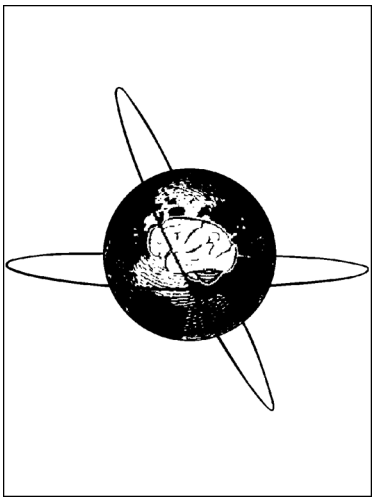

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MOTOR DYSFUNCTION IN MILD COGNITIVE IMPAIRMENT AS TESTED BY KINEMATIC ANALYSIS AND TRANSCRANIAL MAGNETIC STIMULATION

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The study was conducted in laboratories of the Department of Human Neuroscience, Sapienza University of Rome, Italy.

ABSTRACT

Objective: Previous studies have demonstrated voluntary movement alterations as well as motor cortex excitability and plasticity changes in patients with mild cognitive impairment (MCI). To investigate the pathophysiology of movement abnormalities in MCI, we tested possible relationships between movement abnormalities and primary motor cortex alterations in patients.

Methods: Fourteen amnestic MCI (aMCI) patients and 16 healthy controls were studied. Cognitive assessment was performed using clinical scales. Finger tapping was recorded by a motion analysis system. Transcranial magnetic stimulation was used to test the input/output curve of motor evoked potentials, intracortical inhibition, and short-latency afferent inhibition. Primary motor cortex plasticity was probed by theta burst stimulation. We investigated correlations between movement abnormalities, clinical scores, and cortical neurophysiological parameters.

Results: MCI patients showed less rhythmic movement but no other movement abnormalities. Cortical excitability measures were normal in patients, whereas plasticity was reduced. Movement rhythm abnormalities correlated with frontal dysfunction scores.

Conclusion: Our study in MCI patients demonstrated abnormal voluntary movement and plasticity changes, with no correlation between the two. Altered rhythm correlated with frontal dysfunction.

Significance: Our results contribute to the understanding of pathophysiological mechanisms of motor impairment in MCI.

Keywords: Mild cognitive impairment, movement slowness, motor cortex, motor control, TMS.

Abbreviations: active motor threshold (AMT), Alzheimer's disease (AD), amnestic mild cognitive impairment (aMCI), analysis of variance (ANOVA), Beck Depression Inventory (BDI-II), coefficient of variation (CV), electromyography (EMG), first dorsal interosseous (FDI), Frontal Assessment Battery (FAB), healthy controls (HCs), input-output (I/O), interstimulus interval (ISI), long-term potentiation (LTP), primary motor cortex (M1), mild cognitive impairment (MCI), motor evoked potentials (MEPs), MT1mV = intensity required to produce MEPs of ~1 mV in size, Montreal Cognitive Assessment (MoCA), Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS), nonamnestic mild cognitive impairment (naMCI), repeated-measures analysis of variance (rmANOVA), resting motor threshold (RMT), short-interval intracortical inhibition (SICI), short-latency afferent inhibition (SAI), single photon emission computed tomography (SPECT), standard deviation (SD), intermittent theta burst stimulation (iTBS), transcranial magnetic stimulation (TMS).

HIGHLIGHTS

- Voluntary movement abnormalities can be observed in mild cognitive impairment (MCI).
- Abnormal rhythm during repetitive finger movements in MCI patients relates to frontal dysfunction.
- Altered voluntary movement may be an early motor feature in patients with cognitive decline.

