Clinical Neurophysiology 132 (2021) 1163–1172

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Relationship between GABA levels and task-dependent cortical excitability in children with attention-deficit/hyperactivity disorder

Ashley D. Harris [a,b,c,*], Donald L. Gilbert [d], Paul S. Horn [d], Deana Crocetti [e], Kim M. Cecil [f], Richard A.E. Edden [g,h], David A. Huddleston [d], Stewart H. Mostofsky [e,i,j], Nicolaas A.J. Puts [g,h,k]

[a] Radiology, University of Calgary, Calgary, AB, Canada
[b] Child and Adolescent Imaging Research Program, Alberta Children's Hospital Research Institute, University of Calgary, Calgary, AB, Canada
[c] Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada
[d] Division of Pediatric Neurology, Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, Cincinnati, OH, United States
[e] Center for Neurodevelopmental and Imaging Research, Kennedy Krieger Institute, Baltimore, MD, United States
[f] Department of Radiology, Cincinnati Children's Hospital Medical Center and University of Cincinnati, Cincinnati College of Medicine, OH, United States
[g] Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins School of Medicine, Baltimore, MD, United States
[h] F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, United States
[i] Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD, United States
[j] Department of Behavioral Science and Psychiatry, Johns Hopkins School of Medicine, Baltimore, MD, United States
[k] Department of Forensic and Neurodevelopmental Sciences, Sackler Institute for Translational Neurodevelopment, Institute of Psychiatry, Psychology, and Neuroscience, King's College London, London, UK

ARTICLE INFO

Article history:
Accepted 6 January 2021
Available online 10 March 2021

Keywords:
Attention deficit hyperactivity disorder
GABA-edited MRS
Inhibition
Short interval cortical inhibition
Transcranial magnetic stimulation

HIGHLIGHTS

- GABA+ MR spectroscopy, single and paired-pulse TMS and stop signal reaction time tasks were measured in children with ADHD and controls.
- Short-interval intracortical inhibition (SICI) and GABA+ positively correlated in both ADHD and controls, with higher GABA+ level related to lower SICI.
- The GABA+ motor evoked potential relationship changed between task and rest in controls, but was the same in task and at rest in ADHD.

ABSTRACT

Objective: Compared to typically developing (TD) peers, children with attention deficit hyperactivity disorder (ADHD) manifest reduced short interval cortical inhibition (SICI) in the dominant motor cortex measured with transcranial magnetic stimulation (TMS). This multimodal study investigates the inhibitory neurophysiology and neurochemistry by evaluating the relationship between SICI and γ-amino butyric acid (GABA+) levels, measured with magnetic resonance spectroscopy (MRS).
Methods: Across two sites, 37 children with ADHD and 45 TD children, ages 8–12 years, participated. Single and paired pulse TMS to left motor cortex quantified SICI during REST and at times of action selection (GO) and inhibition (STOP) during a modified Slater-Hammel stop signal reaction task. MRS quantified GABA+ levels in the left sensorimotor cortex. Relationships between SICI and GABA+, as well as stopping efficiency and clinical symptoms, were analyzed with correlations and repeated-measure, mixed-models.
Results: In both groups, higher GABA+ levels correlated with less SICI. In TD children only, higher GABA+ levels correlated with larger TMS motor evoked potentials (MEPs) at REST. In GO and STOP trials, higher GABA+ was associated with smaller MEP amplitudes, for both groups. Overall, GABA+ levels did not differ between groups or correlate with ADHD clinical symptoms.
Conclusions: In children with higher motor cortex GABA+, motor cortex is less responsive to inhibitory

Abbreviations: ADHA, attention deficit hyperactivity disorder; TD, typically developing; GABA, γ-aminobutyric Acid; MRS, magnetic resonance spectroscopy; TMS, transcranial magnetic stimulation; SICI, Short Interval Cortical Inhibition.

* Corresponding author at: University of Calgary, Alberta Children's Hospital, B4-512, 28 Oki Drive NW, Calgary T3B 6A8, Canada.
E-mail address: ashley.harris2@ucalgary.ca (A.D. Harris).

https://doi.org/10.1016/j.clinph.2021.01.023

TMS (SICI). Comparing the relationships between MRS-GABA+ levels and responses to TMS at REST vs. GO/STOP trials suggests differences in inhibitory neurophysiology and neurotransmitters in children with ADHD. These differences are more prominent at rest than during response inhibition task engagement.
Significance: Evaluating relationships between GABA+ and SICI may provide a biomarker useful for understanding behavioral diagnoses.

1. Introduction

Disinhibited, impulsive behavior, a core feature of attention-def icit/hyperactivity disorder (ADHD), is highly prevalent and impairing across a broad range of neurological and psychiatric diagnoses. As is the case for most neurodevelopmental disorders, improving treatment is hampered by reliance on subjective rating scales and a lack of understanding of underlying neurobiology (Kapur et al., 2012). Studies aiming to elucidate neurobiology and generate biomarkers useful for future treatment-focused studies often rely on a single technique to measure diagnostic group differences. However, multiple metrics that capture additional domains can provide greater biological insight, possibly allowing for identification of subgroups with distinct treatment responses (Webb et al., 2016). Specifically, to investigate the neurobiology of disinhibited behavior, metabolic inhibition can be quantified using GABA-edited magnetic resonance spectroscopy (MRS) (Harris et al., 2017); physiological inhibition can be quantified with the paired-pulse transcranial magnetic stimulation (TMS) measure short interval cortical inhibition (SICI) (Di Lazzaro et al., 2007; Kujirai et al., 1993); and behavioral inhibition can be quantified using response inhibition tasks (Crosbie et al., 2013). In the current study, we investigated the relationship between the metabolic, physiological and behavioral inhibition with the goal to better understand the underpinnings of these measures and more fully characterize previously identified differences in primary motor cortex inhibitory physiology in children with ADHD.

ADHD hs been studied extensively and inhibitory TMS measures in motor cortex. Compared to typically developing (TD) children, children with ADHD have reduced SICI in resting primary motor cortex (Gilbert et al., 2011; Moll et al., 2001). To quantify SICI, a conditioning pulse is applied immediately prior (1–4 ms) to the suprathreshold TMS test pulse and the degree to which the conditioning pulse modulates the response to the test pulse indicates its inhibitory effect. The relevance of reduced SICI in children with ADHD is supported by findings showing consistent correlations with ADHD symptom severity (Gilbert et al., 2011; Hoegl et al., 2012), sensitivity to ADHD medications (Chen et al., 2014; Moll et al., 2000), and, most recently, persistence during engagement in behaviorally relevant response inhibition Go/No Go (Hoegl et al., 2012) and Stop Signal Reaction Time (SSRT) (Gilbert et al., 2019) tasks. As pharmacological studies have shown that SICI is enhanced by GABA(A) agonists (Di Lazzaro et al., 2006), we hypothesized that, in children with ADHD, since SICI is reduced, γ -amino butyric acid (GABA) in motor cortex should also be reduced. Our initial pilot study to evaluate this possibility supported this notion: using GABA+-edited MRS we found, after controlling for sex, that 8–12 year old children with ADHD had lower GABA+ levels, compared to controls (Edden et al., 2012). However, MRS/TMS studies in healthy adults have not conclusively identified that reduced SICI corresponds to reduced GABA+ (Dyke et al., 2017; Stagg et al., 2011; Tremblay et al., 2013).

