GABAergic activity in autism spectrum disorders: An investigation of cortical inhibition via transcranial magnetic stimulation

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1. Introduction

Autism spectrum disorders (ASD) are neurodevelopmental conditions that involve primary deficits in social relating, communication, and behaviour (i.e., repetitive behaviours, restricted interests) and a host of associated features (e.g., sensorimotor abnormalities, abnormal cognitive profile) (Abrahamson et al., 2010). Although the relatively high prevalence of ASD ensures that it is a significant public health issue, the neurobiological basis of these conditions remains largely unknown. Recent evidence suggests a role for the inhibitory neurotransmitter \(\gamma\)-aminobutyric acid (GABA) in the neuropathophysiology of ASD (e.g., Fatemi et al., 2009a, 2009b; Harada et al., 2011). Indeed, a suppression of GABA as underlying autism was suggested a decade ago (Hussman, 2001).

There have been relatively few investigations of the role of GABA in ASD. Among \textit{in vivo} studies, magnetic resonance spectroscopy (MRS) has revealed both reduced GABA and a reduced GABA-to-glutamate ratio in the frontal lobe among 12 children with ASD; thus, ASD may not only involve reduced GABA activity, but pathological increases in glutamatergic function (glutamate is converted to GABA via glutamic acid decarboxylase [GAD]) (Harada et al., 2011). Recent electroencephalography (EEG) research into ASD has also been interpreted as supporting GABAergic deficits among thalamo-cortical networks (Thatcher et al., 2009). Small post-mortem studies among adults with autism (\(n = 5\)–10) have revealed evidence for GABA\textsubscript{A} receptor abnormalities (e.g., reduced receptor density, reduced benzodiazepine binding sites) in the anterior cingulate cortex (ACC) (Oblak et al., 2009), hippocampus (Guptill et al., 2007), prefrontal cortex, parietal cortex, and cerebellum (Fatemi et al., 2009b). Genetic links, such as those related to migration of GABAergic interneurons, have also been suggested (Grigorenko, 2009; Vincent et al., 2006). More recently, there is emerging evidence for GABA\textsubscript{A} receptor impairments (Fatemi et al., 2009a, 2010) and cerebellar reductions in GAD (Yip et al., 2009) in autism. (See Aitken, 2008 for a review.)

Transcranial magnetic stimulation (TMS) has been widely used as a non-invasive \textit{in vivo} measure of GABAergic activity (e.g., Berardelli et al., 2008; Croarkin et al., 2011; Daskalakis et al., 2002;
A TMS pulse delivered to the primary motor cortex produces a response in peripheral muscle that can be measured via electromyography (EMG). With respect to cortical inhibition, specific paradigms include paired-pulse TMS (ppTMS) and cortical silent period (CSP). ppTMS involves presenting a ‘conditioning’ pulse prior to a ‘test’ pulse. Where a subthreshold conditioning pulse is presented a short period before the test pulse (e.g., 2–5 ms; short interval ppTMS), there is typically a suppressed muscle response to TMS, and pharmacological evidence indicates that this reflects activity at GABA<sub>A</sub> receptors (e.g., Ziemann et al., 1996). Where a suprathreshold conditioning pulse is presented a somewhat longer period before the test pulse (e.g., 100 ms; long interval ppTMS), there is again a suppressed muscle response to the test pulse, but here pharmacological evidence points to the involvement of GABA<sub>B</sub> receptors (McDonnell et al., 2006). By contrast, CSP involves the administration of a TMS pulse during a period of tonic muscle activity, after GABAB receptor activity depending on the stimulus intensity used (e.g., Siebner et al., 1998; Werhahn et al., 1999). It should be noted, however, that CSP has been suggested to reflect activity at both GABA<sub>A</sub> and GABA<sub>B</sub> receptors (e.g., Ziemann et al., 1996), and some studies have failed to show modulation of CSP via a GABA<sub>B</sub> receptor agonist (Inghilleri et al., 1996; McDonnell et al., 2006; Ziemann et al., 1996).

