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Heart Rate Variability and Skin Conductance During Repetitive TMS Course in Children with Autism

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Abstract Autism spectrum disorder (ASD) is a developmental disorder marked by difficulty in social interactions and communication. ASD also often present symptoms of autonomic nervous system (ANS) functioning abnormalities. In individuals with autism the sympathetic branch of the ANS presents an over-activation on a background of the parasympathetic activity deficits, creating an autonomic imbalance, evidenced by a faster heart rate with little variation and increased tonic electrodermal activity. The objective of this study was to explore the effect of 12 sessions of 0.5 Hz repetitive transcranial magnetic stimulation (rTMS) over dorsolateral prefrontal cortex (DLPFC) on autonomic activity in children with ASD. Electrocardiogram and skin conductance level (SCL) were recorded and analyzed during each session of rTMS. The measures of interest were time domain (i.e., R–R intervals, standard deviation of cardiac intervals, NN50-cardio-intervals >50 ms different from preceding interval) and frequency domain heart rate variability (HRV) indices [i.e., power of high frequency (HF) and low frequency (LF) components of HRV spectrum, LF/HF ratio]. Based on our prior pilot studies it was proposed that the course of 12 weekly inhibitory low-frequency rTMS bilaterally applied to the DLPFC will improve autonomic balance probably through improved frontal inhibition of the ANS activity, and will be manifested in an increased length of cardiointervals and their variability, and in higher frequency-domain HRV in a form of increased HF power, decreased LF power, resulting in decreased LF/HF ratio, and in decreased SCL. Our post-12 TMS results showed significant increases in cardiac intervals variability measures and decrease of tonic SCL indicative of increased cardiac vagal control and reduced sympathetic arousal. Behavioral evaluations showed decreased irritability, hyperactivity, stereotype behavior and compulsive behavior ratings that correlated with several autonomic variables.

Keywords Autism spectrum disorder · Autonomic activity · Transcranial magnetic stimulation (TMS) · Heart rate · Heart rate variability · Skin conductance level

Introduction

Autism spectrum disorder is a pervasive developmental disorder of childhood characterized by deficits in social interaction, language, stereotyped behaviors, and restricted range of interests. Symptoms of autonomic dysfunctions are also often found in individuals with ASD diagnosis. The electrophysiological correlates of autonomic dysfunctions in ASD at the resting baseline and during exposure to emotional stimuli are not sufficiently well explored. Though interest to autonomic regulation deficits in autism recently started attracting substantial interests of developmental researchers (Cohen et al. 2000; Klusek et al. 2015; Kushki et al. 2013; Lydon et al. 2014; Patriquin et al. 2013a, b; Schaaf et al. 2015; Smeekens et al. 2015). Our group conducted several pilot studies with affective stimulation where autonomic dysfunctions typical for autism were explored using psychophysiological techniques (Dombroski et al. 2013; Casanova et al. 2014; Hensley et al. 2012, 2013). Important question related to autonomic

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regulation abnormalities in autism is whether there are any applied psychophysiology methodology based techniques that may improve autonomic activity in autism. Non-invasive brain stimulation using either magnetic pulses or electrical currents can be considered as viable candidates for such treatment approaches. Among the newly emerging neuromodulation techniques, repetitive transcranial magnetic stimulation (rTMS) is one of the most promising for the treatment of core symptoms in autism. TMS offers a non-invasive method for altering excitability of the neural circuits and induction of a short-term functional reorganization in the human cortex. TMS is a suitable tool for investigation and modulation of neural plasticity due to its ability to not only stimulate the target cortex, but also induce functional changes in cortical and subcortical areas anatomically and functionally associated with the stimulated regions. In several recent publications we demonstrated positive behavioral, clinical and electrophysiological functional outcomes of rTMS in autism (Baruth et al. 2010, 2011; Casanova et al. 2012, 2014, 2015; Sokhadze et al. 2012, 2014).

Many children with autism exhibit symptoms that should be considered as associated with autonomic nervous system (ANS) function abnormalities. In autism, the ANS dysfunction includes blunted cardiac responses to visual and auditory social stimuli (Hirstein et al. 2001; Palkovitz and Wiesenfeld 1980; Porges 2001; Zahn et al. 1987). Ming et al. (2005, 2011) reported evidence of reduced baseline parasympathetic activity in autistic children in association with evidence of increased sympathetic tone. Reduced cardiac vagal tone, decreased baroreflex sensitivity, and labile respiratory rhythm in autism were reported by Julu et al. (2001). Evidence of more recent studies supports autonomic activity dysregulation in majority of individuals with autism, as evidenced by patterns of sympathetic hyperarousal and dampened parasympathetic vagal tone (Klusek et al. 2015; Patriquin et al. 2013a, b). Heart rate variability (HRV) measures represent reliable assessment of cardiac autonomic responses (Berntson et al. 1997; Cohen et al. 2000; Porges 2003). It should be noted that reduced HRV indicative of limited psychophysiological flexibility is not specific marker for autism, it was found in various psychopathologies (Cohen et al. 2000; Movius and Allen 2005; Shahrestani et al. 2014; Thayer and Friedman 2002; Thayer and Lane 2009). Nevertheless, lower phasic and tonic HRV observed in autism worth further investigation. There are indications that autonomic activity dysfunctions may be related to social functioning in individuals with ASD. Smeekens et al. (2015) explored the relationship of autonomic activity with social functioning in young adult males with ASD compared to young adult males without ASD during rest and social interaction. The ASD group was showing a blunted increase in heart rate (HR) and HRV from resting baseline to social interaction as compared to those without ASD.

Cardiac reactivity difference between autistic and typically developing children usually is more pronounced during sensory stimulation of various modalities (Chang et al. 2012; Schaaf et al. 2015). Stereotyped and repetitive motor behaviors, one of the core features of autism, have been proposed as a response to reduce hyper-responsive sympathetic activity (Toichi and Kamio 2003). Dysfunctions in the parasympathetic system negatively affect social behavior in children with ASD by impacting HR modulation. The inhibitory parasympathetic, n. vagus, acts as a brake and slows HR (Porges 2001, 2003). Functionally, the vagal "brake", which modulates HR, enables rapid engagement and disengagement with objects and people, a skill important for promoting social behaviors (Porges 2001, 2003).

