Substantia nigra echomorphology and motor cortex excitability

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ABSTRACT

The aim of our study was to investigate the relation between substantia nigra (SN) echomorphology and indices of motor cortex excitability. Nigral hyperechogenicity in healthy individuals is thought to represent an SN abnormality or predisposition to Parkinson's disease (PD) and its prevalence is greater in the very old. Our study involved 20 old healthy subjects (aged 72–84 years) known to have normal ($n = 10$) or abnormal ($n = 10$) SN echomorphology. All were in good health with no overt neurological signs. SN morphology was assessed with transcranial sonography through the pre-auricular bone window. Motor cortical excitability and intracortical inhibition were assessed with transcranial magnetic stimulation (TMS) over the first dorsal interosseus motor area. Single stimuli were delivered during relaxation and voluntary contraction and paired stimuli were delivered during relaxation. Each cortical hemisphere was analysed separately. The response to single-pulse TMS (in motor cortex ipsilateral to the target SN) did not differ between groups. However, a significant difference between groups was observed in the paired pulse paradigm (conditioning stimulus intensity: 70% resting motor threshold; interstimulus interval: 2 ms). The conditioned motor evoked potential amplitude was significantly larger ipsilateral to the hyperechogenic SN than in controls ($P = 0.014$). Thus, healthy subjects with SN hyperechogenicity exhibit significantly less intracortical inhibition within the motor cortex than subjects with normal echomorphology. Decreased intracortical inhibition is also observed in PD patients. This study provides further evidence that SN hyperechogenicity in healthy individuals is associated with changes characteristic of PD supporting a role for this feature as a vulnerability marker or state marker for subtle nigral dopaminergic dysfunction.

Introduction

Transcranial ultrasound can be used to identify structural changes in human substantia nigra (SN). The structural changes are characterised by a significant increase of signal intensity termed hyperechogenicity, a feature found in 78–90% of Parkinson's disease (PD) patients (e.g. Doepp et al., 2008; Spiegel et al., 2006; Tsai et al., 2007) and in 9% of healthy individuals aged 20–80 years (for review see Berg, 2007). In PD, SN hyperechogenicity is more pronounced contralateral to the more affected body side (Berg et al., 2005) and remains stable during the course of the disease (Berg et al., 2005). Postmortem studies have revealed a correlation between hyperechogenicity and increased tissue iron content in the SN of PD patients (Berg et al., 2002) but the functional significance of this feature is unclear.

The motor areas of the cerebral cortex are primary targets for output from the basal ganglia and disease of the basal ganglia can affect the activity of cells within the motor cortex. In humans, output from the motor cortex to the spinal cord can be explored with the use of transcranial magnetic stimulation (TMS). The electromyographic response evoked by TMS is sensitive to the level of cortical excitability and reflects activity in intracortical circuits and projections to the spinal cord. Significant changes in the excitability of inhibitory cortical circuits are present in patients with PD (Ridding et al., 1995).

The aim of the current study was to investigate motor cortical excitability in healthy individuals with normal and abnormal SN echogenicity. Subjects were aged 72–84 years, a group in which SN hyperechogenicity is highly prevalent (Behnke et al., 2007). Hyperechogenicity in aged healthy adults is associated with clinical phenomena consistent with preclinical PD (Berg et al., 2001). Based on findings in PD, we hypothesised that SN hyperechogenicity would be associated with reduced intracortical inhibition in healthy adults.

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Materials and methods

Subjects

Motor cortex excitability was examined in 20 healthy subjects known to have normal ($n = 10$) or abnormal ($n = 10$) SN echomorphology. The subjects were selected from a previous study that involved transcranial sonography on 53 aged healthy adults (median age: 77.5 years; age range: 71–83 years) with no or only minor extrapyramidal signs or symptoms not meeting the criteria for the diagnosis of PD. The current subjects were selected by an investigator who was not involved in the scanning and not aware of the results of the clinical examination. The mean age of the subjects involved in this study was 78 ± 4 years (range 72 to 84 years; 12 female; 8 male) and all were in good health with no clinical signs allowing the diagnosis of PD. Fourteen of the 20 subjects (6 with normal SN echomorphology and 8 with abnormal SN echomorphology) underwent standard clinical magnetic resonance imaging (T1 and T2 FLAIR) in a previous study (Duma et al., 2007). The results of the magnetic resonance imaging, performed within 2–15 months of transcranial sonography (median: 11.5 months), were within normal limits for the subjects' ages. All subjects gave written informed consent and the study was approved by the Human Research Ethics Committees of The University of New South Wales and The University of Adelaide. The procedures comply with Australian legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki).