The most common MRS approach to measure GABA+ acquires signal from a pre-defined volume of brain tissue and signal is presented along a chemical shift axis which reflects their chemical environment. Here, our measures focused on a 27 ml volume of tissue in the sensorimotor cortex given its role in motor control and its correspondence to the location for TMS. Because GABA has an inherently low concentration and its peak is overlapped by other, more abundant metabolites, it requires advanced measurement methods. The most common method uses 'editing' that capitalizes on the scalar couplings within the GABA molecule to modulate the GABA signal at 3 ppm with frequency selective editing pulses applied at 1.9 ppm, a method known as GABA-edited MRS. A limitation of this method is that the frequency selective pulses are not completely selective, and the 'GABA' measurement is typically contaminated with a macromolecular signal and is therefore commonly referred to as GABA+. A second factor with all MRS (both edited and non-editing approaches) is signals with different chemical shifts (or frequencies) come from regions with slight spatial offsets, an effect known as chemical shift displacement. While this effect relatively trivial for the metabolite peaks, lipids outside the volume of interest, for example in the dura for cortical measures, can artifactually wrap into the spectrum of interest. This effect can be minimized by selecting the order of the excitation and refocusing pulses of the MRS sequence must be chosen such that lipid contamination of the spectrum is minimized. For more complete reviews on GABA-edited MRS, the reader is directed to (Harris et al., 2017; Mullins et al., 2014).

The objectives of the present, multi-modal study were two-fold. First, we aimed to replicate the prior findings of altered GABA levels (Edden et al., 2012) in this new, larger case control study of 8–12 year old children with ADHD compared to TD children. Second, by combining this with a comprehensive study of ADHD using TMS, we aimed to determine the relationships between multiple different aspects of inhibition, namely metabolic (GABA+ from MRS), physiological (SICI from TMS), cognitive (response inhibition efficiency, SSRT), and clinical (ADHD symptoms). For this paper, "GABA+" levels will refer to concentration of the metabolite GABA as determined from GABA-edited MRS to recognize these MRS measures are contaminated with macromolecules. This is also ensures distinction from discussion of the GABA in a more general context and TMS measures that rely on GABAergic processing (referred to as physiological measures in the present manuscript). "Response inhibition" will refer to performance on a child-friendly, TMS-compatible race-car version of the Slater-Hammel stop signal reaction task (SSRT) (an open access video of this design can be seen in (Guthrie et al., 2018)). The SSRT challenges the participant to inhibit a primed response, i.e., to "*stop*" in a small proportion of trials (Verbruggen et al., 2019), at random, after a cue to "*go*". A TMS-relevant advantage of the Slater-Hammel version is that it clusters GO responses around a target "finish line", allowing for efficient timing of TMS pulses. Thus single and paired pulse TMS can be used to evaluate inhibitory physiology (SICI) during action selection (GO) or suppression/inhibition (STOP). ADHD clinical symptoms are based on standardized symptom rating scales. The global hypothesis of this study is that correlations would be identified between reduced SICI, reduced GABA+, reduced

cognitive control (response inhibition), and greater clinical ADHD symptoms (behavioral disinhibition) in children.

2. Methods

2.1. Participants

Across two sites (Cincinnati Children's Hospital, Cincinnati, and Kennedy Krieger Institute, Baltimore) with the same equipment (3T Philips Achieva scanners, Magstim TMS Bistim200^R), right-handed, 8–12 year old children with previously diagnosed ADHD (n = 66) and TD children (n = 65), were recruited with advertisements in the community and targeted mail solicitations (Gilbert et al., 2019). From this larger project, this current study includes a subset of 82 children who successfully completed the imaging component of the study and have good quality GABA+-MRS data; ADHD n = 37 (17 Cincinnati, 20 Baltimore) and TD n = 45 (28 Cincinnati, 17 Baltimore). Participants had at least a 60 min break between imaging and TMS. Detailed assessments of clinical symptoms, demographics, medical histories, cognition, motor control, and motor physiology (TMS measures, see below) were performed to confirm diagnosis including the ADHD Rating Scale IV (ADHD-RS-IV) (DuPaul and Power, 1998) and the Conners' Parent Rating Scale (CPRS)-Revised (Conners, 1997) or third edition (Conners, 2008). Note that investigators travelled between both sites multiple times to ensure between site data consistency. To ensure site consistency, site was also included in the statistical analyses (see Section 2.5). For children with ADHD, stimulant medications were withheld the day prior to and the day of the TMS/MRI sessions. Exclusion criteria for participation included: 1) the use of non-stimulant ADHD medications and/or any other neuropsychiatric medications; 2) IQ < 85 or reading disability; 3) the presence of any other neurological, psychiatric, of developmental diagnosis identified during structured diagnostic and medical interviews and examinations. The study was approved by the local Institutional Review Boards. Written informed consent was obtained from the legal guardians of study participants, see Table 1 for demographic information.

Table 1
Summary demographic, clinical, cognitive, behavioral, and motor information.

Characteristic	ADHD (n = 37)	TD Children (n = 45)	p-value
Sex	22 M/15F	30 M/15F	0.64
Number by site	Site 1: 17/ Site 2: 20	Site 1: 28 / Site 2: 17	0.18
Race			0.45
African American	9	6	
Asian	1	4	
Biracial	4	4	
Caucasian	23	31	
Age	10.6 ± 1.4	10.5 ± 1.3	0.64
FSIQ	103.8 ± 14.0	118.3 ± 20.8	<0.001
Hollingshead Family SES	50.0 ± 10.4	54.0 ± 10.4	0.11
Conners Inattentive ADHD T score	74.9 ± 12.1	48.2 ± 10.0	<0.0001
Conners Hyper/Impulsive ADHD T score	75.9 ± 16.0	47.5 ± 7.0	<0.0001
ADHD-RS Inattentive Subscore	20.3 ± 4.6	4.0 ± 3.1	<0.0001
ADHD-RS Hyper/Impulsive Subscore	13.7 ± 7.2	2.9 ± 2.5	<0.0001
ADHD-RS Total Score	34.0 ± 10.3	6.9 ± 4.7	<0.0001
Stop Signal Reaction Time (ms)	238 ± 58.4	246 ± 59.2	0.60
PANESS Total Score	28.5 ± 10.0	24.5 ± 11.2	0.11

ADHD = attention deficit hyperactivity disorder. TD = typically developing. FSIQ = Full Scale Intelligence Quotient. SES = socio-economic status. ADHD-RS = ADHD rating scale. ms = millisecond. PANESS Physical and Neurological Examination for Subtle Signs. M/F = male/female; Site 1 = Cincinnati Children's; Site 2 = Kennedy Krieger Institute. Group comparisons by Chi Square for sex, site and race, t test for all others.

2.2. MRS acquisition and analysis

Imaging parameters were identical for both sites. Data at both sites were acquired using a Philips 3T Achieva MRI scanner (Best, the Netherlands) with a 32-channel head coil for receive and body coil for transmit, the total imaging protocol was ~1 h. All participants underwent a mock MRI acquisition prior to being scanned to familiarize the participant with the MRI environment. Children were allowed to watch a movie during the MRI acquisition, and 'points' were given for lying still that could be exchanged for prizes to motivate participant compliance during scanning. First, a 1 mm^3 isotropic T1-weighted image (MP-RAGE) was acquired for the MRS voxel localization (i.e., the volume of tissue in which GABA+ is measured) and voxel segmentation (repetition time, TR = 7.99 ms, echo time, TE = 3.76 ms, Flip angle = 8°, acquisition time 5.5 minutes). GABA-edited MRS data were acquired using a MEGA-PRESS sequence (Harris et al., 2017). Data were acquired from a (3 cm)^3 voxel over the left sensorimotor cortex centered on the central sulcus, posterior to the hand-knob in the axial plane and rotated to align with the cortical surface (Fig. 1 (Yousry et al., 1997)). A total of 320 transients were acquired in 10 min 40 s, using the following parameters: TE/TR = 68/2000 ms, 14 ms editing pulses placed at 1.9 ppm in the ON condition and at 7.46 ppm in the OFF conditions and interleaved over a 16-step phase cycle (OFF-first), 2048 datapoints, 2 kHz spectral width and VAPOR water suppression. Chemical shift direction was optimized to reduce lipid contamination. MRS data were analyzed using the Matlab-based GABA-edited MRS software package 'Gannet' (Edden et al., 2014). Briefly, spectra were frequency- and phase-corrected using spectral registration (Near et al., 2015) and subsequently filtered with Hz exponential line broadening and zero-filled to 32,768 points. GABA+ levels were estimated using a five-parameter Gaussian model, fitting between 2.79 and 3.55 ppm. GABA+ levels were calculated relative to the unsuppressed water signal from the same voxel. For each individual, GABA+ levels were tissue-corrected (Harris et al., 2015) using SPM12 (Ashburner and Friston, 2003) implemented within Gannet. Data were corrected for tissue specific relaxation values, and using an assumed 2:1 ratio of GABA+ in grey matter: white matter (Harris et al., 2015). Subsequently, tissue fractions were group normalized across all data to be representative of 40% grey matter and 60% white matter voxel contents. Due to co-edited macromolecule contamination (Harris et al., 2017), the reported GABA+ values refer to GABA+ co-edited macromolecules.