These TMS paradigms have been used with success in elucidating the neuropathophysiology of psychiatric conditions such as depression (Levinson et al., 2010) and schizophrenia (Farzan et al., 2010; Fitzgerald et al., 2003), but to our knowledge have only been employed twice in ASD. Our preliminary study of cortical inhibition in ASD revealed evidence for reduced GABAergic activity in those diagnosed with DSM-IV autistic disorder, but this impairment did not extend to those diagnosed with DSM-IV Asperger’s disorder (Enticott et al., 2010). ASD is generally considered a heterogeneous group of conditions, at least from a neurobiological perspective, and it may be that GABA is implicated only in specific autism subtypes, as those with autistic disorder, who unlike those with Asperger’s disorder experience significant early language delay. In the other study, Theoret et al. (2005, see that paper’s supplementary material) also examined cortical inhibition via TMS among a small sample of 10 individuals with ASD, but did not discover any significant impairments. They did, however, find a trend toward a reduced CSP in ASD (p = .07). Although CSP was not assessed by Enticott et al. (2010), this is suggestive of a reduction in GABA activity in ASD.

Much of the research examining GABA among individuals with ASD has been characterised by relatively small sample sizes, and the precise role that GABA impairments might play in autism and related disorders therefore remains speculative. It is particularly important to explore GABA in ASD because pharmaceutical organisations are currently conducting Phase II clinical trials using GABA agonists in autism. Building upon our previous study (in which we examined only 2 ms and 15 ms ppTMS among a small sample of young individuals with ASD), we aimed to conduct a more comprehensive investigation of cortical inhibition in ASD, including a much larger sample and a broader range of TMS paradigms.

It was hypothesised that individuals with ASD would demonstrate impairments in cortical inhibition reflecting activity at both GABA<sub>A</sub> and GABA<sub>B</sub> receptors, and that these impairments would be more pronounced for those ASD participants who experienced early language delay (i.e., autism as opposed to Asperger’s disorder). Given the suggestion of laterality effects in ASD (e.g., Pierce, 2011), we examined (and where appropriate compared) both cerebral hemispheres.
and sampled via a CED Micro 1401 mk II analogue-to-digital converting unit (Cambridge Electronic Design, Cambridge, UK).

TMS was firstly used to locate the appropriate site on the primary motor cortex (i.e., M1, the scalp site producing a maximal response in contralateral FDI) and determine resting and active motor thresholds. Consistent with previous TMS research, resting motor threshold (RMT) was defined as the lowest stimulation intensity that produced a peak-to-peak MEP of >50 µV in at least three out of five consecutive trials (e.g., Cirillo et al., 2009). Active motor threshold (AMT) was defined as the lowest stimulation intensity that reduced muscle contraction, producing a peak-to-peak MEP of >100 µV in at least one out of five consecutive trials (e.g., Fitzgerald et al., 2009).

Twenty MEPS were then recorded following single pulse TMS (ten at 115% RMT, ten at 130% RMT). There was a four-second interval between each pulse. Immediately after, CSP (Cantello et al., 1992) was determined via 20 single TMS pulses (10 at 115% AMT, 10 at 130% AMT) during voluntary muscle contraction. This was achieved by having participants depress, with their index finger, a set of scales to 400 g, and maintain this during their 20 TMS pulses. ppTMS (Kujira et al., 1993) was then assessed firstly by administering a randomised block of 45 trials (4s ISI), which included (a) single pulses, (b) paired pulses that were separated by 2 ms (i.e., subthreshold pulse [90% AMT] followed 2 ms later by suprathreshold pulse [120% RMT], thus indexing short-interval cortical inhibition [SICI]), and (c) paired pulses that were separated by 15 ms (i.e., subthreshold pulse [90% AMT] followed 15 ms later by suprathreshold pulse [120% RMT], thus indexing cortical facilitation [CF]), and lastly by a block of 15 trials each involving paired pulses separated by 100 ms (suprathreshold pulse [120% RMT] followed 100 ms after by another suprathreshold pulse [120% RMT], i.e., long-interval cortical inhibition [LICI]).

All of the above measures were completed for both cerebral hemispheres. Specific parameters (e.g., TMS intensity, intervals) were selected based on those that have been used successfully in our lab to examine other clinical populations (e.g., Fitzgerald et al., 2003, 2009).

