Brain Advance Access published March 17, 2005

doi:10.1093/brain/awh473

Excitability of motor cortex inhibitory circuits in Tourette syndrome before and after single dose nicotine

M. Orth,[1,2] B. Amann,[2] M. M. Robertson[2] and J. C. Rothwell[1]

[1]*Sobell Department of Motor Neuroscience and Movement Disorders and* [2]*Department of Neuropsychiatry, The National Hospital for Neurology and Neurosurgery, Royal Free and University College Medical School, Queen Square, London WC1N 3BG, UK*

Correspondence to: Dr M. Orth, The National Hospital of Neurology and Neurosurgery, Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, Box 77, Queen Square, London WC1N 3BG, UK
E-mail: m.orth@ion.ucl.ac.uk

Summary

The pathophysiology underlying the involuntary tics of Gilles de la Tourette syndrome (GTS) remains unknown. Here we used transcranial magnetic stimulation (TMS) to examine the excitability of two different inhibitory systems in the human motor cortex: short interval intracortical inhibition (SICI) and short interval afferent inhibition (SAI) in 10 healthy non-smoking controls and eight untreated non-smoking patients with GTS. Compared with the healthy control group, both SICI (measured at a range of conditioning intensities) and SAI were reduced in patients. This is consistent with the suggestion that reduced excitability of cortical inhibition is one factor that contributes to the difficulty that patients have in suppressing involuntary tics. In addition, the reduced SAI indicates that impaired intracortical inhibition may not be limited to the motor cortex but also involves circuits linking sensory input and motor output. A single dose of nicotine reduced tic severity as assessed by blind video scoring in the majority of patients. In addition, it abolished the difference between patients and controls in SICI and SAI. There was no effect of nicotine, and no difference between controls and patients in measures of motor or SICI threshold. This indicates that cholinergic input can modulate the efficiency of SICI and SAI differently in GTS and healthy controls.

Keywords: transcranial magnetic stimulation; tic treatment; Gilles de la Tourette syndrome; intracortical inhibition; sensory afferent inhibition

Abbreviations: AMT = active motor threshold; CSI = conditioning stimulus intensity; CSP = cortical silent period; FDI = first dorsal interosseus muscle; GCI = global clinical impression; GTS = Gilles de la Tourette syndrome; ICF = intracortical facilitation; ISI = interstimulus interval; MEP = motor evoked potential; MRVS = modified Rush Video Scale; SAI = short interval afferent inhibition; SICI = short interval intracortical inhibition; TMS = transcranial magnetic stimulation

Received August 23, 2004. Revised December 8, 2004. Accepted January 29, 2005

Introduction

The pathophysiology of Gilles de la Tourette syndrome (GTS), a developmental disorder characterized by several motor and one or more phonic tics present for >1 year, is little understood. Simple tics often relieve internal sensory urges felt in the area of the tic (premonitory sensations) (Bliss, 1980), while more complex tics such as echophenomena respond to the perception of external stimuli. These clinical observations suggest that GTS is a sensorimotor disorder where the sensitivity to external stimuli might be increased, and unwanted sensory, motor or emotional stimuli cannot be sufficiently suppressed (Ziemann *et al*., 1997; Greenberg *et al*., 2000). This may lead to tics and perhaps other features of GTS such as habitual motor responses to sensory cues (Leckman, 2002).

There are few electrophysiological studies of GTS. Some have used pre-movement EEG analysis to reveal differences in cortical activity preceding involuntary tics and volitional movement (Obeso *et al*., 1982; Karp *et al*., 1996). More recently Ziemann *et al*. (1997) emphasized possible deficiencies in some forms of cortical inhibition. In the motor cortex, they found that patients had reduced short interval intracortical inhibition (SICI) and a short cortical silent period (CSP). The former is thought to reflect excitability in GABAa-ergic

inhibitory systems, whereas the latter may use a GABAbergic pathway. The authors suggested that reduced activity in inhibitory systems could be one factor that leads to reduced suppression of involuntarily triggered movements.

The first aim of the present project was to confirm this observation in more detail. Ziemann *et al*. (1997) measured SICI using a paired-pulse transcranial magnetic stimulation (TMS) paradigm with only one conditioning stimulus intensity (CSI). However, we and others recently pointed out that this could produce misleading results since the amount of SICI is dependent on the CSI (Ilic *et al*., 2002; Butefisch *et al*., 2003; Orth *et al*., 2003). If the relationship between intensity and amount of SICI differs in patients and controls, a single measure can give an erroneous estimate of the maximum sensitivity of SICI. Therefore, in this study, we measured SICI at a range of conditioning intensities to test the hypothesis of Ziemann *et al*. (1997) more securely.

The second aim of the project was to extend the measures to another form of cortical inhibition. If some tics are an unsuppressed response to sensory urges, then we might expect to see abnormalities in inhibitory pathways that specifically link sensory input and motor output. One such pathway that can be tested in humans is short interval afferent inhibition (SAI: Tokimura *et al*., 2000) in which a transient sensory input leads to a rapid and short-lasting inhibition of motor cortex. We added this test to our electrophysiological study of GTS patients.

The third aim of the project was to test whether any of these physiological measures would be of use in a clinical setting. Clinical evaluation of GTS is notoriously difficult because of the variability of tics, and this includes the assessment of any change in tic severity with treatment. Thus, it would be very useful to have a complementary objective measure that could be used to evaluate the effectiveness of new therapeutics. We therefore tested whether differences between patients and healthy controls in measures of cortical inhibition could be normalized by effective clinical treatments. The treatment we chose was nicotine. Several studies have suggested that nicotinic drugs, such as nicotine itself, the psychoactive alkaloid in tobacco, or mecylamine may influence tics particularly in conjunction with haloperidol (McConville *et al*., 1991, 1992; Silver *et al*., 2001*a*,*b*). In addition, nicotine is easy to apply and is absorbed rapidly when given as a chewing gum. This allowed for repeated measurements on the same day after a single nicotine dose. Nicotine also has the advantage of being safer to test on healthy control subjects than some of the dopaminergic blocking drugs that are also used to treat Tourette patients such as haloperidol. We evaluated the clinical response using video ratings and investigated whether the TMS measures of motor cortex excitability changed following nicotine.