1. INTRODUCTION

Mild cognitive impairment (MCI) is a condition characterized by an objective impairment in cognition that is not severe enough to require help with normal activities of daily living (Petersen, 2004; Albert *et al.*, 2011). MCI is a heterogeneous condition that can be classified according to the type of involved cognitive domain as either amnestic (aMCI) or nonamnestic (naMCI) MCI (Petersen *et al.*, 2014). Studies have found that patients with aMCI have a high probability of developing Alzheimer's disease (AD) (Petersen, 2004; Winblad *et al.*, 2004).

Besides cognitive disturbances, movement studies have variably shown voluntary motor abnormalities in MCI patients, including movement slowness, altered rhythm, impaired fine motor skills, coordination abnormalities, and gait difficulties (Kluger *et al.*, 1997; Schröter *et al.*, 2003; Louis *et al.*, 2005; Aggarwal *et al.*, 2006; Camarda *et al.*, 2007; Yan *et al.*, 2008; Rabinowitz and Lavner, 2014; Roalf *et al.*, 2018; Suzumura *et al.*, 2018). However, some studies have not reported any significant movement abnormalities in MCI (Kluger *et al.*, 1997; Goldman *et al.*, 1999). To date, it is still unclear whether voluntary movement abnormalities in MCI patients reflect cognitive deficits (Rabinowitz and Lavner, 2014; Roalf *et al.*, 2018; Suzumura *et al.*, 2018; Zhang *et al.*, 2018) or whether they are caused by neuropathological involvement of cerebral areas directly implicated in motor control, including the primary motor cortex (M1) (Orta-Salazar *et al.*, 2019), basal ganglia (Burns *et al.*, 2005), and parietal and frontal cortices (Nitrini *et al.*, 2000; Camarda *et al.*, 2007; Okello *et al.*, 2009; Chandra *et al.*, 2019). In this regard, neurophysiological investigations using transcranial magnetic stimulation (TMS) techniques have demonstrated M1 abnormalities in MCI patients, including excitability changes, i.e. reduced short-interval intracortical inhibition (SICI) and short-latency afferent inhibition (SAI), as well as a lack of M1 plasticity (Olazarán *et al.*, 2010; Nardone *et al.*, 2012, 2014; Cantone *et al.*, 2014; Trebbastoni *et al.*, 2015; Padovani *et al.*, 2018, 2019; Di Lorenzo *et al.*, 2020). However, whether these M1 changes play a role in generating voluntary movement abnormalities in MCI patients is unclear.

In this study, we tested possible correlations between voluntary movement abnormalities, M1 neurophysiological parameters, clinical evaluation of cognitive functions, and demographic features of aMCI patients. For this purpose, we objectively assessed voluntary movement execution by kinematic analysis of repetitive finger movements in aMCI patients (Espay *et al.*, 2009, 2011; Bologna *et al.*, 2016, 2018, 2020). Namely, we measured movement amplitude and velocity (degree/s) and amplitude and velocity reduction during movement repetition. Movement rhythm was also measured (Iansek *et al.*, 2006; Bologna *et al.*, 2016, 2018, 2020). Using TMS techniques, we then tested M1 excitability parameters, including resting (RMT) and active (AMT) motor thresholds, the input-output (I/O) curve of motor evoked potentials (MEPs), SICI, SAI, and M1 long-term potentiation (LTP)-like plasticity (Berardelli *et al.*, 2008; Bologna *et al.*, 2020). Data obtained from aMCI patients were compared with those obtained from a group of healthy controls.