### Neurological and neuropsychological assessments

The neurological examination of motor slowing and gait consisted of the third (motor) part of the Unified Parkinson's Disease Rating Scale (UPDRS), a timed 5-m walk, a heel-toe walk of 10 steps, and a glabella tap assessment as described previously (Behnke et al., 2007). Neuropsychological assessment consisted of the logical memory subset (Wechsler, 1987), phonemic and semantic verbal fluency (Benton and Hamsher, 1983), verbal trials (Grigsby and Kaye, 1995), and digit span forwards and backwards tests (Wechsler, 1981).

Transcranial sonography

The sonography examination took place in a dark room with the subjects in a seated position. The insonation was performed through the preauricular acoustic bone window with an ultrasound system equipped with a 2.5 MHz phased-array transducer (Elegra, Siemens, Erlangen, FRG) using previously described parameters and methods (Behnke et al., 2007). The appearance of the SN was categorised as normal or hyperechogenic by two blinded examiners (D. Berg and D. Baumann). SN hyperechogenicity was defined as an area of $\geq 0.20\ cm^2$ of hyperechogenic signal (for review see Berg et al., 2008). Fig. 1 shows an example of a normal unilateral echomorphology of the mesencephalic brainstem in one subject and a hyperechogenic signal in another subject.
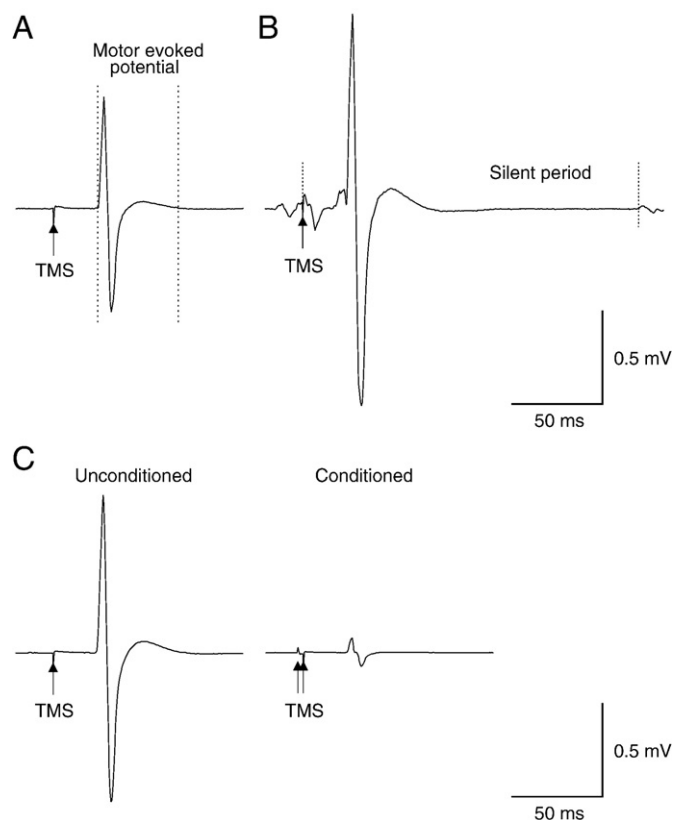
Transcranial magnetic stimulation

Transcranial magnetic stimulation was used to assess indices of motor cortical excitability. Sets of single and paired stimuli were delivered during relaxation or index finger abduction. The evoked compound muscle action potentials were recorded using EMG with surface electrodes (Ag-AgCl, 10 mm diameter) overlying the right and left first dorsal interosseus (FDI) muscles. Surface EMG signals were amplified ($\times 1000$), filtered (16–1000 Hz), and sampled (2000 Hz) for later analysis using a data acquisition system (CED 1401 interface with Signal software, Cambridge Electronic Design, Cambridge, UK).
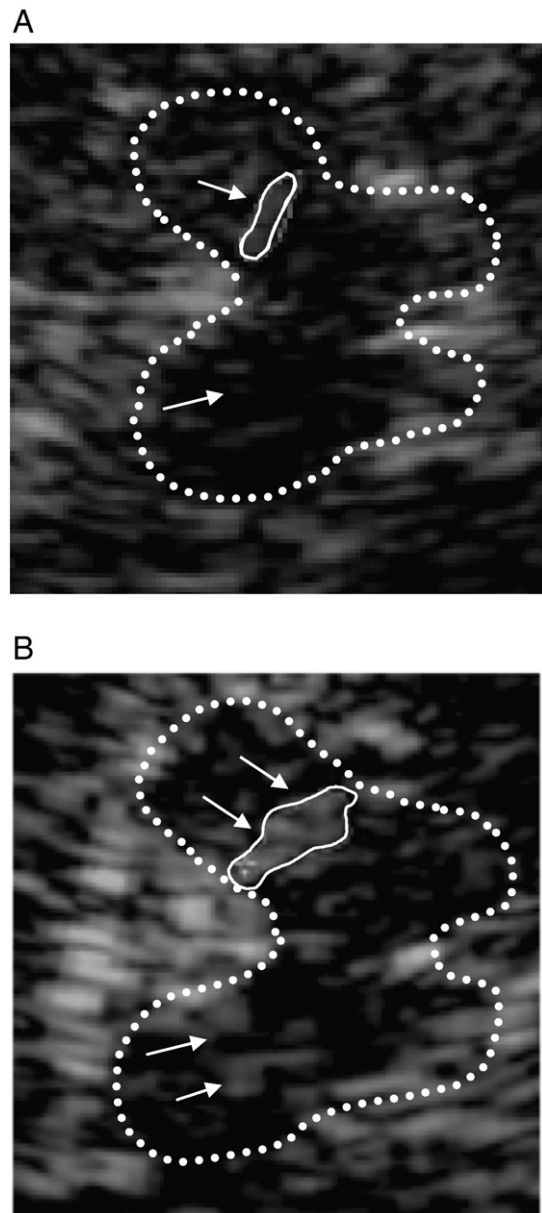
Fig. 1. Echomorphology of the mesencephalic brainstem in two subjects. Dotted white line represents the outer edge of the mesencephalic brainstem, which is encircled on both images. Arrows indicate the anatomical site of the substantia nigra. (A) Normal echomorphology. The substantia nigra ipsilateral to the probe (the side at which the planimetric measurement is done) is encircled with a solid line. (B) Abnormal echomorphology. Solid white line ipsilateral to the probe shows hyperechogenicity of the substantia nigra.

