Title: GABA and Inhibitory Physiology in Dominant Motor Cortex: Altered Moderation of Response Inhibition in Children with ADHD

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ABSTRACT

In children with Attention Deficit/Hyperactivity Disorder (ADHD), a core domain of impaired cognitive control is response inhibition. We hypothesized that measures in dominant motor cortex might moderate efficiency of response inhibition. In 61 right-handed, 8-to-12-year-old children (ADHD n=28; typically developing controls (TDC) n=33), we evaluated 1) response inhibition, using an anticipated-response stop signal reaction time (SSRT) task; 2) left M1 inhibitory physiology, using Transcranial Magnetic Stimulation (TMS) to measure cortical silent period (CSP) duration; and 3) left M1 inhibitory neurochemistry, using Magnetic Resonance Spectroscopy (MRS) to measure gamma-amino butyric acid (GABA+) levels. Diagnostic groups were compared and relationships between SSRT and putative inhibitory moderators were modeled with regression and assessed with bivariate correlations. There were no significant diagnostic-group differences in SSRT, GABA+, or CSP; however, regression modeling showed a robust interaction of diagnosis and GABA+. Within the TDC group (only), better response inhibition (faster SSRT) is associated with both higher GABA+ levels and longer CSP duration in dominant motor cortex. This multi-modal study suggests response inhibition may be reflected by measures of inhibitory physiology and neurochemistry in motor cortex and, further, that these relationships may be disrupted in children with ADHD.

Key words

Neurophysiology
Neurotransmitters
Attention Deficit Hyperactivity Disorder
Cognitive Control
Transcranial Magnetic Stimulation
Magnetic Resonance Spectroscopy

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Introduction

Attention Deficit Hyperactivity Disorder (ADHD) encompasses heterogeneous symptom profiles involving developmentally inappropriate inattentive, hyperactive, and impulsive behaviors. It is diagnosed clinically based on ratings provided by multiple observers of the child[1] and is designated, categorically, as *present* or *absent.* While routine pharmacological treatment based on categorical diagnosis is effective in the short term,[2] negative adult occupational outcomes and psychiatric diagnoses such as substance abuse disorder remain common.[3]

Shortcomings of current treatment approaches may result from our limited knowledge of underlying mechanisms causing impairing symptoms. One approach to improving outcomes is to refocus efforts toward understanding the neurobiological substrate of impaired domains of function[4] and then to incorporate these findings into causal models. A starting point is to seek individual neuropsychological or neurobiological markers correlating with the presence or severity of ADHD. These might be useful in a variety of ways for precision treatment targeting or optimizing treatment doses. However, identifying biomarkers faces multiple challenges. Because ADHD's cardinal symptoms exist on a broad dimension, standard, case-control comparisons of laboratory assessed constructs or neurobiological measures usually yield overlapping findings. Applying a dimensional approach and correlating measures of interest with symptom severity can complement the standard case control approach. However, symptom correlations, which involve subjectively-rated ordinal scales, are moderate at best.[5] Finally, because underlying mechanisms contributing to ADHD are likely heterogeneous, identifying one, unifying biomarker is unlikely.[6]

An alternative approach that could transcend these multiple limitations and provide mechanistic understanding would be to incorporate categorical diagnoses, based on symptom ratings, plus measures of neuropsychological constructs in impaired domains, such as cognitive control or reward salience, with anatomically relevant, neurobiological measures[7, 8] into causal models based on a priori knowledge of neuroanatomy.[9] One widely studied, clinically relevant neuropsychological construct within cognitive control systems is response inhibition,[10] the ability to select to withhold (inhibit) actions that are inappropriate in a particular context.[11] For example, a child with ADHD may, compared to peers, more frequently act out impulsively, leading to injury and higher medical costs.[12] In the laboratory, impulsivity can be studied by evaluating response inhibition with Go/Nogo and Stop Signal Tasks, paradigms corresponding loosely to real world situations involving withholding impulsive, inappropriate responses.[11, 13] One version of this protocol which is well suited to neurobiological studies is the "anticipated response inhibition" stop-signal task.[14] In this task, participants are instructed to make a response at a predictable time, but to withhold this response if a stop cue appears.[8, 15] A short stop signal reaction time (SSRT) indicates efficient response inhibition.[16]

