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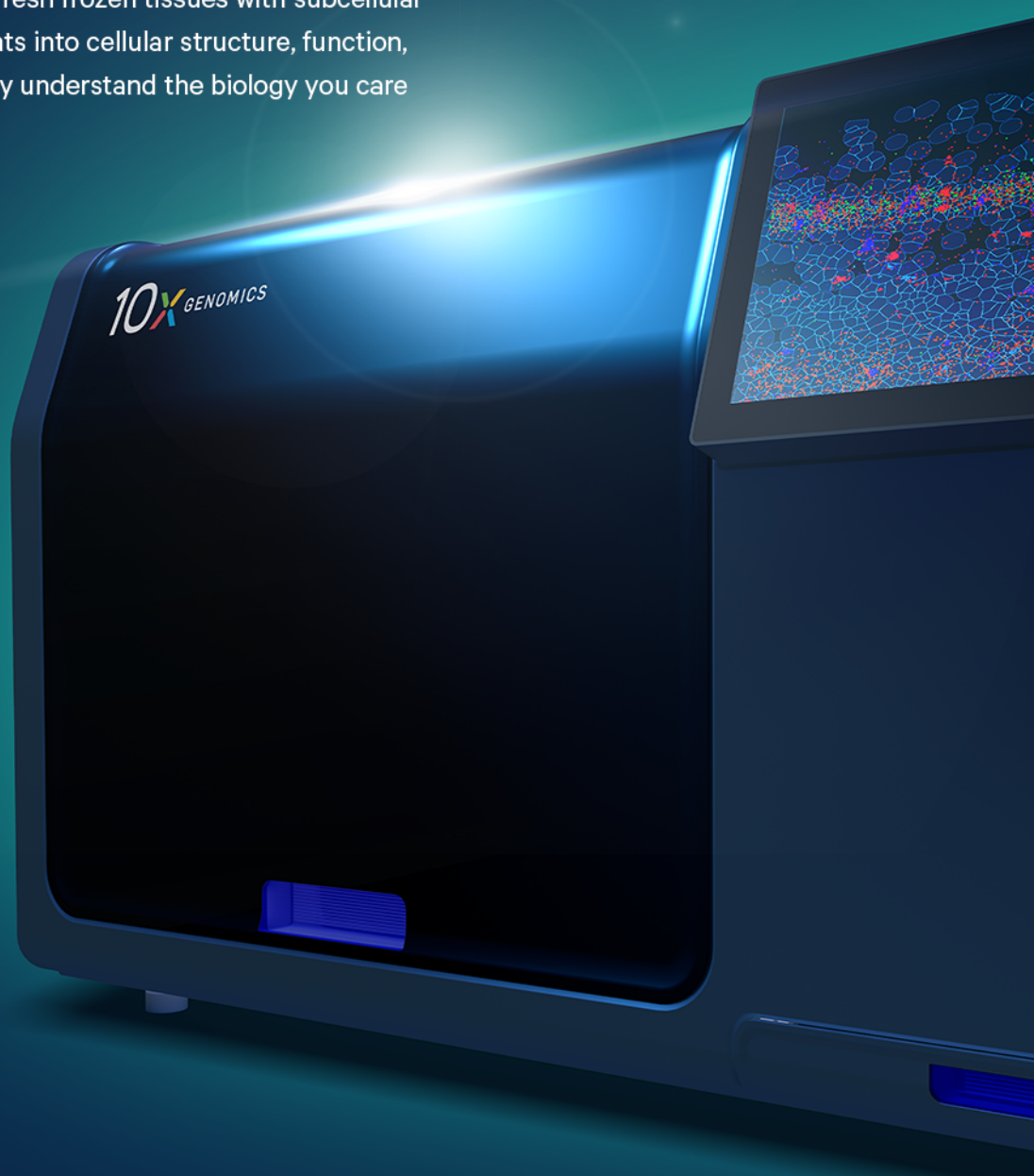

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RESEARCH ARTICLE

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Motor cortical excitability and pre-supplementary motor area neurochemistry in healthy adults with substantia nigra hyperechogenicity

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Abstract

Substantia nigra (SN) hyperechogenicity, viewed with transcranial ultrasound, is a risk marker for Parkinson's disease. We hypothesized that SN hyperechogenicity in healthy adults aged 50–70 years is associated with reduced short-interval intracortical inhibition in primary motor cortex, and that the reduced intracortical inhibition is associated with neurochemical markers of activity in the pre-supplementary motor area (pre-SMA). Short-interval intracortical inhibition and intracortical facilitation in

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primary motor cortex was assessed with paired-pulse transcranial magnetic stimulation in 23 healthy adults with normal (n = 14; 61 ± 7 yrs) or abnormally enlarged (hyperechogenic; n = 9; 60 ± 6 yrs) area of SN echogenicity. Thirteen of these participants (7 SN− and 6 SN+) also underwent brain magnetic resonance spectroscopy to investigate pre-SMA neurochemistry. There was no relationship between area of SN echogenicity and short-interval intracortical inhibition in the ipsilateral primary motor cortex. There was a significant positive relationship, however, between area of echogenicity in the right SN and the magnitude of intracortical facilitation in the right (ipsilateral) primary motor cortex (p = .005; multivariate regression), evidenced by the amplitude of the conditioned motor evoked potential (MEP) at the 10–12 ms interstimulus interval. This relationship was not present on the left side. Pre-SMA glutamate did not predict primary motor cortex inhibition or facilitation. The results suggest that SN hyperechogenicity in healthy older adults may be associated with changes in excitability of motor cortical circuitry. The results advance understanding of brain changes in healthy older adults at risk of Parkinson's disease.

KEYWORDS
motor cortex, pre-supplementary motor area, substantia nigra, transcranial magnetic stimulation

1 | INTRODUCTION

Parkinson's disease is a neurodegenerative disease that affects many parts of the brain, particularly the structure and/or function of movement-related circuitry. The latter includes the death of significant numbers of dopaminergic neurons in the substantia nigra pars compacta (e.g., Fearnley & Lees, 1991) and also of cortico-cortical projecting pyramidal neurons in the pre-supplementary motor area (pre-SMA; MacDonald & Halliday, 2002). The number, and morphological appearance, of pyramidal neurons and interneurons in other motor cortices, such as the primary motor cortex and dorsolateral premotor cortex, appear to be preserved (MacDonald & Halliday, 2002) but changes in motor cortical function are evident. For example, paired-pulse transcranial magnetic stimulation (TMS) over the primary motor cortex shows reduced short-interval ($GABA_A$-mediated) intracortical inhibition within the motor cortex in de novo patients (e.g., Ammann et al., 2020) and patients OFF medication (e.g., Ammann et al., 2020; Bologna et al., 2018; Ridding et al., 1995). The role of such changes in disease etiology, onset, and progression, and treatment efficacy is not fully understood.

The current study explored potential relationships between changes in the structure and function of the substantia nigra, pre-SMA, and primary motor cortex in conscious humans. Investigation and interpretation of such relationships in patients diagnosed with Parkinson's disease is complicated because of uncertainty regarding variability between patients in the extent and diversity of disease-related processes occurring in brain regions that have secondary roles in movement conception and control, such as the prefrontal cortex which has connections with the substantia nigra (e.g., Cacciola et al., 2016). These processes include, for example, the increased and variable presence of Lewy bodies in cortical and subcortical areas (for review, see Surmeier et al., 2017). A potentially less-complicated model for exploring such relationships involves healthy adults with a significant risk marker for Parkinson's disease. The risk marker of interest is an abnormal echogenic appearance of the substantia nigra when viewed with transcranial ultrasound (e.g., Berg et al., 2013, 2015).

The area of echogenic signal in the substantia nigra is abnormally large (termed "hyperechogenicity") in up to 90% of patients with Parkinson's disease (e.g., Doepp et al., 2008; Spiegel

Significance

The primary brain region which degenerates in Parkinson's disease is the substantia nigra. This brain region is also changed in a proportion of healthy older adults who are more likely to be later diagnosed with Parkinson's disease. Here, we show that healthy adults with an altered substantia nigra also exhibit changes in the function of the primary motor cortex. Our study reveals brain changes in healthy persons at risk of Parkinson's disease are not restricted to the substantia nigra but also occur in other movement-associated brain regions. This work increases our understanding of brain changes and risk of Parkinson's disease.

et al., 2006; Tsai et al., 2007) and this abnormality has a high sensitivity (83%–84%) and specificity (85%–87%) for the disease (Li et al., 2016; Prestel et al., 2006; Tao et al., 2019). Substantia nigra hyperechogenicity is an acknowledged risk marker for Parkinson's disease (Berg et al., 2015). Healthy older adults with this abnormality are 17 times more likely to be diagnosed with Parkinson's disease over a 3- year period (Berg et al., 2011) and the abnormality is associated with changes in hand function that resemble those observed in Parkinson's disease patients (Todd et al., 2014). The pathophysiological processes that underlie substantia nigra hyperechogenicity are not fully understood but likely involve local activation of microglia (Berg et al., 2010), decreased neuromelanin (Zecca et al., 2005), and increased tissue iron (Berg et al., 2002) and H- and L-ferritin (Zecca et al., 2005). Impairment of the nigrostriatal pathway, evidenced by reduced ^{18}F-Dopa uptake in the striatum, has also been observed in conjunction with these changes (Behnke et al., 2009; Berg et al., 1999, 2002). Healthy older adults with substantia nigra hyperechogenicity also exhibit changes in the excitability and/or function of circuitry within the primary motor cortex, specifically reduced short-interval ($GABA_A$-mediated) intracortical inhibition (Todd et al., 2010), similar to that observed in patients with Parkinson's disease (e.g., Ammann et al., 2020; Bologna et al., 2018; Ridding et al., 1995).

The aim of this exploratory study was to investigate the relationship between substantia nigra hyperechogenicity, reduced short-interval intracortical inhibition within the primary motor cortex, and neurochemical markers of activity in the pre-SMA obtained using magnetic resonance spectroscopy. A secondary aim was to determine whether the previously documented relationship between substantia nigra hyperechogenicity and reduced short-interval intracortical inhibition within primary motor cortex of healthy adults aged 72–84 years is also evident in healthy adults spanning (aged 50–70 years) the average diagnostic age for Parkinson's disease (60 years, for review see de Lau & Breteler, 2006). We hypothesized that substantia nigra hyperechogenicity in healthy adults aged 50–70 years is associated with reduced short-interval intracortical inhibition in the primary motor cortex and that the reduced intracortical inhibition is associated with neurochemical markers of activity in the pre-SMA. The pre-SMA receives input from the basal ganglia via the ventral anterior thalamus (for review, see Nachev et al., 2008) and the pre-SMA is connected to the primary motor cortex via projections to the caudal supplementary motor area or "SMA proper" (for review, see Tanji, 1994). The pre-SMA is involved in movement preparation, arm reaching and grasping movements, and visually cued movement (e.g., Hoshi & Tanji, 2004; for review see Nachev et al., 2008) and lesions affecting the SMA are associated with a rapidly progressive parkinsonism that is unresponsive to dopaminergic therapies (Haussermann et al., 2001). The results of the current study further understanding of substantia nigra hyperechogenicity and concomitant changes in other brain regions involved in movement control.