Material and methods

Patients and control subjects

Nine patients (six men, mean age 31.3 years, range 19–48) with a DSM-IV diagnosis of GTS and 10 control subjects (seven men, mean age 32.6 years, range 24–38) were recruited. In GTS patients, the severity of tics was rated on the day of the experiments using the Yale Global Tic Severity Scale (Leckman *et al*., 1989); the lifetime history of symptoms indicative of GTS was captured using the diagnostic confidence index (DCI; Robertson *et al*., 1999) (Table 1). All patients and all control subjects were non-smokers, and no patient was on medication at the time of the study.

Table 1 *Demographic and clinical features of Gilles de la Tourette syndrome patients*

Patient	Gender, age (years)	DCI (%)	YGTSS (%)	Co-morbidity
1	M, 31	41	21	None
2	F, 27	76	66	None
3	F, 33	66	67	None
4	M, 48	47	45	None
5	M, 19	59	52	ADHD
6	M, 30	58	45	ADHD
7	F, 20	52	40	None
8	M, 43	56	33	ADHD, OCD
9	M, 31	62	40	None

DCI = diagnostic confidence index; YGTSS = Yale Global Tic Severity Scale; ADHD = attention deficit hyperactivity disorder; OCD = obsessive–compulsive disorder; M = male; F = female.

Patients gave informed written consent according to the Declaration of Helsinki, and the Joint Ethics Committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery approved the study protocol.

Nicotine assay

Before and ~1.5 h after beginning to chew a nicotine gum (2 mg, Nicotinell, Novartis, West Sussex, UK), 5 ml of venous blood were drawn. Samples were centrifuged at 5000 r.p.m. on a benchtop centrifuge and serum separated into a clean screw top container. Samples were stored at −80°C until they were analysed for nicotine concentration using gas chromatography as described (Feyerabend and Russell, 1990). We had planned to include a placebo condition in which patients were given a non-pharmacologically active gum, but the highly distinctive taste of the nicotine gum meant that patients were readily able to distinguish between the placebo and 'real' gums, so that this approach was abandoned.

Electromyography recordings

Surface EMGs were recorded from the right first dorsal interossoeus (FDI) muscle using silver/silver chloride disc surface electrodes (1 cm diameter) in a belly tendon montage. The EMG signal was amplified and analogue filtered (30 Hz to 3 kHz) with a Digitimer D150 amplifier (Digitimer Ltd, Welwyn Garden City, UK). Data (sampling rate 4 kHz) were digitized for off-line analysis using Signal software (Cambridge Electronic Devices, Cambridge, UK). Peak to peak amplitude of motor evoked potentials (MEPs), the area under the curve of the MEP and the silent period duration were measured with in-house software.

Transcranial magnetic stimulation

Patients and controls were seated in a comfortable chair. They were asked to relax as much as possible. Patients had tics throughout the

experiments but were asked not to suppress their tics. Magnetic stimuli were given with a hand-held figure-of-eight coil (outer winding diameter 9 cm) connected to a High Power Magstim 200 stimulator (Magstim Co., Whitland, Dyfed, UK). This stimulator generates a magnetic pulse with monophasic waveform and in the brain induces a current with posterior–anterior flow when the coil handle is positioned at an angle of 45° pointing backwards. The optimal spot for right FDI stimulation was marked with a felt-tipped pen.

Motor thresholds

Resting motor threshold (RMT) was defined as the minimum intensity needed to evoke an MEP of >50 μV in five out of 10 consecutive trials in the relaxed FDI. Active motor threshold (AMT) was defined as the minimum intensity needed to evoke an MEP of >200 μV in five out of 10 trials in the tonically active FDI (∼20% of maximal contraction as assessed visually on an oscilloscope). Thresholds were approached from above threshold in steps of 1% stimulator output. Once no MEPs could be elicited, the intensity was increased in steps of 1% stimulator output until a minimal MEP was observed. This intensity was taken as motor threshold.

Paired pulse paradigm

In each individual, a test stimulus intensity was chosen that elicited an MEP of 0.5–1.5 mV amplitude. The conditioning pulse intensity was varied (60, 70, 80 or 90% of AMT) resulting in four different experimental blocks. With each conditioning pulse intensity and in a randomized order, the 2 and 3 ms interstimulus intervals (ISIs) and the 12 and 15 ms ISIs were examined. The former examine SICI and the latter ICF. With an interval of 4 s between trials, 10 conditioned MEPs were collected for each ISI, and in each experimental block a total of 20 unconditioned MEPs were recorded. The order of data collection for each conditioning pulse intensity was randomized between subjects. Trials recorded while the patients contracted the hand muscles or those coinciding with a tic were excluded on-line. No trials were excluded in the off-line analysis. The average of the amplitudes of each conditioned MEP was expressed as a percentage of the average unconditioned MEP amplitude in the same session. Subjects were asked to refrain from caffeine on the day of the experiment. No major irregularities of sleep, mood or other factors could be elicited by direct questioning.

Cortical silent periods

CSPs were recorded from the tonically active right FDI with the subjects squeezing an object between the thumb and index finger at ∼20% of maximum force output. Ten trials at a fixed test stimulus intensity of 150% AMT were collected in each subject with an interval of 4 s between trials. In each individual trial, the duration of the CSP was measured from the beginning of the MEP evoked by the test stimulus to the resumption of (any level of) sustained EMG activity. In addition, the area under the MEP was determined and a ratio of CSP duration/MEP area calculated (Orth and Rothwell, 2004). The gain of the recordings was set to 1 mV/V in order to measure the end of the CSP, and in a second channel was set to 10 mV/V in order to measure the size of the MEP. Gain settings were the same for all experiments.