Spectral analysis of HRV represents a measure commonly used in psychopathology research (Cohen et al. 2000; Thayer and Friedman 2002) for assessment of cardiac autonomic control (Berntson et al. 1997). Reduced HRV, and in particular attenuated power of high frequency (HF) component of the HRV (index of parasympathetic control) is an indicator of limited psychophysiological flexibility (Berntson et al. 1997; Cohen et al. 2000; Friedman and Thayer 1998; Movius and Allen 2005; Thayer and Lane 2000). Deficits in the modulation of the HRV in HF range (i.e., respiratory sinus arrhythmia, RSA) in different social tasks have been found in autism. Typical children suppressed the HF in HRV more than children with autism (Althaus et al. 1999; Hutt et al. 1975). Autistic children, on the other hand, demonstrated dampened HR reactivity; they showed unusually small deceleratory HR responses and generally low cardiac reactions to auditory stimulation including socially relevant speech, phrases and tones (Chang et al. 2012; Corona et al. 1998; Kushki et al. 2013; Palkovitz and Wiesenfeld 1980; Zahn et al. 1987).

Electrodermal activity (EDA) refers to changes in the electrical activity of the skin. EDA is controlled by sympathetic branch of the ANS (Boucsein 2012), and is therefore often used as a non-invasive measure of autonomic function. EDA can be measured in terms of skin conductance response magnitude, non-specific skin conductance response (NSSCR) frequency, and skin conductance level (SCL). Skin conductance response (SCR) studies in autism have shown a lack of the normal habituation in the SCR to the same stimulus over time (Barry and James 1988; van Engeland 1984). Children with autism had blunted HR and SCR responses to visual or auditory social stimuli (Angus 1970; Hirstein et al. 2001; Palkovitz and Wiesenfeld 1980). Higher basal tonic electrodermal activity as well as larger SCRs to sounds was observed in autistic children compared to controls (Barry and James

1988). In general, majority of studies dedicated to the relationship between autism and the ANS activity abnormalities have also found manifestations of increased sympathetic activity reflected in EDA measures (Dombroski et al. 2013). SCL is controlled solely by the sympathetic inputs (Boucsein 2012); therefore, the above findings are indications of high sympathetic tone and low selectivity of ANS responses in autism.

Several neuropathological studies developed by our group have characterized the ASD as the minicolumnopathy (Casanova et al. 2002, 2006). Our previous research suggests that there is a greater number of minicolumns in the cortex of an individual with ASD, accompanied by increased neuronal density and reduced neuropilspace (periphery of the minicolumn) (Casanova et al. 2002; Casanova 2006). This leads to the decrease of the cortical inhibitory elements contained in the neuropil space. And further this lack of inhibitory elements cause the imbalance between inhibition and excitation in brain cortex and is thought to be the reason of dysfunction of sensory processing and overexcitement in ASD. TMS offers a non-invasive neuromodulation for altering the cortical excitation/inhibition balance. Repetitive TMS (rTMS) has been applied on several neurological or psychiatric conditions, such as depression, bipolar disorders, panic etc. (Kobayashi and Pascual-Leone 2003; Ridding and Rothwell 2007). Studies have pointed out that low frequency (LF) rTMS (<1 Hz) increases inhibition of stimulated cortex, whereas high-frequency rTMS (>5 Hz) increases excitability of stimulated cortex (Pascual-Leone et al. 2000). This point of view is reconsidered as a certain simplification, as some studies consider the frequency of TMS as less important factor compared to other factors related to ability to change functional connectivity in the brain (Fitzgerald et al. 2011; Khedr et al. 2008).

In the case of ASD, the inhibitory elements located at the periphery of the minicolumn will be induced by the magnetic field applied tangentially to the cortex. In particular, Casanova et al. (2012, 2015) proposed that contrary to other inhibitory cells (i.e., basket and chandelier), whose projections keep no constant relation to the surface of the cortex, the geometrically exact orientation of double-bouquet cells and their location at the periphery of the minicolumn (inhibitory surround) makes them an appropriate candidate for induction by a magnetic field applied parallel to cortex. Over a course of treatment, slow rTMS may selectively depotentiate enhanced synaptic weights associated with pathological conditions, and in the case of overactivated cortical structures in ASD it may lower the ratio of cortical excitation to cortical inhibition. The proposed mechanism of rTMS effects on autonomic arousal may include improved tonic fronto-limbic inhibitory influences known to be deficient in autism (Loveland et al. 2008). The limbic system is a complex network of structures central to anxiety and mood regulation (Mayberg 2003). According to recent research studies, frontal cortical areas are involved in ANS control and increased frontal cortical excitability is thought to be a cause of some of the symptoms of ASD. However, few studies have investigated the effects of rTMS on the ANS. In most of our studies using rTMS based intervention in children with autism, we had enrolled only children with high-functioning autism (HFA), as the main functional outcome measures were event-related potential (ERP) and evoked and induced gamma frequency oscillations during performance on selective attention tasks before and after rTMS course (Baruth et al. 2011; Sokhadze et al. 2009, 2012).

In one recent pilot study on a relatively small sample size we reported positive autonomic activity changes in a group of HFA children during 18 session long rTMS course (Casanova et al. 2014). Our current study targeted investigation of effects of intervention that combines rTMS and autonomic monitoring in a pool of children with ASD that included both high and relatively low functioning children, with the only restriction for enrollment was their ability to tolerate rTMS procedure and physiological monitoring. One of the reasons for this choice was the observation that lower functioning children with autism diagnosis are reported to have more clear presentation of autonomic dysregulation symptoms, and more likely to have more prominent changes resulting from neuromodulation.

Therefore, the aim of this research project was to observe the effects of 12 sessions of rTMS on autonomic functions in children with ASD. We hypothesized that 12 sessions of slow rTMS stimulation applied to the DLPFC will have a positive effects on behavioral clinical measures similar to those reported in our prior studies (Baruth et al. 2010; Casanova et al. 2012; Sokhadze et al. 2010, 2014). We predicted that the proposed rTMS therapy would provide for improvements in irritability, hyperactivity and repetitive behavior rating scales on the aberrant behavior checklist (ABC) (Aman and Singh 1994) and Repetitive Behavior Scale (RBS) (Bodfish et al. 1999). We expected that the behavioral improvement will be also manifested in autonomic measures, in particular, lower sympathetic arousal and normalized cardiac autonomic balance. Specifically, HRV and SCL measurements were used to track changes in autonomic balance across the whole course of neuromodulation treatment. We chose to use HRV and SCL as indicators of the effectiveness of rTMS treatment because they are largely controlled by the ANS. The expected outcomes were an increase in average cardiointervals (R–R intervals) from session 1 to session 12, an increase in standard deviation of STDRR increase in pNN50 (percentage of cardiointervals >50 ms different from preceding cardiointerval) across 12 sessions of rTMS,

an increase in the power of HF component of HRV, a decrease in the power of LF component of HRV, that should result in decreased LF/HF ratio, as well as decreased SCL across the course of rTMS.