2 | MATERIALS AND METHODS

2.1 | Experimental subjects

The prevalence of substantia nigra hyperechogenicity in healthy adults in the community is reported to be 8.6% (Berg et al., 1999); in the current study, we used strict and extensive inclusion and exclusion criteria to minimize the effect of confounding variables on the primary outcome measures and analysis. Together, this meant that the proportion of potential participants (healthy older adults with substantia nigra hyperechogenicity) meeting our study criteria comprised only a very small percentage of the community (much less than 8.6%). We screened hundreds of potential participants across two test sites (Sydney, Australia and Tübingen, Germany) to identify healthy individuals aged 50–70 years with substantia nigra hyperechogenicity who also (i) met the health, neuropsychological, and medication eligibility criteria (see below for details), (ii) had a sufficient bone window for performing transcranial ultrasound of the substantia nigra, (iii) had no contraindications for TMS (e.g., pacemaker, use of certain medications, or history of head injury), and (iv) had no contraindications for brain or brainstem MRI (e.g., dental implants). Sydney participants were recruited via community advertisement and Tübingen participants were recruited from a longitudinal study. The final study sample included 23 healthy older adults known to have an abnormally large area of substantia nigra echogenicity (i.e., hyperechogenicity; $n = 9$, aged 60.0 ± 6.1 yrs, 6 M, 3F; "SN+" group) or a normal area of substantia nigra echogenicity ($n = 14$, aged 61.4 ± 6.6 yrs, 7 M, 7F, "SN−" group). Figure 1 illustrates how the final sample was obtained. We aimed to include more than 9 SN+ individuals in the current study. However, the final study sample size ($n = 9$ SN+ and $n = 14$ SN−) was comparable to our previous published study ($n = 10$ SN+ and $n = 10$ SN−) that demonstrated significantly reduced intracortical inhibition in older healthy adults (aged 72–84 yrs) with substantia nigra hyperechogenicity (Todd et al., 2010).

2.2 | Experimental design and procedures

2.2.1 | Screening tests

Each participant completed a brief medical history questionnaire, MRI safety screen, and TMS safety screen (Rossi et al., 2009). Exclusion criteria that related to these questionnaires were contraindications for brain or brainstem MRI (e.g., dental implants), contraindications for TMS (e.g., metal objects in the skull, cardiac pacemaker, use of medications that affect cortical excitability; Rossi et al., 2009), history of neurological damage and/or illness, history of diagnosed medicated mental/psychiatric disease or disorder, and/or self-reported prior or current use of antipsychotic medications (due to uncertainty about whether there is a causal relationship between use of antipsychotic medications and area of substantia nigra echogenicity). Additional exclusion criteria were insufficient

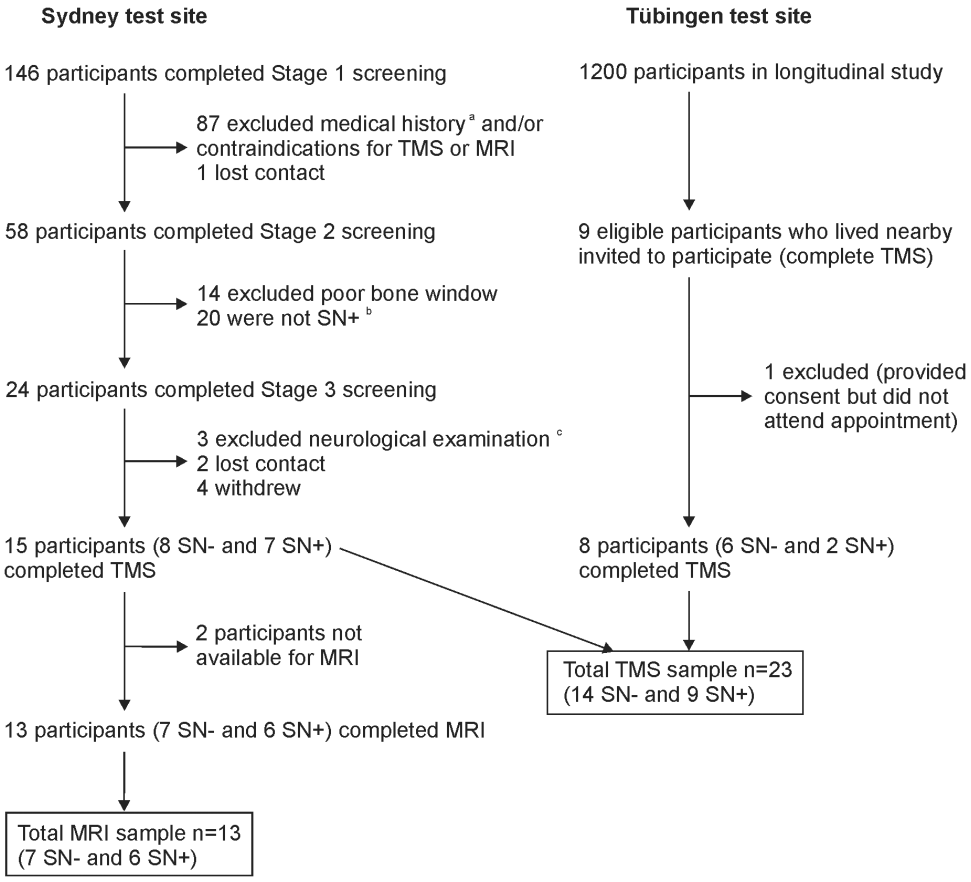

FIGURE 1 Flowchart illustrating the inclusion/exclusion criteria and participants in each group. Stage 1 screening includes medical history questionnaire, magnetic resonance imaging (MRI) safety screen, and transcranial magnetic stimulation (TMS) safety screen. Stage 2 screening includes neuropsychological assessment and transcranial ultrasound of the substantia nigra. Stage 3 screening is the neurological examination performed by a neurologist that specializes in movement disorders. [a]History of neurological damage and/or illness, history of diagnosed medicated mental/psychiatric disease or disorder, and/or self-reported prior or current use of antipsychotic medications. [b]Tested late in study and excluded due to oversupply of SN− participants. [c]Participant disclosed disease/disorder that was not reported on medical history questionnaire, or neurologist identified potential undiagnosed neurological disease/disorder. Abbreviations: SN− group, normal area of substantia nigra echogenicity on the right and left side; SN+ group, abnormally large area of substantia nigra echogenicity on the right and/or left side.

bone window for transcranial ultrasound (determined during a sonographic examination) and poor neuropsychological performance. Details about the transcranial ultrasound and neuropsychological test procedure and exclusion criteria are provided in the following paragraphs.

Transcranial sonography was performed with the participant in a supine position. The 15 Sydney participants (8 SN− and 7 SN+) were examined with a Philips iU22 ultrasound system equipped with a 1–5 MHz transducer (s5-1; Philips Healthcare, Best, the Netherlands). The eight Tübingen participants (six SN− and two SN+) were examined with a Siemens Sonoline Antares ultrasound system equipped with a 1–4 MHz transducer (Siemens, Erlangen, Germany). The transducer was positioned over the pre-auricular acoustic bone window located above the ear. The penetration depth was set at 14–16 cm and the dynamic range was 50–60 dB. A qualitative rating of the bone window was made (1-excellent, 2-good, 3-poor, 4-very poor). Participants with a bone window rating of 4 (very poor) on the right and/or left side were excluded from the study. The area of echogenicity at the anatomical site of the substantia nigra was measured at its greatest extent (e.g., Figure 2a), in line with established guidelines (for review, see Berg et al., 2008). Two additional parameters were also measured: minimum internal diameter of the third ventricle and qualitative rating of the mesencephalic raphe (normal, abnormal-interrupted, abnormal-absent) on one (clearest) side. The examinations were performed by operators experienced with the procedure. The operators were trained by the pioneer of the technique, Prof Daniela Berg. Measurements were made in the B-mode setting. Normal substantia nigra echogenicity was defined as an area of echogenic signal <.22 cm^2 and abnormal substantia nigra echogenicity was defined as an area of echogenic signal ≥.22 cm^2 (termed "hyperechogenicity").

Participants completed a neuropsychological assessment to ensure that only participants with normal memory and cognition were included in the study. Participants were assessed with the Wechsler Memory Scale-Revised Logical Memory subtests I and II (Wechsler, 1987), Verbal Trails (Grigsby & Kaye, 1995), Verbal

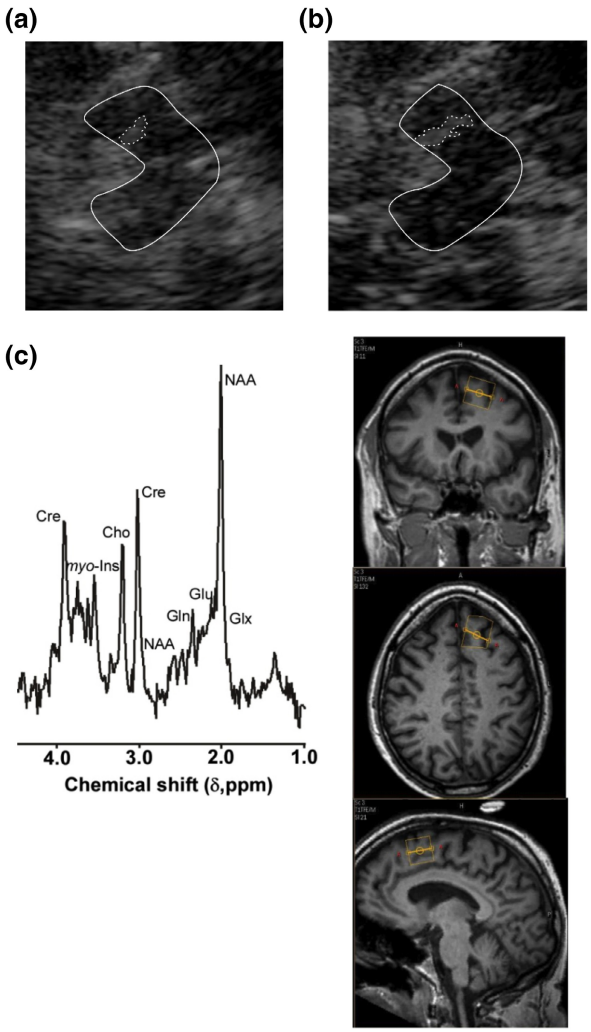

FIGURE 2 Data from two representative participants. (a) and (b) Echomorphology of the mesencephalic brainstem in one participant with normal substantia nigra echogenicity (a) and one participant with abnormal substantia nigra echogenicity (i.e., hyperechogenicity; b). Solid white line represents the outer edge of the mesencephalic brainstem. The substantia nigra ipsilateral to the probe (the side at which the planimetric measurement is collected) is encircled with a dotted line. (c) Section of ^{1}H MRS spectrum obtained from the pre-supplementary motor cortex from the participant shown in panel a. Spectrum was obtained at 3T (Philips Achieva TX, Best, the Netherlands) using an eight channel head coil and the PRESS sequence (TE = 32, TR = 2 s, 1024 data points, 32 transients, VOI 2 cm^3). Spectrum shown transformed with no apodization. The placement of the VOI (yellow cube) over the dorsomedial aspect of the superior frontal gyrus, at the level of Brodmann's area 6, is also displayed along the coronal (top panel), transverse (middle panel), and sagittal (lower panel) planes.