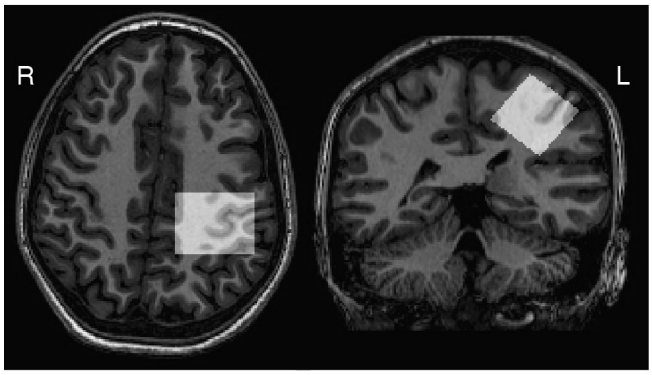

Fig. 1. Exemplar voxel location.

2.3. TMS

TMS measures were standardized across sites as previously described (Gilbert et al., 2019; Gilbert et al., 2011). The TMS protocol was approximately 1 hour. TMS was performed using a Magstim (Magstim Co, New York, NY) Bistim 200^R. In brief, TMS was applied to left primary motor cortex using a 90 mm round coil placed optimally near the vertex with the coil placed tangentially to the skull with the handle angled backward. Motor evoked potentials (MEPs) were measured with electromyography in the right first dorsal interosseous muscle (FDI). Electromyography (EMG) captured first dorsal interosseous muscle activity recorded using disposable Ag/AgCl surface electrodes placed in a belly-tendon montage (Guthrie et al., 2018). Data were amplified, filtered (100–1000 Hz) and digitized at 2 kHz using Signal software and a Micro1401 interface (Signal v6; CED 1401; Cambridge UK). Resting and active motor thresholds (RMT and AMT, respectively) were determined by starting with pulses at 10% maximum stimulator output, increasing by 1% until a consistent MEP was observed, then decreasing the intensity until a point was reached where 3 of 6 pulses produced no MEP and 3 produced an MEP of approximately 50 uV at rest (RMT). While this is fewer pulses than typically applied, in this population reducing testing time is highly desirable. While this may result in a reduction in RMT precision leading to type II error, we did not hypothesize group RMT difference thus find the benefit of reducing experimental time outweighs this risk. The same procedure was followed to determine AMT, in which the MEP is observed above background during ~10% force, as determined visually, tonic muscle contraction. SICI was measured with 10 pulses per condition, inter-trial interval of 6 ± 10% seconds using 0.6*RMT for the conditioning pulse and ~1.2*RMT for the test pulse. As some children had low MEP amplitudes, creating a potential floor effect, the test pulse intensity was adjusted upward by up to 5% maximum stimulator output, as needed, to produce MEPs consistently of 0.5–1.5 mV amplitude (Gilbert et al., 2011; Orth et al., 2003). The measure of interest is the peak-to-peak MEP amplitude, which was captured using an unbiased, automated method with the Signal^R program (v6; Cambridge Electronic Design, Cambridge, UK). REST SICI was calculated as the mean MEP amplitude from the paired-pulse divided by the mean single-pulse MEP amplitudes. This pulse ratio indicates that higher ratios (closer to 1) represent *less* SICI because there is a smaller difference between the paired and single pulse MEPs. For example, a ratio of 0.95 would be very little SICI (5%), and 0.21 would be high SICI (79%). Data were analyzed visually offline by a rater blinded to diagnosis group, and trials were removed with visual EMG artifact ~50 μV above baseline during 200 ms prior to the TMS pulse. Area under the curve was calculated for each tracing for the 200 ms epoch prior to the TMS pulse artifact. This area was included as a repeated measure covariate in regression analyses to account for any remaining motor cortex pre-activation. Artifact amounts for all included trials were also evaluated as dependent variable in a repeated measures regression and there was no significant diagnosis group difference ($p > 0.1$).

2.4. Task description

For REST, TMS was performed while the child was seated comfortably with arms completely relaxed and the ulnar aspects supported by a body-surrounding pillow (The Boppy Company, LLC, Golden, CO) while children watched a quiet video.

For the task of response inhibition (GO/STOP), we utilized the Slater-Hammel Stop Signal Reaction Time paradigm (Coxon et al., 2006; Slater-Hammel, 1960) modified into a child-friendly racecar version (schematic in Fig. 2; video of experimental design published Open Access in (Guthrie et al., 2018). Briefly, participants were again comfortably seated with both arms and hands supported by the body-surrounding pillow, viewing a computer screen. The game was operated using the index finger of the dominant hand, using a Logitech (Newark, CA) gamepad. Surface EMG electrodes recorded from the first dorsal interosseous (FDI) muscle as per Guthrie et al. (2018) Each trial was initiated by the participant adducting their first finger on the game controller which "started the engine" of the race car on the left side of the screen. The racecar then moved in a straight line across the "racetrack" in 1000 ms. During "GO" trials, the goal for participants was to abduct (lift) their finger, activating the FDI, so the car arrived as close to the 800 ms "Finish Line" as possible. "STOP" trials occurred at random 25% of the time. In a "STOP" trial, the car spontaneously stopped before the finish line. In these cases, the participants needed to "stop" their primed action of finger lifting at the 800 ms Finish Line and maintain finger adduction (keep pressing), for greater than 1000 ms. Initially, the STOP cue occurs at 500 ms and is shifted later (more difficult) or earlier (easier) by 50 ms increments depending on STOP success or failure, respectively. The convergence of STOP cue times relative to the mean GO time indicates response inhibition efficiency (Guthrie et al., 2018). Readers are directed to (Guthrie et al., 2018) and video at https://www.jove.com/t/56789/online-transcranial-magnetic-stimulation-protocol-for-measuring for full details of the task.

Testing consisted of three 40 trial blocks including 30 GO trials with 10 STOP trials intermixed. During the first block, the TMS intensity was set at 20% maximum stimulator output (subthreshold). This block familiarized participants with the stimulation procedure and with the auditory artifact. During the second two blocks, single-pulses at an intensity of 1.2*RMT and 3 ms interstimulus interval paired-pulses at intensities of 0.6 and 1.2*RMT were delivered randomly. During GO trials, pulses occurred at 150 ms prior to the expected finger lift. For STOP trials, pulses occurred at 150 ms after the stop cue. Total time for TMS studies was typically 90 minutes or less, including breaks between Rest and Task-TMS.

2.5. Statistical analyses

All models were analyzed using SAS® statistical software version 9.4 (SAS Institute Inc., Cary, NC). Univariate group comparisons were performed using t-test for age and full scale IQ scores and Chi Square to test for differences in sex, site and race. Left sensorimotor GABA+ levels were compared between ADHD and TD children as well as between sexes and sites. Bivariate (Pearson, Spearman) correlations were used to evaluate relationships between GABA+ and the TMS-physiological measurements (primary correlation of interest is SICI). Additional bivariate correlations between GABA+ levels and other physiological measures, response inhibition, clinical symptom severity, and age were also performed on an exploratory basis, without correction for multiple comparisons.