Methods

Subjects

Participants with autism diagnosis were recruited through the University of Louisville Weisskopf Child Evaluation Center (WCEC). Diagnosis was made according to the DSM-IV-TR (APA 2000) and further ascertained with the Autism Diagnostic Interview-Revised (ADI-R) (Le Couteur et al. 2003) by child psychologist Dr. Sears. He also performed scoring of clinical behavioral questionnaires and checklists at pre and post-TMS treatment stages. Totally 33 children with ASD were recruited (28 boys and 5 girls, mean age 12.88 years, SD = 3.76, age range from 7 to 21 years). Among these participants there were 23 high-functioning children with autistic disorder and with full-scale IQs >80 assessed using the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV; Wechsler 2004). Ten participants had lower full-scale IQ (i.e., within 65–79 range) but could tolerate rTMS procedure with autonomic activity monitoring. This was a cohort where TMS effects were never investigated or reported in our prior studies.

The study complied with all required human subjects research regulations and institutional policies and has been approved by the Institutional Review Board (IRB) of University of Louisville. Participating subjects and their parents (or legal guardians) were provided with all information regarding the study. If the individual agreed to participate, both she/he and parent/guardian signed and dated the consent or assent form approved by IRB and received a copy countersigned by the investigator who obtained consent. All subjects enrolled in the study completed all 12 sessions of rTMS. However, three HFA subjects data were either recorded with significant artifacts or had missing behavioral evaluation, and thus only thirty subjects data was entered for statistical analysis and reported in results section. Therefore, our complete useful data retention rate in the study was 90 %, similar to that reported in our prior publications (e.g., Casanova et al. 2014).

Low Frequency Repetitive TMS Procedure

A Magstim Rapid system (Magstim Co., Whitland, UK) was used to deliver magnetic stimulation using a figure-eight shaped coil. Participants were seated in a leather recliner chair and fitted with a swimming head cap. Motor threshold (MT) for the left hemisphere was determined in the following manner: mild supra-threshold stimulations was administered over the left (contra-lateral) motor cortex to determine the optimal area for stimulation of the *first dorsal interossi* (*FDI*) muscle at the right hand. The output of the machine was increased by 7 % each time until the least amount of machine power that induces a 50 μV deflection or a visible twitch is identified in 4 out of 5 trials over the cortical area controlling the contralateral FDI. Three surface pre-geled disposable electrodes were attached over the *FDI* areas. Electromyographic (EMG) responses (motor evoked potentials) were recorded using the C2J&J Engineering Inc., (Poulsbo, WA, USA) physiological monitoring system interfaced with the Magstim TMS device. Similar procedure was applied to determine MT for the right hemisphere starting from the seventh session of rTMS. The TMS treatment course was administered once per week for 12 weeks over the DLPFC (six over the left, six over right hemisphere). The site for stimulation was placed 5 cm anterior to, and in a parasagital plane to the site of maximal FDI stimulation. The figure-eight coil, with a 70-mm wing diameter was kept flat over the scalp. Stimulation was performed at 0.5 Hz and 90 % of resting MT, with a total of 160 pulses per day (session had eight trains by 20 pulses, with a 20-s interval between the trains (for additional procedure detail see Casanova et al. 2012, 2014; Sokhadze et al. 2012, 2014).

Autonomic Activity Monitoring

Physiological monitoring was performed during 2–3 min before the start of rTMS treatment, during ~10 min of rTMS session, and immediately after the completion of the TMS for another 2–3 min. For data analysis for the goals of this study was included only ~10 min long data set collected during the actual administration of TMS. Thus, it was used approximately 10 min long period to calculate HRV variability measures (R–R intervals, standard deviations of R–R, NN50, pNN50, LF and HF of HRV, and LF/HF ratio) derived from an artifact free electrocardiogram (ECG) recording and mean SCL during each rTMS session. The procedure of autonomic monitoring during TMS sessions included presentation of raw ECG, individual HRV measures in a form of bars, floating HRV spectrum, individual HRV components and SCL with both visual and auditory feedback for experimenter. All physiological measures were analyzed both on- and off-line. EMG analysis was not part of this study, as EMG was used only for MT detection and for monitoring of gross motor artifacts and movements.

Measurement of the ANS Dependent Variables

ECG, electromyogram (EMG), pneumogram (PNG), and electrodermal activity (EDA) were acquired (1024 Hz sampling rate for EMG and ECG, 128 Hz for PNG and EDA) by a C-2J&J Engineering Inc., physiological monitoring system with USE-3 software (Physiodata, Poulsbo, WA, USA). Three Ag/AgCl electrodes (El-503, Biopac Systems, Inc., CA, USA) were attached for measurement of Lead II ECG, 3 Ag/AgCl electrodes (EL-501 from Biopac) for EMG recording from the right hand, and PNG was recorded with a strain gauge transducer. EDA was recorded by Ag/AgCl electrodes (EL-507 by Biopac with Unibase isotonic gel) attached to the distal phalanx of index and middle fingers to measure SCL. Average R–R intervals in ECG (R–R), standard deviation of all normal R–R intervals (SDNN), the percentage of intervals >50 ms different from preceding interval (pNN50); frequency domain HRV measures such as power of HF, LF components, and the ratio of the LF over the HF (LF/HF ratio was used as an indirect cardiac autonomic balance index) of HRV were calculated as time domain and frequency domain cardiac activity measures (Kleiger et al. 2005). Artifact-corrected at least 5 min long recording epochs were analyzed with FFT to assess HRV. Integrals of the spectrum in 0.04–0.15 Hz (LF of HRV) and 0.15–0.40 Hz (HF of HRV) bands were measured (in ms^2). All HRV data was analyzed off-line using Kubios HRV software v. 2.0 (University of Kuopio, Finland).

