Brain Neurotransmitters, Cognitive Control, and Resting Heart Rate Variability (rHRV) in the MRI Environment

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ABSTRACT

Determining reliable measures that show how the brain can regulate the body and its physiological states could offer insight into how mental health and wellbeing is related to physiology and self-regulation. This study investigates how heart rate variability (HRV) at rest (rHRV), a measure of autonomic nervous system activity according to several studies (Thayer 2009, Colzato 2017, Shaffer & Ginsberg, 2017), relates to neurotransmitter levels in the prefrontal cortex (PFC), cognitive control over emotional material, and individual traits involved in psychopathology. Across two time points, the study in which this project is embedded takes a multi-perspective approach including brain neurotransmitters, Gamma Aminobutyric Acid (GABA), the main inhibitory neurotransmitter in the central nervous system (CNS), and glutamate, the main excitatory neurotransmitter in the CNS, to neurocircuits and anatomy, to behavior, to self-report about mental states and daily functioning. Resting heart rate (HR) measures were taken via pulse rate measurements in an MRI scanner, and processed into rHRV. Prior research has suggested that increased heart rate variability is associated with a) better emotional regulation and b) individual differences in GABAergic function. We investigated these relationships in a novel manner by examining whether GABAergic function of the prefrontal cortex, which is known to be involved in emotion regulation, is associated with rHRV. To do so rHRV of 21 adult subjects was correlated to levels of GABA in dorsolateral PFC (DLPFC) and ventrolateral PFC (VLPFC) taken from 20 to 24 months prior to the rHRV measurement. In addition, rHRV of adults and children was also associated with their scores on the Emotional Regulation Questionnaire (ERQ) which measures cognitive control of emotional states (Gross, J.J., & John, O.P. 2003). rHRV was additionally associated with psychopathology factors processed from the Mood and Anxiety Symptom Questionnaire (MASQ) and

Pennsylvania State Worry Questionnaire (PSWQ), of Anxious Apprehension, Low Positive Affect, Worry and Common Internalizing factors (Watson et al., 1995, Zhong et al., 2009).

Data was then analyzed to examine relationships between neurotransmitters, rHRV, cognitive emotional control, and psychopathology. No significant correlations were found between rHRV and any of the main measures: prefrontal GABA concentration, emotional regulation or measures related to psychopathology. Potential reasons for these null findings include a) that the GABA concentrations were obtained 20-24 months earlier, b) that the method used to assess rHRV is not as robust as standard measures which are difficult to implement in the magnet, c) the size of our current sample is small and hence may be underpowered to detect such relationships.

INTRODUCTION

The goal of this study is to examine whether resting heart rate variability (rHRV), a measure of autonomic nervous system (ANS) function, is influenced by differences amongst individuals in levels of neurotransmitter in the prefrontal cortex (PFC), and whether rHRV is associated with differences in emotion regulation. The ANS is responsible for controlling many organ functions (e.g. heart function, digestion, breathing rate) (Silverthorn 2004). The two components of the ANS are the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS), which are, respectively, important for maintaining homeostasis within the body, and the fight or flight response, which puts the body in a state to act. For example, in response to an emotionally charged or threatening stimulus, the SNS increases blood flow to limbs, increases heart rate and breathing rate, and increases available energy reserves in order to fight off danger or escape a dangerous situation) (Silverthorn 2004). One critical function of both

components of the ANS is to influence heart rate (Saul, 1990). The effect of ANS influence on the heart can be measured by proxy through HRV (Thayer 2012). HRV measures how intervals between heart beats change over time and rHRV is a consistent and reliable measure of physical and mental health (Thayer 2012, Bourdon et al., 2018). In particular, increased rHRV is associated with higher levels of control over emotions and with better overall physical health.

In the current study, we will be examining how differences amongst individuals in levels of GABA in the PFC may affect rHRV. It is known that the PFC provides inhibitory input into the heart through the nervous system via the vagus nerve, which in turn influences HRV. Greater PNS influence on the heart is associated with higher HRV, and in particular rHRV.. rHRV is often used as a measure as it allows HRV to be examined relatively free from situational variables such as breathing rate or carbon dioxide levels in the blood that might be induced by exercise (Colzato 2017). Neurons containing glutamate project from the PFC to limbic structures, with GABA suppressing systems and connections along the way more locally that reduce the fight or flight responses (e.g. decreasing rHRV), leading to an increase in rHRV overall (Thayer 2009). The PFC helps organize emotional responses into a coherent picture of the environment and body and assists in using that information to complete actions (Kandel 2012). Due to this relationship between the PFC, PNS and heart, it is predicted that greater GABA concentration in the PFC will be associated with higher rHRV.

Interestingly, the brain, specifically PFC regions, are known to control the ANS indirectly through lower brain regions, such as the anterior cingulate, insula, and amygdala (Thayer 2012). These regions in turn are involved in decision making, emotional response, memory formation, and internal bodily states, and are known to influence HR and its variability (Kandel 2012, Thayer 2009, Thayer 2012, Colzato 2017). In addition to controlling aspects of

the ANS, the PFC is also involved in cognitive control, for which one of the hallmark processes is executive function (EF), the ability to guide one's behavior in an effortful manner towards a goal (Banich 2009). Generally, not much is known about GABA concentrations and cognitive control. However, some studies have shown that GABA concentrations in the PFC are associated with performance on competitive selection tasks which are related to cognitive control (de la Vega 2014, Snyder 2010). rHRV is also associated with performance on these cognitive tasks and has been measured and analyzed far more than GABA data (Colzato 2017). Hence, it is plausible that there is an association between GABA concentrations in PFC and rHRV, an issue we investigate in the current study.

It is important to also investigate the relationship between emotional dysregulation and how the cortex is related to autonomic processes such as rHRV, as lower rHRV is associated with anxiety and depression in adults and adolescents (Brunoni 2013, Chalmers et al. 2014). Cognitive control and emotional states may be disrupted in psychopathology and have been thought to be related to other areas of the brain that process emotion and control ANS activity in addition to PFC regions (Thayer 2009, Bourdon et al., 2018). This relationship between psychopathology and autonomic activity can be manifested in rHRV.

