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Humans with Type-2 Diabetes Show Abnormal Long-Term Potentiation-Like Cortical Plasticity Associated with Verbal Learning Deficits

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Abstract.

Background: Type-2 diabetes mellitus (T2DM) accelerates cognitive aging and increases risk of Alzheimer's disease. Rodent models of T2DM show altered synaptic plasticity associated with reduced learning and memory. Humans with T2DM also show cognitive deficits, including reduced learning and memory, but the relationship of these impairments to the efficacy of neuroplastic mechanisms has never been assessed.

Objective: Our primary objective was to compare mechanisms of cortical plasticity in humans with and without T2DM. Our secondary objective was to relate plasticity measures to standard measures of cognition.

Methods: A prospective cross-sectional cohort study was conducted on 21 adults with T2DM and 15 demographically-similar non-diabetic controls. Long-term potentiation-like plasticity was assessed in primary motor cortex by comparing the amplitude of motor evoked potentials (MEPs) from single-pulse transcranial magnetic stimulation before and after intermittent theta-burst stimulation (iTBS). Plasticity measures were compared between groups and related to neuropsychological scores.

Results: In T2DM, iTBS-induced modulation of MEPs was significantly less than controls, even after controlling for potential confounds. Furthermore, in T2DM, modulation of MEPs 10-min post-iTBS was significantly correlated with Rey Auditory Verbal Learning Task (RAVLT) performance.

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Conclusion: Humans with T2DM show abnormal cortico-motor plasticity that is correlated with reduced verbal learning. Since iTBS after-effects and the RAVLT are both NMDA receptor-dependent measures, their relationship in T2DM may reflect brain-wide alterations in the efficacy of NMDA receptors. These findings offer novel mechanistic insights into the brain consequences of T2DM and provide a reliable means to monitor brain health and evaluate the efficacy of clinical interventions.

Keywords: Cognitive aging, neuroplasticity, transcranial magnetic stimulation, type 2 diabetes mellitus, verbal learning

INTRODUCTION

The brain is a target organ in type-2 diabetes mellitus (T2DM) [1]. T2DM affects the central nervous system through neuronal toxicity of hyper- and hypoglycemia episodes, microvascular insults, impaired glucose, and insulin transfer and resistance [2, 3]. Presumably as a consequence of this damage, T2DM accelerates cognitive decline [4] and increases risk of dementia [5, 6]. Cognitive dysfunction in T2DM has been linked to inflammation and altered vasoreactivity [7]. Even in the absence of vascular complications, T2DM can alter synaptic plasticity in the mouse hippocampus resulting in cognitive deficits [8], and mice with T2DM are less likely to recover from stroke due to impaired neuroplastic mechanisms [9]. To our knowledge, no study has directly assessed the mechanisms of brain plasticity or their behavioral significance in humans with T2DM.

Cortical reactivity and plasticity can be measured noninvasively in the human motor cortex using transcranial magnetic stimulation (TMS; Fig. 1). Operational definitions of *reactivity* and *plasticity* can be found in the Materials and Methods; collectively they refer to the process of comparing the motor responses to individual TMS pulses at baseline with those obtained after a repetitive TMS intervention such as theta-burst stimulation (TBS) [10]. TMS-TBS measures have identified age-related changes in plasticity across the lifespan in healthy individuals [11] and revealed altered neuroplastic mechanisms in autism spectrum disorders [12], traumatic brain injury [13], and Alzheimer's disease (AD) [14].

Intermittent TBS (iTBS), which assesses NMDA receptor (NMDAR)-dependent [15] long-term potentiation (LTP)-like plasticity [16], was used to directly investigate whether the mechanisms of brain plasticity are abnormal in T2DM. As the motor system is not specifically affected in T2DM, altered cortico-motor plasticity measures should reflect brain-wide declines in the efficacy of neuroplastic mechanisms. Further, if global changes in brain plasticity are driving deficits in cognitive performance, we measures obtained in the motor cortex should be associated with neuropsychological performance, especially on measures of learning and memory that are also NMDAR-dependent [17].