Short interval afferent inhibition by somatosensory input from the median nerve

SAI of the motor cortex was examined as previously described (Tokimura *et al*., 2000). In brief, a test MEP of ∼1 mV peak-to-peak amplitude was elicited in the FDI by TMS. A paired pulse paradigm examined the influence on MEP size of a supra-threshold electrical stimulus given to the median nerve through bipolar electrodes. The electrical stimulus to the median nerve was delivered at an intensity just above the threshold to elicit a visible contraction in the thenar muscles and preceded the TMS pulse to the FDI hot spot by 14, 18, 20, 22, 24, 26 or 29 ms. Twenty trials of the MEP elicited by TMS alone and 10 trials of conditioned MEPs for each ISI were collected. The amplitude of the MEP in the FDI was measured with in-house software. The average amplitude of the conditioned MEP was expressed as a percentage of the average amplitude of the test MEP alone. Trials recorded while the patients contracted the hand muscles or those coinciding with a tic were excluded on-line. No trials were excluded in the off-line analysis.

Video-recordings

Videos were recorded before and after patients chewed the nicotine gum. Patients were seated comfortably in a quiet room for several minutes Video and audio recordings were then made first of the full frontal body view and then of the head and shoulders only. For each view, patients were filmed for 2 min sitting in the chair with the examiner in the room, for 2 min reading aloud and for 2 min sitting in the chair with no examiner present, so that the total time of the videotaping was 12 min. One of the authors (M.M.R.) who did not know the patients and was blinded to the treatment conditions rated the video recordings.

First, the tic severity on each of the two videos for each patient was compared using global clinical impression (GCI); a difference was rated as one video being 'better' and the other 'worse', or as 'no difference'. Next, for each video, the total number of motor or phonic tics was counted during the 2 min of full body and head and shoulder view with and without the examiner in the room. The total number of motor and phonic tics was related to time and expressed as tics/min. The data were analysed using the Modified Rush Video Scale (MRVS) (Goetz *et al*., 1999). The MRVS consists of five tic domains: the number of body areas involved with tics; motor tic severity; phonic tic severity; frequency of motor tics; and frequency of phonic tics. Within each of the domains, the severity is rated on a scale from 0 to 4. The sum of the five domain scores provides a total tic impairment score (0–20).

Data analysis

For baseline data examining SICI or ICF, we examined whether there was a main effect of 'intensity' (60, 70, 80 or 90% AMT) on the amount of SICI or ICF. This was tested using analysis of variance (ANOVA) statistically. For SICI, ICF or SAI inhibition, we tested whether there was a main effect of 'ISI' on the size of the conditioned MEP using ANOVA. To test whether controls differed from GTS patients, we also used an ANOVA model examining the main effect of 'condition' on the size of the conditioned MEP. In the same way, we statistically evaluated the CSP data. We repeated this analysis with the data recorded after nicotine.

In both controls and GTS patients, the data before and after nicotine were paired observations. Therefore, in order to examine

whether nicotine had an effect on the size of the conditioned MEP, or on the CSP data, we used a repeated measures ANOVA, with 'time' and 'intensity' as within-subject factors and 'group' as between-subjects factor.

In order to assess the correlation of SICI, or SAI, with clinical data, i.e. tic severity, or nicotine serum concentrations, we used linear regression analysis.

A statistical difference in the ANOVAs was followed by a *post hoc* paired *t* test analysis. Mauchley's test was used to test for sphericity in the repeated measures ANOVAs, and the Greenhouse–Geisser correction applied to the degrees of freedom if necessary. Statistical significance levels were set to $P = 0.05$. All statistical analysis was performed using SPSS 11 for Windows software package.

Results

Motor thresholds

Control subjects and GTS patients had similar mean AMTs and RMTs (Table 2). Nicotine had no effect on thresholds (Table 2).

Intracortical inhibition and facilitation

The theoretical threshold for SICI and ICF was extrapolated as described (see Material and methods; Orth *et al*., 2003). This was not possible in one control subject for SICI and in two control subjects for ICF because the data were too variable. The threshold of both SICI and ICF expressed as a percentage of each individual's AMT was similar in controls and GTS patients, and was unaffected by nicotine (Fig. 1A and B).

We went on to examine how SICI or ICF varied at different CSIs (60, 70, 80, 90% AMT). An increase in the CSI increased the effectiveness of both SICI and ICF (Fig. 2A–D). We analysed the data from SICI or ICF separately in two three-way repeated measures ANOVAs, with 'time' and 'intensity' as within-subject factors and 'group' as between-subject factor. For SICI, there was, as expected, a main effect of 'intensity' [$F(3,51) = 15.9$, $P < 0.001$] but also a significant interaction between 'time' and 'group' [$F(1,17) = 5.871$, $P = 0.027$]. This was due to the significantly greater amount of SICI in controls than in GTS patients before nicotine [two-way ANOVA, main effect of 'group' on the data of Fig. 2A; $F(1,68) = 6.9$, $P = 0.011$]. After nicotine, both groups behaved similarly (two-way ANOVA, main effect of 'group' on the data of Fig. 2B: not significant). We then examined the effect of nicotine in controls and GTS patients separately. This revealed that nicotine did not have a significant effect when either group was tested alone (two-way repeated measures ANOVA).

We performed a similar analysis for ICF. There was a significant increase of the amount of ICF with increasing CSIs [repeated measures ANOVA, main effect of ISI, $F(3,51) = 3.8$, $P = 0.015$]. There was no significant effect of nicotine (no main effect of 'time', $P > 0.05$, no interaction of 'group' and 'time').

Table 2 *Motor thresholds*

	RMT		AMT	
Nicotine	–	+	–	+
Controls	38.7 (6.2)	38.7 (5.7)	29.3 (6.8)	29.3 (6.8)
GTS	41.8 (8.1)	41.7 (8.2)	30.9 (6.9)	30.6 (6.8)

There was no significant difference between the motor thresholds of patients with Gilles de la Tourette syndrome (GTS) and controls. Data are means (SD) from nine GTS patients and 10 controls. RMT = resting motor threshold; AMT = active motor threshold.

