasst und keine weiteren als die von mir angegebenen Quellen verwendet zu haben. Künstliche Intelligenz wurde zu keinem Zeitpunkt verwendet.

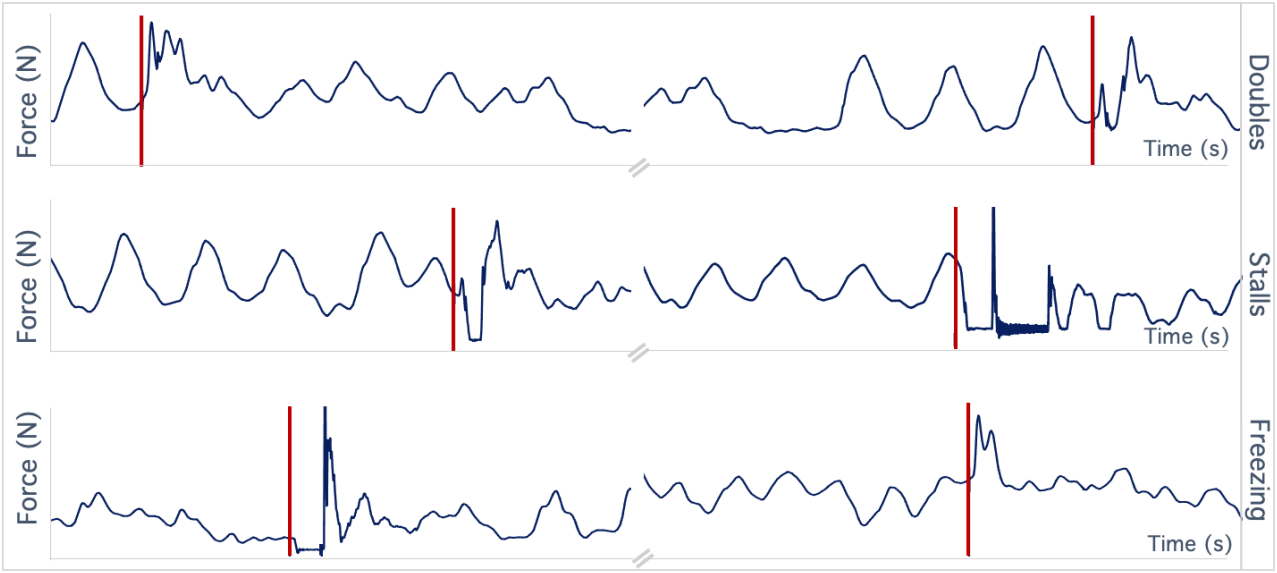
München, den 20.05.2024

[Marlene Topka]

Appendix

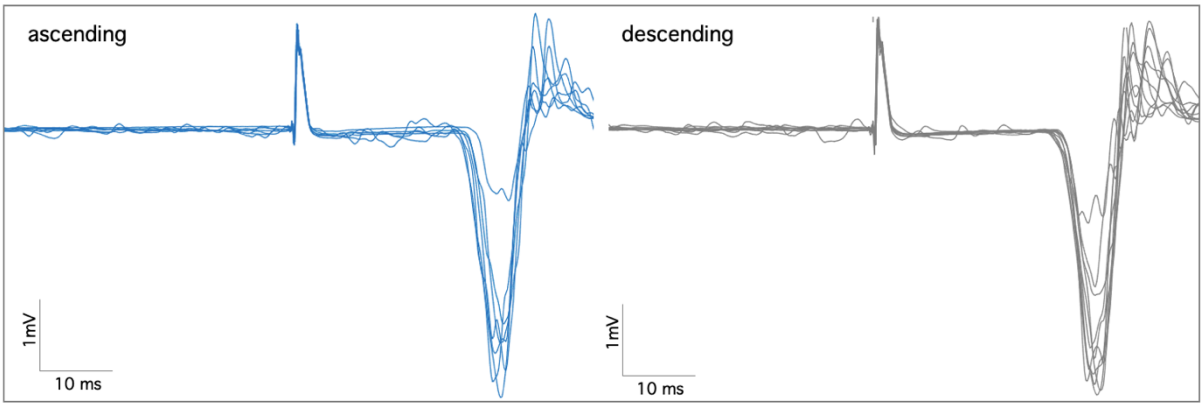
Appendix A: TMS-induced motion artifacts on tapping performance