MATERIALS AND METHODS

Human participants

In a prospective observational cohort study, adults (50–80 y) with and without T2DM were recruited through the Joslin Diabetes Center or responded to flyers posted around Beth Israel Deaconess Medical Center. 83 adults were enrolled, including individuals with well-controlled hypertension and hypercholesterolemia, but excluding significant heart disease (heart attack or stroke). 17 were subsequently excluded for a Mini-Mental State Examination (MMSE) score <27, Geriatric Depression Scale (GDS) score >10, resting tremor, or receiving medications contraindicated for TMS [18]. Seven controls were excluded for indications of pre-diabetes: glycosylated hemoglobin (HbA1c) >5.6% or fasting glucose >100 mg/dL. Two T2DM patients were excluded for HbA1c >10%, indicating uncontrolled T2DM. From saliva-based genotyping, 11 individuals with an *APOE*-ε4 or *BDNF*-Met allele were excluded as these polymorphisms have been shown to alter TBS-based measures of plasticity [19, 20]. A further 10 participants were excluded or withdrew consent for various reasons, including inability to fit in the scanner, discomfort sitting, pending surgery, or failure to show up for study visits. The final cohort consisted of 21 adults with T2DM and 15 demographically-similar controls (Table 1). Most T2DM patients controlled their diabetes with Metformin and the median time since diagnosis was 10 years (range: 2–18 years).

The local Institutional Review Board approved the study. All participants provided written informed consent prior to enrollment according to the Declaration of Helsinki and received monetary compensation upon completion.

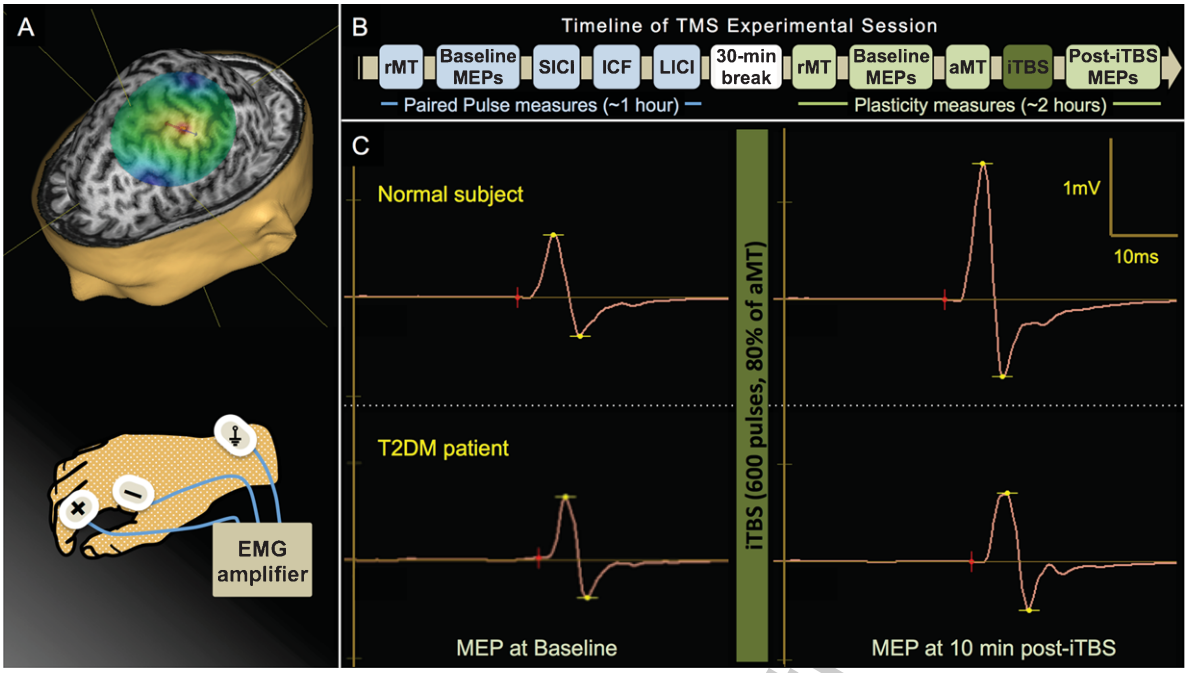

Fig. 1. Cortical reactivity and plasticity can be measured noninvasively in the human motor cortex using TMS. *Reactivity* refers to the average amplitude of MEPs elicited by single-pulse TMS, while *plasticity* is defined as the change in reactivity induced by iTBS. A) MR-guided TMS was applied to the left primary motor cortex and resulting MEPs were recorded from the right FDI muscle by surface EMG. B). The present study assessed TMS-iTBS measures of plasticity as well as paired pulse TMS measures of cortical inhibition and facilitation. After determining resting motor threshold (rMT), 50 single (unconditioned) monophasic TMS pulses were delivered, followed by three sets of 50 pulse-pairs to assess short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and long-interval intracortical inhibition (LICI). After a break, rMT was reassessed and three sets of 30 biphasic pulses were delivered to measure baseline cortico-motor reactivity. The active motor threshold (aMT) was assessed and iTBS was applied. Cortico-motor reactivity was reassessed in six blocks of 30 pulses at 5, 10, 20, 30, 40, and 50 min post-iTBS. C) Example MEP traces from a single control subject (*top*) and T2DM patient (*bottom*) recorded at baseline (*left*) and 10 min after iTBS (*right*).