Single and paired stimuli were delivered using two Magstim 200 stimulators (Magstim Co., Whitland, UK) connected to a Bistim module (Magstim Co.) and a figure-of-eight focal coil (9 cm external diameter of wings). The Bistim module allows pairs of stimuli to be delivered through the same coil and results in a reduction of the stimulus intensity of about one-third that displayed on the individual magnetic stimulators. Stimuli were applied to each hemisphere separately, over the FDI motor area, with the coil held at approximately 45° to the mid-line with the handle pointing posteriorly. This coil orientation induces a current in the brain that flows in a posterior to anterior direction and approximately perpendicular to the central sulcus, an orientation that is optimal for activating the hand region of the motor cortex.

The left and right hemispheres were tested in a pseudorandom order. For each hemisphere, the experiment began by determining

Fig. 2. Raw and average EMG traces from a single subject after single- and paired-pulse transcranial magnetic stimulation (TMS). Stimuli were delivered over the left motor cortex and EMG traces were recorded from the right first dorsal interosseus muscle. (A) The size of the motor evoked potential (MEP) in relaxed muscle after a single stimulus at an intensity of 130% of resting motor threshold. Vertical dashed lines are placed at the boundaries of the MEP. (B) The size of the MEP during a voluntary contraction of the first dorsal interosseus muscle after a single stimulus at the same intensity. 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 substantially larger during voluntary contraction than during relaxation. (C) The size of unconditioned and conditioned MEPs during relaxation. Unconditioned MEPs were evoked by single stimuli whereas conditioned MEPs were evoked by a subthreshold stimulus followed 2 ms later by a suprathreshold stimulus (at the same intensity as that used for the unconditioned MEP). The averages of 8-9 EMG traces are shown.

resting motor threshold. Resting motor threshold was determined at the optimal site for evoking responses in FDI and was defined as the minimum stimulus intensity at which 5 out of 10 consecutive stimuli evoked an MEP of at least 50 μV in amplitude in relaxed muscle. To determine threshold, stimuli were first delivered at a clearly suprathreshold level. The stimulus intensity was then reduced in small decrements until it was clearly below threshold. Fifteen single stimuli were then delivered during relaxation at a frequency of ~0.25 Hz and at an intensity of 130% of resting motor threshold. A further 15 single stimuli, at the same intensity, were delivered during index finger abduction. Subjects were asked to abduct the index finger and hold it in a constant position against a weight (50 g) positioned at the distal interphalangeal joint. Subjects then received a block of 10 single and 40 paired stimuli delivered in a pseudorandom order during relaxation. The paired-pulse technique used here involved a subthreshold conditioning stimulus followed 2, 3, 10, or 12 ms later by a suprathreshold test stimulus to investigate short-interval intracortical inhibition and intracortical facilitation (Kujirai et al., 1993). The inhibition seen at the shorter inter-stimulus intervals most likely represents activation of local GABAergic neurones within the motor cortex (Kujirai et al., 1993). The intensity of the conditioning stimulus was set at 70% of resting motor threshold whereas the intensity of the test stimulus was set to evoke a motor evoked potential (MEP) of

~1 mV in amplitude during relaxation. The block of 10 single and 40 paired stimuli was then repeated with a higher conditioning stimulus intensity (90% resting motor threshold).