The application of TMS over the left M1 impeded finger tapping performance and caused typical motion artifacts. These artifacts induced freezing-like phenomena (*doubles*) or mimicked freezing (*stalls*) but did not meet the criteria for and were not considered ULF. Fig. A shows examples of doubles and stalls. These phenomena were only considered freezing if they were evoked *within* a ULF episode (bottom), that is, when the patient was already freezing.



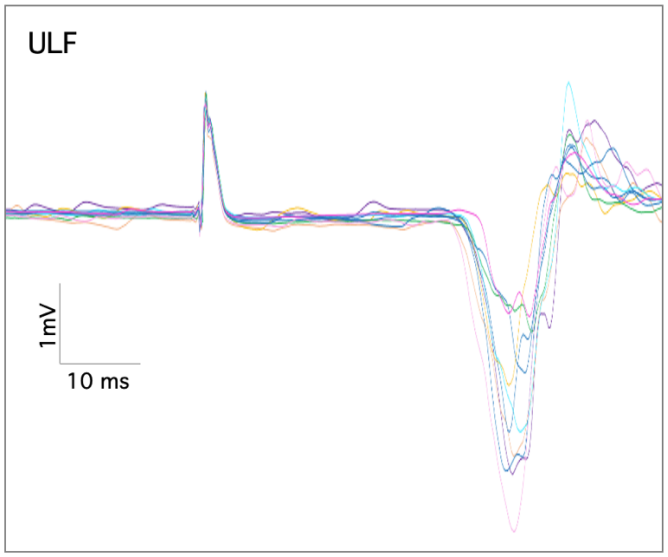
Appx. A: **TMS-induced artifacts on finger tapping performance**. (Levit-Binnun et al., 2007) described two characteristic tapping anomalies caused by stimulation, so-called *doubles,* and *stalls*. **Top**: Illustration of *doubles*; TMS trigger leads to double motion of the finger. **Middle**: Illustration of *stalls*; TMS trigger leads to elongated down movement of the finger. Neither doubles nor stalls represented actual ULF episodes. **Bottom**: Detection of *freezing*; both panels show ULF episodes despite the occurrence of TMS artifacts (*left*: stall; *right*: double); here, the ULF episode was preceded by a characteristic transition period (not shown) and started *before* the pulse, the artifacts emerged within freezing.

Appendix B: Exemplary data, regular tapping. Phase-locked stimulated MEPs during regular tapping and transition.



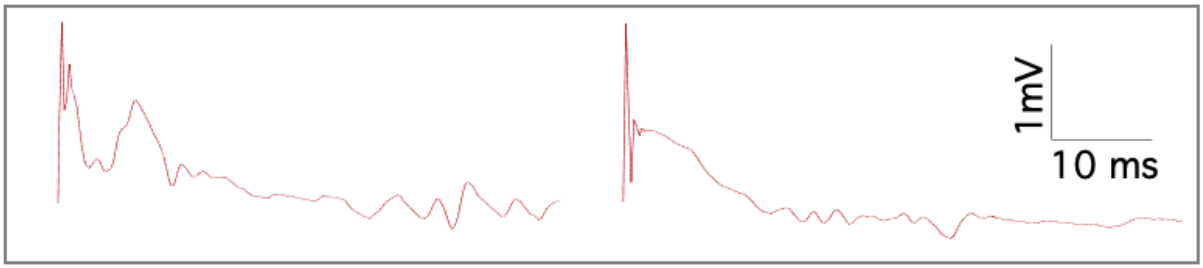
Appx. B: Exemplary data from patient A. MEPs evoked at ascending are shown in grey on the left, MEPs at descending on the left (blue). The large spikes before the MEPs show the TMS pulses. ***Note***: figure shows raw MEPs before normalization. MEP sizes may not be comparable between different figures.