The executive control processes underlying response inhibition are mediated by inhibitory neurotransmission in frontal-subcortical circuits,[17] while the final behavioral output, the action, occurs via descending volleys of nerve action potentials from motor cortex (M1) to muscles.[14] Considering the M1 as a critical, final node in behavioral output network, we and others have used transcranial magnetic stimulation (TMS) to study M1 physiology in children with behavioral disorders, attempting to quantify features of inhibitory networks that reliably reflect clinical symptoms, treatment responses, and associations with domains of impaired

function.[18-20] Based on hypotheses that altered inhibitory physiological properties projecting to or within the motor cortex node might tend to induce disinhibited behavior in ADHD, TMS researchers have focused primarily on paired-pulse TMS-evoked short interval cortical inhibition (SICI).[21, 22]

SICI is a property of inhibitory interneurons in motor cortex for which evidence suggests the inhibitory neurotransmitter GABA acts via ionotropic GABA-A receptors.[23, 24] In a large, ongoing study of children, we reported that SICI measured at rest was reduced in children with ADHD[21] and correlated with both hyperactive and inattentive symptom severity.[22, 25] Supporting a SICI/GABA relationship, in both children with ADHD and typically developing controls, SICI correlated with GABA+ concentration, measured with edited Magnetic Resonance Spectroscopy (MRS), in M1.[26] To probe this relationship further, we and others have measured M1 SICI during a response inhibition task. Although, similar to the resting state findings, SICI was also reduced in children with ADHD during response inhibition,[25, 27] we failed to find any association of SICI with SSRT. This result differs from one reported in 40 adults, in which reduced SICI correlated with slower SSRT.[28]

Another potential candidate for causal modeling of SSRT is the M1 cortical silent period (CSP). CSP is a property of inhibitory interneurons measured using TMS in tonically activated motor cortex. Evidence suggests CSP duration is linked to actions of GABA via slower, ionotropic, G-protein dependent GABA-B receptors.[29, 30] Although, unlike SICI, CSP has not been found to differ robustly in ADHD or correlate with clinical symptom severity,[21, 22] it is easily measurable in children and, as an inhibitory measure, might reflect critical mechanisms necessary for performance of the stop signal task. Therefore, the primary objective of the present study is to evaluate, in children with ADHD and typically developing peers, relationships

between response inhibition, specifically the stop signal reaction time, and two measures in dominant motor cortex: 1) CSP, and 2) GABA+ levels. Identification of such relationships might create a pathway toward a causal model that could illuminate mechanisms of impaired function in ADHD.

Methods

Subjects, Clinical Assessments

Participants in this study were recruited as part of an ongoing study of motor function, motor physiology, and inhibitory neurochemistry in pre-pubertal, right-hand dominant children with ADHD.[20, 26, 27] In brief, children ages 8 years 0 months to 12 years, 11 months were recruited via advertisement at two urban medical centers, from 2011 to 2019. Written informed consent was obtained from the legal guardians of study participants. The study was conducted in accordance with the declaration of Helsinki and approved by the Johns Hopkins Medicine and Cincinnati Children's Hospital Medical Center Institutional Review Boards. All children underwent IQ testing[31] and structured diagnostic interviews.[32] To be included, children had to have full scale IQ > 80, no reading disability, and no serious medical, developmental, or psychiatric diagnoses (except ADHD with or without co-occurring Oppositional Defiant Disorder). ADHD diagnosis was confirmed with available clinical information and ADHD Rating Scale IV - Parent (ADHD-RS-IV)[33] and clinical interview. To enhance generalizability of the clinical cohort, children with ADHD prescribed stimulant medication were included. However, stimulant medication treatment was paused the day prior to and day of the study visit. No other neuropsychiatric medications were permitted.