Neuropsychological testing

A 30-item MMSE, 50-item Wechsler Test of Adult Reading (W-TAR), 15-item GDS, and 78-point activities of daily living (ADL) inventory were administered to characterize general neurocognitive status in the two groups. Additional tests were chosen to assess cognitive domains previously shown [21] to be impaired in T2DM: psychomotor processing speed was assessed with the Digit Symbol Substitution Test (DSST; number of correct substitutions in 90 sec); executive function was measured using the Trail Making Test (difference in time to complete Parts A & B); working memory was assessed with the Digit Span Backwards task (number of correctly-completed trials); and verbal learning and memory was assessed with a 10-item Rey Auditory Verbal Learning Test (RAVLT; percent of correctly recalled words across the five learning trials and after a 30-min delay) [22]. The RAVLT in particular was chosen as it is an NMDAR-dependent [17] measure of cognitive plasticity that is sensitive to prodromal dementia [23].

Magnetic resonance imaging

A T1-weighted anatomical magnetic resonance imaging scan was obtained in all participants on a 3T scanner (GE Healthcare, Ltd., UK) using a 3D spoiled gradient echo sequence: 162 axial-oriented slices for whole-brain coverage; 240-mm isotropic field-of-view; 0.937-mm × 0.937-mm × 1-mm native resolution; flip angle = 15°; TE/TR ≥2.9/6.9 ms; duration ≥432 s. Cortical reconstruction and automatic segmentation were performed with Freesurfer (version 6.0, http://surfer.nmr.mgh.harvard.edu/). Cortical thickness, calculated as the shortest distance between the pial and white matter surfaces [24], was measured for the primary motor cortex (precentral gyrus and central sulcus) in the left hemisphere using a subject-independent probabilistic atlas [25].

Table 1
Demographic and study data

	Controls ($n = 15$) Mean ± SE	T2DM ($n = 21$) Mean ± SE	Pairwise comparison Df†	|t ratio|	$p_{unadjusted}$	Lower CL	Upper CL	$p_{adjusted}$*
Demographics								
Age (y)	63.93 ± 2.2	66.90 ± 1.8	34	1.05	0.299	–2.76	8.70	0.994
Education (y)	15.60 ± 0.6	15.24 ± 0.6	34	0.44	0.660	–2.02	1.29	1.000
# Male (%)	7 (46.7)	12 (57.1)	$N = 36$, df = 1, $p = 0.736$; Fisher's Exact Test, $p_{adjusted} = 1.000$					
# Dextral (%)	15 (100)	18 (85.7)	$N = 36$, df = 2, $p = 0.500$; Fisher's Exact Test, $p_{adjusted} = 1.000$					
# Caucasian (%)	14 (93.3)	17 (81.0)	$N = 36$, df = 3, $p = 0.242$; Fisher's Exact Test, $p_{adjusted} = 0.994$					
Health Indices								
Hemoglobin A1c (%)[a]	5.43 ± 0.1	7.50 ± 0.4	12.6	5.19	**<0.001**	**1.20**	**2.93**	**0.001**
Fasting glucose (mg/dL)[b]	85.50 ± 4.1	144.50 ± 12.0	13.1	4.65	**<0.001**	**31.59**	**86.41**	**0.003**
Creatinine (mg/dL)[c]	0.83 ± 0.1	0.87 ± 0.1	14	0.23	0.823	–0.28	0.34	1.000
Weight (lbs.)[d]	157.10 ± 8.8	187.75 ± 6.8	24	2.77	**0.011**	**7.83**	**53.47**	**0.033**
Height (in.)[e]	65.62 ± 1.3	66.49 ± 0.8	23	0.62	0.543	–2.04	3.77	1.000
Body mass index (lbs./in.2)[e]	25.48 ± 0.9	29.68 ± 1.1	23.0	2.91	**0.008**	**1.22**	**7.18**	**0.032**
Cortical Thickness (mm)								
Left motor cortex	2.39 ± 0.0	2.25 ± 0.0	34	2.92	**0.006**	**–0.25**	**–0.04**	**0.012**
Left hemisphere – mean	2.26 ± 0.0	2.21 ± 0.0	34	1.24	0.223	–0.12	0.03	0.223
Neuropsychological testing								
Mini-mental status examination (# / 30)	29.40 ± 0.2	28.95 ± 0.2	34	1.44	0.158	–1.08	0.18	0.679
Wechsler test of adult reading (age-normed)	112.93 ± 2.6	112.95 ± 2.5	34	0.01	0.996	–7.50	7.54	1.000
Activities of daily living inventory (# / 78)	74.73 ± 0.8	76.48 ± 0.4	34	1.92	0.063	–0.10	3.58	0.377
Geriatric depression scale (# / 15)	0.60 ± 0.3	1.29 ± 0.3	34	1.53	0.136	–0.23	1.60	0.679
Digit Symbol Substitution Test (# / 90)	55.20 ± 2.7	44.67 ± 2.1	34	3.11	**0.004**	**–17.41**	**–3.65**	**0.030**
Trail Making Test (B-A, time in s)	45.40 ± 15.7	53.27 ± 7.1	33	0.50	0.622	–24.33	40.06	1.000
Digit Span Backwards Test (# correct trials)	8.47 ± 0.6	6.67 ± 0.5	34	2.29	**0.029**	**–3.40**	**–0.20**	0.200
Rey auditory verbal learning test (% correct)								
Learning	80.53 ± 1.9	69.71 ± 2.8	32.8	3.20	**0.003**	**–17.69**	**–3.94**	**0.027**
Delayed recall	74.00 ± 5.1	64.76 ± 5.7	34	1.15	0.259	–25.60	7.12	0.778