In addition, cognitive control is mediated by brain structures involved in brain circuits that work to regulate how emotions are felt (Gross 1998, 2003, 2011, Williams 2015). The relationships between autonomic processes such as rHRV and brain control over bodily states are intertwined with neurotransmitters, brain regions, and neural circuits. A model that relates brain substrates and processes involved in autonomic and control circuits, The Neurovisceral Integration Model (see Figure 1), shows how brain structures from the cortex, to the subcortical structures (e.g limbic system, including the amygdala, hypothalamus), to lower structures (e.g.

the brainstem) interact and are modulated by the PFC, and in theory, by GABA levels in the PFC (Thayer 2009). This model is a solid framework from which to draw the connections between PFC, GABA and rHRV.

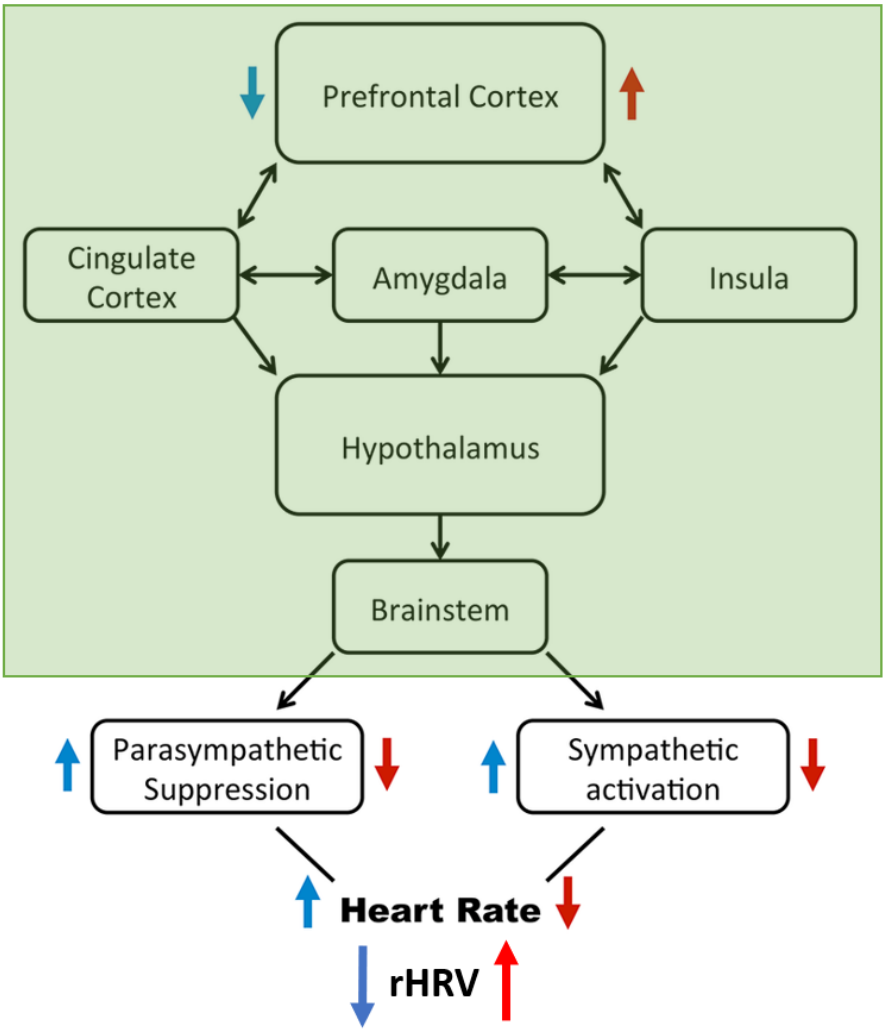

Figure 1: Neurovisceral Integration Model (Thayer 2009). The prefrontal cortex, namely for this study the dorsolateral prefrontal cortex (DLPFC) and ventrolateral prefrontal cortex (VLPFC), influences other brain structures, such as the cingulate cortex and insula (Thayer 2009). The cingulate cortex is an integral part of the limbic system, involved in emotional processing, learning and memory (Kandel 2012). The amygdala has been shown to have a

primary role in detecting salient information in the environment, especially that which is emotional in nature (Kandel 2012). The insula helps create and maintain internal bodily states that are related to emotion. The hypothalamus is crucial in maintaining bodily states and homeostasis (Kandel 2012). The brainstem is crucial to relaying information between the body and brain (Kandel 2012). These structures can activate or suppress the PNS or SNS the vagus nerve to influence rHRV (Thayer 2009).

Within the Neurovisceral Integration Model, there are several anatomical and functional components that process emotional states, affect cognition, and modulate ANS activity (Thayer 2009). The PFC also interconnects with the amygdala, insula, and cingulate cortex (Kandel 2012). The PFC connects with the amygdala, which is involved in detecting salient emotional information, and in learning the emotional significance of information in the environment (Kandel 2012). Similarly, the PFC connects with the cingulate cortex and insula, which respectively are involved in generating effort and motivation for a response, and in creating a mental representation of emotional states and bodily feelings (Banich & Compton, 2018, Kandel 2012). Together, the cingulate cortex, amygdala and insula serve to bridge emotional salience and responses with cognition and autonomic bodily responses. The hypothalamus receives bodily inputs from internal organs, cortical inputs, and limbic inputs from the insula, cingulate cortex and amygdala to relay and regulate physiological responses to emotions and environment (Kandel 2012). These brain areas are crucial in regulating the body and brain and coordinating emotional responses to be appropriate to the context of a given environment, for example, lowering heart rate and increasing digestion when eating a meal and feeling safe and happy, versus increasing heart rate and decreasing appetite when feeling threatened. These anatomical and functional components of the Neurovisceral Integration Model are essential for control of

autonomic and emotional activity by cognitive control processes that allow the brain to integrate emotional and physiological responses. The end result of all this activity can be indexed, in part, by rHRV.

It is important to understand the relationship between cognitive control and physiology in a novel way, starting from the neurotransmitter level. This research will add to the literature regarding rHRV, cognitive control of emotion, psychopathology, and GABA concentration in the PFC. With this study, researchers may be able to further understand GABA's modulatory role on the ANS. rHRV is already used frequently to predict health outcomes, and GABA concentrations in inhibitory circuits are becoming more well understood regarding cognition and mental states. Clinical populations could gain access to better treatment if the relationship of GABA concentrations in the PFC and autonomic function is better understood, particularly those populations with psychopathology such as anxiety and/or depression. Brain neurotransmitter level studies are a newer field, and further studies can build on this study's results between brain GABA concentrations and rHRV.