During HRV interpretation it was assumed that the HF component of HRV could serve as the non-invasive index of parasympathetic influences on the heart (Berntson et al. 1997; Sohn et al. 2001), while the LF component of HRV can be linked to sympathetic nervous system activity and sympatho-vagal balance (Malliani et al. 1994; Pagani et al. 1986). It was also taken in consideration that some studies reported that the LF variability might reflect both sympathetic and vagal influences related to baroreflex mechanisms (Berntson et al. 1997) and therefore the LF cannot be considered exclusively as a sympathetic-only index. It is thought that changes in blood pressure amplitude may cause a vagally-mediated baroreflex responses as well as changes in LF variability. Respiration rate on per minute basis and peak respiration frequency were calculated to control HF peak in HRV related to respiratory frequencies in HRV, but respiration measures were not used as dependent measures. Electrodermal activity in a form of SCL [in μS (μSiemens)] was used as a dependent EDA variable in this study. Considering the fact that rTMS administration is accompanied by audible clicks, it was not considered feasible to calculate non-specific skin conductance responses frequency.

Behavioral Outcomes

Every participant was evaluated before TMS course and within a week following TMS treatment using parents/caregivers questionnaires and surveys, and clinical ratings of improvement (the later not reported in this study). For the evaluation of social and behavioral functioning outcomes in this study we utilized two checklist data. *ABC* (Aman and Singh 1994; Aman 2004) is a clinician administered rating scale to assess Irritability, Lethargy/Social Withdrawal, Stereotypy, Hyperactivity, and Inappropriate Speech based on parent/caregiver report. Each area contains multiple items receiving a rating from 0 to 3. Items are summed and high scores for each area reflect severity of the problem area. The ABC has been shown to be effective in assessing behavior changes in autism (Aman 2004). Specifically, for this study we used the *Irritability, Lethargy/Social Withdrawal and Hyperactivity* subscales of the ABC as outcome measures, as stereotype behavior is more reliably measured by the RBS questionnaire. Repetitive Behavior Scale-Revised (RBS-R, Bodfish et al. 1999) is a caregiver completed rating scale (ratings from 0 to 3) assessing stereotyped, self-injurious, compulsive, ritualistic, sameness, and restricted range (Bodfish et al. 2000). Items from the RBS-R scales are summed to obtain a measure of severity of repetitive behavior. The RBS-R was validated in independent samples and showed high internal consistency and inter-rater reliability (Lam and Aman 2007). Both questionnaires are well established in autism research and treatment clinics.

Statistical Analysis

The primary statistical analyses included linear regression plot estimation of each autonomic dependent variable over 12 sessions of rTMS course, and paired sample Student *t* test of pre- and post-TMS behavioral measures. For each behavioral rating score analyzed using *t* test, normality of distribution test was performed to ensure appropriateness for the *t* test, and 95 % confidence intervals (95 % CI) were included in outcome report. To estimate power of performed test for the linear regression analysis, statistical results included as well values of observed power at $\alpha = 0.05$, and their comparisons to the desired power of 0.80. Actual R, R^2, and adjusted R^2 values are reported along with outcomes of normality test for each dependent variable. Power analysis was performed also for behavioral evaluation outcomes. In addition, there were analyzed changes of autonomic measures from the first 3–4 min of the session 1 (before start of rTMS procedure) and the last 3–4 min of ANS recording (after rTMS at session 12). The changes of physiological variables were entered in correlation analysis with changes of behavioral evaluation

scores. Statistical software packages used in the study were SPSS v.14.0 and SigmaStat v 9.0.

Results

Behavioral Evaluations Post-TMS

As it was expected, the ABC and RBS behavioral checklists did show statistically significant improvements in several domains when analyzed using a paired sample Student's *t* test. *Stereotypy* subscale of the ABC showed a significant score reduction [from 5.45 ± 4.91 down to 3.90 ± 4.32, mean decrease being −1.55 ± 2.78, t(29) = 3.00, $p = 0.006$, 95 % CI from −0.49 to −2.61, power = 0.792 at $\alpha = 0.05$, normality test passed], while *Hyperactivity* score also showed reduction [from 17.00 ± 13.49 down to 13.45 ± 10.82, −3.55 ± 8.01, t(29) = 2.39, $p = 0.024$, 95 % CI −0.50 to −6.59, power = 0.66 at $\alpha = 0.05$]. *Inappropriate Speech* score decreased as well [−1.20 ± 2.61, t(29) = 2.49, $p = 0.019$, 95 % CI from −0.21 to −2.20, power = 0.67 at $\alpha = 0.05$, normality test passed]. Changes of individual subscale rating scores are depicted at the Fig. 1.

We found a significant decrease in stereotype repetitive and restricted behavior patterns following 12 sessions of bilateral rTMS as measured by the RBS-R (Bodfish et al. 1999). *Total RBS-R* score decreased from 24.77 ± 14.39 to 18.90 ± 13.10, with the mean decrease being −5.87 ± 8.22, t(29) = 3.92, $p = 0.001$, 95 % CI from −2.85 to −8.88, power = 0.96 at $\alpha = 0.05$, normality test passed. Changes in individual subscale rating scores are shown in Fig. 2, where *Stereotypic Behavior* Subscale shows significant decrease [from 5.65 ± 4.16 to 4.32 ± 3.71, mean change −1.32 ± 2.40, t(29) = 3.06, $p = 0.005$, 95 % CI −0.44 to −2.20, power = 0.82 at $\alpha = 0.05$] and *Ritualistic/Sameness Behavior* Subscale scores show a significant decrease [−2.09 ± 3.07, t(29) = 3.80, $p = 0.003$, 95 % CI from −0.97 to −3.22, power = 0.94 at $\alpha = 0.05$].

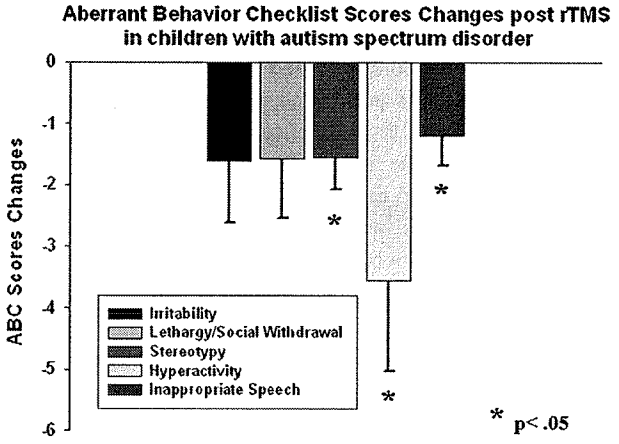

Fig. 1 Changes of Aberrant Behavior Checklist (ABC) scores post-TMS as compared to baseline levels in children with ASD (N = 30). Stereotypy, hyperactivity, and inappropriate speech rating scores decreased significantly post-TMS treatment