The primary between-group statistical analysis was a mixed model, repeated-measures regression with MEP amplitude as the dependent variable. To quantify the relationship between GABA+ levels and SICI, the primary factor of interest was the interaction between GABA+ and Pulse-type (paired pulse paradigm vs. single pulses). REST (20 trials), GO (60 trials), and Successful STOP (~10 trials out of 20) were analyzed separately to allow the GO trial analysis to account for the finger lift/ move-time relative to the TMS test-pulse time as a covariate. Site, sex, artifact, and age were included in all analyses as covariates. In this model, the interaction between GABA+ and pulse-type (single or paired) indicates a relationship between GABA+ and SICI.

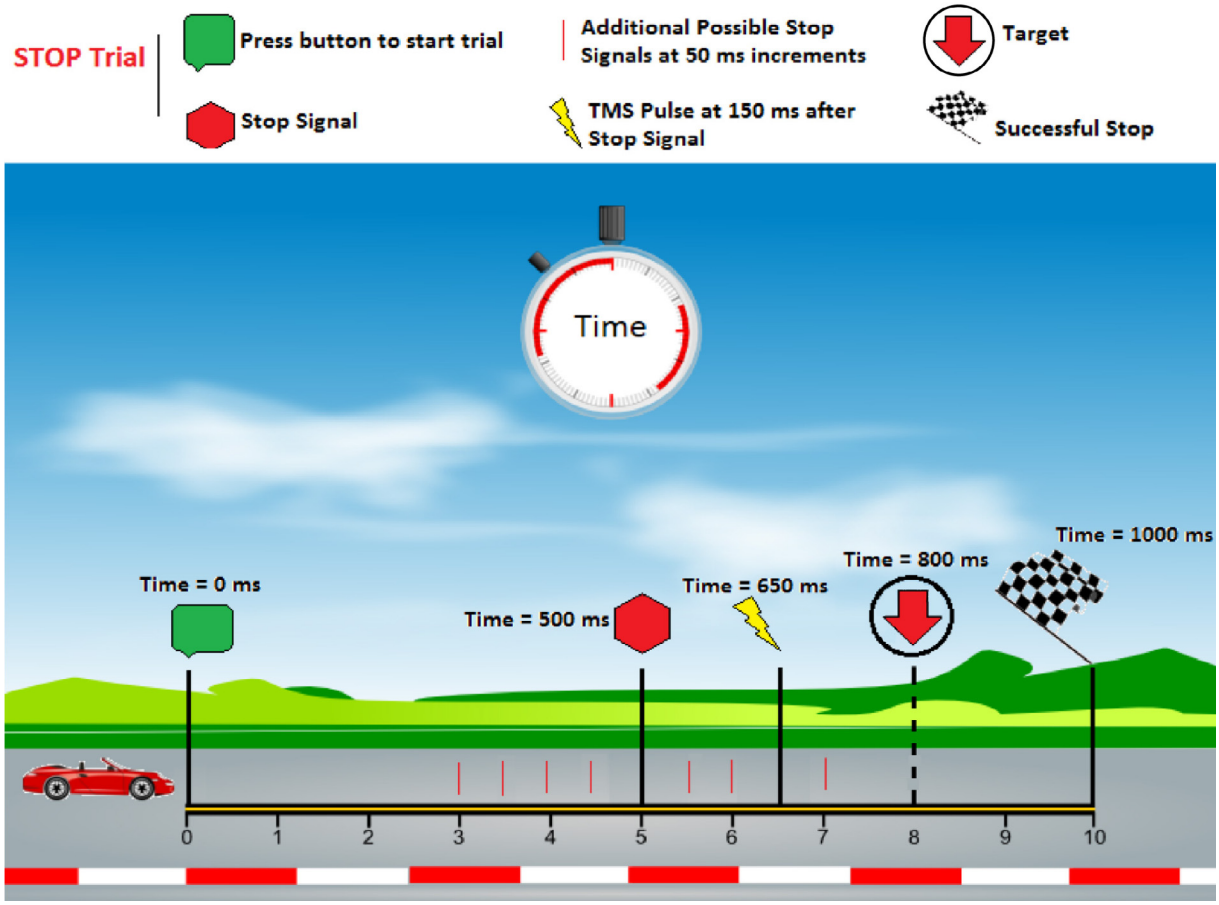

Fig. 2. Schematic of the Slater-Hammel SSRT task. The task is 1000 ms (start at left, ends at flag on right). To initiate the task, the participant presses the button (time = 0 ms). In "GO" trials (75% of trials), the participant tries to stop the car on the target stop line (at 800 ms) by releasing the button. In "STOP" trials (25% of trials), the car spontaneously stops before the target so the participant has to inhibit the primed action of releasing the button and keep pressing the button. This stop is initially at 500 ms (red hexagon) but this time shifts in 50 ms increments. The TMS pulse is indicated in yellow; in GO trials, it is at 650 ms and in STOP trials it 150 ms after the stop signal. TMS = transcranial magnetic stimulation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Group comparisons

Demographic information about participants is shown in Table 1. All superimposed GABA spectra are shown in Fig. 3. Left sensorimotor GABA+ levels did not differ between ADHD (mean 2.82 institutional units, i.u.; SD 0.49 i.u.) and TD (mean 2.95 i.u., SD 0.45 i.u.) groups ($p = 0.21$). Comparing males ($n = 52$) and females ($n = 30$) across both sites of participants, there were no sex differences in GABA+ ($p = 0.12$). Comparing the two sites across both groups of participants, there was no difference in GABA+ ($p = 0.30$). SICI data was not available in one ADHD participant due to high RMT. SICI was diminished in ADHD (mean 0.57, SD

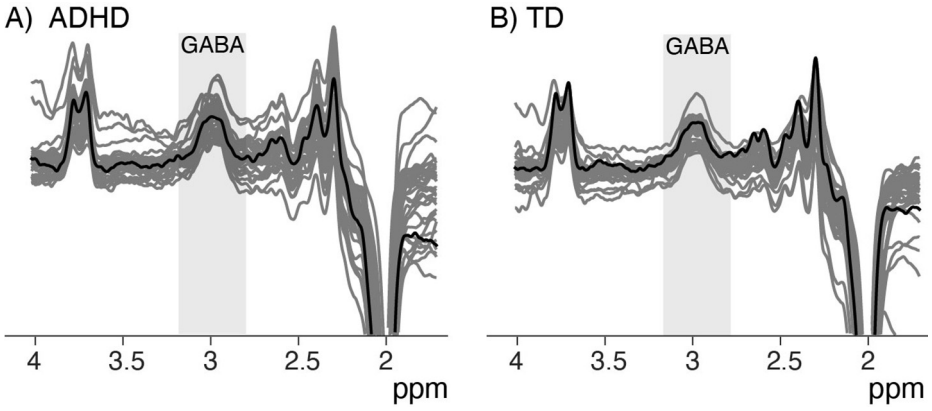

Fig. 3. All GABA+-MRS data from (a) ADHD and (b) TD children superimposed. An example spectrum is highlighted in black. The GABA peak is identified in both the ADHD and the TD groups. ADHD = attention deficit hyperactivity disorder, TD = typically developing.

0.25) compared to TD (mean = 0.47, SD = 0.24), (p = 0.028). In ADHD children, symptom ratings were greater for inattention (p < 0.0001) and hyperactivity (p < 0.0001); however, Stop Signal Reaction Times (SSRTs) did not differ (p = 0.4).

3.2. Bivariate correlations

Across groups, higher GABA+ levels correlated with less SICI (higher ratio; method of means; Pearson correlation) at REST (All: n = 81; r = −0.39; p = 0.0003), and this relationship was found in both the ADHD (n = 36; r = −0.41; p = 0.01) and TD groups (n = 45; r = −0.44; p = 0.003) as shown in Fig. 4 (ratios closer to one indicate less SICI; the single and paired pulse-type MEPs are similar amplitudes). Significance of results were similar using non-parametric Spearman correlations for SICI and GABA+ for All (n = 81; r = −0.35, p = 0.0013); ADHD (n = 36; r = −0.42; p = 0.010) and TD (n = 45; r = −0.35; p = 0.020) children. Removing the two most extreme GABA+ values from each diagnostic group (see Fig. 4 – two female participants with GABA+ > 4 i.u.), correlations remained significant for All (n = 79; r = −0.31, p = 0.005); ADHD (n = 35; r = −0.40; p = 0.017) and TD (n = 44; r = −0.30; p = 0.047).