METHODS

Participants were 51 children of ages 16-25 (M = 19, SD = 1.8), with 26 females and 25 males and their 29 mothers of ages 39-59 (M = 49.8, SD = 6.25). Participants were recruited based on past participation with the GEM (Genes, Environment, and Mood) Lab at the University of Denver, which ran a community-based study focusing on genetics, psychological status, and emotional processing in relation to the development of depression and anxiety. All participants spoke English as their first language and did not have any problems with reading. Participants completed two visits to the University of Colorado Boulder. Participants were

screened before testing to insure they endorsed no previous neurological insult. They complete self-report questionnaires, behavioral and cognitive tasks, and magnetic resonance imaging (MRI) sessions. This longitudinal study also tracks changes in the brain and over development, and has two time points, spaced 20 to 24 months apart, Time Point 1 (TP1) and Time Point 2 (TP2). TP1 is completed and has all of these measures collected except for rHRV. TP2 is currently about one third of the way complete, and many participants' measures still need to be collected, and processed. rHRV measurements were introduced to TP2 and add a physiological component to the multi-level analyses (see Figure 2) of the brain and mood study. Informed consent was obtained from all participants over the age of 18. Adolescent assent as well as parent permission were obtained for participants under 18. All participants were treated in accordance to the policies of the University of Colorado Institution Review Board.

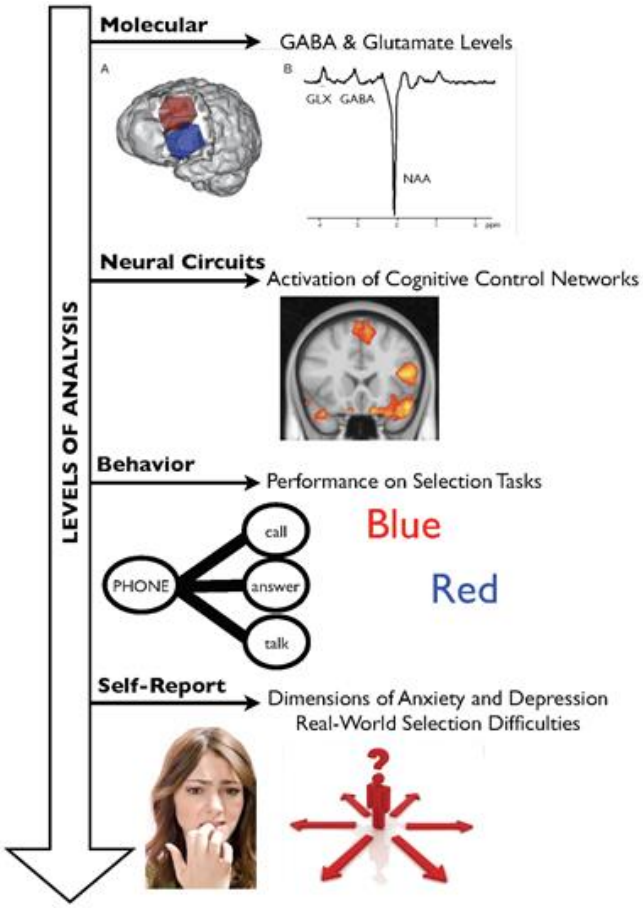

Figure 2: The multi-level structure of the Adolescent GABA Brain and Mood Study. This study offers insight into how molecules of the brain, neural systems and function, behavior, and emotions and feelings relate and change over development in adolescents and over time in their parents (Banich 2014).

Magnetic Resonance Spectroscopy (MRS) was used to obtain information about levels of each individual's GABA concentrations in the PFC. MRS uses magnetic fields to align hydrogen protons in the body and brain in a certain direction, then emits radio frequency pulses to knock them out of this alignment (Berger 2002). As these protons realign to the magnetic field, they emit energy, and this energy is picked up by receivers in the scanner coils (Berger 2002). MRS differs from magnetic resonance imaging (MRI) in that MRS can be tuned to specific protons of

a neurotransmitter (e.g, GABA) (Puts 2011). GABA protons are knocked out of normal spin by radio frequencies in the same way that normal MRI works, and their energy output during realignment with the magnetic field are measured; the key differences are that during MRS water and fat signals are suppressed, and the area scanned is restricted due to the low level of signal. As such, data can only be collected from a very small region of the brain rather than the whole brain (e.g, the DLPFC or VLPFC) (Puts 2011). These measures can be converted into readouts of GABA concentrations of specific areas of the brain. The DLPFC and VLPFC were the brain areas whose GABA concentrations were measured, as these regions are critical for cognitive control. MRS and voxel (the specific 3-dimensional space chosen in the brain to be scanned) specifications can be found in appendix A1.

Two MR spectroscopy voxels were placed manually by visually inspecting each participant's structural image of their brain. The DLPFC voxel was positioned in the middle frontal gyrus (MFG), anterior to the precentral gyrus and posterior to the frontopolar cortex The VLPFC voxel was position in the inferior frontal gyrus anterior to the precentral gyrus and posterior to frontopolar cortex (Helmuth 2018). The voxels were used to determine levels of GABA and GLX in both brain regions as well as water level in those regions (Helmuth 2018). GLX levels were measured using the PRESS sequence and GABA was measured using the MEGA-PRESS sequence as outlined in de la Vega et al (2014). From each voxel the concentration of GABA or GLX was obtained by measuring the levels of GABA and GLX against the baseline of water and dividing by the volume of the respective voxel (Helmuth 2018).

rHRV data was collected during the resting state portion of the MRI scan, which is the period of time where subjects were not undergoing physical or mental activity. Participants were lying in the MRI scanner and looking at a fixation point on a screen and were wearing a

photoplethysmography (PPG) monitor on their left index finger, which optically measures blood volume changes at the microvascular level from the fingertip as pulse rate (Schafer 2013). Blood volume changes in the fingertip are a good proxy of HR as long as the participant remains at rest and immobile, which was the case during resting state when measurements were taken (Elgendi 2010, Schafer 2013). PPG measurements took place over 12 minutes of resting state scanning time, where the participant was not actively doing any mental or physical activity. Since these HR proxy measurements were taken at rest, rPRV was able to be processed into rHRV.

PPG was chosen because it does not require as much time to set up as an electrocardiogram (ECG). ECG is the gold standard for HRV measurement as it obtains direct electrical measurement from the heart being placed on the skin of the chest. In the limited MRI scanning time frame we did not have the time to set the ECG up. Setting up ECG was prohibitive in that the participants were already spending 2 hours in the magnet, and that magnet costs are $550 an hour.