Short interval afferent inhibition

In controls and GTS patients, a supra-threshold electrical stimulus to the median nerve at the wrist before the TMS pulse to the FDI hot-spot reduced the mean amplitude of the test stimulus predominantly at ISIs of 20, 22 and 24 ms (Fig. 3A). Figure 3B shows the time course of SAI after nicotine. It appears that the initial difference between GTS

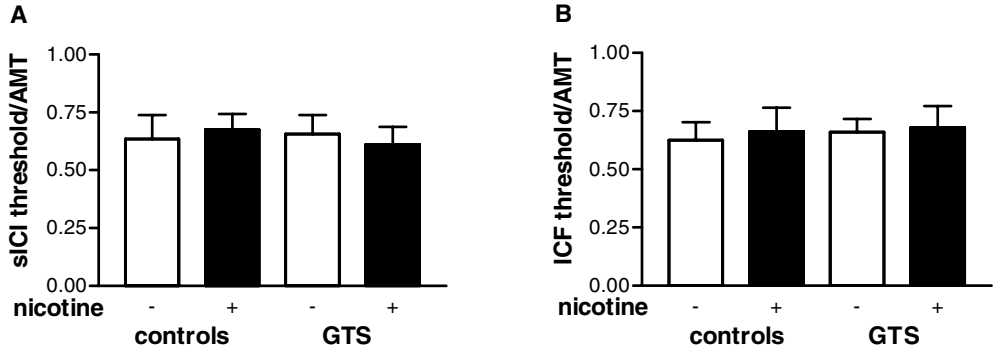

Fig. 1 Thresholds for SICI and ICF. There was no difference between the ratios of the theoretical thresholds for SICI or ICF, respectively, and AMT between patients with GTS and control subjects. In both groups, these ratios remained similar in the presence of nicotine (black bars) compared with baseline values (open bars). Values are means ± SD, $n = 9$ for controls and GTS patients for SICI; $n = 8$ for controls and $n = 9$ for GTS patients for ICF.

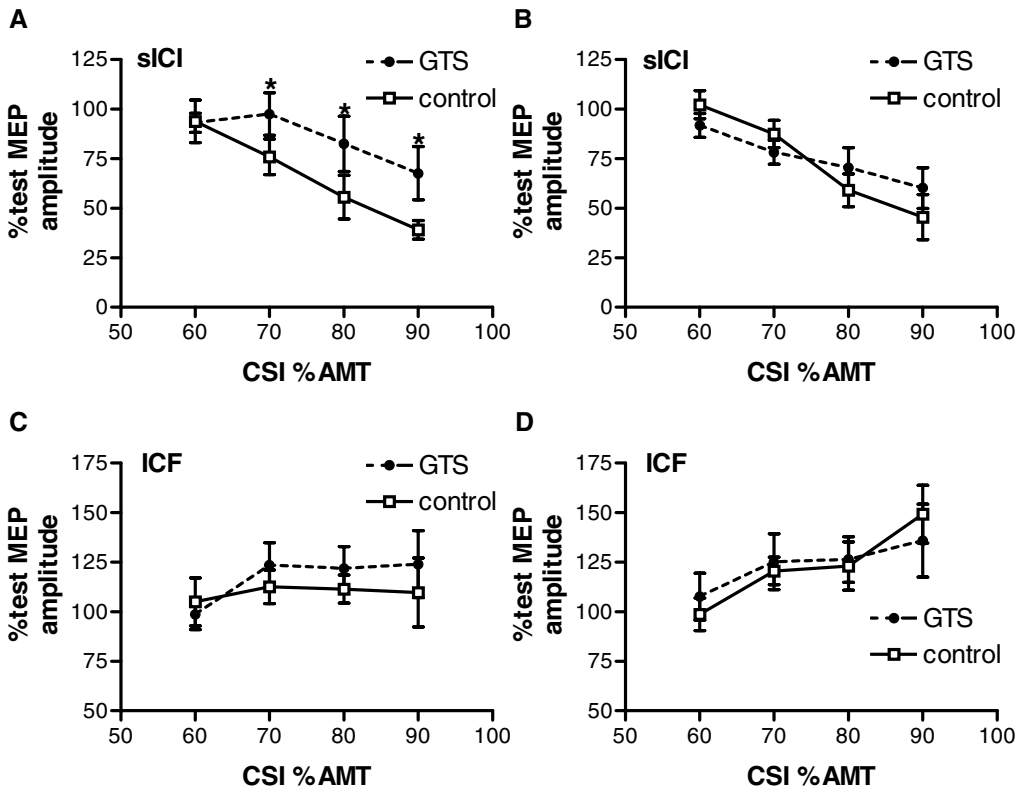

Fig. 2 SICI and ICF with different CSIs. (**A**) Control subjects and GTS patients had increasing amounts of SICI with increasing CSI (main effect of intensity, repeated measures ANOVA, $P < 0.001$) but patients had less SICI (main effect of group, ANOVA, $^{*}P = 0.011$). (**B**) Nicotine markedly reduced the difference between GTS patients and controls. (**C**) With increasing CSI, the amount of ICF increased (main effect of intensity, repeated measures ANOVA, $P = 0.015$); however, there was no statistical difference between GTS patients and controls. (**D**) With nicotine, controls had more ICF than without but this difference was not significant. Values are means ± SEM, $n = 9$ for GTS patients, $n = 10$ for controls.

patients and controls in the amount of inhibition was abolished after nicotine. Since the early period of inhibition is more likely to have a partly cortical origin than later timings (Tokimura *et al*., 2000), we assessed the maximum amount of afferent inhibition in each individual. At baseline, GTS patients had significantly less inhibition than controls [ANOVA, $F(1,17) = 9.7$, $P = 0.006$, Fig. 3C]. Nicotine had a different effect on GTS patients and controls; a two-way repeated-measures ANOVA with 'time' as within-subject factor and 'group' as between subject factor revealed a significant interaction between 'group' and 'time' [$F(1,17) = 8.0$, $P = 0.012$, Fig. 3C]. Following nicotine, the difference between controls and GTS patients disappeared (no main effect of group, $P > 0.1$). We then analysed the effect of nicotine separately in controls and GTS patients. This revealed that there was no significant effect of nicotine on maximal SAI in controls or GTS patients (Fig. 3C).