Data analysis

Descriptive statistics of the data are given as mean, standard deviation, and minimum/maximum as specified. The area, amplitude, duration, and latency of relaxed and active MEPs were measured in each trial (Fig. 2A and B). For measurements obtained during relaxation, trials in which MEPs were preceded by EMG activity were excluded from the analysis. The size of the conditioned MEP was expressed as a percentage of the unconditioned MEP (Fig. 2C). During index finger abduction, the duration of the silent period was measured by cursor and was taken as the interval from the stimulus to the return of continuous EMG (Fig. 2B). One experimenter (GT) measured the duration of the silent period for all subjects. Between-group comparisons were made with unpaired *t*-tests. Spearman Rank Order Correlation was performed to assess the relation between clinical data and measures of intracortical inhibition. Significance was set at the 5% level.

Results

Substantia nigra echomorphology and clinical symptoms

There was no significant difference between the age of subjects in our SN+ (78 ± 4 years) and SN− (78 ± 4 years) groups. Subjects were in good general health and exhibited no overt neurological signs. A movement disorder specialist deemed all subjects to be free of PD according to published criteria (Hughes et al., 1992) and no significant deficits were observed on cognitive testing. Review of medical charts indicated that participants were free of medications impacting on central nervous system integrity with the exception of one control subject who was taking doxepin hydrochloride (Sinequan; 1–2 tablets per night) for the treatment of sleep disturbance.

The acoustic window for transcranial sonography was good to excellent in most subjects. Images of the right and left hemisphere were obtained in 19 subjects and an image of the left hemisphere only was obtained in 1 subject (subsequently classified as SN+). Of the 10 subjects selected for the SN+ group, 4 subjects had unilateral hyperechogenicity and 6 subjects had bilateral hyper-echogenicity. Subjects in the SN+ group had a significantly higher UPDRS score (3.1 ± 2.2) than the SN− group (1.3 ± 0.8; P = 0.03). Performance on the Glabella tap test did not differ between groups. In the SN− group, 6 subjects overcame the glabella tap reflex within 5 taps (i.e. normal), 1 subject overcame the reflex after 5 taps, and 3 subjects required greater than 10 taps to overcome the reflex (i.e. abnormal). In the SN+ group, 7 subjects overcame the glabella tap reflex within 5 taps and 3 subjects required greater than 10 taps to overcome the reflex.

Table 1
Average characteristics of the motor evoked potential evoked during relaxation and weak voluntary contraction for hemispheres with normal substantia nigra echogenicity (SN−; n = 24) and substantia nigra hyperechogenicity (SN+; n = 16).

Muscle state	Parameter	Normal (SN−)	Hyperechogenicity (SN+)
Rest	Area (mV ms)	6.7 ± 6.9	7.9 ± 8.8
	Amplitude (mV)	1.5 ± 1.4	1.8 ± 2.0
	Latency (ms)	23.5 ± 1.1	22.4 ± 2.3
	Duration (ms)	50.5 ± 10.9	46.2 ± 15.9
Active	Area (mV ms)	23.3 ± 10.1	25.9 ± 13.0
	Amplitude (mV)	4.9 ± 2.1	5.5 ± 2.9
	Latency (ms)	22.1 ± 1.2	21.6 ± 2.0
	Duration (ms)	53.6 ± 4.0	52.3 ± 6.6

Each cortical hemisphere was analysed separately (i.e. two data points per subject).

Indices of motor cortical excitability were analysed separately for each hemisphere (i.e. two data points per subject). There was no significant difference in resting motor threshold between groups. The average resting motor threshold (measured through the Bistim) was $59 \pm 10\%$ and $57 \pm 14\%$ of stimulator output for the SN− ($n = 24$) and SN+ ($n = 16$) groups, respectively. The characteristics (area, amplitude, latency, and duration) of MEPs were assessed in response to single stimuli delivered at 130% of resting motor threshold (Fig. 2A and B). During relaxation, there was no significant difference in MEP characteristics between groups (Table 1). However, there was a trend for a shorter resting MEP latency in the SN+ group than in the SN− group ($P = 0.058$). During voluntary contraction, MEPs were larger than during relaxation but there was still no significant difference in MEP characteristics between groups. There was a period of EMG silence after MEPs evoked during voluntary contraction. The duration of the silent period (>100 ms) reflects the strength of intracortical inhibition within the motor cortex. The average duration of the silent period was not significantly different between groups (SN−: 159 ± 37 ms; SN+: 160 ± 31 ms).