After data collection, blood volume changes in the fingertip are processed into PRV by assessing the time between the peaks and valleys of blood volume in the fingertip, seen in Figure 3 (Schafer 2013). BIOPAC monitors were used to collect PPG in the MRI scanner. Kubios analysis software, Kubios HRV Premium (v. 3.2.0), an accepted physiological data processing program, was used to process PPG data and convert rPRV into rHRV before other analyses were conducted (e.g. correlating rHRV and GABA concentrations). Kubios software specifications can be found in appendix A2.

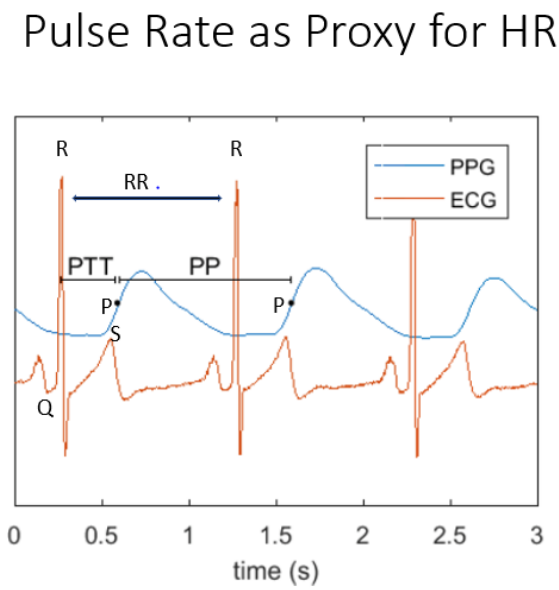

Figure 3 (Kubios 2018): PPG measures blood level changes in the fingertip, known as pulse rate from which variability (PRV) is calculated. PRV is calculated by taking the steepest slope from each wave of the PPG over time and using those point to define the P-P interval between two pulse waves, this represents the time between two heart beats. The Q,R,S waves represent the electrical changes in the heart during a heartbeat (obtained from ECG); the R to R distance is the gold standard for the time between two heart beats (Kubios 2018). The distance between the electrical R peak and pulse volume P peak measures how long it takes for ejected blood of the heart to make a change in the blood volume of the fingertip, where PPG is measured (Kubios 2018). Therefore, blood volume changes in the fingertip can be used to calculate PRV which can be used to reliably estimate HRV and has been used in the past as proxy for HRV as long as the participant is not moving (Kubios 2018, Mather 2017, Shaffer 2013).

Several variables (e.g. lifestyle habits) can affect physiological measures and confound rHRV measurements. Because of this, a survey was administered about behaviors that are related to physiology. The survey was administered directly before participants entered the MRI scanner, with a range of 15 minutes to 30 minutes from the start of HRV data collection. The

survey asked questions about: Exercise per week habits, as athletes and those who exercise regularly tend to have higher rHRV values; questions about sleep quality and quantity, as circadian rhythm and lack of sufficient sleep can affect rHRV; substance use such as caffeine, alcohol, nicotine and sleeping pills, as recent use of these substances can affect rHRV; subjective stress experiences, as stress levels affect rHRV; height and weight as higher BMI is associated with rHRV, and gender can have effects on rHRV because females tend to have higher rHRV (Carnevali 2017, Colzato 2017, Colzato 2018, Quintana 2016). This survey obtained information about potential confounds that could influence rHRV data. Any confounds that were significantly correlated with rHRV in this study were controlled for in final analyses.

Cognitive control over emotional states was indexed via the Emotional Regulation Questionnaire (ERQ) (Gross, J.J., & John, O.P. 2003). The ERQ questionnaire measures the tendency to regulate emotion in two ways: Cognitive Reappraisal or Expressive Selection. Cognitive Reappraisal measures how an individual can change their mindset about experiencing an emotion to affect how they feel, while Expressive Suppression measures how an individual can monitor or regulate their expressions of emotion to affect how they feel (Gross, J.J., & John, O.P. 2003). A 7-point Likert scale is used to measure responses to questions asking about how emotional experiences and situations are handled and expressed by respondents (Gross, J.J., & John, O.P. 2003). Example questions include: a) When I want to feel less negative emotion (such as sadness or anger), I change what I'm thinking about. b) When I am feeling negative emotions, I make sure not to express them. Higher scores mean that the respondent has higher ability to control their emotions, and lower scores mean the participant has less ability to control their emotions. Measurements were taken during TP2, with a range of approximately 1 week to 6 weeks before rHRV measurements.

Psychopathology and emotional dysregulation factors were processed from the Mood and Anxiety Symptoms Questionnaire (MASQ) (Watson et al 1995) and the Penn State Worry Questionnaire (PSWQ) (Meyer et al. 1990). The MASQ, a 39-item self-report scale, measures common and specific aspects of depression and anxiety, while the the PSWQ is a 16-item questionnaire assessing the degree to which an individual worries. For each item, participants are presented with a statement and asked to indicate how true that statement is for them using a 1-5 likert scale: not typical to very typical. From these questionnaires four latent factors related to psychopathology were derived. The first, Common Internalizing Factor, which describes the common symptoms that span anxiety and depression (Watson, Weber, Smith, Assenheimer, Strauss, & McCormick, 1995, Meyer 1990). Then after accounting for this common factor, three more specific factors were derived: Low Positive Affect, measuring low ability to experience positive emotions and feelings; Anxious Arousal, measuring self-reported somatic symptoms in regards to anxiety, such as shaking, sweating, and increasing heart rate in relation to thinking about stressors or going through certain situations; and Worry, measuring how much an individual worries about the future, things that will happen. Higher scores indicate more and worse symptoms, while lower scores indicate less and weaker symptoms. Measurements were taken at TP1 of the Brain and Mood Study, with a range of 20 months to 24 months prior to rHRV measurement for any given participant.

ANALYSES

We controlled for a variety of measures which are known to modulate HRV, such as gender, BMI, stress, heart rate, and smoking (Colzato 2018); see pre-scan questionnaire discussion above. Pre-scan questionnaire measures which were correlated significantly with

rHRV were controlled for in partial correlations and linear models, so any confounds that could influence heart rate were taken out of associations of variables.

All MRS data points were reviewed by an expert in MRS acquisition and analyses (Prof. Mark Brown, CU Anschutz) blind to the analyses to be performed in the remainder of the study. Analyses were performed between TP1 GABA concentrations and TP2 rHRV measures (i.e. ANS activity) to determine any significant correlations in parents but not in children because neurotransmitter levels in the PFC have been shown to be stable over time in adults, but less evidence exists for stability in adolescents (Yasen, Smith, & Christie, 2017). Cognitive control measures were correlated with rHRV to see if any significant relationship had arisen. Psychopathology measures were correlated with rHRV.