Fluency (Benton & Hamsher, 1983), Digit Span forwards and backwards (Wechsler, 1981), Mini-Mental State Examination (Folstein et al., 1975), and/or the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Neuropsychological Test Battery (Morris et al., 1989). Performance on each test was compared to published normative data matched for age and years of education. Participants were excluded if poor performance was observed on two or more of the cognitive domains tested. Poor performance was defined as greater than two standard deviations below the mean of published normative data for Digit Span forwards and backwards (Anstey et al., 2000; Kear-Colwell & Heller, 1978), Verbal Fluency (Tombaugh et al., 1999), Logical Memory I and II (Abikoff et al., 1987), and Mini-Mental State Examination (Kenny et al., 2013) and performance greater than two standard deviations above the mean for Verbal Trails (Mrazik et al., 2010) and Trail Making Task A and B (Nielsen et al., 1995). Depression can influence motor cortical excitability (e.g., Loo et al., 2008; Maeda et al., 2000). Thus, negative emotional states (depression, anxiety, and/or stress) were also assessed (over the past 7–14 days) with a self-report rating scale (Beck Depression Inventory-II; Beck et al., 1996; Depression Anxiety Stress Scales; Lovibond & Lovibond, 1995).

Lastly, all participants underwent a neurological examination of movement performed by a neurologist with expertise in movement disorders. The third (motor) part of the Unified Parkinson's Disease Rating Scale (UPDRS III, Fahn & Elton, 1987) was also performed during this examination. The UPDRS III is a subjective rating scale comprising 13 items that are rated 0 (normal) to 4 (severe/can barely perform the task). The neurologist excluded participants with potential undiagnosed neurological diseases/disorders. Hand dominance was also confirmed with the Edinburgh Handedness Questionnaire (Oldfield, 1971).

The rigorous screening procedure and inclusion and exclusion criteria resulted in exclusion of over 100 participants at the Sydney test site alone (see Figure 1).

2.2.2 | Experimental protocol

Transcranial magnetic stimulation (TMS) was used to assess intracortical inhibition within the motor cortex, and other indices of excitability of the motor cortex and the pathway between the motor cortex and the contralateral first dorsal interosseous muscle. The motor cortex in the left and right hemispheres were tested separately and in a pseudorandom order. The protocol was similar to that used previously by our group (Flavel et al., 2012; Todd et al., 2010). For each hemisphere, single- and paired-pulses were applied to the motor cortex, over the first dorsal interosseous motor area. Stimuli were delivered using two Magstim 200^2 stimulators (part number: 3010-00), a BiStim^2 UI Controller and Connecting Module (part numbers: 3021-00 and 3333-00), and a figure-of-eight coil (part number: 3281-00, 90 mm external diameter of wings, Magstim, Whitland, UK). A posterior-to-anterior current was induced in the brain by positioning the handle of the stimulating coil posteriorly, at approximately 45 degrees to the midline and tangentially to the skull. The electromyographic (EMG) response evoked by stimulation (motor evoked potential or MEP) was recorded from the contralateral first dorsal interosseous muscle. EMG activity was recorded via two surface electrodes (Ag-AgCl, 10 mm diameter) positioned over the muscle belly and tendon (inter-electrode distance: ~3 cm). EMG activity

was amplified (x300–1000), filtered (2nd order Butterworth; high-pass: 16 or 20 Hz; low-pass: 1000 Hz), and sampled at 2000 Hz using a data acquisition system (1902 with Power 1401 Interface and Signal software, Cambridge Electronic Design, Cambridge, UK).

Single stimuli were applied at a rate of ~.2 Hz to determine resting motor threshold. The intensity of stimulation was initially set well above threshold and then reduced in steps of 1%–3% of stimulator output until the intensity was below threshold. Resting motor threshold was defined as the stimulus intensity that produced a MEP greater than 50 μV in amplitude in five out of 10 consecutive stimuli. The intensity of stimulation was then increased to 130% of resting motor threshold and 15 stimuli were delivered at this intensity to assess resting excitability in the motor cortex and the pathway that descends to the contralateral first dorsal interosseous. A further 15 stimuli (at the same intensity) were delivered during weak abduction of the target index finger for assessment of facilitation of excitability during movement and the strength of $GABA_B$-mediated intracortical inhibition in the motor cortex (duration of silent period) (Ziemann, 2004). The contraction was performed with the wrist semi-supinated and the index finger extended. A 53 g weight was positioned at the distal interphalangeal joint, and participants were instructed to hold the weight using index finger abduction. The weight was then removed and participants were instructed to relax.

Participants then received paired-pulse stimulation for assessment of short-interval intracortical inhibition and intracortical facilitation (Kujirai et al., 1993). The intensity of the test pulse was set to produce a resting MEP of ~1 mV in amplitude and the intensity of the conditioning pulse was set at 70% of resting motor threshold. The conditioning pulse preceded the test pulse by 2, 3, 10, or 12 ms. Ten pairs of stimuli were applied at each interstimulus interval and 10–20 single test pulses were also delivered (~.2 Hz). The paired-pulse protocol was then repeated with a higher intensity of conditioning stimulation (90% of resting motor threshold) because the previously observed relationship between area of substantia nigra echogenicity and short-interval intracortical inhibition was dependent on the intensity of the conditioning stimulus in healthy adults aged >70 years (Todd et al., 2010). The reduced intracortical inhibition in healthy adults aged >70 years with substantia nigra hyperechogenicity was more apparent at the 70% conditioning intensity than at the 90% conditioning intensity (Todd et al., 2010). Higher intensities of conditioning stimulation can result in short-interval intracortical facilitation interfering with the measurement of short-interval intracortical inhibition in healthy adults aged 30–43 years (Peurala et al., 2008). The existence and magnitude of this potential source of interference in older adults is unknown. Thus, the two intensities of conditioning stimulation (70% and 90% of resting motor threshold) were retained in the current study to widen understanding and strengthen interpretation of the results. Comparison of the conditioned MEP amplitude evoked by the lower (70% resting motor threshold) and higher (90% resting motor threshold) conditioning intensities, at the 2 and 3 ms interstimulus intervals, in the current study enabled determination of whether this potential source of interference is evident in the data.

Participants at the Sydney test site that did not exhibit contraindications for MRI (7 SN− and 6 SN+) underwent magnetic resonance imaging and spectroscopy to assess neurochemistry in the pre-SMA. All scanning was undertaken at Neuroscience Research Australia using a 3T MRI scanner with an 8 channel SENSE head coil (Achieva TX, Philips, Best, The Netherlands). Following acquisition of a volumetric T1 weighted scan (FFE, 1 mm isotropic resolution), a 2 cm^3 volume of interest was placed in the left pre-SMA (e.g., Figure 2c), as the majority of participants were right-handed. A spectrum was acquired using the PRESS sequence (TE = 32 ms, TR = 2 s, 32 transients acquired across 1024 data point) along with a water reference spectrum (Figure 2c). These spectra were processed using the time-domain fitting program jMRUI (http://sermn02.uab.es/mrui/mrui_Overview.shtml; V3.0) (Naressi et al., 2001). Quantification of the reconstructed signals was performed in the time-domain. The residual water resonance was filtered using Hankel-Lanczos singular values decomposition. The AMARES algorithm (Vanhamme et al., 1997) was used to fit decaying sinusoids, corresponding to Lorentzian line shapes in the frequency domain, to the resonances arising from *N*-acetylaspartate (NAA), choline containing compounds (Cho), creatine-containing compounds (Cre), *myo*-inositol, glutamate, and glutamine using an existing basis set (Singh et al., 2009). Values for glutamate (Glu) and glutamine (Gln) were estimated from individual fits of resonances contributing to the partially resolved γ-CH_2 resonances, while combined glutamate + glutamine (Glx) was determined from the βCH_2 resonances (Singh et al., 2009). Values obtained for these metabolites were expressed relative to that of the unsuppressed reference water signal (assumed to be 80 M). Spectroscopy ROIs were assessed for relative contributions of gray and white matter using a tool developed by Dr. Nia Goulden and Dr. Paul Mullins of Bangor University (Gasparovic et al., 2006) for partial volume estimation of Philips MRI data and found not to be significantly different between groups (Mann–Whitney *U*-test, IBM SPSS Statistics 24).

2.3 | Data analysis and statistics

The data that support the findings of this study are available from the corresponding author upon reasonable request. The latency, peak-to-peak amplitude, and duration of resting and contracting MEPs were measured between cursors manually positioned at the beginning and end of the MEP in each trial (e.g., see Figure 3a). Resting MEPs with preceding voluntary EMG were excluded from the analysis. Voluntary root mean square (RMS) EMG before contracting MEPs (100 ms epoch) and the period of EMG silence following contracting MEPs (silent period) were also measured between cursors. Measurement of the silent period involved placing a cursor at the stimulus onset and at the resumption of voluntary EMG (for example see Figure 3b). Resumption of voluntary EMG was determined by eye with a consistent visual display window. The silent