### 2.3. Data analysis

MEPs were analysed after determining peak-to-peak amplitudes (achieved via Signal 3.8, Cambridge Electronic Design, Cambridge, UK). Resting trials in which there was evidence of tonic muscle activity within 200 ms prior to the TMS pulse were excluded (<0.5% of all trials). CSP was determined by calculating the time (ms) from the beginning of the MEP to the end of the CSP (i.e., upon resumption of tonic EMG activity) (Daskalakis et al., 2003). Mean values were used throughout. Independent samples t-tests were used when comparing ASD and NT groups on most dependent measures (i.e., resting and active motor thresholds, resting and active MEP amplitude following single pulse TMS, CSP). The only exception to this was for 2/15 ms ppTMS, where we conducted a 2 (group: ASD, NT) x 2 (hemisphere) x 3 (TMS condition: single pulse, 2 ms ISI, 15 ms ISI) mixed model ANOVA, and 100 ms ppTMS, where we conducted a 2 (group: ASD, NT) x 2 (hemisphere) x 2 (TMS condition: single pulse, 100 ms ISI) mixed model ANOVA. Independent samples t-tests were also used for comparing SICI (i.e., response to 2 ms ISI as a percentage of response to single pulse), CF (i.e., response to 15 ms ISI as a percentage of response to single pulse), and LICI (i.e., response to 100 ms ISI as a percentage of response to single pulse). Data were screened for normality via inspection of boxplots and a formal test of normality (i.e., Kolmogorov–Smirnov [KS]). Where necessary, data transformations (square root or logarithmic) were conducted to ensure normality. Non-normality was detected for resting and active MEP amplitudes (both 115% and 130% RMT) and LICI in both hemispheres, and in this instance a logarithmic transformation provided the most suitable solution (KS p > .05). Non-normality was also detected for SICI data (both hemispheres), but here a square root transformation was most suitable (KS p > .05).

Given that we have previously found evidence for a dissociation in TMS-indexed GABAergic function based on early language impairment in ASD (Enticott et al., 2010), we further investigated whether there were any differences on these measures between ASD individuals with early language delay (ASD-LD, i.e., no phrase speech before age 3) and ASD without early language delay (ASD-NLD, i.e., phrase speech before age 3). This subtyping approach to ASD has been used in past neurobiological research in ASD (e.g., Lotspeich et al., 2004; McAlonan et al., 2009), and has yielded neurobiological differences. Comparisons within the ASD group (i.e., language delay vs. no language delay vs. NT group) were conducted via ANOVA. We lacked the necessary power for mixed-model ANOVA for the ppTMS paradigms, and only conducted ANOVA on SICI, CF, and LICI data.

### 3. Results

#### 3.1. ASD vs. NT

Summary data, together with t-test results where performed, are presented in Table 3.

The ASD group had higher right (but not left) hemisphere resting, t(64) = 2.39, p = .020, n²p = .071, and active motor thresholds, t(62) = −2.23, p = .029, n²p = .062 (both tests adjusted following unequal variances). One ASD participant did not complete ppTMS. For the 2 ms/15 ms block, there was an effect of condition (Huynh–Feldt correction, based on ε > 0.75 [Girden, 1992]), F(1,96) = 142.87, p < .001, n²p = .681. As expected, ppTMS with a 2 ms ISI produced a significantly reduced MEP compared to single pulse TMS (p < .001), while ppTMS with a 15 ms ISI produced a significantly increased MEP compared to single pulse TMS (p < .001). This reflects SICI and CF, respectively. There was no main effect of group, F(1,67) = 0.12, p = .733, n²p = .002, or hemisphere, F(1,67) = 0.60, p = .440, n²p = .009. There was no interaction effect for group x condition (Huynh–Feldt correction, based on ε > 0.75 [Girden, 1992]), F(2,104) = 0.43, p = .602, n²p = .006. The three way interaction was also not significant (Greenhouse–Geisser correction, based on ε < 0.75 [Girden, 1992]), F(2,103) = 0.24, p = .726, n²p = .004. When converted to a percentage of the response to single pulse TMS, there was no effect of group on SICI for either the right, t(67) = 0.95, p = .346, n²p = .013, or left hemisphere, t(65) = 1.30, p = .198, n²p = .025. Similarly, there was no effect of group on CF for either the right, t(66) = −1.27, p = .210, n²p = .024, or left hemisphere, t(66) = 0.29, p = .774, n²p = .001.