Statistical analyses were conducted using R software version 3.5.1 creaated by the R Core Team (R Core Team 2018). Psychopathology factor analyses were preformed using SPSS version 25 by IBM, by a researcher who was independent from the research team carrying out analyses in the remainder of the study (IBM 2017).

RESULTS

rHRV measurements were processed for n = 80 participants. For analyses between rHRV and other measures, participants with the rHRV measure of the root mean square of successive differences between heart beats (RMSSD) who had values greater than 125 ms were removed, as this is a typically high value that only athletes or those who exercise regularly tend to achieve (Shaffer and Ginsburg 2017). After this cutoff, the total number of subjects was n= 69, with n= 42 children (24 female, 18 male) and 27 adult parents (all females who were the mothers of the children in the study). Subjects who were missing any measures were excluded from analyses

using those measures. Mean heart rate was found to be significantly correlated with rHRV in children, so mean heart rate was controlled for their linear model analyses. Age was found to be significantly correlated with rHRV in parents, so age was controlled for their linear model analyses. Pearson's correlation was used for correlation analyses. Partial correlations used standardized residuals from linear models. Significance was set to $P < 0.05$.

One-Minute Cutoff vs. Five-Minute Cutoff of rHRV Data

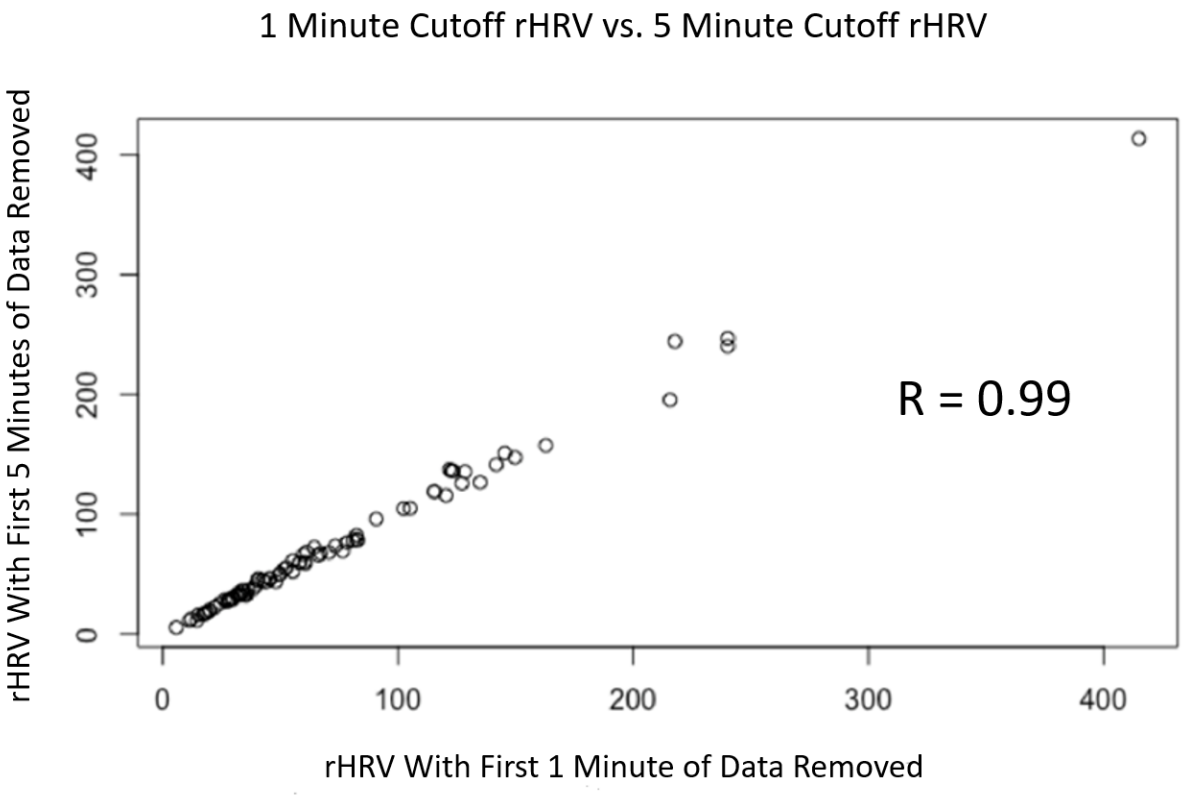

Figure 4: rHRV processed, collected with the first one minute removed, compared to rHRV data with the first five minutes removed. The two data sets were correlated in a linear model, $R^2 = 0.99$, DF = 78.

This study is the first to pilot use of the PPG device in the MRI scanner to get rHRV measures within the Banich Laboratory. As such, software setup and rHRV analyses were exploratory to this environment. A large concern with taking rHRV measurements in the scanner environment was that participants would feel scanner anxiety in an enclosed and loud environment, and that their state may not be resting and may require some acclimation. To examine the potential effects of scanner anxiety, rHRV data were processed in two ways making different assumptions about how much time it takes for any potential acclimation in the scanner due to anxiety: one set cutting off the first one minute of data, and the other set cutting off the first five minutes of data. To determine if there was an optimum amount of data to cut off or ignore, the two data sets were compared. The one minute and five-minute cutoffs of the 12 minute total data set were correlated in a linear model with a resulting r squared value of 0.99 (n=69), as seen in Figure 4. This tells us that 99% of the variance in the one-minute cutoff could be explained by the variance of the five-minute cutoff, so either set is effectively the same. This means that either set can be used to analyze rHRV data without a need for five minutes of acclimation in protocol with collecting HR data at rest in the MRI machine.

rHRV WITH TP1 GABA (PARENTS)

Neurotransmitter in Voxel Associated with rHRV (df = 17)	R^2	P
GABA DLPFC	-0.0767	0.747
GLX DLPFC	0.0217	0.9273
GABA / GLX DLPFC	0.142	0.549

Table 1: Adjusted R^2 and significance of rHRV with GLX/GABA residuals in the DLPFC of parents in a linear model, controlling for age and neurotransmitters (GABA or GLX) for all measures, controlling GLX in the DLPFC when measuring GABA in the DLPFC, and controlling for GABA in the DLPFC when measuring GABA in the DLPFC. Residuals were used as GABA correlates significantly with GLX and age.