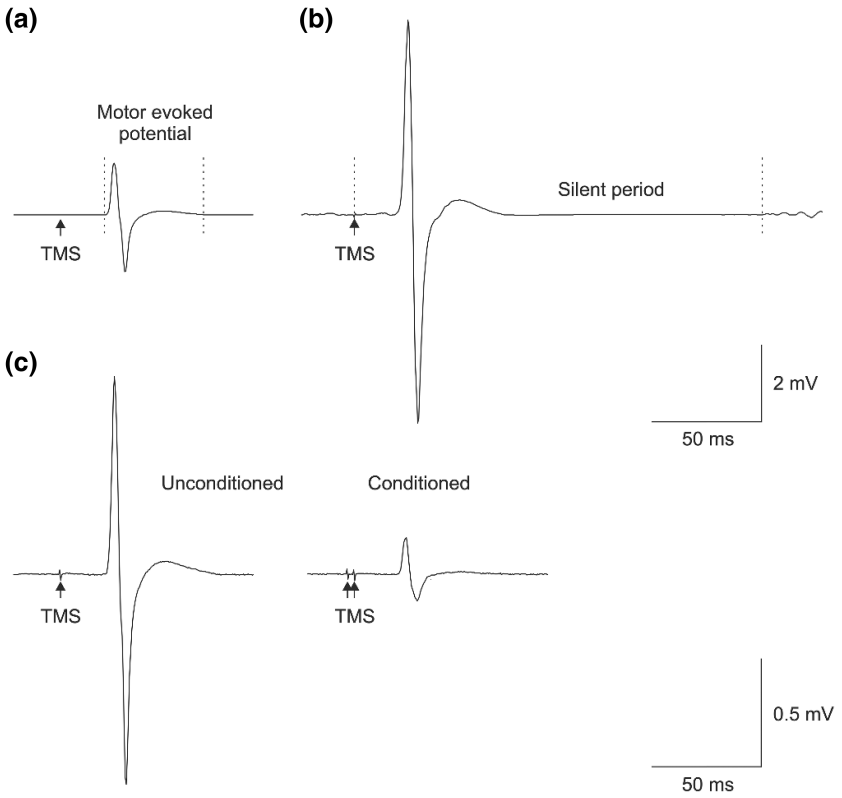

FIGURE 3 Average EMG traces from the right first dorsal interosseous muscle in one participant. The EMG response (motor evoked potential or "MEP") evoked by single- and paired-pulse transcranial magnetic stimulation (TMS) over the left motor cortex are shown. (a) Averaged MEP evoked in relaxed muscle by single-pulse TMS at an intensity of 130% of resting motor threshold. Vertical dashed lines are placed at the boundaries of the MEP. (b) Averaged MEP evoked by single-pulse TMS (at the same intensity) during a weak voluntary contraction. Vertical dashed lines represent the duration of the silent period (i.e., stimulus onset to resumption of voluntary EMG). Note that the size of the MEP is much larger during the voluntary contraction than during relaxation. (c) Average amplitude of the test (unconditioned) and conditioned MEP for paired-pulse TMS during relaxation. A subthreshold stimulus (70% resting motor threshold) was delivered 3 ms before a suprathreshold test stimulus. The averages of 10 EMG traces are shown.

period was defined as the time interval between the stimulus onset and resumption of voluntary EMG. For paired-pulse TMS, the peak-to-peak amplitude of resting MEPs was measured in each trial (e.g., see Figure 3c) and the amplitude of the conditioned MEP was expressed as a percentage of the test (unconditioned) MEP.

Group data are presented as the mean ± standard deviation in the text and figures. Group data for participant characteristics were analyzed with Student's independent *t*-test or Mann–Whitney Rank Sum test. Between-group differences in sex was investigated with a Chi-Square test. A Professor of Biostatistics (author Professor Adrian Esterman) recommended the statistical approach for the TMS, pre-SMA neurochemistry, and substantia nigra echogenicity data. A multivariate regression analysis approach was used to explore the relationship between area of substantia nigra echogenicity (cm^2), pre-SMA glutamate, and short-interval intracortical inhibition and intracortical facilitation within the primary motor cortex. To determine which measure of short-interval intracortical inhibition and intracortical facilitation to select for the model, normality, and homogeneity of variance of raw MEP amplitude (mV) in the paired-pulse TMS paradigm was assessed with Kolmogorov–Smirnov and Levene's tests, respectively. MEP amplitude (mV) was then transformed (square) and a three-way repeated measures analysis of variance (ANOVA) was used for comparison of side (right, left), intensity of conditioning stimulation (70%, 90% of resting motor threshold), and condition (test and 2, 3, 10, 12 ms interstimulus intervals). Mauchly's test of sphericity was performed and the Greenhouse–Geisser method was used to correct for non-sphericity. Post-hoc discrimination was made with the Bonferroni method (IBM SPSS Statistics, Version 25). The average MEP amplitude evoked by paired-pulse stimulation across the 2 and 3 ms interstimulus intervals and across the 10 and 12 min interstimulus intervals was calculated for the higher intensity of conditioning stimulation (90% resting motor threshold) and the average MEP amplitudes were expressed as a percentage of the test MEP amplitude. These normalized MEP amplitudes were included in the multivariate regression model to represent short-interval intracortical inhibition and intracortical facilitation, respectively. To determine which measure of glutamate to select for the multivariate regression model, a series of simple linear regressions of the left substantia nigra area of echogenicity against each left pre-SMA glutamate measure (Glu tot, Glu/NAA, Glu/Cre and Glu tot, Glu/NAA, Glu/Cre normalized to gray matter) was performed (STATA, Version 17 BE) and goodness of fit was measured with Bayesian Information Criteria (BIC; Raftery, 1995).

The difference in the BIC statistic was less than 2 for each regression and therefore the goodness of fit for each outcome measure was similar. This suggests that the glutamate measures are interchangeable. Thus, Glu tot was selected as the measure of pre-SMA glutamate for inclusion in the multivariate regression analysis. Three separate multivariate regressions analyses were performed using STATA's mvreg procedure (STATA, Version 17 BE). This procedure undertakes linear regression with several dependent variables and the same independent variables. In our analyses, only one independent variable was used in each model and the independent and dependent variables for each model are illustrated in Figure 4. Because of multiple testing within each model, and having three separate models, statistical significance for the models was set at $p = .01$.

Normality and homogeneity of variance of single-pulse TMS parameters was assessed with Kolmogorov–Smirnov test (with group as a factor) and Levene's test, respectively. Single-pulse TMS parameters were analyzed with repeated measures ANOVA for comparison of group (SN−, SN+; between-subject factor) and side (right, left; within subject factor; IBM SPSS Statistics, Version 25). The association between area of substantia nigra echogenicity (cm^2) and UPDRS III was explored with Spearman Rank Order Correlation (SigmaPlot for Windows Version 11.0, Systat Software, San Jose CA, USA). Statistical significance was set at $p = .01$ for multivariate regression models. Statistical significance for all other analyses was set at $p = .05$.

3 | RESULTS

3.1 | Participant characteristics

The SN− and SN+ groups did not differ significantly in age ($n = 23$; $p = .626$), years of education ($n = 23$; $p = .439$), sex ($n = 23$; $p = .431$), or UPDRS III score ($n = 23$; $p = .127$), and were well-matched for handedness ($n = 23$, $p = .838$; Table 1). The screening process ensured that all participants exhibited normal memory and cognition. Only one participant (SN+ group) self-reported recent mild signs of depression. No participants were taking medications known to affect excitability of motor circuitry.

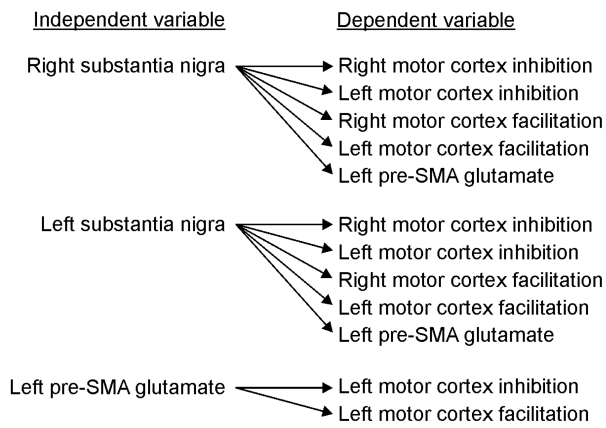

FIGURE 4 Variables and planned regressions in the three multivariate regression models.

The appearance of the substantia nigra was viewed with transcranial ultrasound. The quality of the bone window was rated one (excellent; 61% of observations), two (good; 33% of observations), or three (poor; 4% of observations) and the diameter of the third ventricle was normal in all participants (≤7.6 mm). Figure 2a shows typical images of the substantia nigra in one participant from each group. Five participants in the SN+ group had bilateral substantia nigra hyperechogenicity and four participants had unilateral hyperechogenicity (3 right, 1 left). Group data for area of substantia nigra echogenicity are presented in Table 1. There was no association between area of substantia nigra echogenicity (right, left, or largest hemisphere) and UPDRS III score ($n = 23$, $p \geq .381$). The appearance of the raphe was normal in all SN+ participants and in 11 of the 14 SN− participants (raphe was interrupted in remaining $n = 3$ participants). No overt structural abnormalities were detected on the volumetric T1 weighted scan in the sub-set of participants ($n = 13$) that underwent MRI.

3.2 | Transcranial magnetic stimulation (TMS)

Short-interval intracortical inhibition and intracortical facilitation within the primary motor cortex was assessed with paired-pulse TMS (see Section 2.2). There was no significant main effect of side (right hemisphere, left hemisphere; $n = 21$, $F_{1,20} = .708$, $p = .410$) on the MEP amplitude (mV) evoked by paired pulse stimulation. However, there was a significant main effect of conditioning stimulus intensity (70%, 90% resting motor threshold; $n = 21$, $F_{1,20} = 6.0$, $p = .024$) and condition (test and 2, 3, 10, and 12 ms interstimulus interval; $n = 21$, $F_{2.6,52.2} = 60.023$, $p < .001$) on MEP amplitude (Figure 5). The amplitude of the conditioned MEP at the 2 ms and 3 ms interstimulus intervals was significantly smaller than the test condition ($p < .001$

TABLE 1 Group data ($n = 23$; mean ± SD) showing participant characteristics and area of substantia nigra echogenicity for each group

Characteristic	SN− group	SN+ group
Age (years)	61.4 ± 6.6	60.0 ± 6.1
Education (years)	14.9 ± 3.9	16.6 ± 6.5
Sex	7 male, 7 female	6 male, 3 female
Handedness	13 right, 1 left	8 right, 1 left
UPDRS III score	1.0 ± 1.6	2.8 ± 3.5
Area of substantia nigra echogenicity (cm^2)		
Left hemisphere	.155 ± .042	.252 ± .077
Right hemisphere	.142 ± .036	.252 ± .037
Largest hemisphere	.164 ± .037	.282 ± .049
Average across sides	.148 ± .035	.252 ± .037

Abbreviations: SN− group, normal area of substantia nigra echogenicity on the right and left side; SN+ group, abnormally large area of substantia nigra echogenicity on the right and/or left side; UPDRS III, third (motor) part of the Unified Parkinson's Disease Rating Scale.

and $p = .001$, respectively) and the amplitude of the conditioned MEP at the 10 and 12 ms interstimulus intervals was significantly larger than the test condition ($p = .004$ and $p = .010$, respectively). Overall, the 90% conditioning stimulus intensity produced significantly larger MEPs than the 70% conditioning stimulus intensity ($p = .024$). However, a significant intensity-by-condition interaction ($n = 21$, $F_{4,80} = 6.344$, $p < .001$) revealed that this was due to the 90% conditioning intensity exhibiting significantly larger MEPs at the 10 and 12 ms interstimulus intervals ($p < .001$ and $p = .021$, respectively), but not at the 2 and 3 ms interstimulus intervals or test condition. The results of this three-way repeated measures ANOVA informed parameter selection for the multivariate regression models. The 90% conditioning intensity was selected and the average across the 2 and 3 ms interstimulus intervals (expressed as a % of the test amplitude) and across the 10 and 12 ms interstimulus intervals (expressed as a % of the test amplitude) were selected for inclusion in the model.