Three ASD participants did not complete 100 ms ISI ppTMS. For the 100 ms block, there was an effect of condition, F(1,65) = 187.72, p < .001, n²p = .743, with a 100 ms ISI producing a significantly reduced pulse when compared with the first pulse in the sequence. This reflects LICI. There was no main effect of group, F(1,65) = 0.03, p = .876, n²p = .000, or hemisphere, F(1,65) = 0.66, p = .421, n²p = .010. There was no interaction effect for group x condition, F(1,65) = 0.07, p = .829, n²p = .001, group x hemisphere, F(1,65) = 0.49, p = .488, n²p = .007, condition x hemisphere, F(1,65) = 1.19, p = .279, n²p = .018, or group x condition x hemisphere, F(1,65) = 0.63, p = .429, n²p = .010. When converted to a percentage of the response to single pulse TMS, there was no effect of LICI for either the right, t(66) = −0.61, p = .546, n²p = .006, or left hemisphere, t(65) = −0.24, p = .810, n²p = .001.

Several participants were excluded from CSP analyses (3 ASD and 6 NT) due to technical difficulties when recording the data. There were no group differences in CSP for either the right or left hemisphere. Repeated measures ANOVA (TMS intensity x hemisphere x group) also revealed no effect of group x TMS intensity, F(1,57) = 1.39, p = .243, n²p = .024, group x hemisphere, F(1,57) = 0.03, p = .856, n²p = .001, or

### Table 2

<table>
<thead>
<tr>
<th>Participant</th>
<th>Early language delay</th>
<th>Medication (dosage per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>fluoxetine (5 mg)</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>citalopram (not known)</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>sertraline (50 mg), lorazepam (2 mg), olanzapine (10 mg)</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>venlafaxine (150 mg)</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>fluoxetine (20 mg)</td>
</tr>
<tr>
<td>6</td>
<td>No</td>
<td>fluoxetine (40 mg), risperidone (2 mg)</td>
</tr>
<tr>
<td>7</td>
<td>No</td>
<td>sertraline (100 mg)</td>
</tr>
<tr>
<td>8</td>
<td>Yes</td>
<td>risperidone (2.5 mg)</td>
</tr>
<tr>
<td>9</td>
<td>No</td>
<td>mirtazapine (45 mg)</td>
</tr>
<tr>
<td>10</td>
<td>Yes</td>
<td>fluoxetine (40 mg)</td>
</tr>
<tr>
<td>11</td>
<td>No</td>
<td>sertraline (100 mg)</td>
</tr>
<tr>
<td>12</td>
<td>Yes</td>
<td>quetiapine (not known), lorazepam (PRN, 1 mg)</td>
</tr>
<tr>
<td>13</td>
<td>No</td>
<td>sertraline (140 mg), risperidone (2 mg), lorazepam (PRN, 1 mg)</td>
</tr>
</tbody>
</table>
group × TMS intensity × hemisphere, $F(1,57) = 0.20, p = .656, \eta^2_p = .004$.

The above analyses were repeated without those ASD participants who were medicated. The only change was that the difference in active motor threshold for the right hemisphere was no longer significant, $t(55) = 1.47, p = .149, \eta^2_p = .038$.

### 3.2. ASD subtypes: the impact of early language delay

There were 16 ASD-LD (12 male; mean age: 25.85 [8.13]) and 20 ASD-ND (15 male; mean age: 24.62 [9.57]). There was no difference in the rates of medicated participants per group (ASD-LD: 8 medicated; ASD-ND: 5 medicated; $\chi^2(1) = 2.41, p > .05$). There were no significant differences between these groups in any of the self-report clinical measures of ASD (all $p > .05$), but there was a trend toward a difference in VIQ (ASD-LD: 94.06 [16.68]; ASD-ND: 104.65 [16.56]), $t(34) = -1.90, p = .066$.