When analyzing the MEPs from the two pulse-types separately, higher GABA+ correlated with larger MEP amplitudes evoked by the paired pulse TMS (r = 0.33, p = 0.03) in the TD children. In the ADHD group, however, higher GABA+ correlated with smaller single pulse mean MEP amplitudes approaching significance (r = −0.32, p = 0.06). The opposing signs of these correlations are consistent with the findings from the regression analysis (see Fig. 5).

GABA+ levels did not correlate with RMT or AMT, either across or within diagnostic groups. These were not analyzed further. SSRT correlated negatively with GABA+ (higher GABA+, shorter/better SSRT) though this did not reach statistical significance (r = −0.31, p = 0.08), in TD children only. Across all participants (n = 82), there were no correlations between age and either GABA+ (r = 0.087, p = 0.44) or SICI (r = 0.044, p = 0.62). There were no significant within-group correlations with behavioral rating scales (Supplementary Table).

3.3. Repeated measure analyses of TMS-MEPs with GABA+

With MEP amplitude as the dependent variable and all trials included in repeated-measure, mixed models, SICI was estimated in all models from the regression Least Square Means for the factor "pulse type" (Gilbert et al., 2019). Associations of GABA+ and SICI were assessed via the GABA+*Pulsetype interaction. None of the analyses had a significant effect of site. Among children with higher GABA+ levels, amplitudes of MEPs evoked by paired pulse TMS were increased in size relative to the amplitudes of MEPs evoked by single pulse TMS. That is, across the entire sample of 82 children, there was a robust, relationship between higher GABA+ and less SICI (ratios closer to 1) in REST trials (beta = 0.12 (SE = 0.03); t_{1502} = 4.05; p = 0.0001). This relationship was similarly robust in GO trials (beta = 0.35 (SE = 0.10); t_{1498} = 3.66; p = 0.0003) but not STOP trials (beta = 0.27 (SE = 0.14); $t_{566.5}$ = 1.85; p = 0.06).

To determine if Group (ADHD or TD) influences the association between GABA+ and motor cortex physiology (all MEP amplitudes), the GABA+*Group interaction was assessed in the model. There was a robust interaction between diagnosis and GABA+ for REST trial MEPs, with larger MEP amplitudes in TD children than in children with ADHD (beta = −0.34 (SE = 0.13); $t_{75.4}$ = −2.7; p = 0.009). This relationship between Group (ADHD or TD) and GABA+ was not found in GO (p = 0.54) or STOP (p = 0.40) trials. This indicates that the GABA+/MEP relationship differs primarily with REST trials, where higher GABA+ levels are associated with larger single-pulse and paired-pulse MEPs in TD children but smaller single-pulse and paired-pulse MEPs in children with ADHD.

Given the significant GABA+*Group effect on MEP amplitudes in the REST state, to determine the association between GABA+ and SICI, all REST, GO, and STOP models were repeated, stratified by Group, as shown in Table 2 and Fig. 5. The significance of associations of Pulse-type*GABA+ interaction terms show the relationship of GABA+ to SICI. This shows that, generally, among children with higher GABA+ levels, there is less SICI. That is, the amplitude of the inhibitory paired-pulse MEP increases relative to the amplitude of the single-pulse MEP (see Fig. 5). Put another way, among children with higher GABA+ levels, the conditioning pulse has less effect, relative to the single pulse, and the SICI ratio approaches 1.0. This finding is most statistically robust in TD children during GO trials

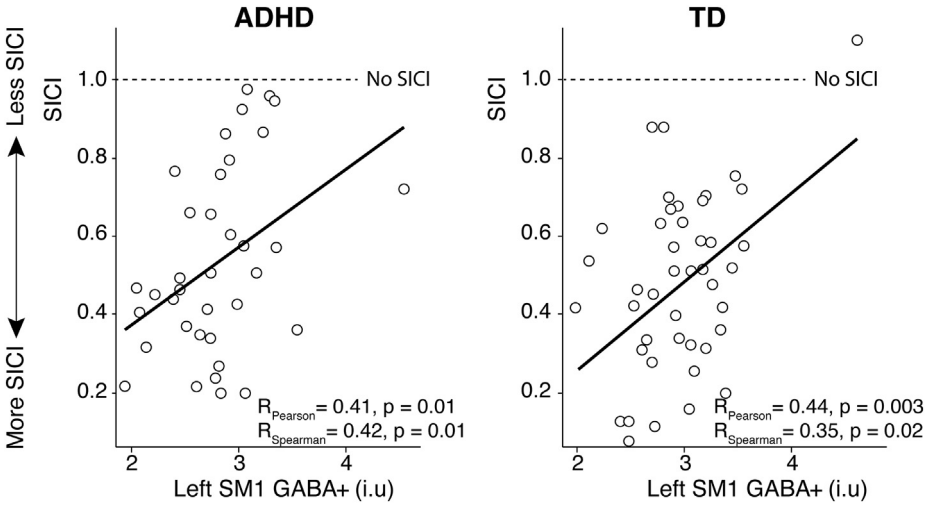

Fig. 4. Relationship between TMS-SICI and MRS-GABA+ for ADHD and TD controls. A SICI value towards zero indicates more SICI (a larger difference in MEP amplitude between single and paired-pulse conditions), a higher value indicates less SICI (a smaller difference in MEP amplitude between single and paired-pulse conditions). Among children with higher left sensorimotor GABA+ levels, paired- and single-pulse evoked amplitudes converge, i.e. SICI is reduced. The dotted line at 1 indicates no SICI. TMS = transcranial magnetic stimulation, SICI = short interval cortical inhibition, MRS = magnetic resonance spectroscopy, ADHD = attention deficit hyperactivity disorder, TD = typically developing, MEP = motor evoked potential.