Response inhibition

Response inhibition was assessed using the Slater-Hammel paradigm, an anticipated response inhibition task,[15] which we modified into a child-friendly race car version.[8] In brief, participants faced a computer monitor running the task in Presentation® (v. 10.0, Neurobehavioral Systems, Albany, CA) while seated in a comfortable chair. Ulnar aspects of both arms and hands rested fully on a body-surrounding pillow (the Boppy Company, LLC) so the palmar surface faced medially. The dominant (right) hand operated a game controller with a fully extended index finger. In each trial, the participant initiated and maintained race car movement by pushing down the finger. The anticipated response ("GO") of this task was lifting the finger as the car reached the 800 ms mark. At random, in one out of 4 trials, the car stopped spontaneously, initially at the 500 ms mark. The response inhibition ("STOP") of this task was withholding lifting the finger past the 800 ms mark until the appearance of a checkered flag at 1000 ms. The difficulty of stopping was adjusted in 50 ms increments based on success or failure. For example, after a failure-to-stop, the next stop trial was 50 ms earlier, giving the participant more time to recognize and inhibit the anticipated response (the finger lift at 800 ms). Thus, the stop cues converged to a time indicating response inhibition efficiency.[8, 15] Over a 40 trial series, the SSRT was calculated from the difference between the mean Go and Stop cue times. We previously reported that Go times were slower and more variable in ADHD, but SSRTs did not differ, allowing us to evaluate at the diagnostic group level M1 physiological predictors or correlates with the same level of performance.

Transcranial Magnetic Stimulation

TMS measures were performed in accordance with accepted safety standards for pediatric use.[34, 35] All protocols were implemented consistently at both sites and were well

tolerated by participating children.[36] Motor cortex physiology was evaluated prior to playing the race car game, with the hand fully at rest, with continuous online monitoring, using identical Magstim 200® TMS machines connected through a Bistim® module to a round 90 mm coil (Magstim Co., New York, NY, USA). EMG data was collected using surface electrodes from the first dorsal interosseous muscle (the muscle activated or inhibited during the race car game), using identical amplifiers, filter settings, and Signal® processing software. TMS coil placement was flat at the vertex, with the induced current flowing in clockwise direction and the coil handle directly posterior. All protocols for active and resting motor thresholds (AMT, RMT),[37] cortical silent periods (CSP),[38] and paired pulse TMS for SICI[24] are in standard use, implemented by our laboratories as previously described.[22, 27] In brief, CSP was measured in strongly contracted muscle by 5 separate pulses administered at an intensity of 1.5*AMT at 0.1 Hz. Trials were rectified and averaged. Onset and offset of CSP was defined visually by one researcher, blinded to diagnosis, and duration was recorded in ms. SICI was measured using 10 conditioning (preceding) pulses with an intensity of 0.6*RMT paired with test pulses with an intensity of 1.2*RMT mixed randomly with 10 single test pulses. The interstimulus interval for pairs was 3 ms, the intertrial interval for single and pairs was 6 +/- 10% seconds.

MRS acquisition and analysis of GABA

Imaging parameters were identical for both sites, as previously described.[26, 39-41] In brief, a Philips 3T Achieva MRI scanner (Best, the Netherlands) with a 32-channel head coil was utilized. First, a 1 mm3 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 from a voxel placed over left sensorimotor cortex. 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'. Spectra were frequency- and phase corrected, filtered with Hz exponential line broadening and zero-filled to 32,768 points. GABA+ levels were estimated using a five parameter Gaussian model and were calculated relative to the unsuppressed water signal from the same voxel. For each individual, GABA+ levels were tissue-corrected for tissue specific relaxation values. Tissue fractions were group normalized across all data to be representative of 40% grey matter and 60% white matter voxel contents. Reported GABA+ values refer to GABA+ co-edited macromolecules.