Short-interval intracortical inhibition and intracortical facilitation were assessed with the paired-pulse technique. The interval between the conditioning and test pulse was 2, 3, 10, or 12 ms and two intensities of conditioning stimulation were investigated (70% and 90% of resting motor threshold). In the 70% paradigm, the average amplitude of the unconditioned MEP (evoked by the test pulse alone) was not significantly different between the SN− (1.1 ± 0.8 mV)

and SN+ (1.2 ± 1.1 mV) groups. The intensity of the test pulse also did not differ between groups (SN−: $77 \pm 15\%$ of stimulator output; SN+: $72 \pm 18\%$ of stimulator output). At the 2 ms interstimulus interval, the amplitude of the conditioned MEP was significantly larger in the SN+ group (92% of unconditioned MEP) than in the SN− group (51% of unconditioned MEP; $P = 0.014$; Fig. 3A). This suggests that the SN+ group had significantly less intracortical inhibition than the SN− group. A significant difference between groups was still evident when data were averaged across hemispheres (i.e. one data point per subject; $P = 0.042$). There was no significant difference between the groups at the 3, 10, and 12 ms interstimulus intervals (Fig. 3A). In the 90% paradigm, the average amplitude of the unconditioned MEP (evoked by the test pulse alone) was not significantly different between the SN− (1.0 ± 0.6 mV) and SN+ (0.9 ± 0.6 mV) groups. The intensity of the test pulse also did not differ between groups (SN−: $78 \pm 14\%$ of stimulator output; SN+: $71 \pm 17\%$ of stimulator output). The amplitude of the conditioned MEP was not significantly different between groups at each interstimulus interval (Fig. 3A).

Spearman Rank Order Correlation was performed to assess the relation between clinical data (UPDRS) and measures of intracortical inhibition. There was a significant correlation between UPDRS and the amplitude of conditioned MEPs at the 2 ms interstimulus interval (conditioning stimulus intensity 70% resting motor threshold: $r = 0.65$, $P < 0.001$; conditioning stimulus intensity 90% resting motor threshold: $r = 0.48$, $P = 0.002$). There was also a significant correlation between UPDRS and the amplitude of conditioned MEPs at the 3 ms interstimulus interval but the correlation was only significant for the lower conditioning stimulus intensity ($r = 0.42$, $P = 0.008$). In general, higher UPDRS scores were associated with reduced intracortical inhibition. There was no significant correlation between UPDRS and silent period duration.

Discussion

The main finding of the current study is that healthy aged individuals with SN hyperechogenicity exhibit reduced intracortical inhibition within the motor cortex. Both SN hyperechogenicity and reduced intracortical inhibition are present in PD but this is the first time that these phenomena have been reported in individuals without clinical disease.

Substantia nigra echomorphology and clinical symptoms

The cellular basis of nigral hyperechogenicity is unknown but animal and human studies strongly suggest that it reflects an increase in tissue iron content (Berg et al., 2002) rather than nigra degeneration (Berg et al., 2005). Studies of young adults with SN hyperechogenicity have reported normal (Berg et al., 1999) or subtle motor dysfunction (Ruprecht-Dorfler et al., 2007), depending on the motor task. In our study, healthy aged adults (72 to 84 years) with SN hyperechogenicity had subtle but statistically significant motor dysfunction compared with aged-matched controls without this echo feature. An association between SN hyperechogencity and subtle motor dysfunction has also been reported in healthy very aged individuals (86–95 years: Behnke et al., 2007) and signs of motor slowing are more common in older relatives of PD patients with hyperechogenicity compared to relatives without this ultrasound feature (Ruprecht-Dorfler et al., 2003). Furthermore, a high proportion of healthy individuals with SN hyperechogenicity also exhibit functional impairment of the nigrostriatal system evidenced by reduced ^{18}F-Dopa uptake in the striatum (Behnke et al., 2009; Berg et al., 1999, 2002). Together, these findings suggest that SN hyperechogenicity reflects subtle nigral dopaminergic dysfunction or morphological differences in the brain which may predispose to PD (Berg, 2007).
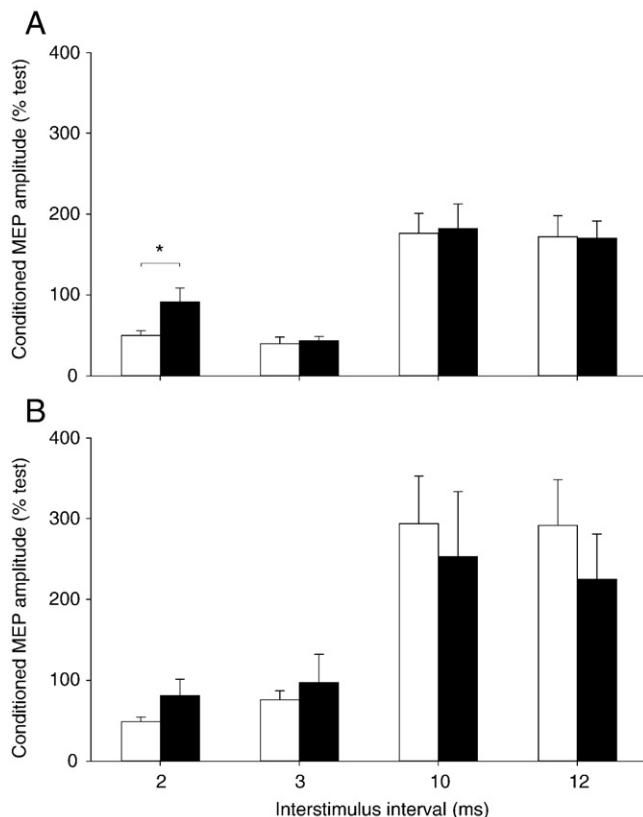
Fig. 3. Group data showing short-interval intracortical inhibition and intracortical facilitation assessed with the paired-pulse technique. The interval between the conditioning and test pulse was 2, 3, 10, and 12 ms and two intensities of conditioning stimulation were investigated (70% and 90% of resting motor threshold). (A) 70% resting motor threshold paradigm. (B) 90% resting motor threshold paradigm. Each hemisphere was analysed separately (i.e. two data points per subject). Conditioned MEPs are expressed as a percentage of the unconditioned test MEP. White bars, SN− group (i.e. normal substantia nigra echogenicity); black bars, SN+ group (i.e. substantia nigra hyperechogenicity). *Significant difference between groups ($P = 0.014$).