Cortical silent periods

We analysed the duration of the CSP and the MEP area, and calculated the ratio (duration)/(MEP area) as described previously (Orth and Rothwell, 2004). We analysed data using a two-way repeated measures ANOVA, with 'time' as within-subject factor and 'group' as between-subject factor. This revealed that nicotine increased the MEP area [$F(1,17) = 5.081$, $P = 0.038$, Fig. 4A] with a similar effect in both groups (no significant interaction between 'group' and 'time). There was a tendency for MEP area to be smaller in GTS patients, but this was not significant (no main effect of 'group', Fig. 4A). There was no significant difference between the groups and no significant effect of nicotine on either CSP duration or the ratio (duration)/(MEP area) (Fig. 4B and C), although CSP duration was slightly shorter in patients as reported by Ziemann *et al*. (1997).

Behavioural effect of nicotine

No participant had side effects from the nicotine chewing gum or the TMS. No blood sample taken before the nicotine chewing gum contained nicotine. After the chewing gum, controls had mean nicotine plasma concentrations of 4.4 ng/ml (SD 1.26). This was similar to GTS patients (mean 4.2, SD 1.62). The analysis of the videos revealed that on GCI, tics were better following nicotine in six patients, while

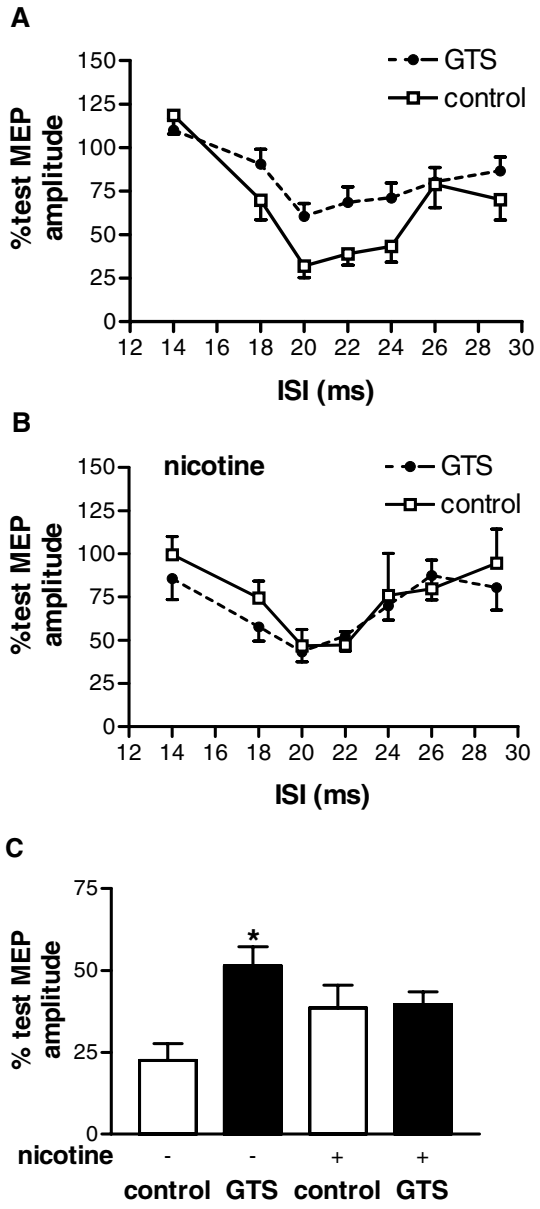

Fig. 3 Short interval afferent inhibition curves. (**A**) In the absence of nicotine, both controls and GTS patients showed significant inhibition at ISIs of 20, 22 and 24 ms (repeated measures ANOVA, $P = 0.001$). (**B**) In the presence of nicotine, the amount of inhibition was similar in controls and GTS patients. (**C**) The analysis of the cortical inhibitory effects at maximum inhibition reveals that patients had less inhibition than controls ($^*P = 0.006$). Nicotine had a different effect in controls and GTS patients (repeated measures ANOVA, interaction between 'group' and 'time', $P = 0.012$). In the presence of nicotine, controls had less inhibition while GTS patients had more inhibition, but these effects were not significant. Values are means ± SEM, $n = 9$ for GTS patients, $n = 10$ for controls.

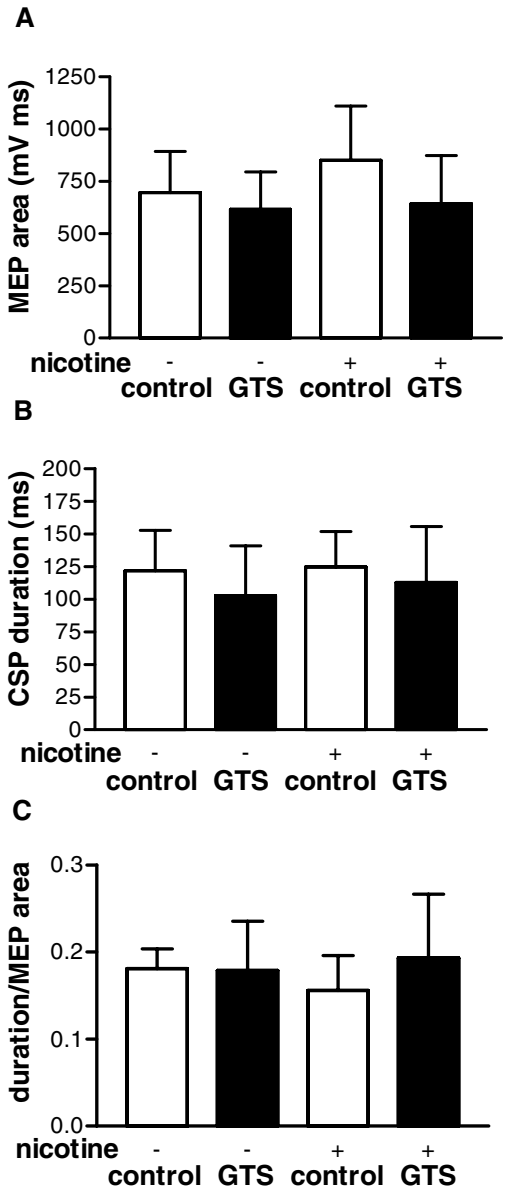

Fig. 4 Cortical silent period. (**A**) Nicotine increased the MEP size (repeated measures ANOVA, $P = 0.038$) but the effect was similar in controls and GTS patients. (**B** and **C**) Silent period duration (**B**) and the ratio (MEP size/CSP duration) (**C**) were similar between controls and GTS patients, and there was no significant effect of nicotine. Values are means ± SD, $n = 9$ for GTS patients, $n = 10$ for controls.

in two patients there was no change and one patient was worse. MRVS total scores revealed a mild but significant improvement of the objective tic video analysis [mean 11.2 (SD 1.7) before versus 10 (1.4) after nicotine, paired samples t test, $t = 3.1$, $P = 0.016$].