Statistical Analysis

All models and correlations were performed using SAS^R statistical software version 9.4 (SAS Institute Inc., Cary, NC). The primary objective of this study was to estimate the relationship of CSP to SSRT and compare this by diagnosis. Therefore, SSRT was regressed over CSP and Diagnosis, accounting for age, sex, and study-site, for the full cohort and then stratifying separately within diagnostic groups. GABA+ levels were included in the regression. Models were calculated with and without CSP*Diagnosis and GABA+*Diagnosis interaction terms. Post hoc, simple bivariate correlations, adjusted for age, were calculated between 1) SSRT; 2) CSP duration; and 3) GABA+ levels. As these correlations were exploratory, all were performed conservatively using the Spearman (non-parametric) statistic, with $p < 0.05$ considered

significant, without adjustment for multiple comparisons. As for regression, these were performed in the full cohort as well as separately by diagnosis.

Results

Participants

Demographic, clinical, physiological, and behavioral variables were compared by group (see Table 1). Participants included 61 children ages 8-12 years (28 ADHD, 33 TDC). Demographic characteristics were well-matched. As expected, ratings for ADHD symptoms were significantly greater in ADHD for all scales, $p < 0.0001$. Experimental measures did not differ; however, CSP was longer in the TDC group, at the trend level ($p = 0.067$).

Table 1 Demographic characteristics, clinical ratings, and experimental measures

Characteristic	N	ADHD, n = 28[1]	TDC, n = 33[1]	p-value[2]
Sex	61			>0.9
Female		9 (32%)	12 (36%)	
Male		19 (68%)	21 (64%)	
Race	61			0.3
African American		7 (25%)	4 (12%)	
Asian		1 (3.6%)	4 (12%)	
Biracial		4 (14%)	3 (9.1%)	
Caucasian		16 (57%)	22 (67%)	
Ethnicity	61			0.8
Hispanic		3 (11%)	2 (6.1%)	
non-Hispanic		25 (89%)	31 (94%)	
Age	61	10.7 (1.4)	10.2 (1.2)	0.15
ADHD Scales				
Conners: Inattentive	60	75.9 (12.5)	48.3 (9.6)	<0.001
Conners: Hyper/Impulsive	60	75.5 (15.9)	48.4 (7.2)	<0.001
ADHD-RS: Inattentive	59	20.3 (4.4)	3.9 (2.9)	<0.001
ADHD-RS: Hyper/Impulsive	59	13.6 (7.4)	3.1 (2.5)	<0.001
ADHD-RS: Total	59	33.9 (10.2)	7.0 (4.7)	<0.001
Experimental Data				
SSRT (ms)	61	240.6 (58.7)	245.6 (58.3)	0.7
CSP (ms)	57	63.4 (46.1)	86.5 (45.6)	0.067
Left M1 GABA+ (iu)	61	2.78 (0.52)	2.93 (0.51)	0.2

ADHD = Attention Deficit/Hyperactivity Disorder; TDC = typically developing controls. RS = rating scale. SSRT = stop signal reaction time in milliseconds.; CSP = Cortical Silent Period in milliseconds. M1 = motor cortex. GABA+ indicates gamma amino butyric acid levels, international units (see methods).

Regressions

SSRT Full cohort, not accounting for diagnosis With SSRT as the dependent variable, associations of left motor cortex CSP duration and GABA+ concentrations were evaluated together in the full cohort (see Table 2). Age, sex, and city were included in all models but had no effect (not shown). There was no strong evidence of association of SSRT with either CSP duration ($p = 0.076$) or GABA+ ($p = 0.96$).

*SSRT Full cohort, model accounting for diagnosis and Diagnosis*CSP interaction* This model evaluated whether a CSP association with SSRT might differ by diagnosis, while accounting for GABA+. There was no primary effect of diagnosis ($p = 0.20$) or GABA+ ($p = 0.74$) on SSRT. However, better (shorter) SSRT was marginally associated with longer CSP ($p = 0.044$). There was no interaction of the CSP duration association by diagnosis (Dx*CSP $p = 0.43$).