Substantia nigra echomorphology and motor cortex excitability

A single TMS pulse produces multiple descending volleys in corticospinal neurones that result in a muscle response which is recorded as a MEP. Paired-pulse paradigms enable investigation of intracortical inhibition within the motor cortex. The technique involves a subthreshold conditioning stimulus followed 1–5 ms later by a suprathreshold test stimulus (Kujirai et al., 1993). The inhibition seen at these inter-stimulus intervals, known as short-interval intracortical inhibition, is likely due to activation of GABA$_A$ receptors on neurones within the motor cortex (Kujirai et al., 1993). We observed short-interval intracortical inhibition in our SN− group and the magnitude was similar to that reported in young adults (Oliviero et al., 2006; Peinemann et al., 2001). With a conditioning stimulus intensity of 70% resting motor threshold, the amplitude of the conditioned MEP at the 2 ms interstimulus interval was significantly larger in the SN+ group (92% of unconditioned MEP) than in the SN− group (51% of unconditioned MEP). Thus, individuals with SN hyperechogenicity exhibit significantly less short-interval intracortical inhibition than subjects with normal SN echomorphology. The disparity between groups was also apparent with the higher conditioning stimulus intensity (90% resting motor threshold) but the difference did not reach significance.

The association between SN hyperechogenicity and reduced short-interval intracortical inhibition within the motor cortex is unlikely to be due to age related changes in the central nervous system such as cerebral atrophy. There was no significant difference between the age of subjects in our SN+ (78±4 years) and SN− (78±4 years) groups and the 14 subjects who previously underwent standard clinical magnetic resonance imaging were deemed to be normal. The association between SN hyperechogenicity and reduced intracortical inhibition is consistent with observations in PD patients. PD is associated with reduced short-interval intracortical inhibition (Bares et al., 2003; Ridding et al., 1995) that is correlated with reduced blood flow in the region of the basal ganglia (Hanajima et al., 1996). Short-interval intracortical inhibition is reduced in PD patients when tested with a 2 ms interstimulus interval but is normal when tested with a 3 ms interstimulus interval (Ridding et al., 1995), a pattern that is consistent with our observations in SN+ healthy individuals. Interestingly, short-interval intracortical inhibition is reduced in normal subjects after a single dose of a dopaminergic antagonist (Haloperidol) and augmented after a dopaminergic agonist (Bromo-criptine) (Ziemann et al., 1997).

It is unknown whether the reduced short-interval intracortical inhibition seen in PD is due to pathology within the motor cortex or to secondary changes reflecting primary damage in the basal ganglia. There is a rich dopaminergic innervation of rat (Awenowicz and Porter, 2002) and primate (Goldman-Rakic et al., 1989) motor cortex and pyramidal tract neurones decrease spontaneous firing with application of dopamine (Awenowicz and Porter, 2002). Furthermore, there is substantial cell loss in the pre-SMA (Camicioli et al., 2007; MacDonald and Halliday, 2002) and caudal intralaminar thalamus (Henderson et al., 2000), and Lewy bodies are present in the cerebral cortex (e.g. anterior cingulate gyrus) (Hughes et al., 1992) of patients with PD. Our results imply that nigral hyperechogenicity in healthy aged individuals is associated with a Parkinsonian-like decrease in intracortical inhibition within the motor cortex of old healthy individuals. Importantly, although the mean score on the UPDRS Part III was 3.1±2.2, no subject demonstrated signs of early PD sufficient for a neurologist to make a clinical diagnosis. The reduced intracortical inhibition in our healthy subjects with SN hyperecho-gencity could be due to a downstream effect of abnormal SN morphology or compensatory changes within the motor cortex which allow normal movement despite abnormal SN morphology. Significant central compensatory changes are known to occur in PD. Indeed normal motor function is maintained for an estimated 5 years

of preclinical PD despite massive neuronal loss within the SN (Fearnley and Lees, 1991). The impact of advancing disease pathology upon compensatory cortical mechanisms may be critical for the development of significant motor dysfunction in this disorder.