CL, confidence level; T2DM, type-2 diabetes mellitus; iTBS, intermittent theta-burst stimulation; TMS, transcranial magnetic stimulation. †Integers reflect pooled variance, non-integers reflect unequal variance. *Significance values for each set of tests were adjusted using Holm-Bonferroni method. [a]Obtained from 12 T2DM and 3 controls. [b]Obtained from 12 T2DM and 4 controls. [c]Obtained from 10 T2DM and 6 controls. [d]Obtained from 16 T2DM and 10 controls. [e] Obtained from 15 T2DM and 10 controls.

Electromyography

To measure the amplitude of TMS-induced MEPs, Ag-AgCl surface electrode-pairs (Ambu A/S, Denmark) were placed on the belly and tendon of the right first dorsal interosseous (FDI) and a ground on the right ulnar styloid process (Fig. 1A).

Transcranial magnetic stimulation

All parameters used in the study conformed to current recommended guidelines for the safe application of TMS endorsed by the International Federation of Clinical Neurophysiology (IFCN) [18]. Following IFCN guidelines [26], resting motor threshold (rMT) and active motor threshold (aMT) were measured individually and used to set the intensity of subsequent stimulation. MEP trials were randomly jittered (5000–6000 ms) to avoid train effects. A Navigated Brain Stimulation system (Nexstim Plc, Finland) was used to identify the hand region of the primary motor cortex and ensure consistent targeting throughout the experimental session (Fig. 1A). We operationally define *reactivity* as the average amplitude of motor evoked potentials (MEPs) elicited by suprathreshold single-pulse TMS, and *plasticity* as the change in reactivity induced by subthreshold TBS [10].

Paired-pulse TMS

Neuronavigated paired-pulse TMS was applied using a handheld monophasic figure-of-eight focal coil (Nexstim Plc, Finland). Three protocols were utilized: short-interval intracortical inhibition (SICI; 80%-rMT conditioning pulse, 120%-rMT test pulse, 3-ms interval), intracortical facilitation (ICF; 80%-rMT conditioning pulse, 120%-rMT test pulse, 12-ms interval); and long-interval intracortical inhibition (LICI; 80%-rMT conditioning pulse, 120%-rMT test pulse, 100-ms interval) [27, 28]. A preceding block of single TMS pulses at 120% rMT provided a measure of unconditioned cortico-motor reactivity. Each block consisted of 50 trials and individual MEP amplitudes >2.5 SD from the mean were excluded. Measures of SICI, LICI, and ICF were calculated as the percent change of the conditioned MEPs from the unconditioned block.

Theta-burst TMS

Neuronavigated iTBS was applied to participants using a handheld passive-cooling fluid-filled figure-of-eight coil attached to a MagPro X100 stimulator (MagVenture A/S, Denmark). Intensity was 80% aMT. The pattern was a two-second train of biphasic bursts (three pulses at 50 Hz) repeated every 200 ms. Trains were repeated 20 times with an eight-second inter-train interval (600 pulses, 192 seconds). This protocol has been shown to potentiate cortico-motor reactivity for up to 40 minutes in healthy individuals [10, 29].

Figure 1B depicts the timeline of the TMS experimental session. Prior to iTBS, participants received three blocks of 30 pulses at 120% rMT. The peak-to-peak amplitudes of all recorded MEPs (Fig. 1C) were measured and averaged for each individual as a measure of baseline cortico-motor reactivity. Cortico-motor reactivity was reassessed in blocks of 30 TMS pulses at 5, 10, 20, 30, 40, and 50 min post-iTBS. For each block, individual MEPs >2.5 SD from the mean were excluded. Plasticity was calculated as the percent change of each post-iTBS block from baseline.