SSRT Full cohort, accounting for diagnosis, modeling GABA+ interaction This model evaluated whether a GABA+ effect on SSRT might differ by diagnosis, while accounting for CSP. In this model, better (shorter) SSRTs were marginally associated higher M1 GABA+ concentration ($p = 0.047$) as well as with longer CSP ($p=0.019$). A primary effect of diagnosis was now highly significant ($p = 0.0052$), as was the interaction of diagnosis with GABA+ levels (Dx*GABA+ $p = 0.0086$).

Table 2. Regression models of associations of cortical silent period and motor cortex GABA+ with Stop Signal Reaction Time

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Full Cohort					
Intercept	266.21	43.35	54	6.14	<.0001
CSP	-0.30	0.16	54	-1.81	0.0763
GABA+	-0.77	14.49	54	-0.05	0.9579
Include Diagnosis in model					
Interaction Model: Dx|CSP + GABA+					
Intercept	299.73	49.50	52	6.06	<.0001
Dx	-39.33	30.07	52	-1.31	0.1966
CSP	-0.46	0.22	52	-2.07	0.0435
Dx*CSP	0.28	0.34	52	0.8	0.4246
GABA+	-4.87	14.78	52	-0.33	0.7432
Interaction Model: Dx|GABA+ + CSP					
Intercept	390.29	58.10	52	6.72	<.0001
Dx	-235.67	80.86	52	-2.91	0.0052
CSP	-0.39	0.16	52	-2.41	0.0194
GABA+	-37.99	18.68	52	-2.03	0.0472
Dx*GABA+	76.30	27.96	52	2.73	0.0086

CSP=Cortical Silent Period, measured in dominant motor cortex using transcranial magnetic stimulation. Dx=Diagnosis. GABA+ Gamma amino butyric acid, measured in left sensorimotor voxel using magnetic resonance spectroscopy.

Correlations

In the full cohort, SSRT did not correlate with CSP or GABA+ concentrations. Stratifying by diagnosis showed that within TDC, shorter (better) SSRT correlated marginally with longer CSP ($p = 0.03$) and significantly with higher GABA+ ($p = 0.008$). While correlations were not significant within the ADHD group, there was a suggestion ($p = 0.10$) of an inverted SSRT GABA+ relationship compared to TDC. GABA+ did not correlate with CSP (see Table 3, Figure 1). Correlations were recalculated with outliers removed. Significance was not affected (see supplemental appendix).

Table 3. Bivariate correlations with SSRT

Measures correlated with SSRT		ALL	ADHD	TDC
CSP	n	61	28	33
	r	-0.21	-0.014	**-0.38**
	p	0.12	0.95	**0.03**
GABA+	n	61	28	**33**
	r	-0.08	0.32	**-0.46**
	p	0.54	0.10	**0.008**

All measures in left motor cortex (M1). *TMS (Transcranial Magnetic Stimulation) measures:* CSP cortical silent period duration. Magnetic Resonance Spectroscopy (MRS) measure: GABA+ gamma amino butyric acid concentration. SSRT is stop signal reaction time. Number is n; Spearman correlation is r. A negative correlation indicates association with shorter (better) SSRT.

Figure 1. Bivariate relationships between Experimental Measures.