2. MATERIALS AND METHODS

2.1 Participants

Fourteen patients with aMCI (10 males, mean age ± 1 standard deviation (SD): 74.6 ± 5.8 years) and 16 healthy controls (HCs) (8 males, mean age: 71.1 ± 11.1 years) were enrolled in the study. All participants were right-handed, as evaluated by the Edinburgh Handedness Inventory (Oldfield, 1971). MCI diagnosis was based on clinical criteria (Albert *et al.*, 2011). All patients underwent laboratory blood tests and magnetic resonance imaging of the brain to exclude secondary forms of dementia, and a complete neuropsychological battery to identify the pattern of cognitive alterations. Patients with epilepsy, migraine, or other psychiatric disturbances were also excluded. Treatment with drugs potentially influencing corticospinal excitability or plasticity was discontinued at least 72 h prior to the evaluation (Ziemann *et al.*, 2015). Patients were clinically evaluated through the Movement Disorder Society (MDS)-sponsored revision of the Unified Parkinson's Disease Rating Scale, motor section (UPDRS-III) (Antonini *et al.,* 2013). Clinical assessment also included the Montreal Cognitive Assessment (MoCA) (Freitas *et al.*, 2013), the Frontal Assessment Battery (FAB)

(Dubois *et al.*, 2000), and the Beck Depression Inventory (BDI-II) (Beck *et al.*, 1961). The study conformed to the Declaration of Helsinki and international safety guidelines (Rossi *et al.*, 2009, Rossini *et al.*, 2015*a*) and was approved by the local ethics committee. All subjects provided written informed consent for their participation in the study.

2.2 Movement analysis

Subjects sat comfortably on a chair and were asked to tap their index finger repetitively on their thumb as widely and quickly as possible for 15 s with the dominant hand (finger-tapping task) (Bologna *et al.*, 2016, 2018, 2020). Three finger-tapping trials were recorded, and movements were performed with a 60-s rest interval between trials in order to avoid fatigue. In addition, one practice trial was required before kinematic recording began in order to familiarize participants with the motor task. An optoelectronic system (SMART motion, BTS Technology, Italy) was used for kinematic measurements. Reflective markers with negligible weight and a 5-mm diameter were taped to the participant's upper limb. The three-dimensional displacement of these markers was followed by three infrared cameras (sampling rate of 120 Hz). Two markers were positioned on the tips of thumb and the index finger and 3 additional markers were placed on the hand (one on the head of the 2nd metacarpal bone, one on the base of the 2nd metacarpal bone, and one on the base of the 5th metacarpal bone). This montage allowed to determine a reference plane and mathematically eliminate possible interference of undesired movements of the hand during recordings (Bologna *et al.*, 2016, 2018, 2020). Movement analysis was performed using specialized software (SMART Analyzer, BTS Engineering, Italy). Linear regression techniques to determine the intercept reflecting movement amplitude (degree) and velocity (degree/s) were used to quantify repetitive finger movement kinematics, as well as the slope representing amplitude and velocity decrement during movement repetition. We also used the coefficient of variation (CV), as calculated by SD/mean value of the intertap intervals, to measure movement rhythm, with higher values corresponding to lower repetitive movement regularity (the higher the CV value, the less rhythmic the movement performed) (Iansek *et al.*, 2006; Bologna *et al.*, 2016, 2018, 2020).

2.3 TMS

Single- and paired-pulse TMS was provided via a Magstim BiStim^2 and a figure-of-eight coil delivering monophasic pulses (Magstim Company Limited, UK), to study cortical excitability. The hotspot of the right first dorsal interosseous (FDI) muscle (i.e. optimal scalp site to elicit MEPs) was identified with the TMS coil handle oriented backwards and laterally to the midline. We estimated RMT (minimum stimulus intensity producing an MEP of ≥50 μV peak-to-peak amplitude in at least 50% of 10 trials with the tested muscle at rest) and AMT (minimum stimulus intensity producing an MEP of ~200 μV peak-to-peak amplitude in at least 50% of 10 trials during mild isometric contraction of the tested muscle) (Rossini *et al.*, 2015*a*), as well as the intensity required to produce MEPs of ~1 mV in size (MT_{1mV}) (Curra *et al.*, 2002). Ten single TMS pulses at six different stimulation intensities (total of 60 pulses) ranging in 20% increments from 80-180% RMT were delivered in order to measure the I/O curve. We randomized the order of intensity to avoid hysteresis effects (Möller *et al.*, 2009; Bologna *et al.*, 2015). Standardized protocols were used to evaluate SICI and SAI (Kujirai *et al.*, 1993; Tokimura *et al.*, 2000). We tested SICI by delivering paired TMS pulses with a subthreshold conditioning stimulus at 80% AMT, an MT_{1mV} suprathreshold test stimulus, and two interstimulus intervals (2 and 4ms ISIs). For SAI, we used a 0.1 ms electrical rectangular pulse (DS7 stimulator; Digitimer, UK) at the intensity inducing a painless twitch of the thumb to perform median nerve stimulation at the wrist. We set TMS intensity at MT_{1mV}, and tested two intervals between the median nerve and cortical stimulation (22 and 24ms ISIs). SICI and SAI were evaluated in separate blocks. Fifteen trials were recorded for each ISI for both SICI and SAI. Trials were randomized with 15 single-pulse stimuli delivered at MT_{1mV} (unconditioned MEPs). SICI and SAI were expressed as a ratio (unconditioned/conditioned MEPs). The intertrial interval of MEP recordings in the I/O curve, SICI and SAI was 4.5-5.5 s.