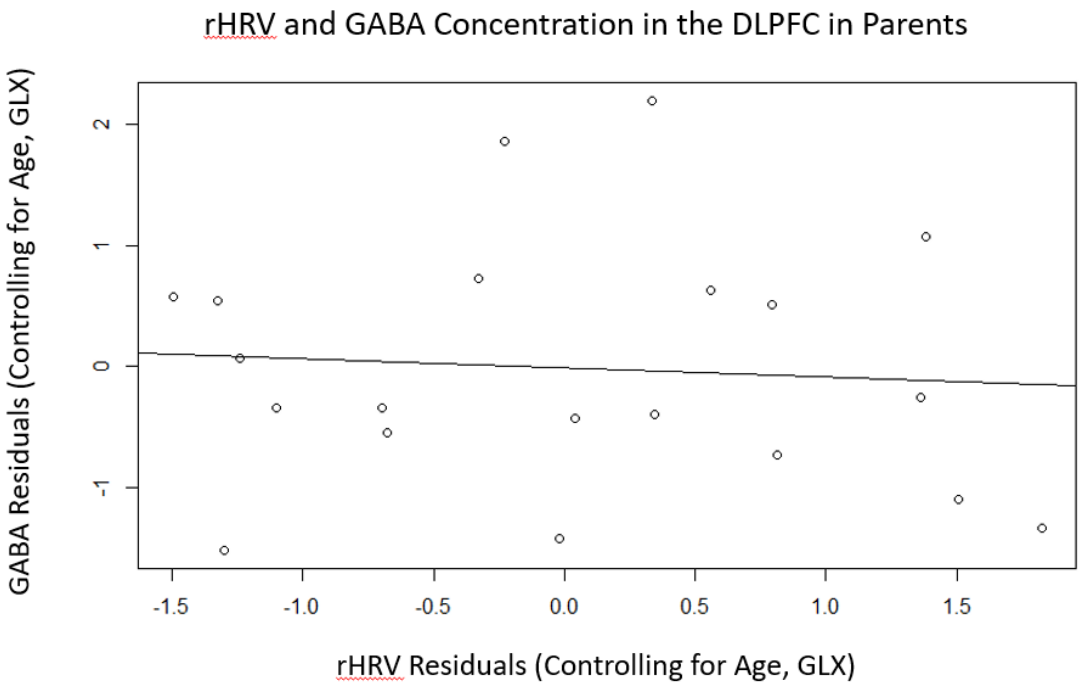

Figure 5: Association of TP2 GABA DPLFC from TP1and rHRV standardized residuals; the effects of Age and GLX removed.

DF = 17, R^2= -0.0767, P = 0.549

Due to the ongoing nature of the study, TP2 GABA spectroscopy data was not available by the time of the analyses. As such, TP1 GABA spectroscopy data for parents was associated with TP2 rHRV values, as seen in Table 1 and Figure 5. Adults have been shown to have more

consistent GABA and glutamate levels in the PFC over time, so they were included while children (since their GABAergic systems are still in development) is excluded until TP2 spectroscopy data is available (Yasen, Smith, & Christie, 2017). Across 19 parents (not all 27 parents had useful TP1 Gaba data and TP2 rHRV data), there was no significant association between DLPFC GABA nor GLX and rHRV while controlling for age (see Table 1). In both linear models, we controlled for GLX concentration to examine the specific effect of GABA (de la Vega, 2014). Additionally, we ran a linear model to compare the ratio of DLPFC GLX to GABA with rHRV while controlling for age. None of these linear models yielded any significant relationships. We reran all the same linear models as above, except that we used VLPFC GABA and GLX concentrations instead of these concentrations in the DLPFC. None of these yielded any significant relationships either, and these results are similar to those found in Table 1. However, we found a significant association between age and rHRV in the parents. This latter significant correlation has been found in the literature previously and served as a validation of our measurements (Stein 2009).

rHRV WITH COGNITIVE CONTROL SCORES

ERQ TP2 Associated with rHRV in Parents (DF = 17)	R^2	P
Cognitive Reappraisal	.0092	0.29
Expressive Suppression	-0.0373	0.602

Table 2: Adjusted R^2 and significance of rHRV with ERQ TP2 measures in parents.

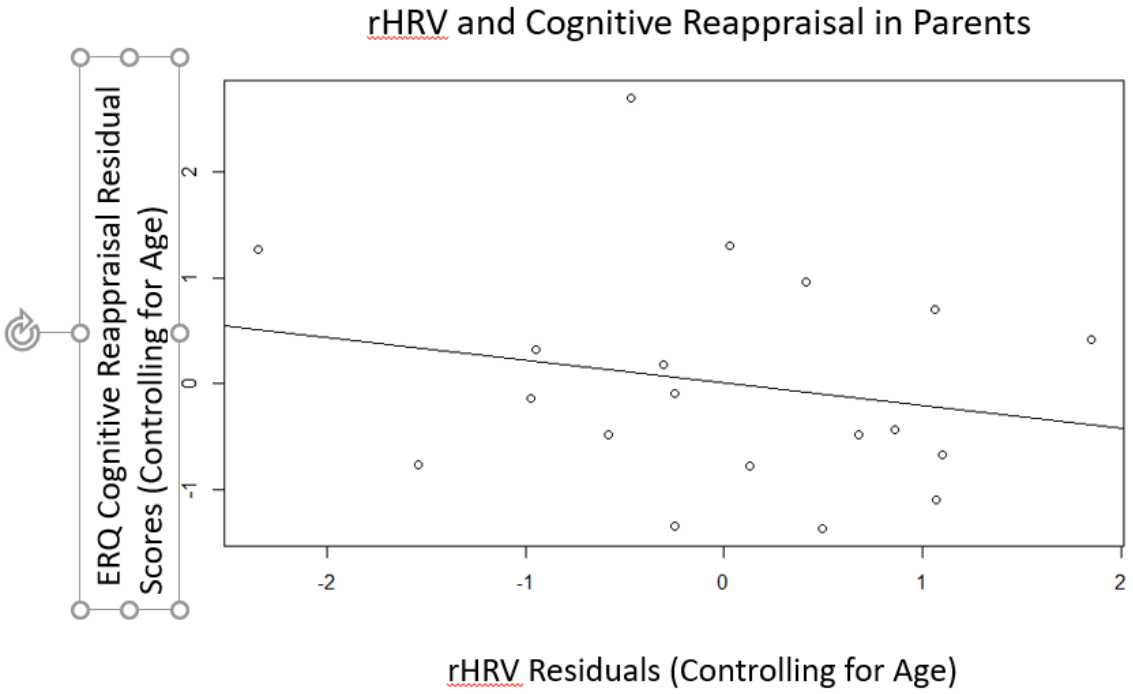

Figure 6: Association of ERQ TP2 Cognitive Reappraisal standardized residuals and rHRV residuals; the effect of age removed. DF = 19, adjusted R2 = .0092,