Summary data, together with ANOVA results where performed and not reported in text, are presented in Table 4, Figs. 1 and 2. We again used the transformed data where appropriate (i.e., logarithmic transformation for resting/active MEP amplitudes and LICI, square root transformation for SICI).

There was an effect of group on RMT in the right hemisphere, $F(2,66) = 3.54, p = .035, \eta^2_p = .097$. Post-hoc tests (Bonferroni corrected) revealed an increased resting motor threshold among the ASD-ND group relative to controls ($p = .031$). There were no other between-group differences in motor threshold, and no group effects on MEP amplitude.

For ppTMS, there was an effect of group on SICI for the left hemisphere, $F(2,64) = 4.55, p = .014, \eta^2_p = .125$. Post-hoc tests (Bonferroni corrected) revealed that the ASD-LD group had reduced SICI compared with both the ASD-ND ($p = .027$) and NT groups ($p = .027$). By contrast, there was no effect of group on SICI for the right hemisphere, $F(2,66) = 1.60, p = .210, \eta^2_p = .046$. There was no effect of group on CF for either the right, $F(2,65) = 0.92, p = .405, \eta^2_p = .027$, or left hemisphere, $F(2,65) = 0.55, p = .581, \eta^2_p = .017$.

There was no effect of group on LICI for either the right, $F(2,65) = 0.21, p = .813, \eta^2_p = .006$, or left hemisphere, $F(2,64) = 0.63, p = .539, \eta^2_p = .019$ (see Fig. 1).

For CSP, there was an effect of group on right hemisphere CSP (115% AMT), $F(2,57) = 3.26, p = .046, \eta^2_p = .103$. Post-hoc comparisons suggested a trend toward an increase in CSP among the ASD-ND group, but comparisons with both the ASD-LD ($p = .079$) and NT groups ($p = .095$) were not significant following Bonferroni correction. There was no effect of group for right hemisphere CSP (130% AMT), $F(2,58) = 1.63, p = .205, \eta^2_p = .053$, left hemisphere CSP (115% AMT), $F(2,60) = 0.56, p = .574, \eta^2_p = .018$, or left hemisphere CSP (130% AMT), $F(2,60) = 1.58, p = .215, \eta^2_p = .050$ (see Fig. 2). As with data comparing ASD and NT groups, repeated measures ANOVA (TMS intensity × hemisphere × group) also revealed no effect of group × TMS intensity, $F(2,56) = 1.02, p = .367, \eta^2_p = .035$, group × hemisphere, $F(2,56) = 0.03, p = .969, \eta^2_p = .001$, or group × TMS intensity × hemisphere, $F(2,56) = 0.24, p = .789, \eta^2_p = .008$.

### 4. Discussion

The current findings do not support the hypothesis that ASD, as broadly defined, is associated with deficits in cortical inhibition. This is despite an emerging literature suggesting that GABA may be closely involved in the neuropathophysiology of ASD. When considering subtypes, however, there was evidence for some reductions in cortical inhibition, presumably reflecting GABAergic impairments, among those with early language delay (i.e., high-functioning autism); specifically, where there was an early language delay, there was some evidence for a cortical inhibition deficit in the left hemisphere (SICI).

These findings indicate that GABA_A (SICI) deficits may exist in individuals diagnosed with an ASD who have exhibited a delay in language acquisition, but that there may be laterality effects. That
is, evidence of a GABA_A deficit was only found in the left hemisphere. As recently reviewed by Levinson et al. (2010), these inferences are based on numerous pharmacological studies demonstrating that GABAergic agents have a modulating effect on TMS cortical inhibition paradigms; for example, GABAergic agonists seem to enhance SICI (Ziemann et al., 1996). The current results are partly consistent with our previous study, where bilateral GABA_A deficits were found among those with ASD and an early language delay (Enticott et al., 2010). To a degree, this is consistent with the emerging body of evidence concerning the possible role of GABA in ASD, where both GABA_A and GABA_B have been implicated, but again suggests that it is only a specific subtype (i.e., those with early language delay) that are affected, and that any impairments are not particularly extensive. Interestingly, many previous studies that implicate GABA have employed groups of individuals diagnosed with autistic disorder (e.g., Fatemi et al., 2009b; G updill et al., 2007; Oblak et al., 2009), where in line with diagnostic criteria there were presumably language delays (American Psychiatric Association, 2000).