Statistical analysis

Statistical analyses were performed in JMP Pro (version 12.0, http://www.jmp.com) and Stata (version 14.1, http://www.stata.com) using a normal distribution, Levene's test for homoscedasticity, and a two-tailed 95% confidence interval ($\alpha = 0.05$). Individual significance values for each set of tests were adjusted for multiple comparisons using Holm-Bonferroni correction.

Pairwise comparisons were made against the null hypotheses that demographic, health, cortical thickness, neuropsychological, and neurophysiological measures were equivalent between T2DM and controls. The proportions of gender, handedness, and racial-ethnic composition were compared using Fisher's Exact tests, while all continuous variables were compared with Student's *t*-tests.

To test the null hypothesis that the after-effects of iTBS are equivalent between groups, post-iTBS changes in MEP amplitudes were entered into a 2 (*diagnosis*) × 6 (*time*) full-factorial linear mixed-effects model. However, as the peak modulation of MEP amplitudes typically occurs immediately after iTBS [29], planned pairwise comparisons between T2DM and controls for each time-point were conducted using Student's *t* tests.

To evaluate the behavioral significance of altered plasticity, correlation analyses were performed between MEP amplitudes 10-min post-iTBS (POST10; %Δ from baseline) and scores on the

DSST, TMT, Digit Span, and RAVLT. POST10 was selected *post hoc* (see Results) as the time-point when plasticity measures were most altered in T2DM relative to controls. Correlations were thus performed separately for T2DM and controls to avoid confounding group differences in neuropsycholgical and TMS measures.

RESULTS

Table 1 details group means ± standard error of continuous variables, numbers and proportions of categorical variables, and pairwise comparisons, including adjusted *p*-values. Unless otherwise indicated, *p*-values reported in the text are unadjusted.

Demographics, health, cortical thickness, and neuropsychological testing

Fisher's Exact Tests yielded with no group differences in the proportion of gender, handedness, or ethnic composition, while student's *t* tests indicated T2DM and control participants were similarly aged and educated (p's > 0.2). These results indicate that the two groups had equivalent demographic composition.

As expected, the T2DM group had significantly worse health indices, including greater HbA1c and fasting glucose levels, weight, and body-mass index (p's < 0.02), though the groups had similar creatinine levels ($p = 0.823$) and were of similar height ($p = 0.543$). All differences remained significant after Holm-Bonferroni correction. These results indicate that measures of blood sugar and obesity were higher in the T2DM group, while height and creatinine, a marker of kidney function, were equivalent.

Analysis of cortical thickness found that the mean thickness across the left hemisphere did not differ significantly ($p = 0.223$), however the left motor cortex (precentral gyrus and central sulcus) was thinner for T2DM than controls ($p = 0.012$).

In the neuropsychological measures, there were no significant group differences in the MMSE, WTAR, or GDS (p's > 0.1), though the T2DM group had slightly higher ADLs ($p = 0.063$). These results indicate T2DM did not differ from control participants in terms of overall neurocognitive status, premorbid IQ, functional independence, or levels of depression, respectively. Despite these similarities and the lack of subjective cognitive complaints, the T2DM group exhibited reduced psychomotor processing speed, working memory, and verbal learning. Specifically, T2DM made fewer correct substitutions on the DSST ($p = 0.004$), completed fewer trials on the Digit Span Backwards task ($p = 0.029$), and recalled fewer words on the RAVLT learning trials ($p = 0.003$). After applying Holm-Bonferroni correction, the DSST and RAVLT remained significant.

Measures of cortico-motor reactivity and plasticity

All participants tolerated TMS and iTBS with no complications or unexpected side effects. Student's *t* tests yielded no significant differences in baseline neurophysiological measures, including motor thresholds and baseline MEP amplitudes and latencies (p's > 0.1). Similarly, there were no group differences in the paired pulse TMS measures (p's > 0.1). These results indicate T2DM did not differ from controls in cortico-motor reactivity, the corticospinal response to TMS, or the efficacy of inhibitory and excitatory intracortical circuits (Supplementary Table 1 and Supplementary Figures 1–3).

Across all post-iTBS time-points, the mean ± standard error percent change in MEP amplitude was 36.21 ± 7.2 for controls and 7.22 ± 6.0 for T2DM. This effect in controls is consistent with a recent meta-analysis of TBS in healthy subjects [29].