Table 2 shows other TMS parameters recorded during the TMS session with single-pulse TMS. Only one single-pulse TMS parameter (active MEP amplitude) had a significant main effect of side ($n = 22$, $F_{1,21} = 8.576$, $p = .008$; right hemisphere: 6.0 ± 3.5 mV, left hemisphere: 4.8 ± 2.7 mV) and there was no significant main effect of group or group-by-side interaction on single-pulse TMS parameters.

3.3 | Pre-SMA neurochemistry: Selection of glutamate parameter for multivariate regression model

Simple linear regression of the left substantia nigra area of echogenicity against each left pre-SMA glutamate measure (Glu tot, Glu/NAA, Glu/Cre and Glu tot, Glu/NAA, Glu/Cre normalized to gray matter) was performed ($n = 13$). The Bayesian Information Criteria (BIC) statistic for each regression was −55.2, −54.5, −56.0, −55.2, −54.5, and − 56.0, respectively. The difference in the Bayesian Information Criteria (BIC) statistic was less than 2 for each regression. This indicates that the goodness-of-fit for each outcome measure was similar and the measures of glutamate are interchangeable. Thus, Glu tot was selected for inclusion in the model.

3.4 | Relationship between substantia nigra echogenicity, pre-SMA glutamate, and short-interval intracortical inhibition and intracortical facilitation in primary motor cortex: multivariate regression model

There was a significant positive relationship between the area of echogenicity in the right substantia nigra and the magnitude of intracortical facilitation in the right primary motor cortex (amplitude of the conditioned MEP at the 10–12 ms interstimulus intervals; $p = .005$, Table 3 and Figure 5b). This relationship was not significant on the left side, and there was no significant relationship between area of substantia nigra echogenicity and short-interval intracortical inhibition on the right or left side (Table 3). There was also no significant relationship between area of substantia nigra echogenicity and glutamate level (Glu tot) in the pre-SMA, and glutamate level (Glu tot) in the pre-SMA was not associated with the magnitude of short-interval intracortical inhibition or intracortical facilitation in the primary motor cortex (Table 3).

TABLE 2 Group data ($n = 23$; mean ± SD) showing characteristics of the motor evoked potential (MEP) evoked by single-pulse transcranial magnetic stimulation (TMS) over the primary motor cortex during relaxation (rest) and weak voluntary contraction (active) of the target first dorsal interosseus muscle

	SN− group			SN+ group		
Side	**Right**	**Left**	**Average**	**Right**	**Left**	**Average**
RMT (%)	46.6 ± 8.3	45.7 ± 9.3	46.2 ± 8.6	46.8 ± 11.4	45.9 ± 10.9	46.3 ± 10.5
Resting MEP						
Amplitude (mV)	2.4 ± 2.5	1.7 ± .9	2.1 ± 1.6	2.1 ± 1.7	1.5 ± 1.3	1.8 ± 1.3
Latency (ms)	23.0 ± 1.7	22.6 ± 1.4	22.8 ± 1.5	23.2 ± 1.9	23.0 ± 1.8	23.1 ± 1.7
Duration (ms)	42.1 ± 12.3	43.9 ± 9.0	43.0 ± 9.7	44.7 ± 8.9	42.2 ± 15.0	43.4 ± 11.1
Active MEP						
Amplitude (mV)	6.1 ± 4.2	5.4 ± 2.9	5.8 ± 3.3	5.8 ± 2.2	3.8 ± 2.2	4.8 ± 1.9
Amplitude (% rest)	410 ± 329	358 ± 227	384 ± 211	482 ± 507	412 ± 325	447 ± 400
Latency (ms)	21.0 ± 1.8	20.7 ± 1.6	20.8 ± 1.6	21.7 ± 2.3	22.1 ± 2.1	21.9 ± 2.1
Duration (ms)	48.1 ± 9.4	48.4 ± 7.5	48.2 ± 8.1	53.2 ± 4.9	49.3 ± 9.6	51.2 ± 7.1
Silent period (ms)	175.2 ± 34.8	176.1 ± 29.1	175.6 ± 29.1	174.6 ± 36.7	166.0 ± 31.4	170.3 ± 29.8

Note: Data for the right hemisphere, left hemisphere, and average across the right and left hemisphere are shown.

Abbreviations: RMT, resting motor threshold (% stimulator output); SN− group, normal area of substantia nigra echogenicity on the right and left side; SN+ group, abnormally large area of substantia nigra echogenicity on the right and/or left side.

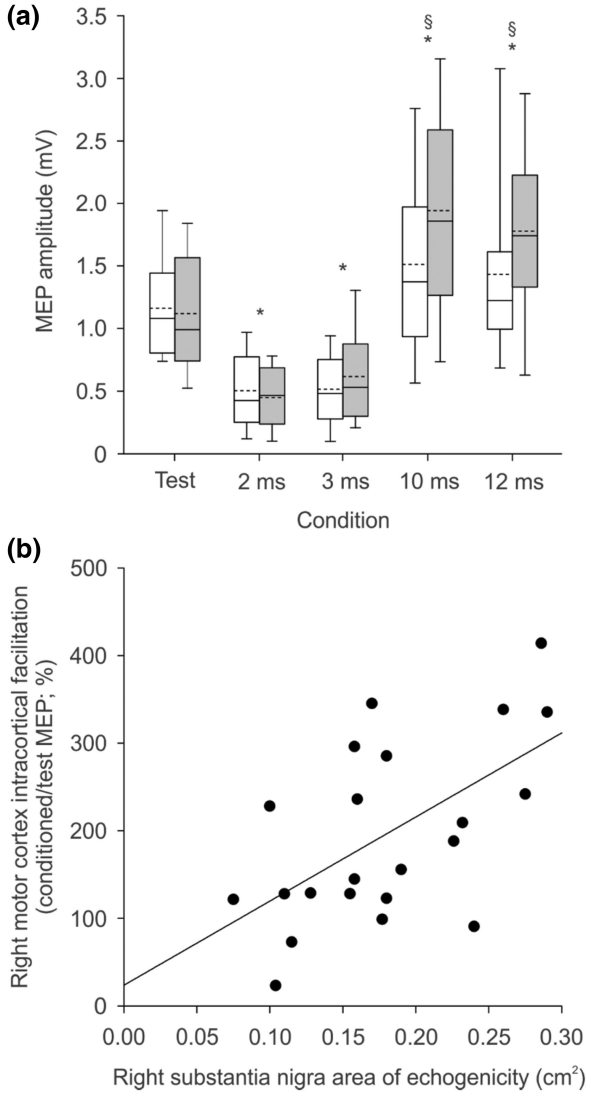

FIGURE 5 Single-subject and group data showing short-interval intracortical inhibition and intracortical facilitation within the primary motor cortex. (a) Group data ($n = 22$) for the average amplitude of the motor evoked potential (MEP) across the right and left hemisphere during paired-pulse transcranial magnetic stimulation (TMS). The boundary of each box indicates the 25th and 75th percentile and the whiskers (error bars) indicate the 10th and 90th percentiles. The solid and dashed lines within each box indicate the median and mean values, respectively. Data illustrate the significant main effects of conditioning stimulus intensity (white boxes: 70% resting motor threshold; gray boxes: 90% resting motor threshold) and condition (test and 2, 3, 10, and 12 ms interstimulus intervals) on MEP amplitude. The significant main effects were observed in a three-way repeated measures analysis of variance (ANOVA; within subject factors: Side, conditioning stimulus intensity, and condition). *Significant difference between test MEP and conditioned MEP at 2, 3, 10, and 12 ms interstimulus intervals ($p \leq .010$). §Significant difference between the two intensities of conditioning stimulation within a given interstimulus interval ($p \leq .021$). (b) Single subject data ($n = 22$) showing the relationship between intracortical facilitation within the right primary motor cortex and area of echogenicity in the right substantia nigra ($p = .005$, multivariate linear regression). Intracortical facilitation is the average across the 10 and 12 ms interstimulus intervals (expressed as a % of the test amplitude) when the intensity of the conditioning stimulation was set at 90% resting motor threshold.

4 | DISCUSSION

We previously demonstrated that substantia nigra hyperechogenicity is associated with reduced short-interval intracortical inhibition in the ipsilateral primary motor cortex of healthy elderly adults aged 72–84 yrs (Todd et al., 2010). The results of the current study suggest that this is not the case for healthy adults aged 50–70 years; spanning the average diagnostic age for Parkinson's disease. This result does not support the first part of the hypothesis. Instead, we observed a significant positive relationship between the area of echogenicity in the right substantia nigra and the magnitude of intracortical facilitation in the ipsilateral primary motor cortex. The second part of the hypothesis, that reduced short-interval intracortical inhibition in the primary motor cortex is associated with glutamatergic neurotransmission in the pre-SMA, was also not supported. The amount of glutamate in the pre-SMA was not associated with the magnitude of short-interval intracortical inhibition in the primary motor cortex. Our data suggest that substantia nigra hyperechogenicity, a risk marker for Parkinson's disease (Berg et al., 2015), may not be an isolated phenomenon and that additional specific, and potentially age-related, changes are evident in human motor circuitry. This is consistent with findings that healthy individuals with substantia nigra hyperechogenicity exhibit subtle changes in movement performance (Berg et al., 2001; Todd et al., 2014), although the relationship between these changes and the marked degeneration of substantia nigra dopaminergic neurons characterizing Parkinson's disease is unclear.

Normal short-interval intracortical inhibition was observed in the primary motor cortices of the SN− and SN+ group, when measured with the lower or higher intensity of conditioning stimulation. The normal short-interval intracortical inhibition was evidenced by the amplitude of the conditioned MEP at the 2 and 3 ms interstimulus intervals being similar to that previously observed in healthy young adults (Oliviero et al., 2006; Peinemann et al., 2001) and healthy older adults (Todd et al., 2010). The potential interference of the short-interval intracortical inhibition measurement by short-interval intracortical facilitation at the higher intensity of conditioning stimulation (Peurala et al., 2008) was likely to be minimal in the current study because the conditioned MEP amplitude evoked by the lower (70% resting motor threshold) and higher (90% resting motor threshold) conditioning intensities was comparable at the 2 and 3 ms interstimulus intervals. The normal short-interval intracortical inhibition in the sample suggests that the TMS protocol was appropriate to observe an association between substantia nigra echogenicity and short-interval intracortical inhibition if such a relationship existed in healthy adults aged 50–70 years.