Fig. 1. ppTMS results (absolute mV response to ppTMS expressed as a percentage of absolute mV response to single pulse TMS that was included in each ppTMS protocol) for ASD-LD, ASD-ND, and NT groups.

### Table 4
Mean TMS outcome measures (untransformed) for ASD-LD and ASD-ND groups (SD in parentheses; NT values included where comparisons performed).

<table>
<thead>
<tr>
<th></th>
<th>ASD-LD</th>
<th>ASD-NLD</th>
<th>NT</th>
<th>F</th>
<th>df</th>
<th>p</th>
<th>( \eta^2 )</th>
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<tr>
<td><strong>Resting motor threshold (%)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Right hemisphere</td>
<td>43.69 (8.00)</td>
<td>46.53 (7.98)</td>
<td>41.09 (6.28)</td>
<td>3.54</td>
<td>2.66</td>
<td>.035</td>
<td>.097</td>
</tr>
<tr>
<td>Left hemisphere</td>
<td>45.88 (8.55)</td>
<td>45.37 (9.21)</td>
<td>42.88 (6.29)</td>
<td>1.09</td>
<td>2.66</td>
<td>.342</td>
<td>.032</td>
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<td><strong>Active motor threshold (%)</strong></td>
<td></td>
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<tr>
<td>Right hemisphere</td>
<td>35.44 (6.56)</td>
<td>37.05 (7.19)</td>
<td>33.09 (5.04)</td>
<td>2.76</td>
<td>2.66</td>
<td>.070</td>
<td>.077</td>
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<tr>
<td>Left hemisphere</td>
<td>37.06 (6.41)</td>
<td>36.95 (6.44)</td>
<td>34.62 (6.72)</td>
<td>1.15</td>
<td>2.67</td>
<td>.323</td>
<td>.033</td>
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<tr>
<td><strong>Motor evoked potential amplitude (mV)</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Resting right hemisphere 115% RMT</td>
<td>0.71 (0.53)</td>
<td>0.75 (1.17)</td>
<td>0.62 (0.60)</td>
<td>0.14</td>
<td>2.66</td>
<td>.869</td>
<td>.004</td>
</tr>
<tr>
<td>Resting right hemisphere 130% RMT</td>
<td>0.70 (0.58)</td>
<td>0.57 (0.33)</td>
<td>0.70 (0.43)</td>
<td>0.06</td>
<td>2.66</td>
<td>.940</td>
<td>.002</td>
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<td>Resting left hemisphere 115% RMT</td>
<td>1.01 (1.26)</td>
<td>0.81 (0.71)</td>
<td>0.85 (1.39)</td>
<td>0.16</td>
<td>2.66</td>
<td>.855</td>
<td>.005</td>
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<td>Resting left hemisphere 130% RMT</td>
<td>2.23 (2.38)</td>
<td>1.38 (0.82)</td>
<td>1.86 (2.03)</td>
<td>1.08</td>
<td>2.65</td>
<td>.924</td>
<td>.002</td>
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<td>Active right hemisphere 115% AMT</td>
<td>0.33 (0.23)</td>
<td>0.43 (0.37)</td>
<td>0.49 (0.50)</td>
<td>0.50</td>
<td>2.66</td>
<td>.607</td>
<td>.015</td>
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<td>Active right hemisphere 130% AMT</td>
<td>0.80 (0.52)</td>
<td>0.78 (0.42)</td>
<td>2.79 (2.09)</td>
<td>0.18</td>
<td>2.66</td>
<td>.832</td>
<td>.006</td>
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<tr>
<td>Active left hemisphere 115% AMT</td>
<td>1.05 (0.81)</td>
<td>1.06 (0.57)</td>
<td>2.28 (1.08)</td>
<td>0.25</td>
<td>2.67</td>
<td>.780</td>
<td>.007</td>
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<td>Active left hemisphere 130% AMT</td>
<td>2.47 (2.19)</td>
<td>2.16 (1.22)</td>
<td>2.77 (2.13)</td>
<td>0.44</td>
<td>2.65</td>
<td>.648</td>
<td>.013</td>
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<td>ppTMS right hemisphere single pulse</td>
<td>0.90 (0.68)</td>
<td>1.14 (1.30)</td>
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<tr>
<td>ppTMS right hemisphere 2 ms ISI</td>
<td>0.55 (0.66)</td>
<td>0.39 (0.38)</td>
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<tr>
<td>ppTMS right hemisphere 15 ms ISI</td>
<td>1.14 (0.93)</td>
<td>1.41 (1.48)</td>
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<tr>
<td>ppTMS right hemisphere single pulse (–100)</td>
<td>0.94 (0.65)</td>
<td>1.12 (1.63)</td>
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<tr>
<td>ppTMS right hemisphere 100 ms ISI</td>
<td>0.23 (0.17)</td>
<td>0.23 (0.32)</td>
<td></td>
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</tr>
<tr>
<td>ppTMS left hemisphere single pulse</td>
<td>1.37 (1.75)</td>
<td>0.86 (0.68)</td>
<td></td>
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<tr>
<td>ppTMS left hemisphere 2 ms ISI</td>
<td>0.60 (0.72)</td>
<td>0.28 (0.24)</td>
<td></td>
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<tr>
<td>ppTMS left hemisphere 15 ms ISI</td>
<td>1.77 (2.47)</td>
<td>1.17 (0.79)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>ppTMS left hemisphere single pulse (–100)</td>
<td>1.29 (1.51)</td>
<td>0.99 (0.87)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ppTMS left hemisphere 100 ms ISI</td>
<td>0.30 (0.36)</td>
<td>0.16 (0.16)</td>
<td></td>
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</table>
behaviours that form part of the diagnostic criteria (although this would presumably be expected for ASD-ND also), or even broader neurobiological (e.g., neural connectivity) or regulatory processes related to, for example, motor function, social relating, or sensory integration. This would be consistent with, for example, studies of motor function that suggest increased variability among those with high-functioning autism (compared with Asperger’s disorder) (Rinehart et al., 2006). That GABAergic deficits were not found in a clinically similar group is consistent with the notion that there are different neurobiological pathways to ASD; that is, different neurobiological impairments might produce characteristically similar clinical presentations, or even have similar effects at a neural level. There is general agreement that ASDs are a highly heterogeneous group of disorders, and this heterogeneity is perhaps the reason that we failed to find any overall between-group effects when comparing ASD and NT groups. While aetiologies remain unclear, there appear to be numerous genetic factors that can be associated with ASD, although at present these only explain 10–20% of individuals with an ASD diagnosis (State, 2010). Thus, it is highly likely that ASDs comprise separate neurobiological profiles that together produce a clinically identifiable syndrome of impairments.