Appendix C: Exemplary data, freezing. MEPs evoked during ULF.



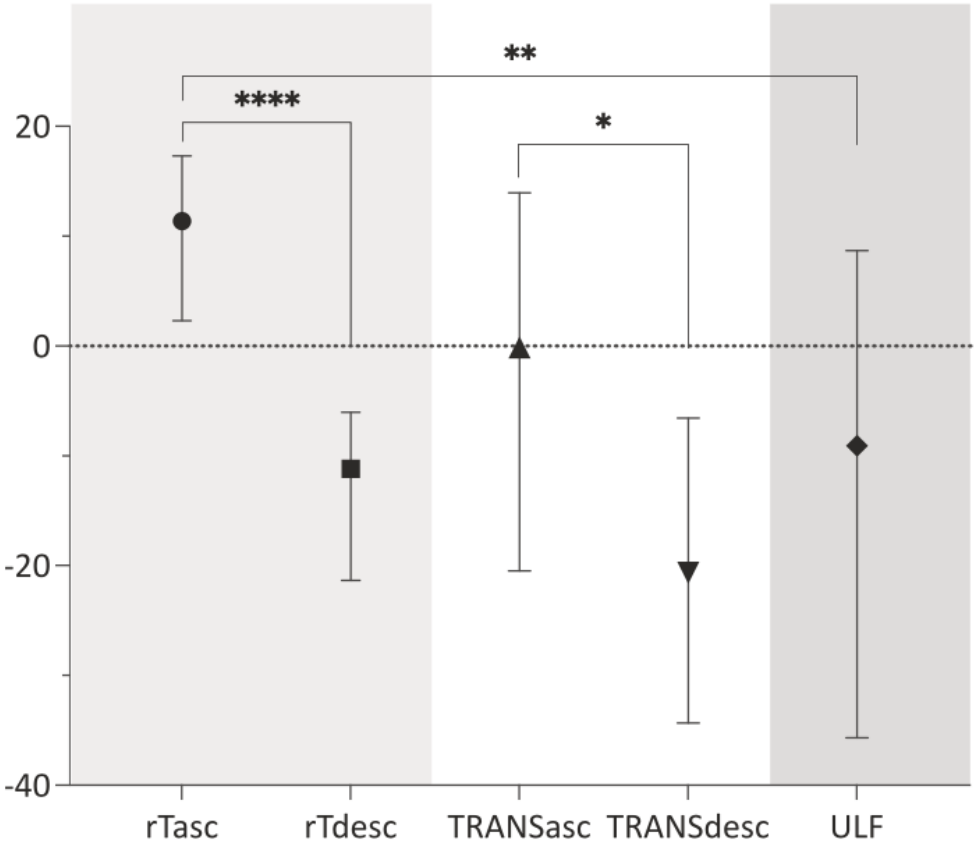
Appx. C: Exemplary data from patient B during freezing. Note: Figure shows raw MEPs before normalization. MEP sizes may not be comparable between different figures.

Appendix D: Exemplary data. No MEP evoked (<200µV threshold).



Appx. D: Exemplary data when no MEP was evoked through stimulation and the value was set to *zero* to be included in the statistical analyses as "*no MEP*", that is, the lowest level of cortical excitability. The spike at the beginning shows the TMS pulse.

Appendix E: Summary of results (from Topka et al., 2022).



Appx. E: Motor cortex excitability in regular tapping, transitions, and freezing. Overview of all conducted Mann-Whitney U tests on normalized MEP sizes (ascending slope vs. descending slope) during regular tapping (rTasc vs. rTdesc), the transition (TRANSasc vs. TRANSdesc), and regular

tapping ascending (rTasc) with ULF. MEP sizes were greater at ascending (downward press) compared to descending (upward release) during both regular tapping and transition. MEPs evoked at rTasc (successful motor output) were also greater than during ULF (unsuccessful motor output), but overall MEP size variability increased when freezing (ULF). X-axis: % change from block average as median ± 95%-confidence interval; Y-axis: experimental condition. Modified from (Topka et al., 2022) and reproduced with permission from Springer Nature.

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