In the right hemisphere, there was a significant positive relationship between area of substantia nigra echogenicity and the magnitude of intracortical facilitation in primary motor cortex. A larger area of echogenicity was associated with greater intracortical facilitation in the ipsilateral primary motor cortex. This is consistent with the

TABLE 3 Multivariate regression models

Dependent variable	Independent variable	Coefficient	Std err	*t*	*p* > |*t*|	95% CI
Left pre-SMA glutamate		−1.77	.79	−2.24	.052	−3.56, .02
Right motor cortex inhibition		152.95	104.39	1.47	.177	−83.2, 389.1
Left motor cortex inhibition		13.22	150.57	.09	.932	−327.4, 353.8
Right motor cortex facilitation		1235.30	332.67	3.71	.005	482.8, 1987.8
Left motor cortex facilitation		216.27	310.25	.70	.503	−485.6, 918.1
Right substantia nigra						
Left pre-SMA glutamate		.25	.74	.33	.746	−1.4, 1.9
Right motor cortex inhibition		−68.84	85.25	−.81	.440	−261.7, 124.0
Left motor cortex inhibition		−36.72	113.81	−.32	.754	−294.2, 220.7
Right motor cortex facilitation		319.04	387.94	.82	.432	−558.5, 1196.6
Left motor cortex facilitation		−65.64	241.05	−.27	.792	−610.9, 479.6
Left substantia nigra						
Left motor cortex inhibition		.94	50.91	.02	.986	−114.2, 116.1
Left motor cortex facilitation		−78.11	104.45	−.75	.474	−314.4, 158.2
Left pre-SMA glutamate						

Note: Two of the 13 participants that completed both MRI and TMS had a missing TMS data point. Thus, the results of the multivariate regression model are for $n = 11$. Inhibition, average across the 2 and 3 ms interstimulus intervals (expressed as a % of the test amplitude) when the intensity of the conditioning stimulation was set at 90% resting motor threshold. Facilitation, average across the 10 and 12 ms interstimulus intervals (expressed as a % of the test amplitude) when the intensity of the conditioning stimulation was set at 90% resting motor threshold. Glutamate, Glu tot. Substantia nigra, area of substantia nigra echogenicity (cm^2).

Abbreviation: CI, confidence interval.

existence of a significant ipsilateral structural connection between the substantia nigra and primary motor cortex in the adult human brain (e.g., Cacciola et al., 2016). The clinical relevance of greater intracortical facilitation in the ipsilateral primary motor cortex is unclear because intracortical facilitation (measured at 10 and 15 ms interstimulus intervals) and short-interval intracortical facilitation (measured at 1.2, 1.4, and 1.6 ms interstimulus intervals) appear to be unaltered in Parkinson's disease, both in de novo patients (Ammann et al., 2020) and patients OFF medication (Ammann et al., 2020; Bologna et al., 2018; Ridding et al., 1995). One must be cautious when interpreting the above relationship because the correlation between area of substantia nigra echogenicity and intracortical facilitation in the primary motor cortex was modest and thus other factors may contribute to this association. An association between substantia nigra echogenicity and short-interval intracortical inhibition would have been more clinically relevant because reduced short-interval intracortical inhibition is present in patients with Parkinson's disease (e.g., Ammann et al., 2020; Bologna et al., 2018; Leon-Sarmiento et al., 2013; Ridding et al., 1995) who typically present with substantia nigra hyperechogenicity (for review, see Berg et al., 2008).

In the left hemisphere, there was no significant relationship between area of substantia nigra echogenicity and the magnitude of short-interval intracortical inhibition or intracortical facilitation in the left (ipsilateral) primary motor cortex. The different relationship observed in the right and left hemispheres could be associated with use of a small heterogenous SN+ sample. Four of the nine participants in the SN+ group had unilateral substantia nigra hyperechogenicity, of which three were hyperechogenic on the right side but only one expressed unilateral hyperechogenicity on the left side. The remaining five participants in the SN+ group had bilateral hyperechogenicity. The different relationship observed in the right and left hemisphere could also be due to the connections between the right and left sides of the basal nuclei and/or motor cortices, and the ipsilateral and contralateral connections between these structures and the substantia nigra, being complex (e.g., Gerfen et al., 1982; McColgan et al., 2020; Schmitt et al., 2016) and not necessarily identical in each hemisphere.

The association between substantia nigra echogenicity and intracortical facilitation in primary motor cortex is unlikely to result from neuropsychological factors. All participants had normal memory and cognition and only one (SN+) participant self-reported recent mild signs of depression. The relationship is also unlikely to arise from overt pathological changes in brain structure because no structural abnormalities were evident in the 13 participants who underwent MRI and the diameter of the third ventricle (viewed with transcranial ultrasound) was normal in all participants (i.e., <7.6 mm, Seidel et al., 1995). The observed association between substantia nigra echogenicity and intracortical facilitation could feasibly arise from pathophysiological changes within the substantia nigra or primary motor cortex, or as a consequence of alterations in the basal ganglia or premotor cortices (premotor cortex, supplementary motor area, and/or pre-SMA). Histological studies demonstrate the number, and morphological appearance, of pyramidal neurons and interneurons in the primary motor cortex is unaffected in patients

with Parkinson's disease (MacDonald & Halliday, 2002). Based on this, one would assume that the number and morphology of these neurons is also unaffected in the current cohort of healthy adults. Thus, characteristics of the basal ganglia or premotor cortices are more likely to underlie the association with intracortical facilitation in the primary motor cortex. We focused on the potential role of the pre-SMA in this process because the pre-SMA undergoes neurodegeneration in Parkinson's disease (MacDonald & Halliday, 2002), is connected to the primary motor cortex via projections to the SMA proper (for review see Tanji, 1994), and receives input from the basal ganglia via the ventral anterior thalamus (for review, see Nachev et al., 2008).

We investigated neurochemistry in the pre-SMA with magnetic resonance spectroscopy and explored the relationship between the level of glutamate in the pre-SMA and excitability of circuitry within the primary motor cortex. The level of glutamate in the pre-SMA did not predict the magnitude of short-interval intracortical inhibition or intracortical facilitation in the primary motor cortex. The result is in line with observations of unaffected cellular activity markers (NAA/Cr and Cho/Cr) in pre-SMA in untreated patients with early Parkinson's disease (Martin et al., 2008). However, reduced levels of the neuronal marker NAA/Cr are present in the pre-SMA of patients with Hoehn and Yahr stage 2–3 Parkinson's disease tested ON medication (Camicioli et al., 2007) and unilateral administration of MPTP to rhesus monkeys results in lower levels of glutamate in the motor cortex ipsilateral to MPTP administration than in the contralateral motor cortex (Fan et al., 2014).

Hundreds of healthy adults were screened in the current study to identify the healthy participants with substantia nigra hyperechogenicity. Nevertheless, the proportion of recruited participants with substantia nigra hyperechogenicity that subsequently met our stringent study criteria was small and this is a limitation of the current study. The small sample size is primarily due to only 8.6% of healthy older adults in the community exhibiting substantia nigra hyperechogenicity (e.g., Berg et al., 2008) and the tendency for older adults to have contraindications for TMS (e.g., previous head injury) or contraindications for brain and brainstem MRI (particularly dental implants). Furthermore, the bone window for transcranial ultrasound is insufficient to view the substantia nigra in 10%–20% of adults that identify as Caucasian and >20% of adults that identify as Asian (Skoloudik & Walter, 2010). The prevalence of insufficient bone window further increases the number of potential participants that need to be recruited and screened for participation eligibility. Despite the challenges of recruiting participants that met all criteria for inclusion, we were able to study 9 SN+ and 14 SN− subjects using our detailed study design. Multivariate regression revealed a significant association (at a significance level of $p = .01$) between substantia nigra echogenicity and intracortical facilitation within the primary motor cortex. It should be noted, however, that a post priori power analysis based on the observed effect size between groups for intracortical inhibition (G*Power, independent two-group t-test, $\alpha = .05$) provided a medium power (.6) suggesting the current study should be considered to be exploratory. Differences in the reported relationship between area of substantia nigra echogenicity and excitability of intracortical circuitry within the primary motor cortex in the current study and a previous study (Todd et al., 2010) may be due to participant age (50–70 years vs. 72–84 years, respectively). There is a progressive loss of neurons in the substantia nigra pars compacta with normal aging and this is estimated to occur at a rate of 4.7% per decade between the ages of 20 and 90 years (e.g., Fearnley & Lees, 1991). Variability in the extent of neuronal loss is also considerably greater in healthy adults aged over 70 years than in healthy adults aged 50–70 years (e.g., Fearnley & Lees, 1991).

Another limitation of the current study relates to the two test sites. Comparable TMS and EMG equipment was used at the two sites but the ultrasound operators and machines (Philips iU22 vs. Siemens Sonoline Antares) were different. The German research team (lead by Professor Daniela Berg) provides an international training course on transcranial ultrasound of the substantia nigra and the team member who performed the Australian ultrasounds (Associate Professor Gabrielle Todd) has completed this course. Professor Berg has also performed multiple blinded evaluations of Associate Professor Todd's image quality and measurements (e.g., Aoun et al., 2021; Todd et al., 2013, 2014). Agreement between the two ultrasound machines and operators was not investigated. Both SN− and SN+ participants were tested at each site and thus any subtle difference between ultrasound machines and operators would be present in both groups.

In summary, our detailed study of a small sample of healthy adults with substantia nigra hyperechogenicity, who were of the age (50–70 years) when Parkinson's disease is typically diagnosed, do not exhibit an association between area of substantia nigra echogenicity and short-interval intracortical inhibition in the primary motor cortex. Rather, we demonstrate a positive association between the area of substantia nigra echogenicity and the magnitude of intracortical facilitation in the ipsilateral primary motor cortex of the right hemisphere. The excitability of circuitry within the primary motor cortex was not related to glutamatergic neurotransmission in the pre-SMA. Further research is required to verify the results and to elucidate the functional and clinical relevance of these findings in relation to motor function and risk of Parkinson's disease.