Correlation of clinical parameters with TMS parameters and nicotine readings

We expressed the nicotine-induced change of electrophysiological parameters, i.e. SICI and SAI, or tic severity as judged by video scores, as a percentage of baseline (pre-nicotine) measures (see Table 3). The percentage change of SICI or SAI did not correlate with the percentage change of tic severity (linear regression analysis). We then correlated the percentage change of either SICI, SAI or tic severity with serum nicotine concentration. This revealed that there was no

Table 3 *Effect of nicotine on electrophysiological parameters and tic severity in individual GTS patients*

Patient	SICI	SICI nicotine	SAI	SAI nicotine	Tics GCI	MRVS (%change)	Nicotine (ng/ml)
1	61.4	67.6	79	41.8	Better	9.1	5.2
2	58.9	55.3	47	49.9	Better	9.1	7.2
3	28.4	66.4	51.4	26.3	Better	20	2.4
4	32.5	22.1	61.1	50.1	Same	8.3	2.3
5	110.4	67.6	70.4	48.2	Better	16.7	5.4
6	116.6	53.4	56.5	21.2	Same	0	2.9
7	21.8	14.4	37.1	52.9	Better	0	4.5
8	49.4	43.9	34.6	30.9	Better	30	4.4
9	70	45.9	25.5	35.2	Worse	0	3.3
Pooled controls	32.5 (15)	35 (18.6)	26 (18.1)	38.6 (21.8)			

SICI and SAI are expressed as a percentage of unconditioned MEP. Values represent the maximum SICI obtained with different conditioning stimulus intensities, or the maximum SAI with different interstimulus intervals as described in Material and methods. The data of controls were pooled for comparison; values are the means (SD). SICI = short interval intracortical inhibition; SAI = short latency afferent inhibition; GCI = global clinical impression= MRVS: modified Rush Video Score.

correlation of any of these parameters. Next, we looked at those patients that improved by GCI on tic rating. Improvement of any of the electrophysiological parameters did not correlate with clinical change (Table 3).

We went on to evaluate the responses of individual patients. To this end, we took the maximum SICI or SAI of controls and calculated the mean and the SD. We then rated the patients' maximum measures of SICI or SAI as either 'normal' or 'abnormal' (greater than the mean plus 1 SD; see Table 3). At baseline, six patients had 'abnormal' SICI and six patients had 'abnormal' SAI; only one patient had both 'normal' SICI and SAI (see Table 3). We then repeated this with the data after nicotine. First, we looked at how many patients were now in the 'normal' pre-nicotine range; four patients were now 'normal' for SICI and five for SAI. Only one patient still had both 'abnormal' SICI and SAI, but only two patients had both 'normal' SICI and SAI (Table 3). We then calculated the mean and SD of controls after nicotine and rated the patient data. This showed that five patients were now in the 'normal' range for SICI, and all patients were now in the 'normal range' for SAI.

Discussion

The present study shows that the excitability of SICI is reduced in GTS patients at all intensities of conditioning stimulus. In addition, we show that a measure of inhibitory interactions between afferent input and motor ouput, SAI, is also reduced. A single dose of nicotine, at serum nicotine levels similar to those seen after smoking a single cigarette, adjusts electrophysiological measures of the excitability of circuits within the motor cortex to normal levels in GTS and reduces tics.

Baseline (pre-nicotine) electrophysiology in GTS patients

As reported previously (Ziemann *et al*., 1997), we found that in the basal state (pre-nicotine), motor thresholds of untreated and non-smoking GTS patients were similar to those of controls and that SICI was smaller than normal. In addition, the duration of the CSP tended to be shorter in patients than in controls, but this was not significant in our group of individuals. The shorter CSP duration was accompanied by a smaller MEP size in patients so that the ratio of CSP duration/MEP size was similar in patients and controls (Orth and Rothwell, 2004).

Our results extend previous data in two ways. First we distinguished between the threshold intensity needed to produce SICI and the amount of SICI at suprathreshold intensities of conditioning shock (Orth *et al*., 2003). This showed that patients had normal thresholds for SICI, but that recruitment of inhibition at suprathreshold intensities was reduced. It is thought that TMS pulses recruit SICI by exciting axons and that this secondarily leads to synaptic release of inhibitory neurotransmitters. Thus, normal thresholds in the presence of decreased recruitment would be compatible with the idea that in GTS, axonal excitability is normal whereas the recruitment of synaptic inhibition in the SICI circuit is reduced. Effectively, the motor system might use SICI to shape patterns of motor output. If the sensitivity of this system were reduced, then a given input would lead to less effective output, and hence less effective shaping of the motor command.

The second new result was that our electrophysiological measure of inhibitory interactions between sensory input and motor output, SAI, was reduced in the baseline state in patients. Again this is consistent with a reduced efficiency of synaptic inhibition. Given the possible influence of sensory inputs in triggering the release of tics, our electrophysiological data suggesting impaired sensory motor inhibition may be a direct physiological reflection of increased access of sensory input to motor output in GTS. In essence, we imagine that sensory input can influence motor output through a variety of channels, some inhibitory and some excitatory. If the motor system needs to reduce the possibility of sensory input triggering movement, then it may be necessary to shift the balance of excitability towards inhibition. If some of the

inhibitory pathways (such as SAI tested here) are less responsive than normal, a given input will lead to less effective suppression of sensory influences than expected, and may contribute to release of involuntary movements.