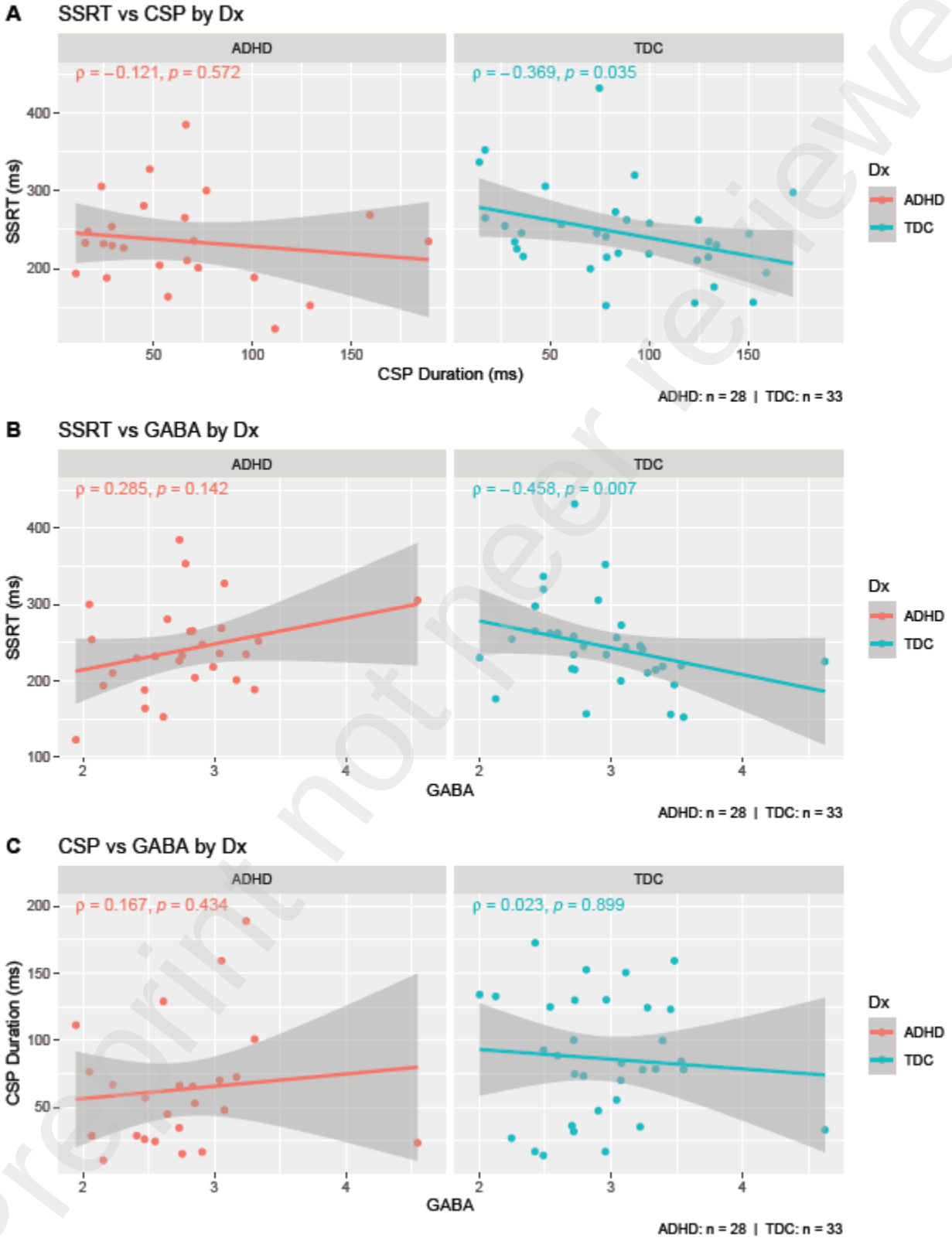