Although healthy individuals with SN hyperechogenicity and patients with PD both present with altered activation of local GABA$_A$ receptors on neurones within the motor cortex, the similarity does not persist across other indices of motor cortical excitability. The significant reduction in short-interval intracortical inhibition was the only difference that we observed between the SN+ and SN− groups. However, the use of single-pulse TMS has revealed other abnormal-ities in PD patients compared to healthy age-matched controls. Patients with PD and other movement disorders present with a normal or shorter MEP latency (Cantello, 2002; Cantello et al., 2002), although this may reflect difficulty in achieving complete muscle relaxation resulting from extra subliminal excitation of alpha motoneurones (Ikoma et al., 1994). PD patients also have a longer MEP duration due to prolonged TMS evoked volleys (Kleine et al., 2001). The prolonged corticospinal volleys could be due to disturbed generation of corticospinal volleys within a hyperexcitable motor cortex (Kleine et al., 2001). PD is also associated with larger MEPs during relaxation and reduced facilitation of MEPs during voluntary contractions (Cantello et al., 2002). The larger resting MEP suggests greater excitability in the cortico-motoneuronal pathways during relaxation. Reduced MEP facilitation during movement is consistent with known neuronal circuitry whereby an abnormal morphology or cell loss in the SN pars compacta should decrease motor cortex activation during movement (Utter and Basso, 2008). During voluntary contraction, the MEP is followed by a period of relative EMG silence, known as the 'silent period.' The duration of the silent period evoked by motor cortex stimulation can be up to 200–300 ms during voluntary contractions of upper limb muscles (Orth and Rothwell, 2004; Taylor et al., 2000; Wu et al., 2002). The effect of PD on silent period duration is unclear. There are reports of normal silent period duration in PD patients (e.g. Ridding et al., 1995) and abnormal (shorter) silent periods on the more affected side relative to age-matched controls (Cantello, 2002; Cantello et al., 2002). A shorter silent period likely reflects reduced intracortical inhibition mediated by GABA$_B$ receptors (Ziemann, 2004). In PD patients with a short silent period, the duration of the silent period returns to normal after levodopa administration (Priori et al., 1994). The contradictory findings may in part be explained by differences in the magnitude of pathology within the small patient groups studied.

In summary, we observed reduced short-interval intracortical inhibition in healthy aged subjects with SN hyperechogenicity, similar to that known to occur in PD patients. Our data suggest that SN hyperechogenicity in healthy individuals is associated with physio-logical changes in functionally connected brain regions. The ability of some individuals with SN hyperechogenicity to maintain clinically normal movement suggests the presence of central compensatory mechanisms despite structural and function changes within the motor circuit.

References

Awenowicz, P.W., Porter, L.L., 2002. Local application of dopamine inhibits pyramidal tract neuron activity in the rodent motor cortex. J. Neurophysiol. 88, 3439–3451.

Bares, M., Kanovsky, P., Klajblova, H., Rektor, I., 2003. Intracortical inhibition and facilitation are impaired in patients with early Parkinson's disease: a paired TMS study. Eur. J. Neurol. 10, 385–389.

Behnke, S., Double, K.L., Duma, S., Broe, G.A., Guenther, V., Becker, G., Halliday, G.M., 2007. Substantia nigra echomorphology in the healthy very old: correlation with motor slowing. Neuroimage 34, 1054–1059.

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.

Benton, A.L., Hamsher, K., 1983. Multilingual Aphasia Examination. AJA Associates, Iowa City.

Berg, D., 2007. Ultrasound in the (premotor) diagnosis of Parkinson's disease. Parkinsonism Relat Disord 13 (Suppl. 3), S429–S433.

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.

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.

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. Arch. Neurol. 59, 999–1005.

Berg, D., Merz, B., Reiners, K., Naumann, M., Becker, G., 2005. Five-year follow-up study of hyperechogenicity of the substantia nigra in Parkinson's disease. Mov. Disord. 20, 383–385.

Berg, D., Godau, J., Walter, U., 2008. Transcranial sonography in movement disorders. Lancet Neurol. 7, 1044–1055.

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. Mov. Disord. 22, 382–386.

Cantello, R., 2002. Applications of transcranial magnetic stimulation in movement disorders. J. Clin. Neurophysiol. 19, 272–293.

Cantello, R., Tarletti, R., Civardi, C., 2002. Transcranial magnetic stimulation and Parkinson's disease. Brain Res. Brain Res. Rev. 38, 309–327.

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. Mov. Disord. 23, 405–410.