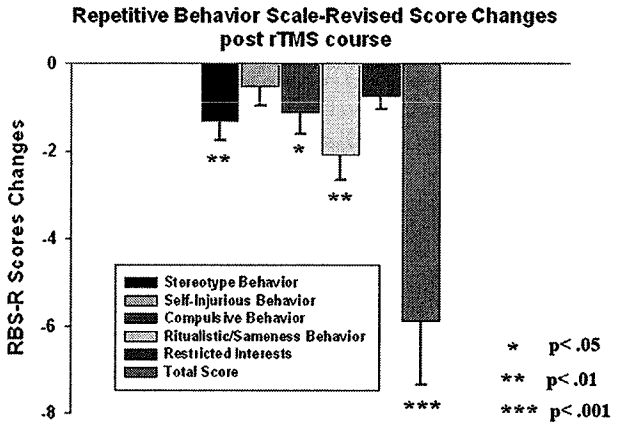

Fig. 2 Changes of Repetitive Behavior Scale (RBS-R) scores post-TMS as compared to baseline levels in children with ASD (N = 30). Stereotype behavior, ritualistic and ritualistic behaviors and total RBS scores decreased significantly following 12 sessions of rTMS

Autonomic Activity Measures

Time-Domain Measures of HRV (R–R Intervals, SDNN, RMSSD, pNN50)

Cardiointervals in ECG (R–R intervals) showed a statistically significant linear regression over 12 sessions of rTMS (R = 0.59, $R^2 = 0.34$, adjusted $R^2 = 0.28$, F = 5.34, $p = 0.043$, observed power = 0.53 at $\alpha = 0.05$, below desired power of 0.80, normality test passed, Fig. 3). Standard deviations of R–R (SDNN) intervals showed strong statistically significant linear increase over 12 sessions of rTMS (R = 0.83, $R^2 = 0.70$, adjusted $R^2 = 0.67$, F = 23.44, $p < 0.001$, observed power = 0.95 at $\alpha = 0.05$, normality test passed, Fig. 4). Number of R–R intervals over 50 ms (NN50 index) showed linear increase (R = 0.69, $R^2 = 0.47$, F = 9.10, $p = 0.013$), and their percentage (pNN50) also increased linearly (R = 0.76, $R^2 = 0.58$, adjusted $R^2 = 0.55$, F = 14.28, $p = 0.004$, observed power = 0.86 at $\alpha = 0.05$, well over the desired power of 0.80; normality test passed) (Table 1).

Frequency-Domain Measures of HRV (LF and HF of HRV, LF/HF Ratio Index)

The power of LF component of HRV showed a marginal trend towards linear regression to reach statistical

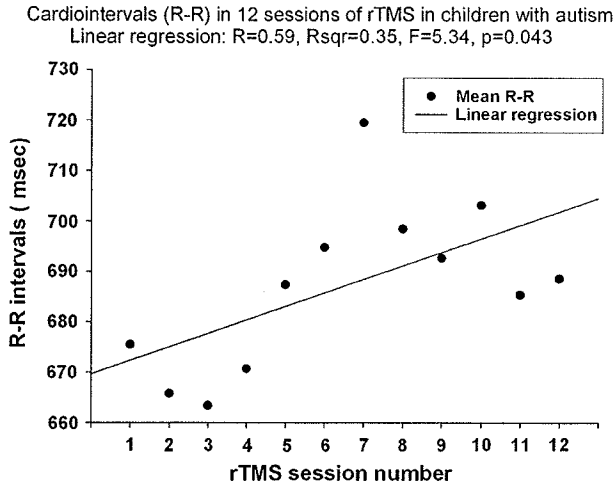

Fig. 3 Scattergram of linear regression of R–R intervals in ECG across 12 sessions of rTMS in 30 children with ASD. The mean values of R–R intervals depicted as a *dots* for each session show linear increase over the rTMS course (R = 0.59, $p = 0.043$)

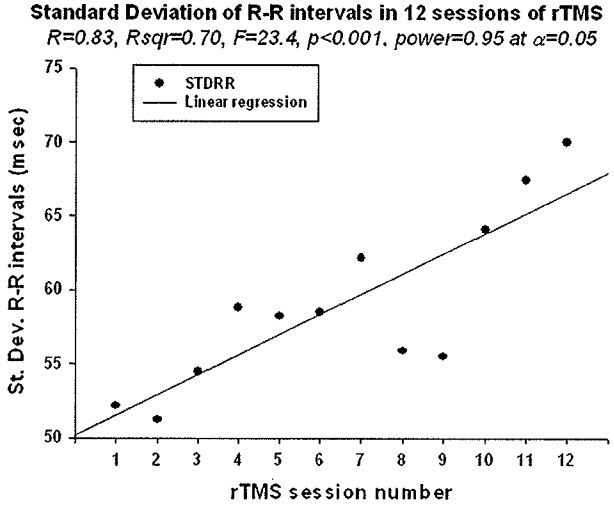

Fig. 4 Scattergram of linear regression of standard deviations of R–R intervals (SDNN) across 12 sessions of rTMS course in 30 children with autism shows linear increase (R = 0.83, $p < 0.001$)

significance (R = 0.58, $R^2 = 0.34$, adjusted $R^2 = 0.27$, F = 4.94, $p = 0.044$ observed power = 0.52 at $\alpha = 0.05$, significant, though well below the desired power of 0.80; normality test passed, Fig. 5).

Power of HF component of HRV showed a statistically significant linear increase (R = 0.63, $R^2 = 0.39$, adjusted $R^2 = 0.34$, F = 6.65, $p = 0.027$, power = 0.61 at $\alpha = 0.05$, normality test passed, Fig. 6).

The LF/HF ratio of HRV (cardiac balance index) showed linear regression that was statistically significant (R = 0.66, $R^2 = 0.44$, adjusted $R^2 = 0.38$, observed power = 0.67 at $\alpha = 0.05$), but it was below the desired power of 0.80. Normality test passed for LF/HF ratio, $p = 0.41$.

Skin Conductance Level

SCL showed statistically significant linear regression over 12 sessions of rTMS(R = 0.68, $R^2 = 0.47$, adjusted $R^2 = 0.42$, F = 9.03, $p = 0.013$, observed power = 0.72 at $\alpha = 0.05$, still below the desired power of 0.80; normality test passed).

Changes of dependent autonomic variables and their correlation with behavioral scores.