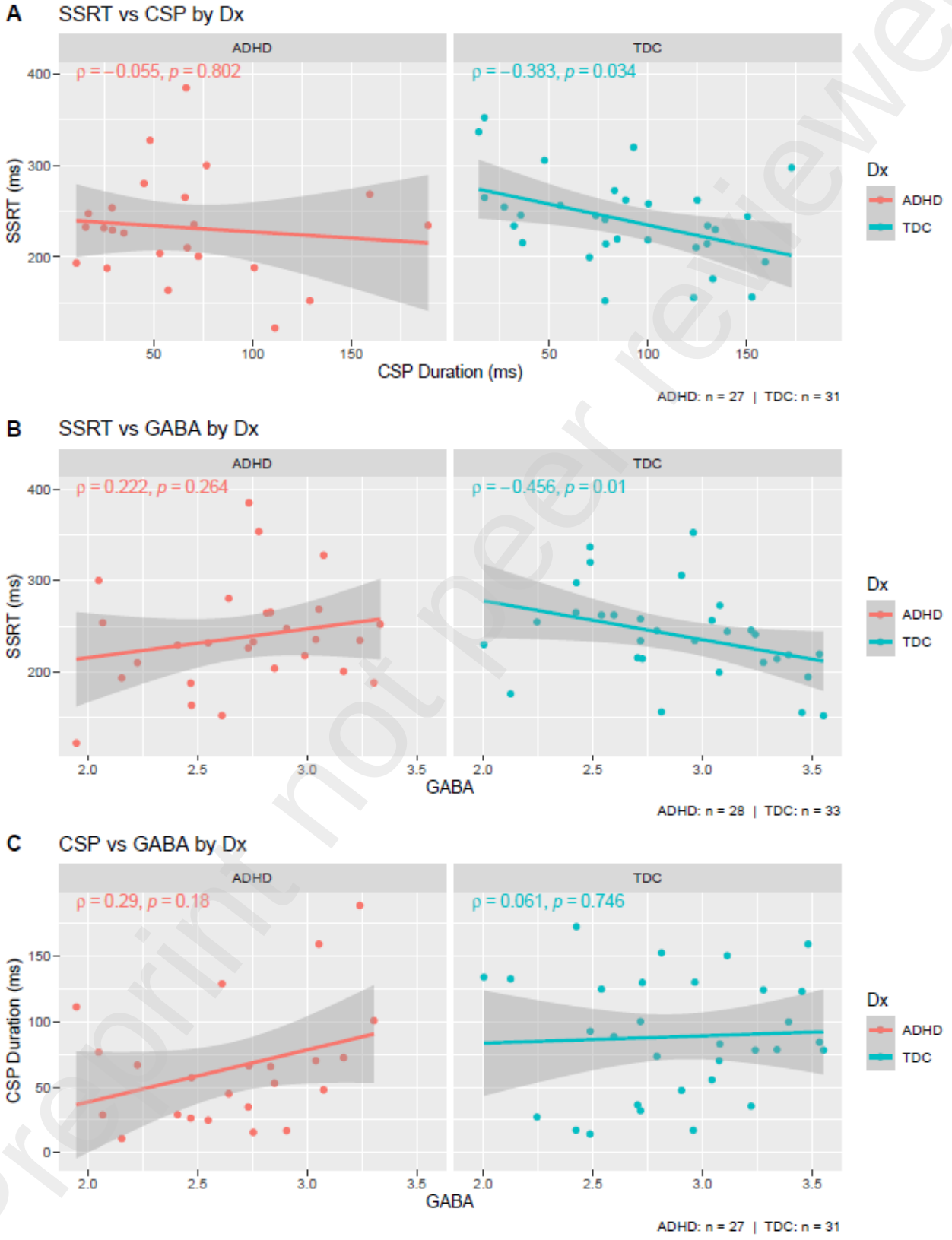