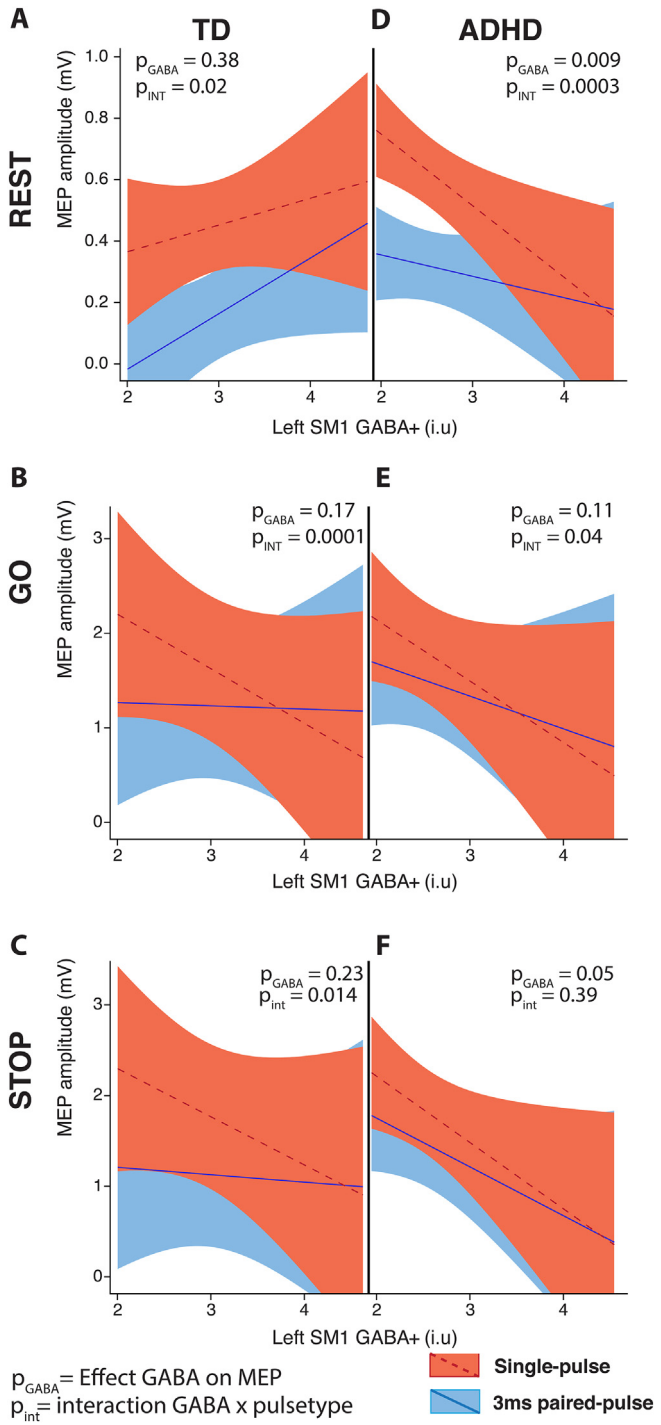

Fig. 5. Relationship between TMS - MEP amplitudes and MRS measured GABA+ in TD (A, B and C) and ADHD (D, E F) participants. Results are divided across task; REST (A, D), GO (B, E) and STOP (C, F). In each plot, quantified GABA+ level is on the x-axis and the TMS evoked MEP amplitude is along the y-axis. Shown are the results from both the paired- (blue) and single- (red) pulses, with the colored regions indicating the 95% confidence bands and the lines are the fits. For each plot, the p-values (threshold for significance is p = 0.05) are shown; pGABA indicates the significance of the relationship between GABA and MEP across both ADHD and TD, and pINT, (for interaction) indicates the level of significance between the two slopes. The full statistical model results are in Table 2. TMS = transcranial magnetic stimulation, MEP = motor evoked potential, MRS = magnetic resonance spectroscopy, TD = typically developing, ADHD = attention deficit hyperactivity disorder. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and in children with ADHD at rest. It is absent in STOP trials in children with ADHD.

As shown in Fig. 5D-F, relationships between GABA+ levels and MEP amplitudes appear to be qualitatively similar for ADHD children in all states (REST, GO, STOP). Similar relationships appear to hold for TD children during GO and STOP trials but differ for the REST trials. TD children do not show a statistically significant relationship between GABA+ levels and MEP amplitudes in any trial-type (Table 2), unless the pulse-type is considered. In TD children during action selection and inhibition (STOP and GO trials), the paired-pulse MEP is not affected by GABA+, as seen in Fig. 5. Conversely, in the single-pulse trials there is a negative relationship between GABA+ and MEPs during action selection and inhibition. In ADHD, there is a negative relationship between GABA+ and MEPs for both STOP and GO trials, though this relationship is non-significant in GO trials and marginally significant in STOP trials (p = 0.045), and there is no effect of pulse-type. Taken together, the significance of GABA+*Group effects (p = 0.009) in REST trials (but not GO or STOP trials) and the similar relationships between GABA+ and MEPs in STOP and GO trials across diagnostic groups (Figs. 5B – 4F) but not at REST (Figs. 5A/4D), supports a finding that ADHD and TD children manifest different GABA-related inhibitory physiology at rest but similar inhibitory physiology during action selection and inhibition.

4. Discussion

This multimodal study combined GABA+-edited MRS in the sensorimotor cortex, TMS measures of motor cortex inhibition, stop signal reaction times, and clinical symptoms in 8 to 12 years old children with ADHD and TD controls. We quantify and compare cortical GABA+ across diagnostic groups and analyze relationships with motor cortex short interval cortical inhibition (SICI) both at REST and during action selection/inhibition. The major findings of this study are that motor cortex GABA+ levels correlate inversely with SICI (more GABA+/less SICI) in children, and that there appears to be an anomalous GABA+/SICI relationship in ADHD at REST but not during action. If validated, these findings and this multimodal approach may hold promise in developing future biomarker-defined subtypes of ADHD.

With regard to the primary objective of this study, comparing sensorimotor GABA+ in TD children with ADHD to controls, in this larger sample, we failed to confirm group differences in GABA+, as seen previously (Edden et al., 2012), despite the current study using rigorous GABA+ approaches and analyses. ADHD is heterogeneous, and failure to identify and replicate group differences with a single technique occurs routinely in biomarker studies of this nature. Nonetheless, we have again shown that this technique is feasible even in hyperactive/hyperkinetic children, and that consistent data can be generated across multiple research locations. It appears at least for ADHD, sensorimotor GABA+ MRS utility may be greater in multi-modal and correlational studies rather than in a traditional single variable hypothesis-testing study with groups based on cases and controls.

The anomalies we identified are most clear at REST: *as GABA + increases*, TMS-evoked MEPs are larger in TD and smaller in ADHD, (Fig. 5A and D). We also found in this response inhibition task, that in performing action selection and inhibition, GABA+/SICI differences appear to resolve (Fig. 5B/E; C/F). It may be important to note that the response inhibition task was designed to be only moderately difficult, allowing for measurements of physiology while achieving comparable success in both groups (Gilbert et al., 2019; Guthrie et al., 2018). Looking at TMS-SICI alone, ADHD vs.

Table 2
Relationship of GABA+ to TMS-MEPs for REST, GO, and STOP trials.

		TD Children n = 45					Children with ADHD n = 36				
Task	Factor	effect on MEP (mV)	SE	t	df	p value	effect on MEP (mV)	SE	t	df	p value
REST	Pulse Type (single -> paired)	−0.57	0.12	−4.8	825.2	**<0.001**	−0.72	0.13	−5.5	674.3	**<0.001**
	GABA+	0.09	0.10	0.88	44.0	0.38	−0.23	0.08	−2.8	37.6	**0.009**
	GABA*Pulse Type	0.09	0.04	2.3	825.0	**0.02**	0.16	0.05	3.6	674.3	**<0.001**
GO	Pulse Type (single -> paired)	−2.02	0.40	−5.0	770.7	**<0.001**	−1.07	0.42	−2.6	701.7	**0.01**
	GABA+	−0.58	0.41	−1.4	28.9	0.17	−0.65	0.39	−1.7	25.2	0.11
	GABA*Pulse Type	0.54	0.13	4.1	770.5	**<0.001**	0.30	0.15	2.05	701.4	**0.04**
STOP	Pulse Type (single -> paired)	−1.99	0.55	−3.7	289	**<0.001**	−0.85	0.63	−1.3	275.4	0.18
	GABA+	−0.53	0.43	−1.2	30.6	0.23	−0.73	0.35	−2.1	29.1	0.045
	GABA*Pulse Type	0.45	0.18	2.5	288.9	**0.014**	0.19	0.22	0.86	274.8	0.39

Data are results of mixed model regression within group (ADHD or TD), with MEP amplitude as the dependent variable. Analyses are stratified by REST (baseline) and by tasks – GO (action selection), and STOP (response inhibition). For each Task (REST, GO, STOP) the factors of pulse-type (single-pulse or 3 ms paired-pulse) GABA+ level and the interaction of GABA+ and pulse type (indicated as GABA*Pulse-type) are tested and the full statistical model results including the effect size, standard error (SE), test-statistic (t), degrees of freedom (df) and statistical significance (p value) are reported. The arrow in the factor "single -> paired" indicates the direction of change in the estimate of the dependent variable, MEP amplitude (e.g., in the REST task, the −0.57 effect in the TD group indicates paired-pulse-evoked MEPs are 0.57 mV smaller than single-pulse-evoked MEPs). Across the full cohort, the factor "Group" interacted with GABA+ levels for REST MEPs only (p = 0.009), not GO or STOP (see results). The "Pulse-type" factor shows the effect of the paired-pulse versus the single- pulse, i.e. SICI. ADHD = attention deficit hyperactivity disorder, TD = typically developing, MEP = motor evoked potential, TMS = transcranial magnetic stimulation.

TD differences at rest (less SICI in ADHD) persisted in the action selection and inhibition trials (Gilbert et al., 2019). In a future study with a more difficult task, it is possible that, similar to REST in this study, there would be group differences in the GABA+/SICI associations during response selection and inhibition that reflect poorer performance.