AUTHOR CONTRIBUTIONS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Conceptualization*: G.T., C.D.R. and K.L.D.; *Methodology*: G.T., C.D.R., J.L.T., J.E.B., M.H., R.A.W., and K.L.D.; *Formal Analysis*: G.T., C.D.R., K.A. and A.E.; *Investigation*: G.T., C.D.R., J.L.T., N.C.R., J.E.B., M.H., R.A.W., K.A., S.J.G.L., J.M.H., E.M., D.B., C.P., A-K.V., C.C. and K.L.D.; *Resources*: G.T., C.D.R., J.L.T., J.E.B., S.C.G., D.B., and K.L.D.; *Writing – Original Draft*: G.T.; *Writing – Review & Editing*: G.T., C.D.R., J.L.T., N.C.R., J.E.B., M.H., R.A.W., S.C.G., K.A., S.J.G.L., J.M.H., E.M., J.G., D.B., C.P., A-K.V., C.C. and K.L.D.; *Visualization*: G.T. and C.D.R.; *Supervision*: G.T., C.D.R. and K.L.D.; *Project Administration*: G.T. and K.L.D.; *Funding Acquisition*: G.T., C.D.R. and K.L.D.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Abikoff, H., Alvir, J., Hong, G., Sukoff, R., Orazio, J., Solomon, S., & Saravay, S. (1987). Logical memory subtest of the Wechsler Memory Scale: Age and education norms and alternate-form reliability of two scoring systems. *Journal of Clinical and Experimental Neuropsychology*, *9*, 435–448. https://doi.org/10.1080/01688638708405063

Ammann, C., Dileone, M., Pagge, C., Catanzaro, V., Mata-Marin, D., Hernandez-Fernandez, F., Monje, M. H. G., Sanchez-Ferro, A., Fernandez-Rodriguez, B., Gasca-Salas, C., Manez-Miro, J. U., Martinez-Fernandez, R., Vela-Desojo, L., Alonso-Frech, F., Oliviero, A., Obeso, J. A., & Foffani, G. (2020). Cortical disinhibition in Parkinson's disease. *Brain*, *143*, 3408–3421. https://doi.org/10.1093/brain/awaa274

Anstey, K. J., Matters, B., Brown, A. K., & Lord, S. R. (2000). Normative data on neuropsychological tests for very old adults living in retirement villages and hostels. *The Clinical Neuropsychologist*, *14*, 309–317. https://doi.org/10.1076/1385-4046(200008)14:3;1-P;FT309

Aoun, K., Double, K. L., Pearson-Dennett, V., Yilmaz, R., Berg, D., & Todd, G. (2021). Measurement of the adult human midbrain with transcranial ultrasound. *PLoS One*, *16*, e0247920. https://doi.org/10.1371/journal.pone.0247920

Beck, A. T., Steer, R. A., & Brown, G. K. (1996). *Manual for the Beck Depression Inventory-II*. The Psychological Corporation.

Behnke, S., Schroeder, U., Dillmann, U., Buchholz, H. G., Schreckenberger, M., Fuss, G., Reith, W., Berg, D., & Krick, C. M. (2009). Hyperechogenicity of the substantia nigra in healthy controls is related to MRI changes and to neuronal loss as determined by F-Dopa PET. *NeuroImage*, *47*, 1237–1243. https://doi.org/10.1016/j.neuroimage.2009.05.072

Benton, A. L., & Hamsher, K. (1983). *Multilingual aphasia examination*. AJA Associates.

Berg, D., Becker, G., Zeiler, B., Tucha, O., Hofmann, E., Preier, M., Benz, P., Jost, W., Reiners, K., & Lange, K. W. (1999). Vulnerability of the nigrostriatal system as detected by transcranial ultrasound. *Neurology*, *53*, 1026–1031. https://doi.org/10.1212/wnl.53.5.1026

Berg, D., Behnke, S., Seppi, K., Godau, J., Lerche, S., Mahlknecht, P., Liepelt-Scarfone, I., Pausch, C., Schneider, N., Gaenslen, A., Brockmann, K., Srulijes, K., Huber, H., Wurster, I., Stockner, H., Kiechl, S., Willeit, J., Gasperi, A., Fassbender, K., … Poewe, W. (2013). Enlarged hyperechogenic substantia nigra as a risk marker for Parkinson's disease. *Movement Disorders*, *28*, 216–219. https://doi.org/10.1002/mds.25192

Berg, D., Godau, J., Riederer, P., Gerlach, M., & Arzberger, T. (2010). Microglia activation is related to substantia nigra echogenicity. *Journal of Neural Transmission*, *117*, 1287–1292. https://doi.org/10.1007/s00702-010-0504-6

Berg, D., Godau, J., & Walter, U. (2008). Transcranial sonography in movement disorders. *Lancet Neurology*, *7*, 1044–1055. https://doi.org/10.1016/S1474-4422(08)70239-4

Berg, D., Postuma, R. B., Adler, C. H., Bloem, B. R., Chan, P., Dubois, B., Gasser, T., Goetz, C. G., Halliday, G., Joseph, L., Lang, A. E., Liepelt-Scarfone, I., Litvan, I., Marek, K., Obeso, J., Oertel, W., Olanow, C. W., Poewe, W., Stern, M., & Deuschl, G. (2015). MDS research criteria for prodromal Parkinson's disease. *Movement Disorders*, *30*, 1600–1611. https://doi.org/10.1002/mds.26431

Berg, D., Roggendorf, W., Schroder, U., Klein, R., Tatschner, T., Benz, P., Tucha, O., Preier, M., Lange, K. W., Reiners, K., Gerlach, M., & Becker, G. (2002). Echogenicity of the substantia nigra: Association with increased iron content and marker for susceptibility to nigrostriatal injury. *Archives of Neurology*, *59*, 999–1005. https://doi.org/10.1001/archneur.59.6.999

Berg, D., Seppi, K., Behnke, S., Liepelt, I., Schweitzer, K., Stockner, H., Wollenweber, F., Gaenslen, A., Mahlknecht, P., Spiegel, J., Godau, J., Huber, H., Srulijes, K., Kiechl, S., Bentele, M., Gasperi, A., Schubert, T., Hiry, T., Probst, M., … Poewe, W. (2011). Enlarged substantia nigra hyperechogenicity and risk for Parkinson disease: A 37-month 3-center study of 1847 older persons. *Archives of Neurology*, *68*, 932–937. https://doi.org/10.1001/archneurol.2011.141

Berg, D., Siefker, C., Ruprecht-Dorfler, P., & Becker, G. (2001). Relationship of substantia nigra echogenicity and motor function in elderly subjects. *Neurology*, *56*, 13–17. https://doi.org/10.1212/wnl.56.1.13

Bologna, M., Guerra, A., Paparella, G., Giordo, L., Alunni Fegatelli, D., Vestri, A. R., Rothwell, J. C., & Berardelli, A. (2018). Neurophysiological correlates of bradykinesia in Parkinson's disease. *Brain*, *141*, 2432–2444. https://doi.org/10.1093/brain/awy155

Cacciola, A., Milardi, D., Anastasi, G. P., Basile, G. A., Ciolli, P., Irrera, M., Cutroneo, G., Bruschetta, D., Rizzo, G., Mondello, S., Bramanti, P., & Quartarone, A. (2016). A direct cortico-nigral pathway as revealed by constrained spherical deconvolution tractography in humans.

Frontiers in Human Neuroscience, *10*, 374. https://doi.org/10.3389/fnhum.2016.00374

Camicioli, R. M., Hanstock, C. C., Bouchard, T. P., Gee, M., Fisher, N. J., & Martin, W. R. (2007). Magnetic resonance spectroscopic evidence for presupplementary motor area neuronal dysfunction in Parkinson's disease. *Movement Disorders*, *22*, 382–386. https://doi.org/10.1002/mds.21288

de Lau, L. M., & Breteler, M. M. (2006). Epidemiology of Parkinson's disease. *Lancet Neurology*, *5*, 525–535. https://doi.org/10.1016/S1474-4422(06)70471-9

Doepp, F., Plotkin, M., Siegel, L., Kivi, A., Gruber, D., Lobsien, E., Kupsch, A., & Schreiber, S. J. (2008). Brain parenchyma sonography and 123I-FP-CIT SPECT in Parkinson's disease and essential tremor. *Movement Disorders*, *23*, 405–410. https://doi.org/10.1002/mds.21861

Fahn, S., & Elton, R. L. (1987). The unified Parkinson's disease rating scale. In S. Fahn, C. D. Marsden, D. B. Calne, & M. Goldstein (Eds.), *Recent developments in Parkinson's disease* (pp. 153–163, 293–304). MacMillan Healthcare Information.

Fan, X. T., Zhao, F., Ai, Y., Andersen, A., Hardy, P., Ling, F., Gerhardt, G. A., Zhang, Z., & Quintero, J. E. (2014). Cortical glutamate levels decrease in a non-human primate model of dopamine deficiency. *Brain Research*, *1552*, 34–40. https://doi.org/10.1016/j.brainres.2013.12.035

Fearnley, J. M., & Lees, A. J. (1991). Ageing and Parkinson's disease: Substantia nigra regional selectivity. *Brain*, *114*, 2283–2301. https://doi.org/10.1093/brain/114.5.2283

Flavel, S. C., White, J. M., & Todd, G. (2012). Motor cortex and corticospinal excitability in humans with a history of illicit stimulant use. *Journal of Applied Physiology*, *113*, 1486–1494. https://doi.org/10.1152/japplphysiol.00718.2012

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

Gasparovic, C., Song, T., Devier, D., Bockholt, H. J., Caprihan, A., Mullins, P. G., Posse, S., Jung, R. E., & Morrison, L. A. (2006). Use of tissue water as a concentration reference for proton spectroscopic imaging. *Magnetic Resonance in Medicine*, *55*, 1219–1226. https://doi.org/10.1002/mrm.20901

Gerfen, C. R., Staines, W. A., Arbuthnott, G. W., & Fibiger, H. C. (1982). Crossed connections of the substantia nigra in the rat. *The Journal of Comparative Neurology*, *207*, 283–303. https://doi.org/10.1002/cne.902070308

Grigsby, J., & Kaye, K. (1995). Alphanumeric sequencing and cognitive impairment among elderly persons. *Perceptual and Motor Skills*, *80*, 732–734. https://doi.org/10.2466/pms.1995.80.3.732

Haussermann, P., Wilhelm, T., Keinath, S., Stolzle, C., Conrad, B., & Ceballos-Baumann, A. (2001). Primary central nervous system lymphoma in the SMA presenting as rapidly progressive parkinsonism. *Movement Disorders*, *16*, 962–965. https://doi.org/10.1002/mds.1193

Hoshi, E., & Tanji, J. (2004). Differential roles of neuronal activity in the supplementary and presupplementary motor areas: From information retrieval to motor planning and execution. *Journal of Neurophysiology*, *92*, 3482–3499. https://doi.org/10.1152/jn.00547.2004

Kear-Colwell, J. J., & Heller, M. (1978). A normative study of the Wechsler Memory Scale. *Journal of Clinical Psychology*, *34*, 437–442. https://doi.org/10.1002/1097-4679(197804)34:2<437::AID-JCLP2270340239>3.0.CO;2-K

Kenny, R. A., Coen, R. F., Frewen, J., Donoghue, O. A., Cronin, H., & Savva, G. M. (2013). Normative values of cognitive and physical function in older adults: Findings from the Irish longitudinal study on ageing. *Journal of the American Geriatrics Society*, *61*(Suppl 2), S279–S290. https://doi.org/10.1111/jgs.12195

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferber, A., Wroe, S., Asselman, P., & Marsden, C. D. (1993). Corticocortical inhibition in human motor cortex. *The Journal of Physiology*, *471*, 501–519. https://doi.org/10.1113/jphysiol.1993.sp019912

Leon-Sarmiento, F. E., Rizzo-Sierra, C. V., Bayona, E. A., Bayona-Prieto, J., Doty, R. L., & Bara-Jimenez, W. (2013). Novel mechanisms underlying inhibitory and facilitatory transcranial magnetic stimulation abnormalities in Parkinson's disease. *Archives of Medical Research*, *44*, 221–228. https://doi.org/10.1016/j.arcmed.2013.03.003

Li, D. H., He, Y. C., Liu, J., & Chen, S. D. (2016). Diagnostic accuracy of transcranial sonography of the substantia Nigra in Parkinson's disease: A systematic review and meta-analysis. *Scientific Reports*, *6*, 20863. https://doi.org/10.1038/srep20863

Loo, C. K., Sachdev, P., Mitchell, P. B., Gandevia, S. C., Malhi, G. S., Todd, G., & Taylor, J. L. (2008). A study using transcranial magnetic stimulation to investigate motor mechanisms in psychomotor retardation in depression. *The International Journal of Neuropsychopharmacology*, *1-12*, 935–946. https://doi.org/10.1017/S1461145708008821

Lovibond, S. H., & Lovibond, P. F. (1995). *Manual for the depression anxiety stress scales* (2nd ed.). Pscyhological Foundation.