The linear mixed-effect model indicated the change in MEP amplitudes did not vary significantly (at the 0.05 level) by *diagnosis*, $F(1,34.1) = 2.59$, $p = 0.117$, *time*, $F(5,167.2) = 1.97$, $p = 0.086$, or their interaction, $F(5,167.2) = 1.45$, $p = 0.209$. However, planned *t* tests showed T2DM subjects had significantly less potentiation of MEP amplitudes 5-min post-iTBS (POST5; $p = 0.042$) and POST10 ($p < 0.007$) (Fig. 2), with the latter remaining significant after Holm-Bonferroni correction. From 20–50 min post-iTBS, the change in MEP amplitudes was statistically equivalent between groups (p's > 0.3). These results indicate it is the initial impact of iTBS on cortico-motor reactivity that is selectively altered in T2DM relative to controls.

Follow-up linear regression analyses demonstrated *diagnosis* remained a significant predictor of POST10 plasticity (p's < 0.02) after controlling for age, gender, BMI, HbA1c, fasting glucose, or motor cortex thickness, resting/active motor thresholds, or baseline MEP amplitude. Table 2 lists the significance of each covariate as a predictor of POST10 plasticity, as well as changes in the regression coefficient of

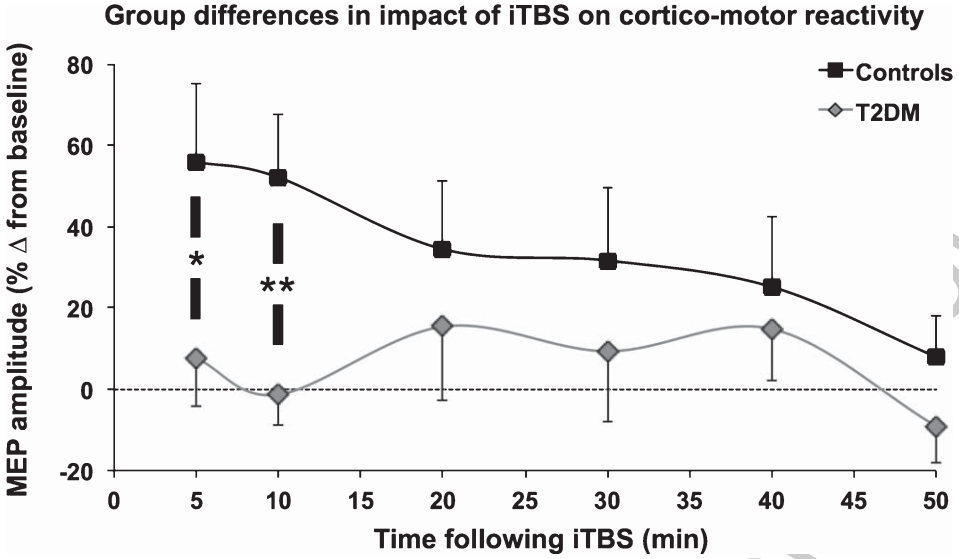

Fig. 2. Comparison of TMS-plasticity measures by group. Mean and standard error of the percent change in MEP amplitude are shown for each post-iTBS time-point. Pairwise comparisons between controls and T2DM for each time-point were made with Student's *t* tests ($^{*}p < 0.05$, $^{**}p < 0.01$). 5–10 min after iTBS, the change in MEP amplitudes was significantly reduced in individuals with T2DM relative to controls.

Table 2
Change in regression coefficients of Diagnosis on POST10 after adding covariates

	$\%\Delta\beta_{\text{diagnosis}}$	$\Delta P_{\text{diagnosis}}$	$\Delta R^2_{\text{model}}$	$P_{covariate}$
Diagnosis plus *Weight*	–0.17	0.02	0.03	0.313
Diagnosis plus *M1 thickness*	–0.14	0.02	0.02	0.378
Diagnosis plus *Race*	–0.09	0.01	0.01	0.219
Diagnosis plus *BMI*	–0.07	0.01	0.01	0.697
Diagnosis plus *Age*	–0.07	0.00	0.03	0.233
Diagnosis plus *Fasting glucose*	–0.06	0.00	0.00	0.680
Diagnosis plus *Height*	–0.03	0.00	0.02	0.409
Diagnosis plus *Baseline MEP*	–0.03	0.00	0.06	0.123
Diagnosis plus *Gender*	–0.02	0.00	0.01	0.537
Diagnosis plus *RMT*	–0.02	0.00	0.05	0.148
Diagnosis plus *Creatinine*	–0.01	0.00	0.01	0.569
Diagnosis plus *AMT*	0.00	0.00	0.06	0.126
Diagnosis plus *Handedness*	0.03	0.00	0.07	0.235
Diagnosis plus *HbA1c*	0.25	0.00	0.06	0.189