However, it is important to note that a deficit in these two inhibitory pathways at rest does not preclude the possibility that input from other areas of the brain can compensate for their function, at least temporarily. It is very clear, for example, that patients can sometimes perform at extremely high levels of efficiency despite this lack of inhibition. Patients can also suppress their tics with effort of will and, in these conditions, imaging studies show activation of circuits linking striatum, frontal lobe and those cortical areas involved in movement execution (Peterson *et al*., 1998; Stern *et al*., 2000). EEG-coherence analysis indicates that movement inhibition increases cortico-cortical coupling more in GTS patients compared with normal controls, suggesting that the increased activity may compensate for abnormal input into the motor cortex (Serrien *et al*., 2005). The degree to which patients are able to suppress their tics may thus reflect the balance between underlying deficits and adaptive, compensatory changes in other parts of cortico-subcortical networks involving the basal ganglia, motor and pre-motor cortex, thalamus and pre-frontal cortex. Much as tics take a waxing and waning course in intensity, and can occur in bouts, the interactions between different parts of these cortico-subcortical networks, and ultimately their influence on shaping motor output, are not static but change continuously. Curiously, many patients experience that engaging in specific tasks can abolish their tics, and the preceding urges, altogether and thus enable them to perform normally in highly complex and demanding motor activities; it seems therefore that activity in brain areas relevant to attention such as the prefrontal cortex may have a particularly strong compensatory influence on motor output via complex neuronal networks.

Effects of single nicotine dose

The present data confirm that a single nicotine dose in GTS can reduce tic severity in the majority of the patients tested. However, while significant, the improvement was small and it is not certain that this will be clinically meaningful. In addition, the highly distinctive taste of the nicotine chewing gum meant that we were not able to include a blinded control condition so that a placebo effect could have contributed to the clinical effect that we observed. Therefore, while our data are in agreement with previous studies showing that modulation of the acetylcholine system may be promising as a treatment for GTS (Silver *et al*., 2001*a*,*b*), we do not think that our data provided conclusive evidence to recommend nicotine chewing gum. This study, assessing the short-term effect of a single low dose of nicotine, was not intended to evaluate nicotine as a treatment for tics; this needs to be the subject of further research. Thus, although it is interesting to note that overall the electrophysiological data were consistent with the clinical results, we also found that there was no correlation in individual patients between improvements in clinical and electrophysiological scores. This suggests either that there is too much variation in the scoring systems to obtain significance with the numbers of patients that we examined, or that other factors, such as a placebo effect, may have influenced the clinical response. Further studies are needed to address these questions perhaps using other agents with an established effect on tics such as dopamine receptor antagonists.

While it is conceivable that the clinical effect of nicotine may be due to a placebo effect, we think it is unlikely that such a placebo effect could account for the effect on SICI or SAI that we measured because clinical and electrophysiological effects did not correlate. However, placebo may increase release of striatal dopamine (de la Fuente-Fernandez *et al*., 2001), suggesting that placebo may in some circumstances even be able to modify physiological measures. Future studies will be required to address this point.

The electrophysiological data suggest (i) that these two inhibitory pathways in the motor cortex can be modulated by cholinergic inputs; and (ii) that this effect differs between GTS patients and normal controls. There is good evidence from *in vitro* studies in tissue of animals and humans for cholinergic modulation of inhibitory pathways in the brain. Nicotinic and muscarinic acetylcholine receptors (AchRs) are widely distributed throughout the human CNS (Kimes *et al*., 2003; Podruchny *et al*., 2003) and the effects mediated at these receptors not only have direct synaptic effects themselves but also modulate synaptic transmission at GABAergic and glutamatergic synapses (McCormick and Prince, 1985; Lena *et al*., 1993; Jones and Yakel, 1997; Lena and Changeux, 1997; Fisher *et al*., 1998; Xiang *et al*., 1998; Zhong *et al*., 2003). Indeed, recent *in vivo* experiments in humans have shown that the muscarinic antagonist scopolamine reduced SAI, consistent with cholinergic modulation of inhibitory transmission (Di Lazzaro *et al*., 2000). Although Di Lazzaro *et al*. (2000) did not find any effect of scopolamine on SICI, they did not test for nicotinic effects on either SICI or SAI.

If the effects on SICI and SAI in the present experiments are due to a cholinergic action of nicotine on the excitability of two classes of inhibitory interactions in the motor cortex, then the fact that differences between patients and controls can be improved by administration of nicotine suggests that there is no structural deficiency in these connections in patients. This would be consistent with the normal threshold for recruitment of inhibition. This implies that the deficiencies in GTS are likely to be caused by a subtle loss of neuromodulatory function in cortical circuits.

Our finding that cholinergic stimulation can remove the neurophysiological differences between GTS and controls does not necessarily indicate that GTS is primarily due to cholinergic deficits. Others, for example, have suggested a role for dopamine. However, since both transmitters can serve as neuromodulators, it is conceivable that one can substitute

to some extent for the other or that the functional cholinergic deficit in GTS patients is a consequence of abnormal neuromodulation by dopamine. Clearly, further studies are needed to address this issue more fully.

In conclusion, we show that in GTS patients, inhibitory control over motor output was reduced. This was not restricted to SICI, a measure of motor–motor inhibition, but included SAI, thus supporting clinical observations indicating a role for sensory symptoms in provoking tics. Nicotinic modulation of inhibitory cortical pathways differed between controls and GTS patients and provides further insight into possible mechanisms that underlie these physiological changes.

Acknowledgements

We wish to thank the patients and control subjects for participating in this study, Mark Richardson for critical reading of the manuscript, and Michael O'Sullivan for help with the video recordings.

References

Bliss J. Sensory experiences of Gilles de la Tourette syndrome. Arch Gen Psychiatry 1980; 37: 1343–7.

Butefisch CM, Netz J, Wessling M, Seitz RJ, Homberg V. Remote changes in cortical excitability after stroke. Brain 2003; 126: 470–81.

de la Fuente-Fernandez R, Ruth TJ, Sossi V, Schulzer M, Calne DB, Stoessl AJ. Expectation and dopamine release: mechanism of the placebo effect in Parkinson's disease. Science 2001; 293: 1164–6.

Di Lazzaro V, Oliviero A, Profice P, Pennisi MA, Di Giovanni S, Zito G, et al. Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. Exp Brain Res 2000; 135: 455–61.