MacDonald, V., & Halliday, G. M. (2002). Selective loss of pyramidal neurons in the pre-supplementary motor cortex in Parkinson's disease. *Movement Disorders*, *17*, 1166–1173. https://doi.org/10.1002/mds.10258

Maeda, F., Keenan, J. P., & Pascual-Leone, A. (2000). Interhemispheric asymmetry of motor cortical excitability in major depression as measured by transcranial magnetic stimulation. *The British Journal of Psychiatry*, *177*, 169–173. https://doi.org/10.1192/bjp.177.2.169

Martin, W. R., Wieler, M., Gee, M., Hanstock, C. C., & Camicioli, R. M. (2008). Intact presupplementary motor area function in early, untreated Parkinson's disease. *Movement Disorders*, *23*, 1756–1759. https://doi.org/10.1002/mds.22101

McColgan, P., Joubert, J., Tabrizi, S. J., & Rees, G. (2020). The human motor cortex microcircuit: Insights for neurodegenerative disease. *Nature Reviews. Neuroscience*, *21*, 401–415. https://doi.org/10.1038/s41583-020-0315-1

Morris, J. C., Heyman, A., Mohs, R. C., Hughes, J. P., van Belle, G., Fillenbaum, G., Mellits, E. D., & Clark, C. (1989). The consortium to establish a registry for Alzheimer's disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. *Neurology*, *39*, 1159–1165. https://doi.org/10.1212/wnl.39.9.1159

Mrazik, M., Millis, S., & Drane, D. L. (2010). The oral trail making test: Effects of age and concurrent validity. *Archives of Clinical Neuropsychology*, *25*, 236–243. https://doi.org/10.1093/arclin/acq006

Nachev, P., Kennard, C., & Husain, M. (2008). Functional role of the supplementary and pre-supplementary motor areas. *Nature Reviews. Neuroscience*, *9*, 856–869. https://doi.org/10.1038/nrn2478

Naressi, A., Couturier, C., Castang, I., de Beer, R., & Graveron-Demilly, D. (2001). Java-based graphical user interface for MRUI, a software package for quantitation of in vivo/medical magnetic resonance spectroscopy signals. *Computers in Biology and Medicine*, *31*, 269–286. https://doi.org/10.1016/s0010-4825(01)00006-3

Nielsen, H., Lolk, A., & Kragh-Sorensen, P. (1995). Normative data for eight neuropsychological tests, gathered from a random sample of Danes aged 64 to 83 years. *Nordisk Psykologi*, *47*, 241–255. https://doi.org/10.1080/00291463.1995.11863861

Oldfield, R. C. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia*, *9*, 97–113. https://doi.org/10.1016/0028-3932(71)90067-4

Oliviero, A., Profice, P., Tonali, P. A., Pilato, F., Saturno, E., Dileone, M., Ranieri, F., & Di Lazzaro, V. (2006). Effects of aging on motor cortex excitability. *Neuroscience Research*, *55*, 74–77. https://doi.org/10.1016/j.neures.2006.02.002

Peinemann, A., Lehner, C., Conrad, B., & Siebner, H. R. (2001). Age-related decrease in paired-pulse intracortical inhibition in the

human primary motor cortex. *Neuroscience Letters*, *313*, 33–36. https://doi.org/10.1016/s0304-3940(01)02239-x

Peurala, S. H., Muller-Dahlhaus, J. F., Arai, N., & Ziemann, U. (2008). Interference of short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF). *Clinical Neurophysiology*, *119*, 2291–2297. https://doi.org/10.1016/j.clinph.2008.05.031

Prestel, J., Schweitzer, K. J., Hofer, A., Gasser, T., & Berg, D. (2006). Predictive value of transcranial sonography in the diagnosis of Parkinson's disease. *Movement Disorders*, *21*, 1763–1765. https://doi.org/10.1002/mds.21054

Raftery, A. E. (1995). Bayesian model selection in social research. *Sociological Methodology*, *25*, 111–163. https://doi.org/10.2307/271063

Ridding, M. C., Inzelberg, R., & Rothwell, J. C. (1995). Changes in excitability of motor cortical circuitry in patients with Parkinson's disease. *Annals of Neurology*, *37*, 181–188. https://doi.org/10.1002/ana.410370208

Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology*, *120*, 2008–2039. https://doi.org/10.1016/j.clinph.2009.08.016

Schmitt, O., Eipert, P., Kettlitz, R., Lessmann, F., & Wree, A. (2016). The connectome of the basal ganglia. *Brain Structure & Function*, *221*, 753–814. https://doi.org/10.1007/s00429-014-0936-0

Seidel, G., Kaps, M., Gerriets, T., & Hutzelmann, A. (1995). Evaluation of the ventricular system in adults by transcranial duplex sonography. *Journal of Neuroimaging*, *5*, 105–108. https://doi.org/10.1111/jon199552105

Singh, S., Mistry, S., Jefferson, S., Davies, K., Rothwell, J., Williams, S., & Hamdy, S. (2009). A magnetic resonance spectroscopy study of brain glutamate in a model of plasticity in human pharyngeal motor cortex. *Gastroenterology*, *136*, 417–424. https://doi.org/10.1053/j.gastro.2008.10.087

Skoloudik, D., & Walter, U. (2010). Method and validity of transcranial sonography in movement disorders. *International Review of Neurobiology*, *90*, 7–34. https://doi.org/10.1016/S0074-7742(10)90002-0

Spiegel, J., Hellwig, D., Mollers, M. O., Behnke, S., Jost, W., Fassbender, K., Samnick, S., Dillmann, U., Becker, G., & Kirsch, C. M. (2006). Transcranial sonography and [123I]FP-CIT SPECT disclose complementary aspects of Parkinson's disease. *Brain*, *129*, 1188–1193. https://doi.org/10.1093/brain/awl042

Surmeier, D. J., Obeso, J. A., & Halliday, G. M. (2017). Selective neuronal vulnerability in Parkinson disease. *Nature Reviews. Neuroscience*, *18*, 101–113. https://doi.org/10.1038/nrn.2016.178

Tanji, J. (1994). The supplementary motor area in the cerebral cortex. *Neuroscience Research*, *19*, 251–268.

Tao, A., Chen, G., Deng, Y., & Xu, R. (2019). Accuracy of transcranial sonography of the substantia Nigra for detection of Parkinson's disease: A systematic review and meta-analysis. *Ultrasound in Medicine & Biology*, *45*, 628–641. https://doi.org/10.1016/j.ultrasmedbio.2018.11.010

Todd, G., Haberfield, M., Faulkner, P. L., Rae, C., Hayes, M., Wilcox, R. A., Taylor, J. L., Gandevia, S. C., Godau, J., Berg, D., Piguet, O., & Double, K. L. (2014). Hand function is impaired in healthy older adults at risk of Parkinson's disease. *Journal of Neural Transmission*, *121*, 1377–1386. https://doi.org/10.1007/s00702-014-1218-y

Todd, G., Noyes, C., Flavel, S. C., Della Vedova, C. B., Spyropoulos, P., Chatterton, B., Berg, D., & White, J. M. (2013). Illicit stimulant use is associated with abnormal substantia nigra morphology in humans. *PLoS One*, *8*, e56438. https://doi.org/10.1371/journal.pone.0056438

Todd, G., Taylor, J. L., Baumann, D., Butler, J. E., Duma, S. R., Hayes, M., Carew-Jones, F., Piguet, O., Behnke, S., Ridding, M. C., Berg, D., & Double, K. L. (2010). Substantia nigra echomorphology and motor cortex excitability. *NeuroImage*, *50*, 1351–1356. https://doi.org/10.1016/j.neuroimage.2010.01.088

Tombaugh, T. N., Kozak, J., & Rees, L. (1999). Normative data stratified by age and education for two measures of verbal fluency: FAS and animal naming. *Archives of Clinical Neuropsychology*, *14*, 167–177. https://doi.org/10.1016/S0887-6177(97)00095-4

Tsai, C. F., Wu, R. M., Huang, Y. W., Chen, L. L., Yip, P. K., & Jeng, J. S. (2007). Transcranial color-coded sonography helps differentiation between idiopathic Parkinson's disease and vascular parkinsonism. *Journal of Neurology*, *254*, 501–507. https://doi.org/10.1007/s00415-006-0403-9

Vanhamme, L., van den Boogaart, A., & Van Huffel, S. (1997). Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *Journal of Magnetic Resonance*, *129*, 35–43. https://doi.org/10.1006/jmre.1997.1244

Wechsler, D. (1981). *Wechsler adult intelligence scale—Revised*. Psychological Corporation.

Wechsler, D. (1987). *Wechsler Memory Scale—Revised*. Psychological Corporation.

Zecca, L., Berg, D., Arzberger, T., Ruprecht, P., Rausch, W. D., Musicco, M., Tampellini, D., Riederer, P., Gerlach, M., & Becker, G. (2005). In vivo detection of iron and neuromelanin by transcranial sonography: A new approach for early detection of substantia nigra damage. *Movement Disorders*, *20*, 1278–1285. https://doi.org/10.1002/mds.20550

Ziemann, U. (2004). TMS and drugs. *Clinical Neurophysiology*, *115*, 1717–1729. https://doi.org/10.1016/j.clinph.2004.03.006

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