It is interesting that, despite a larger sample, the GABA impairments were seemingly not as widespread as in our earlier study (Enticott et al., 2010), where bilateral deficits were found. The major difference between these studies is that the current sample was older, and that may simply reflect developmental effects whereby the magnitude of these impairments dissipates as people with ASD and early language delay get older.

Interestingly, when comparing the ASD and NT groups, there was some evidence for differences in motor threshold. This refers to the intensity of the magnetic field required to generate a MEP of a predetermined amplitude. This difference was significant only for the right hemisphere. When examining subgroups, this only held for the ASD-ND group (although this analysis was likely underpowered). This finding is inconsistent with Theoret et al. (2005), although this likely reflects the heterogeneity of ASD. While increased motor threshold may be interpreted as an excitatory deficit, or even enhanced inhibition, there are many factors that contribute to one’s motor threshold. One such factor is the scalp to cortex distance (McConnell et al., 2001); thus, it might be determined that ASD is overall associated with a greater distance between the scalp and the cortex, and this might relate to altered patterns of brain development (Courchesne et al., 2011). Cortical thinning in ASD might also contribute to this difference (e.g., Hadjikhani et al., 2006). Furthermore, if this finding was indeed related to neurophysiological differences in excitability or inhibition then we would have expected to see this on our other measures (e.g., MEP amplitude), but this was not the case. Interestingly, there have been inverse relationships found between motor threshold and the integrity of white matter pathways in numerous regions (including motor regions and the internal capsule; Kloppel et al., 2008), and there is also evidence of white matter abnormalities in ASD (Stigler et al., 2011); thus, our results might reflect abnormalities in the autistic brain’s microarchitecture. These possibilities, however, are highly speculative, and further research that combines brain stimulation and neuroimaging techniques in ASD will be critical to uncovering the true nature of this relationship.