Cortical plasticity was assessed using intermittent theta-burst stimulation (iTBS – Huang *et al.*, 2005) delivered through a biphasic stimulator (Magstim SuperRapid; Magstim Company Limited, UK) connected to a figure-of-eight coil positioned over the hotspot. The intensity of stimulation was 80%

AMT. Ten bursts of three pulses at 50 Hz were repeated at 200ms intervals and delivered in short 2s trains, with a pause of 8s between consecutive trains (20 trains, 600 pulses in total) (Huang *et al.*, 2005).

We recorded MEPs via surface electrodes with a standard belly-tendon montage. Digitimer D360 (Digitimer, UK) was used to amplify and filter (20 Hz-1 kHz) electromyographic (EMG) signals, which were then stored on a computer (5 kHz sampling rate) through an analog-digital converter AD1401 plus (Cambridge Electronic Design, UK) and analyzed offline with specialized software (Signal version 5.08, Cambridge Electronic Design, UK). We rejected trials with background EMG activity >100 μV in the 200 ms before TMS. The amplitude of MEPs was calculated peak-to-peak in the 20-40 ms following the TMS artifact.

2.4 Experimental design

Subjects participated in a single experimental session that included clinical evaluation and kinematic recording of finger movements. TMS assessment began with RMT, AMT, and MT_{1mV} estimation, followed by the measurement of corticospinal and intracortical excitability parameters at rest. Finally, 15 MT_{1mV} MEPs (intertrial interval 4.5-5.5 s) were recorded before (T0) and 5 (T1), 15 (T2), and 30 min (T3) after iTBS in order to assess the LTP-like plasticity of M1. Thus, the interval between motor threshold estimation and pre-iTBS baseline MEP recording was ~ 20 min.

2.5 Statistical analysis

The Mann-Whitney U test was used to evaluate possible differences in age and MoCA, FAB, BDI-II, and UPDRS-III scores between aMCI patients and HCs, while Fisher's exact test was applied to evaluate possible differences in gender distribution between groups. Unpaired t-tests were used to compare kinematic variables, motor thresholds, and the amplitude of MEPs evoked by single TMS pulses (i.e. unconditioned MEPs for SICI and SAI and MEPs before iTBS).

A repeated-measures analysis of variance (rmANOVA) with 'GROUP' (2 levels: aMCI, HC) and 'TMS intensity' (6 levels: 80%, 100%, 120%, 140%, 160%, and 180% RMT) as factors was used to

assess possible differences in the I/O curve between aMCI patients and HCs. The same analysis with 'GROUP' and 'ISI' (2 levels: 2 and 4 ms for SICI, 22 and 24 ms for SAI) was applied to compare SICI and SAI between aMCI patients and HCs. An rmANOVA with 'GROUP' and 'TIME POINT' (3 levels: T1, T2, and T3) as factors was adopted to verify possible differences in iTBS effects between groups. For this analysis, MEP amplitude recorded at T1, T2, and T3 was normalized to T0.