Differences of R–R intervals from the first minutes of the initial session to the last minutes of the treatment course was significant [18.89 ± 31.32 ms, t(29) = 2.39, $p = 0.017$, power = 0.63 at $\alpha = 0.05$]. This measure showed negative correlation with *Hyperactivity* rating of the ABC (r = −0.487, $p = 0.016$). Hyperactivity scores changes also showed negative correlation with HF of HRV (r = −0.439, $p = 0.017$). Power of HF component did show moderate increase [1012 ± 2145 ms^2, t(29) = 2.41, $p = 0.016$, power = 0.68 at $\alpha = 0.05$]. The same trend was demonstrated by STDRR measure [18.34 24.72, t(29) = 2.20, $p = 0.035$, power = 0.64 at $\alpha = 0.05$]. Therefore, the most prominent was correlation of the *Hyperactivity* scores with HRV measures post rTMS course.

Changes of SCL from the beginning to the end of rTMS course were significant and well powered [−4.94 5.57 μS, t(29) = 4.70, $p < 0.001$, 95 % CI from −6.99 to −2.90 μS, power = 0.93 at $\alpha = 0.05$]. Electrodermal activity changes showed positive correlation with several RBS rating scores (with *Total RBS* score r = 0.460, $p = 0.033$; and with *Stereotype Behavior* r = 0.54, $p = 0.007$). The SCL changes also did show correlation with *Hyperactivity* scores on the ABC questionnaire (r = 0.45, $p = 0.009$).

Discussion

Neuromodulation based on rTMS is a neurotherapy approach, which could have potential as a non-invasive intervention in autism. Considering the fact that treatment might be effective in children with ASD due to a higher neural plasticity, more research is needed to understand how TMS effects are mediated and what are the vital physiological signs that undergo changes. There is still a need for pilot and exploratory studies to define feasibility, magnetic stimulation frequency and power, preferred number of sessions, frequency of laboratory visits, and duration of intervention, along with other parameters of treatment. Current study showed that positive effects of rTMS on autonomic activity and behavioral evaluation

Table 1 Regression equations and statistics of linear regression of autonomic dependent variables over the 12 session long rTMS course in 30 children with ASD

Measures	Units	t	p	R	R^2	Regression equation	Power at $\alpha = 0.05$
R–R intervals	ms	2.31	0.043	0.59	0.35	$y = 2.68x + 669.67$	0.53
pNN50	%	3.77	0.004	0.76	0.59	$y = 0.51x + 16.33$	0.86
NN50	ms	3.01	0.013	0.69	0.48	$y = 1.89x + 51.34$	0.72
SD R–R intervals	ms	4.84	<0.001	0.83	0.70	$y = 1.36x + 50.19$	0.95
Power of LF of HRV	ms^2	−2.27	0.047	0.58	0.34	$y = -343.38x + 5075.8$	0.51
Power of HF of HRV	ms^2	2.57	0.027	0.63	0.39	$y = 75.46x + 905.57$	0.61
LF/HF ratio		−2.08	0.019	0.66	0.38	$y = 0.35x + 4.54$	0.67
Skin conductance level	μS	−3.00	0.013	0.68	0.47	$y = -0.38x + 8.25$	0.71

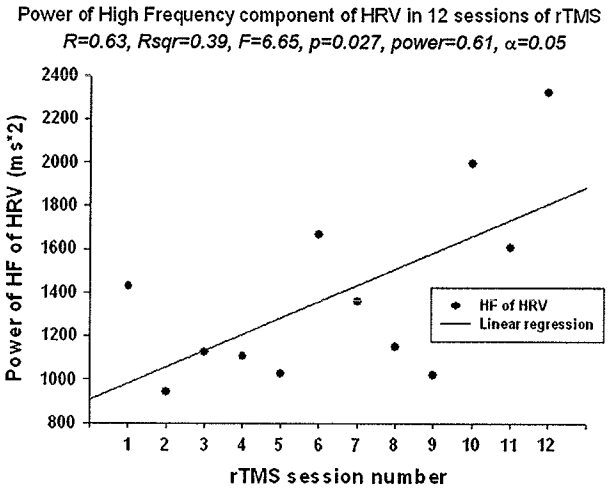

Fig. 5 Scattergram of linear regression of the LF component of HRV across 12 sessions of rTMS in 30 children with ASD. The mean values of LF show tendency to decrease over the rTMS course and the trend was marginally reaching significance level (R = 0.58, $p = 0.047$)

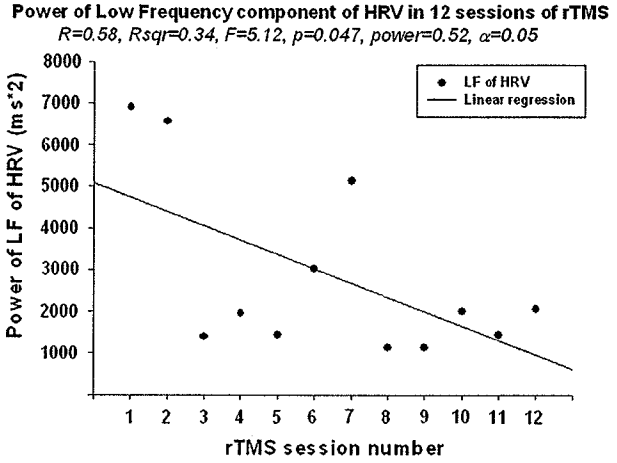

Fig. 6 Scattergram of linear regression of the HF component of HRV across 12 sessions of rTMS in 30 children with ASD. The mean values of HF show linear increase over the rTMS course (R = 0.63, $p = 0.027$)

ratings can be seen not only in 18 session long rTMS treatment course (Casanova et al. 2014), but even in 12 weekly sessions. In addition, it also showed that these effects can be detected not only in children with HFA diagnosis, but as well in lower functioning children (i.e., IQ <80). The study demonstrated reproducibility of autonomic changes trends (e.g., decrease of HR, increased variability, decreased SCL, etc.) in a different pool and in more representative cohort of children with ASD.

It was also shown that behavioral evaluation outcomes correlate with autonomic changes during 12 session long rTMS course in children with ASD.

The findings of the study contribute to a series of proof-of-concept studies of our group aimed at demonstration of feasibility and usefulness of rTMS-based neuromodulation targeting behavioral symptoms, executive function deficits and autonomic activity dysfunctions symptoms present in children with ASD (Baruth et al. 2010, 2011; Casanova et al. 2012, 2014; Sokhadze et al. 2009; 2014). The study represents continuation of efforts to find support for the hypothesis that LF, inhibitory rTMS applied bilaterally over the DLPFC improves the cortical excitation/inhibition balance in autism, and normalizes autonomic balance by restoring normative inhibitory influences of the prefrontal cortex (PFC) on the limbic system, paralimbic, and other cortical and sub-cortical structures known to control autonomic arousal (for review see Thayer and Lane 2009).