P = 0.29

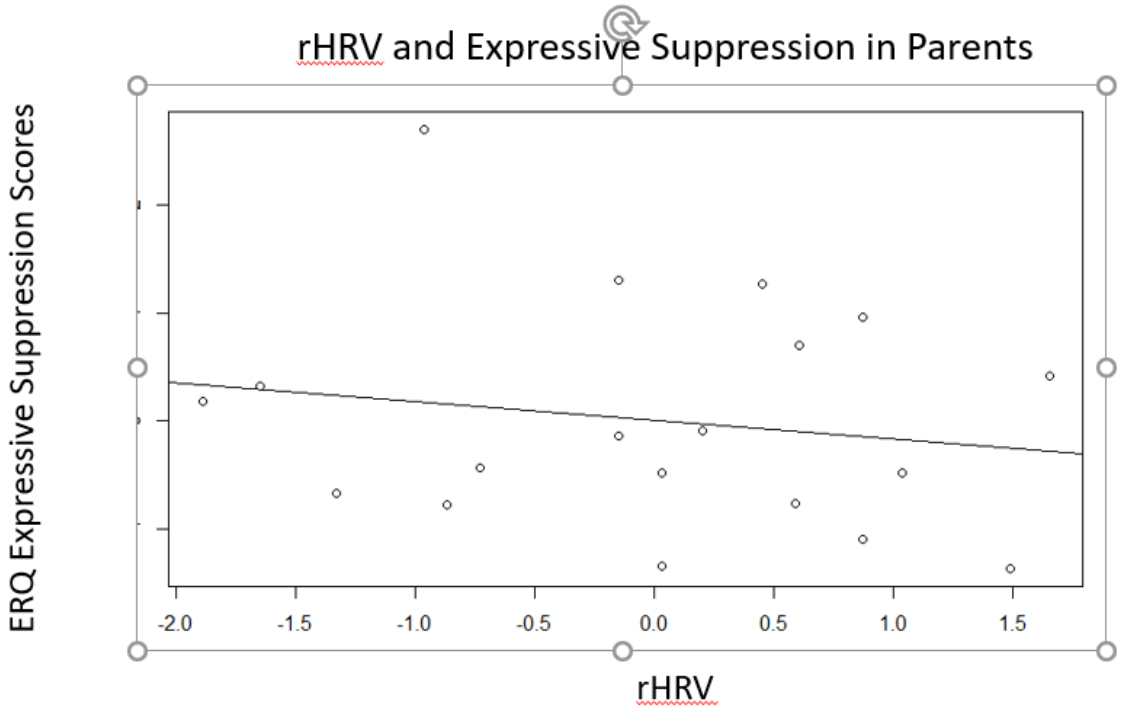

Figure 7: Association of ERQ Expressive Suppression (from TP2) and rHRV; parents. DF = 19, R^2 = -0.0373, P = 0.602

ERQ TP2 in Children Associated with rHRV (DF =39)	R^2	P
Cognitive Reappraisal	-.0138	0.504
Expressive Suppression	0.0358	0.123

Table 3: Adjusted R^2 and significance of rHRV residuals with ERQ TP2 residuals in children, controlling for mean heart rate. Residuals were used as mean heart rate was significantly associated with rHRV in children.

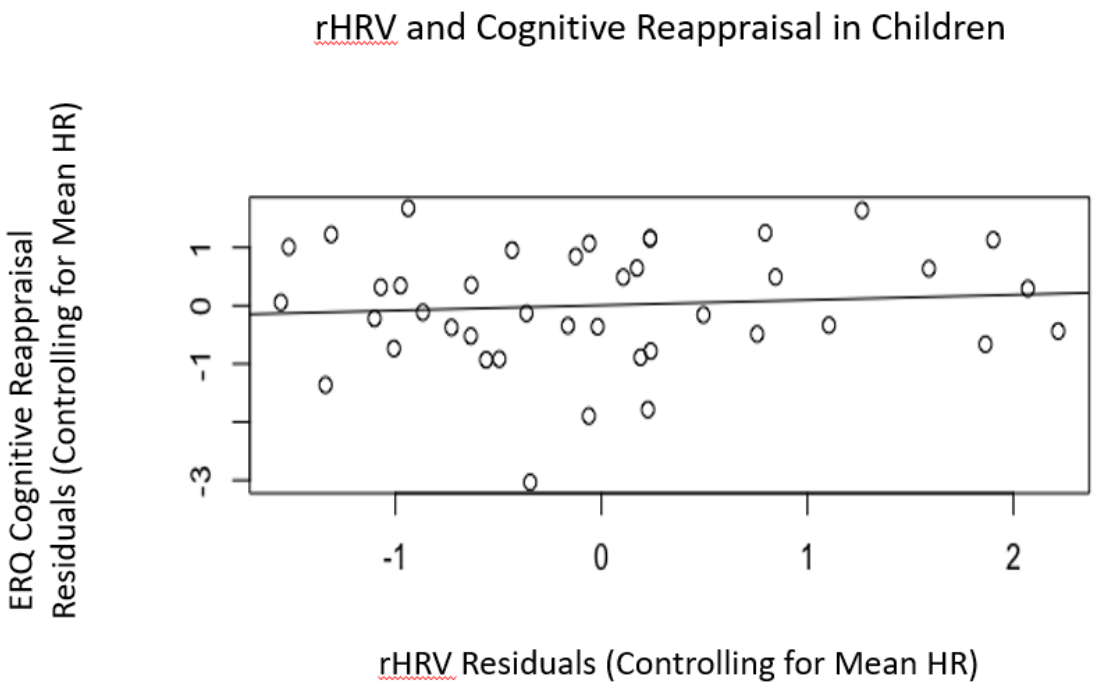

Figure 8: Adjusted R^2 of ERQ TP2 Cognitive Reappraisal residual and HRV standardized residuals; (with the effects of Mean HR removed). DF = 19, R^2 = -.0138, P = 0.504