Feyerabend C, Russell MA. A rapid gas-liquid chromatographic method for the determination of cotinine and nicotine in biological fluids. J Pharm Pharmacol 1990; 42: 450–2.

Fisher JL, Pidoplichko VI, Dani JA. Nicotine modifies the activity of ventral tegmental area dopaminergic neurons and hippocampal GABAergic neurons. J Physiol (Paris) 1998; 92: 209–13.

Goetz CG, Pappert EJ, Louis ED, Raman R, Leurgans S. Advantages of a modified scoring method for the Rush Video-Based Tic Rating Scale. Mov Disord 1999; 14: 502–6.

Greenberg BD, Ziemann U, Cora-Locatelli G, Harmon A, Murphy DL, Keel JC, et al. Altered cortical excitability in obsessive–compulsive disorder. Neurology 2000; 54: 142–7.

Ilic TV, Meintzschel F, Cleff U, Ruge D, Kessler KR, Ziemann U. Short-interval paired-pulse inhibition and facilitation of human motor cortex: the dimension of stimulus intensity. J Physiol 2002; 545: 153–67.

Jones S, Yakel JL. Functional nicotinic ACh receptors on interneurones in the rat hippocampus. J Physiol 1997; 504: 603–10.

Karp BI, Porter S, Toro C, Hallett M. Simple motor tics may be preceded by a premotor potential. J Neurol Neurosurg Psychiatry 1996; 61: 103–6.

Kimes AS, Horti AG, London ED, Chefer SI, Contoreggi C, Ernst M, et al. 2-[18F]F-A-85380: PET imaging of brain nicotinic acetylcholine receptors and whole body distribution in humans. FASEB J 2003; 17: 1331–3.

Leckman JF. Tourette's syndrome. Lancet 2002; 360: 1577–86.

Leckman JF, Riddle MA, Hardin MT, Ort SI, Swartz KL, Stevenson J, et al. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. J Am Acad Child Adolesc Psychiatry 1989; 28: 566–73.

Lena C, Changeux JP. Role of Ca2+ ions in nicotinic facilitation of GABA release in mouse thalamus. J Neurosci 1997; 17: 576–85.

Lena C, Changeux JP, Mulle C. Evidence for 'preterminal' nicotinic receptors on GABAergic axons in the rat interpeduncular nucleus. J Neurosci 1993; 13: 2680–8.

McConville BJ, Fogelson MH, Norman AB, Klykylo WM, Manderscheid PZ, Parker KW, et al. Nicotine potentiation of haloperidol in reducing tic frequency in Tourette's disorder. Am J Psychiatry 1991; 148: 793–4.

McConville BJ, Sanberg PR, Fogelson MH, King J, Cirino P, Parker KW, et al. The effects of nicotine plus haloperidol compared to nicotine only and placebo nicotine only in reducing tic severity and frequency in Tourette's disorder. Biol Psychiatry 1992; 31: 832–40.

McCormick DA, Prince DA. Two types of muscarinic response to acetylcholine in mammalian cortical neurons. Proc Natl Acad Sci USA 1985; 82: 6344–8.

Obeso JA, Rothwell JC, Marsden CD. The neurophysiology of Tourette syndrome. Adv Neurol 1982; 35: 105–14.

Orth M, Rothwell JC. The cortical silent period: intrinsic variability and relation to the waveform of the transcranial magnetic stimulation pulse. Clin Neurophysiol 2004; 115: 1076–82.

Orth M, Snijders AH, Rothwell JC. The variability of intracortical inhibition and facilitation. Clin Neurophysiol 2003; 114: 2362–9.

Peterson BS, Skudlarski P, Anderson AW, Zhang H, Gatenby JC, Lacadie CM, et al. A functional magnetic resonance imaging study of tic suppression in Tourette syndrome. Arch Gen Psychiatry 1998; 55: 326–33.

Podruchny TA, Connolly C, Bokde A, Herscovitch P, Eckelman WC, Kiesewetter DO, et al. In vivo muscarinic 2 receptor imaging in cognitively normal young and older volunteers. Synapse 2003; 48: 39–44.

Robertson MM, Banerjee S, Kurlan R, Cohen DJ, Leckman JF, McMahon W, et al. The Tourette syndrome diagnostic confidence index: development and clinical associations. Neurology 1999; 53: 2108–12.

Serrien DJ, Orth M, Evans AH, Lees AJ, Brown P. Motor inhibition in patients with Gilles de la Tourette syndrome: functional activation patterns as revealed by EEG coherence. Brain 2005; 128: 116–25.

Silver AA, Shytle RD, Philipp MK, Wilkinson BJ, McConville B, Sanberg PR. Transdermal nicotine and haloperidol in Tourette's disorder: a double-blind placebo-controlled study. J Clin Psychiatry 2001a; 62: 707–14.

Silver AA, Shytle RD, Sheehan KH, Sheehan DV, Ramos A, Sanberg PR. Multicenter, double-blind, placebo-controlled study of mecamylamine monotherapy for Tourette's disorder. J Am Acad Child Adolesc Psychiatry 2001b; 40: 1103–10.

Stern E, Silbersweig DA, Chee KY, Holmes A, Robertson MM, Trimble M, et al. A functional neuroanatomy of tics in Tourette syndrome. Arch Gen Psychiatry 2000; 57: 741–8.

Tokimura H, Di Lazzaro V, Tokimura Y, Oliviero A, Profice P, Insola A, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol 2000; 523: 503–13.

Xiang Z, Huguenard JR, Prince DA. Cholinergic switching within neocortical inhibitory networks. Science 1998; 281: 985–8.

Zhong P, Gu Z, Wang X, Jiang H, Feng J, Yan Z. Impaired modulation of GABAergic transmission by muscarinic receptors in a mouse transgenic model of Alzheimer's disease. J Biol Chem 2003; 278: 26888–96.

Ziemann U, Paulus W, Rothenberger A. Decreased motor inhibition in Tourette's disorder: evidence from transcranial magnetic stimulation. Am J Psychiatry 1997; 154: 1277–84.