More broadly, our study has implications for fundamental mechanistic conceptions regarding SICI, cortical interneurons, and GABA. A reasonable conceptual framework is that greater levels of inhibitory physiology in cerebral cortex might correspond to increased GABA (either metabolic or activity) (Di Lazzaro et al., 2012). This is supported empirically by studies that showed administration of GABA(A) agonists (benzodiazepines) increased SICI (Di Lazzaro et al., 2006; Ziemann et al., 2015). However, these pharmacological studies were single-dose probes, generally open-label, with small recruited samples of healthy adults. In addition to GABA(A) receptor function, we also cannot exclude the possibility that GABA(B) receptor mechanisms contribute to the relationship between brain GABA levels, cortical inhibition, and behavior. Further, the pharmacological administration of GABA may be expected to have a different impact on TMS-measure compared to resting GABA levels. Indeed, the three previously published studies evaluating the associations of SICI and GABA+ measured with MRS, with analyzable data from a total of 71 healthy adults, found little evidence to support a relationship between SICI and GABA+ (Dyke et al., 2017; Stagg et al., 2011; Tremblay et al., 2013). Data from one experiment in 11 healthy subjects suggested there might be a relationship between 1 ms SICI (evaluated via a slope of an input/output SICI curve) and higher GABA+ levels, though this relationship was not present with 2.5 ms SICI in the same study (Stagg et al., 2011). Thus, while it may seem intuitive that higher metabolic GABA+ would correlate with higher SICI-inhibition, there is little evidence to support any relationship between GABA+ and SICI in the adult literature. This project provides new information about the relationship between GABA+ measured using MRS and TMS-SICI in pediatric populations.

Our data suggests that, in contrast to the largely negative MRS-GABA+/TMS-SICI results in adults (Dyke et al., 2017; Stagg et al., 2011; Tremblay et al., 2013), in children, GABA+ measured using MRS, correlates significantly with SICI, but in the opposite direction than was expected. That is, for children ages 8–12 years, both those with ADHD and TD children, whether resting (REST) or in behavioral selection or inhibition (GO or STOP), higher MRS-GABA+ levels is associated with less TMS-SICI. Understanding this phenomenon requires considering separately the "numerator" (paired-pulse TMS-evoked MEP amplitudes) and the "denominator" (single-pulse TMS-evoked MEP amplitudes). This finding may indicate that among children with higher GABA+, the inhibitory conditioning pulse is "less efficient." As a result, the paired- and single-pulse TMS-evoked MEP amplitudes converge, and the calculated SICI ratio approaches 1.0, i.e. higher GABA+ results in reduced SICI. In Fig. 5, this is visualized as the convergence of the MEPs for the single- and paired-pulse TMS conditions as GABA+ levels increase. This robust and paradoxical finding in our sample of 81 children should induce further studies to provide empirical evidence for mechanistic conceptualizations of relationships of inhibitory interneurons to motor cortex excitability. It is reasonable to speculate that differences between results of our study and prior ones in adults may be due in part to differences in TMS or MRS techniques, ages of the participants, and statistical power in the small convenience-samples (Dyke et al., 2017; Stagg et al., 2011; Tremblay et al., 2013). Consequently, future studies in children, adolescents and adults should recruit larger samples and seek to identify effects of different experimental techniques.

Our analytic method of repeated measures within-subjects was critical to supporting these findings. Using the traditional calculation of SICI as a single value ratio of mean amplitudes identified a significant GABA+/SICI correlation in the same direction, but it obscures whether a statistical relationship with MRS-measured GABA+ corresponds to effects related to the paired pulses (the ratio numerator), single pulses (the denominator), or both. In addition, the traditional method of means does not account for inter-trial variability in MEPs, which may differ between subjects and between diagnostic groups. Here, critically, we found the same result in the correlational and regression analyses: More GABA+, Less SICI. However, the regression analysis is more informative. In analyzing all paired-pulse and single-pulse MEPs, the regression analysis shows that the SICI reduction with higher GABA+ levels shown in Fig. 4 results from a convergence of MEP amplitudes from the paired-pulse and single-pulse types that are both enlarging in the TD group but both are diminishing in the ADHD group (see Fig. 5A). It appears in both groups at REST that among children with higher GABA+ levels, there is less SICI specifically because there is less responsiveness to the paired-pulse, relative to the single-pulse as measured with TMS. In one study of 20 healthy adults, GABA+ levels in the motor cortex correlated with larger single TMS single-pulse evoked MEP amplitudes (Greenhouse et al., 2017). Our repeated-measures analysis of TD children (Fig. 5A,

red dashed curve) is largely consistent with the finding that GABA+ (as determined with GABA-edited MRS) may be linked to larger MEP amplitudes as seen in mature adults. The reason for this is unclear, but the speculation that the GABA+ pool (i.e., that measured with MRS) may provide a homeostatic mechanism that could offset a more excitable cerebral cortex (Greenhouse et al., 2017) is reasonable. This speculation may be consistent with our results, that with more GABA available the cortex is less excitable during action selection, as we saw in both GO and STOP trials.

Strengths of this multi-modal study include its relatively large sample size, recruitment with consistency of results across two cities, and highly detailed phenotyping of participants (Gilbert et al., 2019). Further, this is the only pediatric study combining these techniques and the only study of its kind in ADHD. Limitations of this study include a relatively small number of TMS trials per condition. A smaller number of trials would likely decrease our precision and lead to type II error, not type I error. Increasing the number of TMS trials per condition, particularly in resting motor cortex and during the stop trials, might yield more accurate estimates of cortical excitability. In addition, all of our action selection, or GO trials, occurred in the context of possibly needing to shift to inhibition. In future, it would be interesting to collect TMS data during GO-only trials first before engaging in any response inhibition. Our TMS measures are less precise for stop trials, due to the 3:1 GO to STOP ratio. Future studies might attempt to include additional trials; although in children, particularly with ADHD who have stopped medications, lengthening sessions is challenging. A second limitation is need to select an intensity of the paired-pulse; for the current study we used 60% RMT for the paired-pulse based on our prior studies as well as "dose effect" studies (Orth et al., 2003) and other ADHD studies (Hoegl et al., 2012). We recognize others sometimes use other parameters, which could impact the findings. Future studies may also consider interleaving TMS with paradigms probing other domains of impaired function besides response inhibition. Finally, a limitation of the GABA+-MRS measures is the necessity to acquire data from a relatively large voxel placed in the sensorimotor cortex to obtain sufficient signal-to-noise ratio to reliably measure GABA+ levels. This however, results in GABA+ measures in tissue beyond the primary motor cortex tissue targeted by TMS. While we correct for different types of tissue within the voxel of interest between individuals (i.e., cerebrospinal fluid, white matter and grey matter), we are unable to look at specific contributions from primary motor cortex.

In conclusion, we compared inhibition between ADHD and TD children by quantifying GABA+ levels in the cortex and examining the relationship between GABA+ levels and paired-pulse TMS-SICI measures at rest and during a Stop Signal Reaction Time task (which assesses behavioral response inhibition). Across both groups, we show that children with lower levels GABA+ show more TMS-evoked SICI at rest. Further, children with ADHD showed an anomalous association between of the single and paired-pulse MEPs and GABA+ level by task state when comparing the REST, GO, and STOP trials. The combined data provide, for the first time, clear physiological evidence for how GABAergic mechanisms might contribute to an abnormally highly excited, "less relaxed" REST state in ADHD, which further appears as differences in task-state and action selection. The findings may point us toward novel biomarkers with strong potential for guiding ADHD diagnosis and therapy.

Funding

This project was supported by funding from the National Institutes of Health; R01 MH078160, R01 MH085328 and R00MH107719. This study applies tools developed under NIH R01 EB016089 and P41 EB015909; RAEE also receives salary support from these grants.