POST10, 10-min post-iTBS; AMT, active motor threshold; RMT, resting motor threshold; MEP, motor evoked potential; BMI, body mass index; M1, primary motor cortex.

the model, and the significance and beta coefficient of diagnosis (β_1) after adding each covariate. While no covariate contributed significantly to the model (p's > 0.1), adding weight or motor cortex thickness reduced β_1 by more than 10%, suggesting group differences in these factors may account for some of the observed association between T2DM and POST10 plasticity. By comparison, β_1 increased by 25% after adding HbA1c, suggesting POST10 plasticity may be even more altered in T2DM once HbA1c is taken into consideration.

Relationship between cortico-motor plasticity and cognitive function

For the control group, there were no significant correlations between POST10 and any of the cognitive measures (all p's > 0.6). In the T2DM group by comparison, there were significant positive associations between POST10 plasticity and performance on the digit span backwards task ($R_{19} = 0.49$, $p = 0.025$), RAVLT-learning ($R_{19} = 0.55$, $p = 0.009$; Fig. 3), and RAVLT-delayed recall ($R_{19} = 0.44$, $p = 0.047$). After

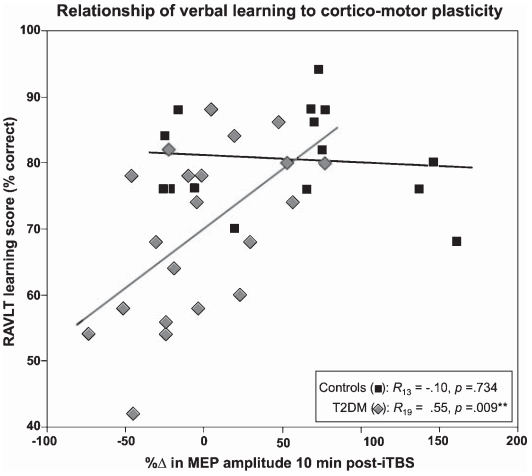

Fig. 3. Relationship between cortico-motor plasticity and verbal learning. Pearson's correlation coefficients were calculated separately for T2DM and control groups to assess the relationship between the change in MEP amplitude 10-min post-iTBS (x-axis) and performance (y-axis) on the Rey Auditory Verbal Learning Test (RAVLT) learning trials (% correct). T2DM participants whose MEPs increased following iTBS demonstrated better verbal learning performance than those whose MEPs remained unchanged or decreased.

Holm-Bonferroni adjustment, only the relationship between POST10 and RAVLT-learning remained significant. These results indicate that individuals with T2DM whose MEPs increased at POST10 tended to perform better on the cognitive measures than those whose MEPs remained unchanged or decreased.

DISCUSSION

The present study compared older adults with and without T2DM on TMS-measures of brain plasticity assessed in the motor cortex. Our major novel finding is that individuals with T2DM, unlike their non-T2DM counterparts, did not show significant potentiation of MEP amplitudes 5–10 minutes post-iTBS. This period corresponds with the peak effect of TBS in normal individuals [10, 29] and demonstrates the highest test-retest reliability [30]. Using similar TMS measures, altered mechanisms of brain plasticity have been demonstrated in autism spectrum disorder [12], traumatic brain injury [13], and AD [14, 31, 32], and used to track changes over the lifespan in healthy adults [11]. In the future, similar plasticity measures might be obtained from higher-order association areas more directly linked to cognition using real-time integration of TMS with electroencephalography. Nonetheless the present findings suggest that TMS-based assessments of motor cortex plasticity offer a clinically relevant marker of central nervous system changes in T2DM.

It is unlikely that differences in TMS measures of brain plasticity result from a direct impact of T2DM on the motor cortex. While the motor cortex was thinner in T2DM participants, diagnosis remained a significant predictor of the impact of iTBS even after accounting for these macrostructural differences. Magnetic resonance spectroscopy has shown evidence in T2DM of abnormal metabolism in non-motor regions [33]; future studies could investigate if the motor cortex is similarly altered. T2DM has been associated with altered integrity of the cortico-spinal pathway [34]. However, a structured neurological exam or medical history review found no evidence of neuropathy in any of our T2DM participants. Moreover, motor thresholds, baseline MEP amplitudes and latencies were all equivalent, indicating that T2DM does not alter cortico-motor reactivity or the ability of TMS-induced activity to propagate along the cortico-spinal pathway and elicit a muscle contraction. Thus, alterations in the response to iTBS likely reflect T2DM-related changes to the efficacy of neuroplastic mechanisms within the cortex. Indeed, using invasive techniques to monitor brain activity, Di Lazzaro and colleagues [16] demonstrated that iTBS assesses intracortical mechanisms of plasticity. In rodents' neocortex, iTBS has been shown to increase pyramidal cell output by reducing parvalbumin expression in fast-spiking inhibitory interneurons [35]. Importantly, the present study found no differences between T2DM and controls in any of the paired-pulse TMS measures of intracortical inhibition and facilitation. T2DM in humans does not therefore appear to alter intracortical circuits within the motor cortex, but the ability of the synapses therein to be potentiated.