Figure 1 Legend. Bivariate Pearson correlations for experimental variables in children with ADHD (attention deficit hyperactivity disorder) and TDC (typically developing controls). SSRT = Stop Signal Reaction Time (longer is less efficient). Ms = milliseconds. CSP = cortical silent period. GABA = gamma amino butyric acid levels in international units.

Supplemental Figure 1: Bivariate relationships between experimental variables, outliers removed



DISCUSSION

This study provides preliminary evidence that more of two inhibitory properties in domiant motor cortex, i.e. longer cortical silent periods (CSPs) and higher available GABA, correlates with more efficient response inhibition in typically children (TDC). That is, the efficiency of responding and withholding responses, as indexed by the SSRT, may be moderated independently by the available inhibitory neurotransmitter GABA and by the physiological properties responsible for the cortical silent period. While these associations between behavioral inhibition and neurophysiological and neurochemical measures of inhibition were present in typically developing children, we failed to find evidence of this association in children with ADHD.

This result requires cautious interpretation. The construct of response inhibition can be assessed in various ways, and the anticipated response version of the stop signal task employed in this study may not generalize to other response inhibition tasks or to behavioral inhibition in the natural environment. Moreover, only a fairly small number of trials, forty per participant, was used to estimate SSRT and its associations with CSP and GABA.[27] However, if we assume that faster SSRTs in these 61 children provide some metric of better cognitive, inhibitory control, it is notable to find associations with two inhibitory biomarkers in motor cortex. As we did not find evidence that these biomarkers correlate with each other, they may index processes that contribute independently to response inhibition.

One possible explanation for independent, uncorrelated biomarkers each relating to the same behavioral task is that the stop signal task involves multifaceted interplay between efficiency and speed of the go action, attentiveness and recognition of the stop cue, and capacity for response-withholding.[16] Future investigations should attempt to differentiate relationships

between physiological markers and specific components of this or other response inhibition tasks. For example, the relatively slower, GABA-B, G-protein coupled receptor system believed to underlie CSP might play a critical role in performance of the anticipated response – the finger lift at 800 ms. In contrast, higher GABA+ concentrations in the voxel may indicate tonically available inhibitory substrate deployed for rapid implementation of cued, response inhibition.

To our knowledge, this is the first study to explore these relationships in the developing child. Generally, our GABA findings are consistent with adult studies using MRS in the elderly and in population s with dementia, showing reduced GABA correlates with worse (slower) SSRTs.[42] In contrast, our CSP findings are not consistent with a recent report in 27 healthy adults showing worse (slower) SSRTs with longer CSPs.[43, 44] Multiple reasons, including the nature of the response inhibition paradigm, the age of the participants, and the TMS technique, could all contribute to this difference. However, our CSP findings harmonize with those in several studies which administered repetitive TMS and reported post-treatment lengthening of the CSP duration.[45-48] That is, TMS treatment protocols aiming to improve various neuropsychiatric symptoms have tended to increase the duration of this form of cortical inhibition. Thus, CSP duration might serve as a surrogate predictor or correlate of beneficial changes in response to repetitive TMS. While the association of CSP was less robust than that of GABA+ levels, it has another substantial advantage: it can be measured much more quickly in the same room, using the RTMS equipment.

Limitations of this study include the possibility of diagnostic misclassification and imprecise assessment of SSRT. However, imprecision should bias findings toward the null, whereas we found differences in associations of both biomarkers and SSRT between ADHD (no or inverted correlations) and typically developing children (consistent correlations between more

inhibition and better performance). Another possibly important factor is method of TMS. The intensity we selected for CSP, indexed to active motor threshold (AMT), has been utilized in multiple other studies.[21, 29, 30] However, AMT assessment can be technically challenging (evaluating gradual disappearance of small MEPs on a background of muscle activity). Another reasonable approach might be to index CSP to RMT and evaluate several intensities, e.g. 1.0*RMT (which is close to 1.5*AMT used in this study) and 1.2*RMT. Higher intensities induce longer CSPs, resulting from greater engagement of cortical inhibitory function.[30] A larger CSP might create a possibility for more sensitive correlation across a range of SSRT values. However, higher intensity stimulation can be challenging in young children for multiple technical and practical reasons.

In summary, this preliminary evaluation suggests that CSP, measured with TMS in dominant motor cortex, and GABA+ concentration, measured with MRS, may independently reflect inhibitory cortical capacities engaged during response inhibition. This supports the possibility that these inhibitory properties in motor cortex moderate response inhibition performance. These relationships appeared to be disrupted in children ages 8 to 12 years diagnosed with ADHD. While the magnitudes of these associations were modest, if replicated, these could, in combination with other measures, contribute to multi-dimensional assessments to enhance our understanding of the mechanisms of impaired response inhibition and identify clinically meaningful diagnostic subgroups for treatment studies.

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