The Duncan test was used as a post-hoc analysis in the various rmANOVAs. We applied Greenhouse-Geisser corrections when a violation of sphericity was detected using Mauchly's test. Pearson's coefficient was used to test possible correlations between kinematic and TMS measures, whereas Spearman's rank correlation coefficient was adopted to verify possible relationships between patient clinical data and neurophysiological measures (kinematic and TMS parameters). Unless otherwise stated, results are shown as mean values ± 1 standard error of the mean. The significance level was set at $p<0.05$ and data were analyzed using STATISTICA (TIBCO Software Inc., Palo Alto, California, US).

3. RESULTS

3.1 Clinical and demographic data

Age (p=0.42), gender distribution (p=0.28), and BDI-II scores (p=0.55) were comparable between aMCI patients and HCs. In contrast, MoCA (aMCI patients: 21.0 ± 3.9 vs. HCs: 25.1 ± 0.9, p=0.01) and FAB scores (aMCI patients: 14.2 ± 2.5 vs. HCs: 16.0 ± 0.4, p=0.04) were both lower in patients than in HCs. The mean ± 1 SD) of the UPDRS-III score in MCI patients was 2.8 ± 2.6 (Table 1) due to the presence of very mild impairment in finger and foot tapping (7/14 patients), rigidity of the upper limb (3/14 patients), slightly impaired posture (3/14 patients), slight postural and kinetic tremor (2/14 patients), and gait imbalance (1/14 patients). However, no patient had bradykinesia associated with rigidity or tremor that would have met the definition for parkinsonism (Postuma *et al.*, 2015).

3.2 Movement kinematics

The analysis demonstrated altered movement rhythm in aMCI patients, as demonstrated by the higher CV values with respect to HCs (aMCI patients: 0.16 ± 0.05, HCs: 0.11 ± 0.04, p<0.01). In contrast, the number of performed movements (aMCI patients: 45.8 ± 15.21, HCs: 51.48 ± 13.63, p=0.29), movement amplitude (aMCI patients: 45.9 ± 13.54 degrees, HCs: 48.32 ± 15.7 degrees, p=0.65), movement velocity (aMCI patients: 871.06 ± 231.62 degrees/s, HCs: 858.62 ± 232.45 degrees/s, p=0.88), amplitude decrement (aMCI patients: -0.25 ± 0.28 degrees/n mov, HCs: 0.26 degrees/n mov, p=0.92), and velocity decrement (aMCI patients: -5.47 ± 6.3 (degrees/s)/n mov, HCs: -3.11 ± 4.44 (degrees/s)/n mov, p=0.24) were all similar between patients and HCs (**Fig. 1**).

3.3 TMS measures

AMT, RMT, MT_{1mV}, unconditioned MEP amplitude in SICI and SAI protocols, and MEP amplitude before iTBS did not differ between groups (**Table 2**). The I/O curve was also similar between aMCI patients and HCs ('GROUP': $F_{1,28}$=0.70, p=0.41; 'GROUP' x 'TMS intensity': $F_{5,140}$=1.67, p=0.15) (**Fig. 2a**), as was the degree of SICI ('GROUP': $F_{1,28}$=0.01, p=0.92; 'GROUP' x 'ISI': $F_{1,28}$=0.16, p=0.69) and SAI ('GROUP': $F_{1,28}$=0.59, p=0.45; 'GROUP' x 'ISI': $F_{1,28}$=1.53, p=0.23) (**Fig. 2b**). When comparing SICI, a significant effect of the factor 'ISI' emerged ($F_{1,28}$=6.26, p=0.02), indicating lower values at ISI 2 ms than 4 ms.