Declaration of Competing Interest

Dr. Gilbert has received honoraria and/or travel support from the Tourette Association of America/ Centers for Disease Control and Prevention, the American Academy of Pediatrics, the Child Neurology Society, the Texas Neurological Society, and the American Academy of Neurology. He has received compensation for expert testimony for the U.S. National Vaccine Injury Compensation Program, through the Department of Health and Human Services, and for the US Armed Services/US Attorney's Office of VA. Dr. Gilbert has received research support from the NIH (NIMH, NINDS). He has received funding for work as a clinical trial site investigator from Ecopipam Pharmaceuticals (clinical trial, Tourette Syndrome) and EryDel (clinical trial, Ataxia Telangiectasia). He has received book royalties from Elsevier and Wolters Kluwer. Dr. Edden has received grants from Siemens.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2021.01.023.

References

Ashburner J, Friston KJ. Image Segmentation. In: Frackowiak RSJ, Friston KJ, Frith C, Dolan R, Friston KJ, Price CJ, editors. Human Brain Function, 2nd ed.. Academic Press; 2003.
Chen TH, Wu SW, Welge JA, Dixon SG, Shahana N, Huddleston DA, et al. Reduced short interval cortical inhibition correlates with atomoxetine response in children with attention-deficit hyperactivity disorder (ADHD). J Child Neurol 2014;29(12):1672–9.
Conners CK. Conners' Rating Scales-Revised. North Tonawanda, NY: Multi-Health Systems; 1997.
Conners CCK. Manual. 3rd ed. Toronto, Ontario, Canada: Multi-Health Systems; .
Coxon JP, Stinear CM, Byblow WD. Intracortical inhibition during volitional inhibition of prepared action. J Neurophysiol 2006;95(6):3371–83.
Crosbie J, Arnold P, Paterson A, Swanson J, Dupuis A, Li X, et al. Response inhibition and ADHD traits: correlates and heritability in a community sample. J Abnorm Child Psychol 2013;41(3):497–507.
Di Lazzaro V, Pilato F, Dileone M, Profice P, Ranieri F, Ricci V, et al. Segregating two inhibitory circuits in human motor cortex at the level of GABAA receptor subtypes: a TMS study. Clin Neurophysiol 2007;118(10):2207–14.
Di Lazzaro V, Pilato F, Dileone M, Ranieri F, Ricci V, Profice P, et al. GABAA receptor subtype specific enhancement of inhibition in human motor cortex. J Physiol 2006;575:721–6.
Di Lazzaro V, Profice P, Ranieri F, Capone F, Dileone M, Oliviero A, et al. I-wave origin and modulation. Brain Stimul 2012;5(4):512–25.
DuPaul GJ, Power TJ. ADHD Rating Scale-IV: Checklist, norms, and clinical interpretations. New York: Guilford Press; 1998.
Dyke K, Pepes SE, Chen C, Kim S, Sigurdsson HP, Draper A, et al. Comparing GABA-dependent physiological measures of inhibition with proton magnetic resonance spectroscopy measurement of GABA using ultra-high-field MRI. Neuroimage 2017;152:360–70.
Edden RA, Crocetti D, Zhu H, Gilbert DL, Mostofsky SH. Reduced GABA Concentration in Attention-Deficit/Hyperactivity Disorder. Arch Gen Psychiatry 2012;69(7):750–3.
Edden RA, Puts NA, Harris AD, Barker PB, Evans CJ. Gannet: A batch-processing tool for the quantitative analysis of gamma-aminobutyric acid-edited MR spectroscopy spectra. J Magn Reson Imaging 2014;40(6):1445–52.
Gilbert DL, Huddleston DA, Wu SW, Pedapati EV, Horn PS, Hirabayashi K, et al. Motor cortex inhibition and modulation in children with ADHD. Neurology 2019;93(6):e599–610.
Gilbert DL, Isaacs KM, Augusta M, Macneil LK, Mostofsky SH. Motor cortex inhibition: a marker of ADHD behavior and motor development in children. Neurology 2011;76(7):615–21.
Greenhouse I, King M, Noah S, Maddock RJ, Ivry RB. Individual Differences in Resting Corticospinal Excitability Are Correlated with Reaction Time and GABA Content in Motor Cortex. J Neurosci 2017;37(10):2686–96.
Guthrie MD, Gilbert DL, Huddleston DA, Pedapati EV, Horn PS, Mostofsky SH, et al. Online Transcranial Magnetic Stimulation Protocol for Measuring Cortical Physiology Associated with Response Inhibition. J Vis Exp 2018:e56789.
Harris AD, Puts NA, Edden RA. Tissue correction for GABA-edited MRS: Considerations of voxel composition, tissue segmentation, and tissue relaxations. J Magn Reson Imaging 2015;42(5):1431–40.

Harris AD, Saleh MG, Edden RA. Edited (1) H magnetic resonance spectroscopy in vivo: Methods and metabolites. Magn Reson Med 2017;77(4):1377–89.
Hoegl T, Heinrich H, Barth W, Losel F, Moll GH, Kratz O. Time course analysis of motor excitability in a response inhibition task according to the level of hyperactivity and impulsivity in children with ADHD. PLoS ONE 2012;7(9) e46066.
Kapur S, Phillips AG, Insel TR. Why has it taken so long for biological psychiatry to develop clinical tests and what to do about it?. Mol Psychiatry 2012;17 (12):1174–9.
Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. J Physiol 1993;471:501–19.
Moll GH, Heinrich H, Trott G, Wirth S, Rothenberger A. Deficient intracortical inhibition in drug-naive children with attention-deficit hyperactivity disorder is enhanced by methylphenidate. Neurosci Lett 2000;284(1–2):121–5.
Moll GH, Heinrich H, Trott G-E, Wirth S, Bock N, Rothenberger A. Children With Comorbid Attention-Deficit-Hyperactivity Disorder and Tic Disorder: Evidence for Additive Inhibitory Deficits Within the Motor System. Ann Neurol 2001;49 (3):393–6.
Mullins PG, McGonigle DJ, O'Gorman RL, Puts NA, Vidyasagar R, Evans CJ, et al. Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. Neuroimage 2014;86:43–52.
Near J, Edden R, Evans CJ, Paquin R, Harris A, Jezzard P. Frequency and phase drift correction of magnetic resonance spectroscopy data by spectral registration in the time domain. Magn Reson Med 2015;73(1):44–50.
Orth M, Snijders AH, Rothwell JC. The variability of intracortical inhibition and facilitation. Clin Neurophysiol 2003;114(12):2362–9.
Slater-Hammel AT. Reliability, accuracy, and refractoriness of a transit reaction. Res Q 1960;31:217–28.
Stagg CJ, Bestmann S, Constantinescu AO, Moreno LM, Allman C, Mekle R, et al. Relationship between physiological measures of excitability and levels of glutamate and GABA in the human motor cortex. J Physiol 2011;589:5845–55.
Tremblay S, Beaule V, Proulx S, de Beaumont L, Marjanska M, Doyon J, et al. Relationship between transcranial magnetic stimulation measures of intracortical inhibition and spectroscopy measures of GABA and glutamate +glutamine. J Neurophysiol 2013;109(5):1343–9.
Verbruggen F, Aron AR, Band GP, Beste C, Bissett PG, Brockett AT, et al. A consensus guide to capturing the ability to inhibit actions and impulsive behaviors in the stop-signal task. Elife 2019;8.:8.
Webb CA, Dillon DG, Pechtel P, Goer FK, Murray L, Huys QJ, et al. Neural Correlates of Three Promising Endophenotypes of Depression: Evidence from the EMBARC Study. Neuropsychopharmacology 2016;41(2):454–63.
Yousry TA, Schmid UD, Alkadhi H, Schmidt D, Peraud A, Buettner A, et al. Localization of the motor hand area to a knob on the precentral gyrus. A new landmark. Brain 1997;120:141–57.
Ziemann U, Reis J, Schwenkreis P, Rosanova M, Strafella A, Badawy R, et al. TMS and drugs revisited 2014. Clin Neurophysiol 2015;126(10):1847–68.