Our second major findings was that reduced measures of brain plasticity in T2DM participants were associated with lower verbal learning scores on the RAVLT and fewer correct trials of the Digit Span Backwards task. These results bring human evidence of T2DM-associated cognitive impairment in line with genetic mouse models of impaired insulin signaling and insulin resistance. Mice engineered without the glucagon-like peptide 1 (GLP-1) receptor had reduced LTP in area CA1 of the hippocampus, showed impaired discrimination of learned and novel objects and performed poorly on a water maze task [36]. Similarly, reducing insulin receptor expression globally by means of β-subunit haploinsufficiency [37] or in the hippocampus using a

lentiviral vector [38] severely curtailed hippocampal LTP and impaired spatial memory. What makes the present results notable is that plasticity was assessed in the motor cortex, while learning and memory are hippocampal-dependent and working memory is most closely associated with lateral prefrontal and posterior parietal cortices. While it is possible that all three systems are independent targets of T2DM-related damage, the more parsimonious explanation is that T2DM affects a common substrate. In rodents, N-methyl-D-aspartate receptors (NMDARs) are known to be crucial for induction of theta burst-driven LTP in the hippocampus [39] and iTBS after-effects in the neocortex [40] as well as for behavioral measures of working memory [41] and learning and memory [42]. Similarly, in humans, iTBS after-effects, working memory and RAVLT performance have all been shown to be NMDAR-dependent [15, 17, 43–45]. Thus, the relationship of reduced verbal learning and working memory to altered LTP-like plasticity in the present study may reflect a T2DM-associated brain-wide reduction in the density or efficacy of NMDARs. Given T2DM is associated with upregulation of the GLUT1 glucose transporter [46], and glucose provides the original source of glutamate in the brain [47, 48], chronic hyperglycemia could lead to excessive glutamate and increased risk of excitotoxicity. Any reduction in post-synaptic NMDARs to moderate this risk would consequently reduce the efficiency of LTP and alter any NMDAR-dependent measures.

Since the RAVLT in particular is sensitive to age-related cognitive decline [23] and T2DM is an important risk factor for dementia [5, 49], the present findings suggest impairments in the mechanisms underlying neuroplasticity may predicate learning and memory deficits. An alternative interpretation is that preserved brain plasticity might provide protection against cognitive decline. The relationship between plasticity and cognitive resilience deserves further investigation. Nonetheless, our results would lead to the prediction that T2DM individuals with normal TMS plasticity measures would be less likely to develop dementia. In future studies, these assessments of brain plasticity could be used to chart the progress of T2DM-related brain changes and evaluate the therapeutic efficacy of interventions.

The present findings provide neurobiological support for the epidemiological link between T2DM and AD [5, 50]. Several TMS studies have shown similar patterns of reduced LTP-like plasticity in AD patients [14, 31, 32]. In particular, Koch and colleagues have demonstrated that TMS measures of plasticity are associated with the severity of Tau neuropathology in AD [51] but independent from the age that cognitive symptoms first appear [32]. Furthermore, a 4-week treatment with a dopamine agonist was shown to rescue LTP-like plasticity in early AD [31], a finding that both provides mechanistic insight into altered cortical plasticity and offers a potential therapeutic intervention to recover it. Similarly, intranasal insulin therapy has been shown to improve cognition in healthy individuals [52], as well as patients with mild cognitive impairment/early AD [53–55] or T2DM [56]. Future studies could examine how plasticity relates to Tau levels or dopaminergic function in T2DM or investigate whether cognitive improvement following intranasal insulin administration is mediated through enhancement of LTP-like plasticity.

Several factors may limit the generalizability of our findings. While the sample size is consistent with recently-published work on diabetes and cognitive aging [7, 56, 57], it is relatively small when compared to large-scale epidemiological studies [58]. Further, we enrolled a relatively homogenous population of non-demented adults. Thus it is not possible to know if our findings extend to patients with significant comorbidities or evident dementia. Lastly, it was not possible to obtain recent HbA1c or fasting glucose levels in all participants.

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Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/16-0505r1).

SUPPLEMENTARY MATERIAL

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