When comparing the effects of iTBS between aMCI patients and HCs, rmANOVA showed a significant 'GROUP' x 'TIME POINT' interaction ($F_{2,56}$=3.87, p=0.03) and no effect of the main factor 'GROUP' ($F_{1,28}$=1.93, p=0.17). As expected, the analysis also showed a significant effect of 'TIME POINT' ($F_{2,56}$=3.20, p=0.04), indicating peak MEP facilitation after iTBS at T2. Interestingly, post-hoc analysis revealed that iTBS-induced MEP facilitation was lower in aMCI patients than in HCs at T2 (p=0.03), while values at T1 (p=0.46) and T3 (p=0.18) were comparable between groups (**Fig. 3**).

3.4 Correlation analysis

We found a negative relationship between movement rhythm (CV) and FAB scores (r=-0.68, p<0.01),

meaning that a higher CV (less rhythmic movement) was associated with a lower FAB score (greater cognitive impairment). In addition, a trend toward correlation was present between movement rhythm and MoCA score (r=-0.43, p=0.11). No relationship was found between movement rhythm and iTBS-induced MEP facilitation at T2 in patients (r=0.10, p=0.72), or between iTBS-induced MEP facilitation at T2 and FAB (r=-0.05, p=0.86) or MoCA scores (r=-0.15, p=0.61).

4. DISCUSSION

In the present study, we examined voluntary movement execution in aMCI patients through kinematic analysis of repetitive finger tapping. We then tested whether movement abnormalities correlated with clinical cognitive scores or altered neurophysiological measures of M1. We found that finger-tapping movements in aMCI patients were characterized by less rhythmic movement (increased CV) as compared to HCs. We also found decreased synaptic M1 plasticity in aMCI patients. Movement rhythm abnormalities correlated with FAB score but not with altered M1 plasticity in aMCI patients. Our results provide evidence of motor dysfunction and insight into its possible pathophysiological mechanisms in MCI.

The less rhythmic movement observed in aMCI patients during finger-tapping execution is in line with previous studies that used computerized (though not kinematic) methods of analysis and reported increased intraindividual variability (Roalf *et al.*, 2018) or rhythm fluctuation during finger tapping (Suzumura *et al.*, 2018). The possible pathophysiological mechanisms of altered motor rhythm in MCI are unclear. When investigating excitability mechanisms of M1 using TMS techniques, we found that motor thresholds, the I/O curve, and SICI were comparable in aMCI patients and HCs. These results confirm previous observations (Nardone *et al.*, 2012, 2014; Tsutsumi *et al.*, 2012) and support evidence of normal corticospinal excitability and normal intracortical excitability of M1 in aMCI patients. We also found comparable SAI between groups. Neurophysiological observations in HCs linked SAI to M1 cholinergic activity (Di Lazzaro *et al.*, 2000; Tokimura *et al.*, 2000; Ferreri *et al.*, 2012), a measure that is altered in AD (Di Lazzaro *et al.*, 2002; Guerra *et al.*, 2011; Di Lorenzo