Physiological monitoring during neuromodulation treatments such as rTMS therapy should be considered as an important addition not only for safety purposes but also for the better interpretation of possible neurobiological mechanisms of rTMS action. Our study one more time supported assumption that HRV and SCL measures are

very informative variables for interpretation of the balance of the inputs of sympathetic and parasympathetic ANS branches in autism. Shorter cardiointervals along with attenuated HRV indices (i.e., low HF of HRV, high LF/HF ratio in HRV, low standard deviation of R–R intervals), and higher than normative levels of skin conductance found in children with ASD at the resting baseline are indicative of over-activated sympathetic and under-activated parasympathetic system in ASD. Such profile is reflecting limited psychophysiological flexibility and behavioral rigidity (Thayer and Lane 2000, 2009).

Our study explored pattern of ANS activity changes across 12 weekly sessions of LF prefrontal rTMS sessions in children with ASD. Based on outcomes of our prior pilot study (Casanova et al. 2014) using 18 sessions of rTMS we expected that children with ASD in this 12 session-long rTMS study would also show improved HRV measures (lower HR, longer R–R intervals, increased STDRR, higher pNN50 index, increased HF power, decreased LF power, decreased LF/HF ratio) and lower SCL measures. Our results showed that all dependent HRV variables changed in the predicted way, as indexed by statistically significant liner regression coefficients over TMS sessions.

It should be noted, that like in a prior study (Casanova et al. 2014) time-domain HRV measures (i.e., standard deviation of R–R intervals, pNN50) demonstrated the most significant changes during rTMS course. However, frequency-domain HRV measures (HF and LF of HRV, LF/HF ratio) showed only very moderate power of changes (e.g., within 0.52–0.67 range). Electrodermal activity also showed a moderately powered effect in a form of a decrease of the tonic SCL. Future studies, based on power analysis and sample size of the current pilot study, should target larger sample size for the stronger power of observed effects. These studies shold also use more advanced statistical methods to assess treatment effect size.

The increased length and variability of R–R intervals accompanied by increased power of the HF of HRV and decreased autonomic cardiac index value (i.e., LF/HF ratio) are suggesting increased vagal activity input in cardiac control. We did observe only marginally significant decrease of the LF component of HRV. The resulting profile of cardiac autonomic control post-TMS could be interpreted as the restoration of autonomic control of heart rhythm mainly through an increased of the parasympathetic cardiac neural control. Even though observed the change in the LF component were very marginal, we did find a decrease in SCL over the 12 sessions in a way similar to the one recorded in a 18 session-long rTMS course. This result suggests an attenuation of overall sympathetic arousal as it is well known (Boucsein 2012) that skin conductance is controlled solely by the sympathetic inputs.

There are several plausible explanations how LF inhibitory prefrontal rTMS may affect autonomic functions in autism. So far, only few studies investigated effects of rTMS on autonomic system or on frontal cortical areas known to be are directly implicated in regulation of the autonomic activity (Czeh et al. 2002; Filippi et al. 2000). Neurohumoral changes after treatment with rTMS might be considered as yet another potential mediator of neuromodulation (Ben-Shachar et al. 1997). Some of anxiolytic effects of rTMS may result from the normalization of the hypothalamic–pituitary–adrenocortical (HPA) activity (Holsboer 2000). In animal model studies it was demonstrated that rTMS of frontal area may result in decrease of stress-induced corticotropin and corticosterone levels and attenuated activity of the HPA system (Hedges et al. 2002; Keck et al. 2000). It was shown that LF rTMS can influence autonomic balance assessed using HRV measures as outcome (Yoshida et al. 2001). Another study (Udupa et al. 2007) reported that HRV measures indicated that rTMS produced significant reduction in the cardiac sympathetic/parasympathetic ratio, suggesting improvement in sympatho-vagal balance. Lower post-TMS sympathetic activity was reported in Jenkins et al. (2002) even in healthy volunteers.

It is very likely that rTMS effects are mediated through the fronto-limbic connections. The limbic system is a complex network of structures central to anxiety and mood regulation (Mayberg 2003; Seminowicz et al. 2004). Originally rTMS was investigated as a potential antidepressant therapeutic device under the assumption that magnetic stimulation of the PFC would engage the connected limbic regions involved in mood and anxiety regulation (George et al. 1999). The hypothesis is consistent with the PFC rTMS modulating the function of fronto-limbic circuits. Over a course of treatment rTMS may selectively lower the ratio of cortical excitation to cortical inhibition known to be high in autism (Casanova 2006; Rubenstein and Merzenich 2003). LF rTMS over DLPFC may therefore lead to improvement in frontal functions, including tonic fronto-limbic inhibitory function. We already reported positive effects of rTMS in autism and discussed possible mechanisms of observed improvements (Baruth et al. 2010, 2011; Casanova et al. 2012, 2015; Sokhadze et al. 2009, 2010, 2012, 2014). The mechanism of low-frequency TMS involves increasing inhibition of the stimulated cortex. For this study the stimulated region was the DLPFC, which is linked to the tonic inhibitory control of the ANS activity. The findings of our study indicate that TMS applied to the DLPFC was successful in the positive modulation of the autonomic balance in ASD through activation of the parasympathetic tone and withdrawal of sympathetic tone.

Sympathetic over-activation is often associated with anxiety disorders. Children and adolescents with ASD are known to present anxiety symptoms, as this is very common problem in clinical settings. Majority of children with ASD meet criteria for at least one anxiety disorder (de Bruin et al. 2007; McPheeters et al. 2011). Symptoms of anxiety may be also related to reduced functional connectivity between frontal cortex and limbic system, as a weakened normative frontal inhibition of limbic system is compromised in ASD. Development of new neuromodulation and neurotherapy methods aimed at reducing autonomic arousal in children with autism is thus an important clinical research objective. Excess of sympathetic arousal on a background of attenuated parasympathetic tonus in autism may provide an explanation of several social, emotional and communication deficits observed in individuals with ASD. It might be hypothesized that cardiac under-reactivity during socially engaging situations results in lower behavioral flexibility and reduced attentional capacity to attend to relevant stimuli critical for social communication development. Althaus et al. (1999) showed that children with serious problems in communication and interaction with others are features with significantly less cardiac responsiveness to the demands of attention task, and show greater limitation in behavioral control of attention than do healthy children. There are also other studies suggesting that lower executive control of behavior is associated with decreased vagal modulation of cardiac responses and generally attenuated ANS responsiveness and blunt autonomic reactivity to task-related information processing in individuals with autism (Mezzacappa et al. 1998; Zahn et al. 1987). Most of these studies have found that children with ASD are less capable of flexible allocation of energy to cope with task requirements. Thus deficient vagal responses in autistic subjects are probably reflecting a reduced ability to mobilize processing resources appropriately and may result in a flattened affect and general detachment from environment (Porges 2003 Zahn et al. 1987; van Engeland 1984). Beside decreased vagal tone and reduced ability to mobilize processing resources, Althaus et al. (2004) suggested that resulting decreased peripheral feedback (e.g., via vagal afferents) may fail to form coherent somatic representations (so called "somatic markers", Damasio 1994) required for development of mental representations of somatic states. Therefore, reduced autonomic feedback may represent yet another functional aspect of unbalanced autonomic responsiveness in autism which may negatively affect normal development of social communication skills.