Duma, S.R., Piguet, O., Broe, G.A., 2007. Activation is reduced in the pre-supplementary motor area in pre-clinical Parkinson's disease. 37th Annual Meeting of the Society for Neuroscience, San Diego, USA, p. 796.799/N727.

Fearnley, J.M., Lees, A.J., 1991. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 114, 2283–2301.

Goldman-Rakic, P.S., Leranth, C., Williams, S.M., Mons, N., Geffard, M., 1989. Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. Proc. Natl. Acad. Sci. USA 86, 9015–9019.

Grigsby, J., Kaye, K., 1995. Alphanumeric sequencing and cognitive impairment among elderly persons. Percept. Mot. Skills 80, 732–734.

Hanajima, R., Ugawa, Y., Terao, Y., Ogata, K., Kanazawa, I., 1996. Ipsilateral cortico-cortical inhibition of the motor cortex in various neurological disorders. J. Neurol. Sci. 140, 109–116.

Henderson, J.M., Carpenter, K., Cartwright, H., Halliday, G.M., 2000. Loss of thalamic intralaminar nuclei in progressive supranuclear palsy and Parkinson's disease: clinical and therapeutic implications. Brain 123, 1410–1421.

Hughes, A.J., Daniel, S.E., Kilford, L., Lees, A.J., 1992. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J. Neurol. Neurosurg. Psychiatry 55, 181–184.

Ikoma, K., Mano, Y., Takayanagi, T., 1994. Pulsed magnetic stimulation and F waves in Parkinson's disease. Intern. Med. 33, 77–81.

Kleine, B.U., Praamstra, P., Stegeman, D.F., Zwarts, M.J., 2001. Impaired motor cortical inhibition in Parkinson's disease: motor unit responses to transcranial magnetic stimulation. Exp. Brain Res. 138, 477–483.

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. J. Physiol. 471, 501–519.

MacDonald, V., Halliday, G.M., 2002. Selective loss of pyramidal neurons in the pre-supplementary motor cortex in Parkinson's disease. Mov. Disord. 17, 1166–1173.

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. Neurosci. Res. 55, 74–77.

Orth, M., Rothwell, J.C., 2004. The cortical silent period: intrinsic variability and relation to the waveform of the transcranial magnetic stimulation pulse. Clin. Neurophysiol. 115, 1076–1082.

Peinemann, A., Lehner, C., Conrad, B., Siebner, H.R., 2001. Age-related decrease in paired-pulse intracortical inhibition in the human primary motor cortex. Neurosci. Lett. 313, 33–36.

Priori, A., Berardelli, A., Inghilleri, M., Accornero, N., Manfredi, M., 1994. Motor cortical inhibition and the dopaminergic system. Pharmacological changes in the silent period after transcranial brain stimulation in normal subjects, patients with Parkinson's disease and drug-induced parkinsonism. Brain 117, 317–323.

Ridding, M.C., Inzelberg, R., Rothwell, J.C., 1995. Changes in excitability of motor cortical circuitry in patients with Parkinson's disease. Ann. Neurol. 37, 181–188.

Ruprecht-Dorfler, P., Berg, D., Tucha, O., Benz, P., Meier-Meitinger, M., Alders, G.L., Lange, K.W., Becker, G., 2003. Echogenicity of the substantia nigra in relatives of patients with sporadic Parkinson's disease. Neuroimage 18, 416–422.

Ruprecht-Dorfler, P., Klotz, P., Becker, G., Berg, D., 2007. Substantia nigra hyperechogenicity correlates with subtle motor dysfunction in tap dancers. Parkinsonism Relat. Disord. 13, 362–364.

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.

Taylor, J.L., Allen, G.M., Butler, J.E., Gandevia, S.C., 2000. Supraspinal fatigue during intermittent maximal voluntary contractions of the human elbow flexors. J. Appl. Physiol. 89, 305–313.

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. J. Neurol. 254, 501–507.

Utter, A.A., Basso, M.A., 2008. The basal ganglia: an overview of circuits and function. Neurosci. Biobehav. Rev. 32, 333–342.

Wechsler, D., 1981. Wechsler Adult Intelligence Scale - Revised. Psychological Corporation, New York.

Wechsler, D., 1987. Wechsler Memory Scale - Revised. Psychological Corporation, New York.

Wu, L., Goto, Y., Taniwaki, T., Kinukawa, N., Tobimatsu, S., 2002. Different patterns of excitation and inhibition of the small hand and forearm muscles from magnetic brain stimulation in humans. Clin. Neurophysiol. 113, 1286–1294.

Ziemann, U., 2004. TMS and drugs. Clin. Neurophysiol. 115, 1717–1729.

Ziemann, U., Tergau, F., Bruns, D., Baudewig, J., Paulus, W., 1997. Changes in human motor cortex excitability induced by dopaminergic and anti-dopaminergic drugs. Electroencephalogr. Clin. Neurophysiol. 105, 430–437.