et al., 2016; Bologna *et al.*, 2020*)*. Our results are consistent with previous studies in MCI and point to a preserved cholinergic inhibitory drive in M1 in the pre-dementia stage of AD (Sakuma *et al.*, 2007; Di Lorenzo *et al.*, 2020). The observation of normal M1 excitability parameters in aMCI patients imply that motor abnormalities in this condition are not related to M1 neurophysiological dysfunction. We also demonstrated weaker MEP facilitation at T2 after iTBS in aMCI patients as compared to HCs, which suggests altered LTP-like plasticity in M1. One recent study assessed iTBS-induced M1 plasticity in MCI patients and found that MCI patients showed impaired LTP-like aftereffects as compared to HCs (Di Lorenzo *et al.*, 2020). In addition, the authors demonstrated that MCI patients who progressed to dementia at follow-up had weaker LTP-like plasticity at the time of first evaluation (Di Lorenzo *et al.*, 2020). These data, along with our findings, further support altered LTP-like plasticity in M1 as a relevant pathophysiological process underlying AD and aMCI. These findings also confirm previous TMS and TMS-EEG studies demonstrating impaired M1 functionality in all stages of dementia (Ferreri *et al.*, 2003, 2016; Julkunen *et al.*, 2008; Guerra *et al.*, 2015; Di Lorenzo *et al.*, 2016; Trebbastoni *et al.*, 2015). Amyloid beta and phosphorylated tau protein deposition are key neuropathological processes in AD (Hardy, 2002; Busche and Hyman, 2020) and are thought to underlie the disruption of synaptic plasticity mechanisms in this condition (Selkoe, 2008; Koch *et al.*, 2011). Since aMCI is considered the predementia stage of AD *(*Petersen *et al.,* 2014*),* we hypothesize that altered LTP-like plasticity in aMCI is due to early amyloid beta and phosphorylated tau protein deposition at the cortical level. However, we found that abnormal LTP-like plasticity in M1 did not correlate with altered movement rhythm in aMCI. This result is not surprising. Similar to what has been observed here in aMCI, we recently demonstrated a lack of correlation between these two neurophysiological abnormalities in AD patients (Bologna *et al.*, 2020). Overall, these data suggest that altered movement rhythm is independent of M1 plasticity changes, and instead reflects other mechanisms. Thus, altered M1 plasticity does not necessarily constitute a pathophysiological substrate of motor impairment in these conditions. Instead, a recent

study found that impaired LTP-like cortical plasticity in AD was selectively associated with less efficient verbal memory (Di Lorenzo *et al.*, 2019).

An alternative explanation for motor impairment in aMCI is that less rhythmic movement during finger tapping reflects dysfunction in frontal areas, especially in relation to defective attentive processes (Rabinowitz and Lavner, 2014). In fact, performance of the finger-tapping task as regularly and precisely as possible requires a high level of attention, which may already be impaired in MCI patients (Albert *et al.*, 2011; McLaughlin *et al.*, 2014; Kirova *et al.*, 2015). The results of the present study support the hypothesized relationship between voluntary movement abnormalities and frontal lobe dysfunction by providing evidence of a negative correlation between movement rhythm and FAB score, i.e. the less rhythmic the movement, the greater the cognitive impairment in executive functions located in the frontal lobes (Dubois *et al.*, 2000). Indeed, in patients with aMCI (Kume *et al.*, 2011) or other conditions such as frontotemporal dementia (Guedj *et al.*, 2008), FAB scores were found to correlate with SPECT perfusion in lateral and medial frontal areas. Moreover, even in the earliest stages of AD the pathological deposition of amyloid beta and phosphorylated tau protein is known to occur in the hippocampus as well as in other regions, including the frontal cortices (Okello *et al.*, 2009; Chandra *et al.*, 2019), which may underlie executive function impairment. Although not statistically significant, we also found a trend toward an association between MoCA scores and altered movement rhythm. This result is in line with other studies (Roalf *et al.*, 2018; Suzumura *et al.*, 2018) and may suggest that deficits in cognitive functions other than executive ones (e.g. memory) (Rabinowitz and Lavner, 2014) also contribute to finger tapping rhythm alterations. In summary, we may hypothesize that dysfunctions of brain areas involved in cognitive processes underlie the generation of altered motor rhythm in aMCI. This hypothesis, however, requires validation by further studies using structural and functional neuroimaging and molecular imaging.