Recent studies by our group have characterized the neuropathology of autism as that of a minicolumnopathy (reviewed in Casanova et al. 2015). Postmortem studies have found that the brains of autistic individuals have smaller minicolumns with a reduction in its peripheral neuropil space (Casanova 2006). Deficits within the inhibitory elements that surround the cell minicolumn suggest a mechanistic explanation to the cortical inhibitory/excitatory imbalance in autism (Rubenstein and Merzenich 2003). Oscillations and synchronization of pyramidal cells in and across minicolumns are maintained by networks of inhibitory GABA interneurons. The use of LF rTMS was meant to increase the inhibitory tone of cellular elements surrounding the minicolumns of autistic individuals. Our group (Baruth et al. 2011; Casanova et al. 2002, 2012, 2015) proposed the hypothesis that contrary to other inhibitory cells (i.e., basket and chandelier), whose projections keep no constant relation to the surface of the cortex, the geometrically exact orientation of double-bouquet cells and their location at the periphery of the minicolumn (inhibitory surround) makes them an appropriate candidate for induction by a magnetic field applied parallel to cortex. Over a course of treatment LF rTMS may restore the balance between cortical excitation and cortical inhibition by activation of GABAergic interneurons and lead to improved long-range cortical connectivity. This may explain some of the autonomic effects of LF inhibitory rTMS on autonomic activity.

Thayer and Lane (2009) reviewed neuroanatomical and neuroimaging studies that implicate inhibitory GABAergic pathways from the prefrontal lobe to the limbic system, including amygdale, along with inhibitory pathways between the amygdala and the sympathetic and parasympathetic neurons in medulla neurons known to be involved in modulation of HRV. This group of authors described earlier (Lane et al. 2007; Lane 2008; Thayer and Lane 2000, 2005) a neurovisceral integration model that is directly involved in regulation of emotion, and proposed role of dysregulation that may result in various psychopathologies, including anxiety disorder (Friedman 2007).

HRV is controlled by the joint activity of the sympathetic nerves and vagus nerves at the sinoatrial node. Both branches of the ANS are tonically active with sympathetic activity associated with HR acceleration and parasympathetic activity associated with HR deceleration (reviewed in Levy 1990). Importantly, HR is under tonic parasympathetic inhibitory control (Uijtdehaage and Thayer 2000). Benarroch (1997) used the term "the central autonomic network (CAN)" and proposed connections of the CAN with to the sinoatrial node of the heart via the stellate ganglia and the vagus nerve. As it was outlined by Thayer (2015) control and regulation of the output of the CAN is under tonic inhibition via GABAergic neurons in the nucleus of the solitary tract (NTS). Thus PFC in healthy subjects is tonically inhibiting limbic activity via pathways to intercalated GABAergic neurons in the amygdala

(Shekhar et al. 2003; Thayer and Lane 2009). Disinhibition of the amygdala if the frontal inhibition is weak may lead to increased HR and decreased HRV. Potential mechanisms according to Thayer and Lane (2009) might be a disinhibition of tonically active sympatho-excitatory neurons in the rostral ventrolateral medulla (RVLM), or inhibition of neurons in the nucleus tractus solitaries (NTS) resulting in inhibition of tonically active nucleus ambiguus (NA) and dorsal vagal motor nucleus (DVN) neurons leading to a net decrease of parasympathetic activity (Saha et al. 2000). Thus, decreased tonic inhibitory output of the PFC can lead to disinhibition of the tonically inhibited structures of central autonomic control (i.e., CAN) and result in a simultaneous disinhibition of sympathoexcitatory neurons and an inhibition of parasympathoexcitatory neurons accompanied by an increase in HR and a concomitant decrease of vagally mediated HRV. Sympathetic branch activity circuits are under tonic inhibitory control by the PFC (Amat et al. 2005; Thayer 2015), in particular are under tonic inhibitory control via GABAergic mediated projections from the PFC (Davidson 2000; Thayer and Lane 2009). Activation of inhibitory interneurons of the frontal cortex using rTMS is therefore a potential mechanisms how neuromodulation may restore normative prefrontal inhibitory tone resulting in normalization of the cardiac autonomic balance in autism.

Conclusion

This is a proof-of-concept type study aimed to replicate the existence of positive behavioral and autonomic functional outcomes of our procedure of rTMS intervention in a larger sample of ASD individuals, this time not limiting eligibility diagnosis to high functioning autism only, and exploring effects of 12 weekly session-long rather than 18 session-long TMS course (Casanova et al. 2014). It is hoped that the study will establish the potential to pursue future trials of adequate sample size using a sham control population. In this regard the present study does not constitute a clinical trial as such. Our study brings attention to usefulness of the ANS activity monitoring during rTMS, as it not only provides additional information about safety of the procedure in children with ASD, but also contributes to understanding potential neurobiological mechanisms of neuromodulation, specifically focusing on potential mediators of positive outcomes. There should be considered also other prospective implications of this study, in particular potential feasibility of using autonomic biofeedback immediately after TMS session. The rationale for such combination is obvious, as it was shown that rTMS lowers autonomic arousal, specifically improves HRV and SCL measures. Application of HRV biofeedback or even simultaneous HRV and SCL biofeedback immediately after each individual rTMS treatment session in autism may facilitate reinforcing low autonomic activation in an operant conditioning paradigm. Our plans include integration of LF rTMS with HRV biofeedback training to reward post-TMS changes in children with autism. Future studies of adequate sample size and sham controls are needed to explore the use of rTMS as a novel treatment for improving autonomic balance in ASD and even consider using rTMS in combination with autonomic biofeedback.

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