The current findings are particularly relevant to contemporary autism research and clinical practice. There is an increasing trend toward the study of ‘autism spectrum disorders,’ which incorporates a host of pervasive developmental disorders, all with impaired social relating at their core, rather than ‘splitting’ disorders according to DSM-IV-TR nosology (e.g., autistic disorder, Asperger’s disorder). It appears likely that DSM-V will not include these separate diagnoses, but instead refer to ASD. This is despite, as indicated, ASD generally being regarded as a highly heterogeneous disorder with multiple underlying aetiologies and pathophysiologicals. We are clearly in the initial stages of forging a more complex understanding of the neurobiology of ASD, but an approach that utilises subtypes, be they phenotypical or genotypical, might provide the greatest insights and opportunities for individualised treatment. By contrast, examining broadly defined groups of individuals means that differences between subgroups of individuals, particularly subtle differences, are likely to be missed (as demonstrated in the present study). Without reliable subtyping of ASD, this will arguably further obscure attempts at elucidating the neurobiological basis of these conditions, and thus prevent the development of biomedical treatments. Related to these findings are also particularly timely given current clinical trials assessing the use of GABA agonists among individuals with ASD. It
is clear that more investigative work is needed to establish the precise nature of GABAergic function in ASD, and to therefore determine the appropriateness of GABA-based therapeutic approaches to ASD.

There are several limitations to this research that must be considered when interpreting these findings. Perhaps most importantly, the current paradigm allows measurement of cortical inhibition within the motor cortices, but this does not necessarily inform as to cortical inhibition in other brain regions (e.g., prefrontal cortex). It is possible that GABAergic deficits in ASD, as broadly defined, only exist in specific brain regions, such as those identified in histological studies. Future TMS studies in this population should adopt a combined TMS–EEG approach, which has been successfully used to measure cortical inhibition in non-motor sites (Daskalakis et al., 2008; Fitzgerald et al., 2008).

Some of the clinical participants were medicated, which may have an effect on our EMG measures. Based on the medication types, however, it seems fairly unlikely that medications would have served to have a significant effect on GABAergic function. As indicated in Table 2, there was also an even split between ASD-LD and ASD-ND with respect to medications that might be expected to modulate measures of GABAergic function (i.e., benzodiazepines and antipsychotics; Daskalakis et al., 2002). In addition, the sample comprised adolescents and adults, and any deficits may be more pronounced among younger samples. Sub-analyses (i.e., ASD-LD vs. ASD-ND) were characterised by relatively small sample sizes, and it is possible that several analyses were underpowered (particularly RH SCI and CSP measures). The use of only ‘high-functioning’ participants limits generalisability; although there are additional considerations in attempting to include individuals with concurrent intellectual disability (e.g., capacity to provide informed consent, tolerability of TMS), it remains that we cannot gain a full understanding without including these individuals in research projects. Finally, the administration of the various TMS paradigms might be considered repetitive TMS, which can affect corticospinal excitability (and therefore the measured obtained). We consider this unlikely, however, as there was a relatively small number of pulses administered to each hemisphere (55 single pulses, 45 paired pulses), and short breaks implemented between the various paradigms. In addition, stimulation at less than 1 Hz has not been found to consistently affect cortical excitability.

In summary, the current study indicates that GABAergic deficits may not be common to all individuals with high-functioning ASD, but do affect a subset of these individuals (i.e., those with early language delay). Future studies (e.g., TMS–EEG, MRS) will allow us to determine the larger extent and functional relevance of these impairments, and assess whether GABAergic agonists might be of some utility in the treatment of some forms of ASD.

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