In contrast to the present results in aMCI patients, we have recently demonstrated that less rhythmic movement during finger tapping is combined with movement slowness in AD patients (Bologna *et al.*, 2020). We also found that movement slowness in AD correlated with cholinergic M1 dysfunction

(Bologna *et al.*, 2020), a neurophysiological alteration that was not present in our aMCI cohort. Therefore, we now speculate that altered movement rhythm is the only motor abnormality occurring in the pre-dementia stage (aMCI), while altered movement rhythm occurring in combination with motor slowness occurs in clinically overt dementia as a result of a marked dysfunction in cholinergic neurotransmission in M1. Taken as a whole, the results of the present study in aMCI together with those of our previous study in AD (Bologna *et al.*, 2020) suggest that voluntary movement, as assessed by kinematic techniques, may be an indirect biomarker of cognitive dysfunction within the aMCI and AD spectrum. Longitudinal studies are needed to elucidate whether kinematic methods may be used to predict the clinical course in MCI patients.

Our study has some limitations that must be considered. First, the sample size was relatively small, although the objective techniques used to quantify finger-tapping movements provided accurate and reproducible measurements of motor impairment (Heldman *et al.*, 2011). Second, although we recruited aMCI patients based on accurate neuropsychological diagnoses and according to current clinical criteria (Albert *et al.*, 2011), we acknowledge that we did not perform biomarker assessments (e.g. amyloid cerebrospinal fluid or positron emission tomography). Therefore, we cannot fully exclude that mild cognitive deficits in some patients of our sample were due to alternative aetiologies. However, since previous evidence has shown that aMCI patients have a higher probability of developing AD dementia than naMCI patients (Petersen, 2004; Winblad *et al.*, 2004), we recruited only aMCI patients in our study in order to minimize the likelihood that cognitive impairment in MCI was due to types of dementia other than AD. Finally, in our assessment of M1 plasticity we did not use a neuronavigation system during TMS recording or collect more than 20 MEPs, methodological procedures that could have provided a more precise estimate of MEP amplitude as suggested by other studies (Chang *et al.*, 2016; Goldsworthy *et al.*, 2016).

In conclusion, our study provides new information on fine voluntary movement impairment in aMCI patients. In our patient sample, we found only less rhythmic movement, and no movement slowness. Altered movement rhythm was unrelated to changes in M1 excitability or LTP-like

plasticity and was likely dependent on frontal area abnormalities. Our results suggest that altered rhythm in fine voluntary movements, as assessed by kinematic techniques, may be an early motor feature in patients with cognitive decline. Future longitudinal studies combining kinematic methods with TMS measures may provide a clearer understanding of the neurophysiological abnormalities that may predict the clinical course of MCI patients.

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Potential Conflicts of Interest

None of the authors have any potential conflicts of interest.

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Figure Legends

Fig. 1

MCI: mild cognitive impairment; HCs: healthy controls. Dots denote individual data. White diamonds denote average values. Horizontal lines indicate the median value (50th percentile). Boxes indicate the 25-75th percentiles of the dataset. Asterisks denote significant between-group differences at post hoc analyses.

Fig. 2

Panel A. The input-output curve of motor evoked potentials (MEPs) in mild cognitive impairment (MCI) patients and healthy controls (HCs). The y-axis displays MEP amplitude (mV), while the x-axis displays the six tested stimulation intensities (80, 100, 120, 140, 160, and 180% of the resting motor threshold – RMT). *Panel B.* Short-interval intracortical inhibition (SICI) and short-latency afferent inhibition (SAI) in MCI patients and HCs. The y-axis provides the ratio between conditioned/unconditioned MEP amplitudes, while the x-axis shows the tested interstimulus intervals (2 and 4 ms for SICI, 22 and 24 ms for SAI). *Panel C.* Changes in MEP amplitude after the intermittent theta burst stimulation (iTBS) protocol in MCI patients and HCs. The y-axis displays MEP amplitudes normalized to baseline (T0), while the x-axis shows measurements at the four time points: before iTBS (T0) and 5 (T1), 15 (T2), and 30 min (T3) after iTBS. Asterisks denote significant between-group differences at post hoc analyses.

Fig. 3

Correlation between the rhythm of movement, as measured by the coefficient of variation (x-axis), and the Frontal Assessment Battery (FAB) score (y-axis).