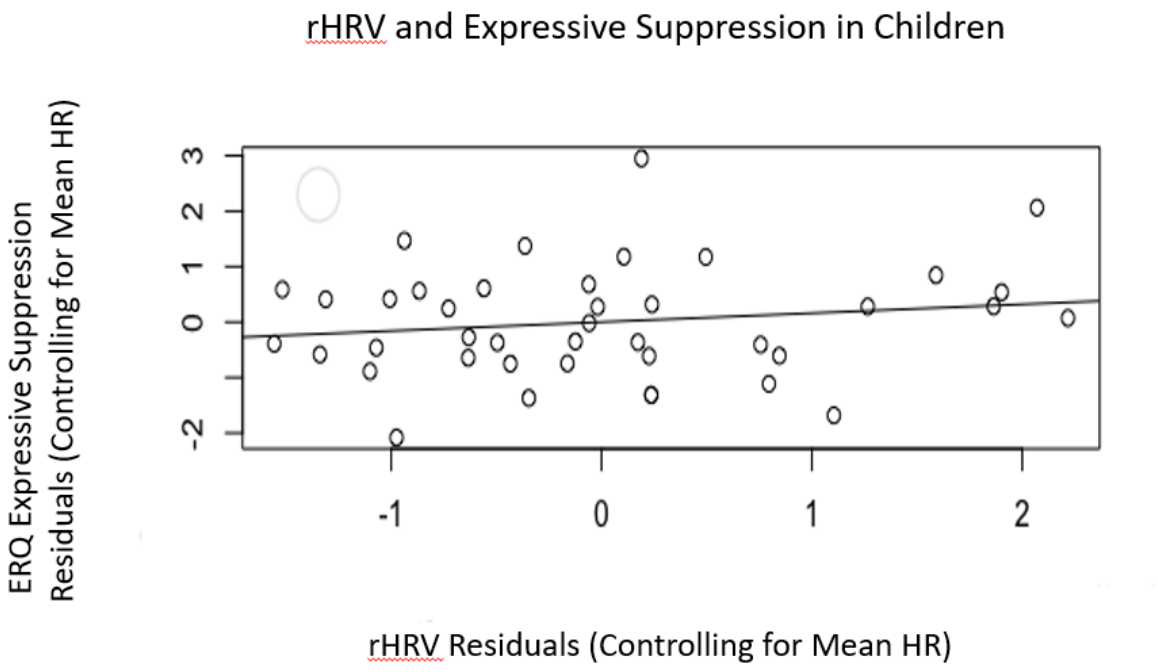

Figure 9: Adjusted R^2 of ERQ TP2 Expressive Suppression standardized residuals and rHRV; while controlling for mean heart rate in children. DF = 19, R^2 = 0.0358, P = 0.123

Neither adult nor adolescent correlations were statistically significant while controlling for age and mean HR (children only), as seen in Figures 6-9 and Tables 2-5.

rHRV WITH TP1 PSYCHOPATHOLOGY MEASURES (Parents)

MASQ/PSWQ Associated with rHRV in Parents (df = 17)	R^2	P
Low Positive Affect	0.0214	0.245
Anxious Arousal	-0.0442	0.0773
Worry	-0.0334	0.559
Common Internalizing Measure	-0.0428	0.677

Table 4: Adjusted R^2 and significance of rHRV with MASQ/PSWQ TP1 measures in parents.

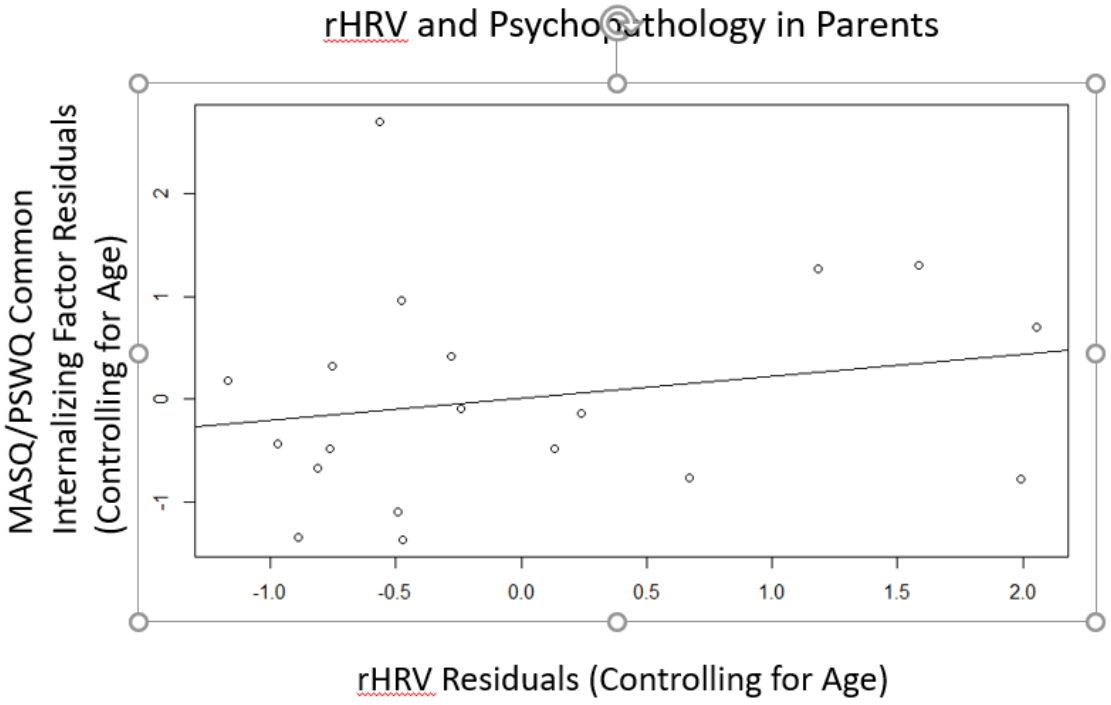

Figure 10: Association Common Internalizing Measure Residuals TP1 with rHRV standardized residuals of parents in a linear model; the effect of age removed DF = 17, adjusted R2 = -0.0428, P = 0.677

MASQ/PSWQ Associated with rHRV in Children (df = 37)	R^2	P
Low Positive Affect	0.0494	0.09
Anxious Arousal	-.00997	0.438
Worry	-0.0242	0.781
Common Internalizing Measure	0.0526	0.7473

*Table 5***:** Adjusted R^2 of rHRV residuals with MASQ/PSWQ TP1 measure residuals in children; the effect of mean heart rate removed.

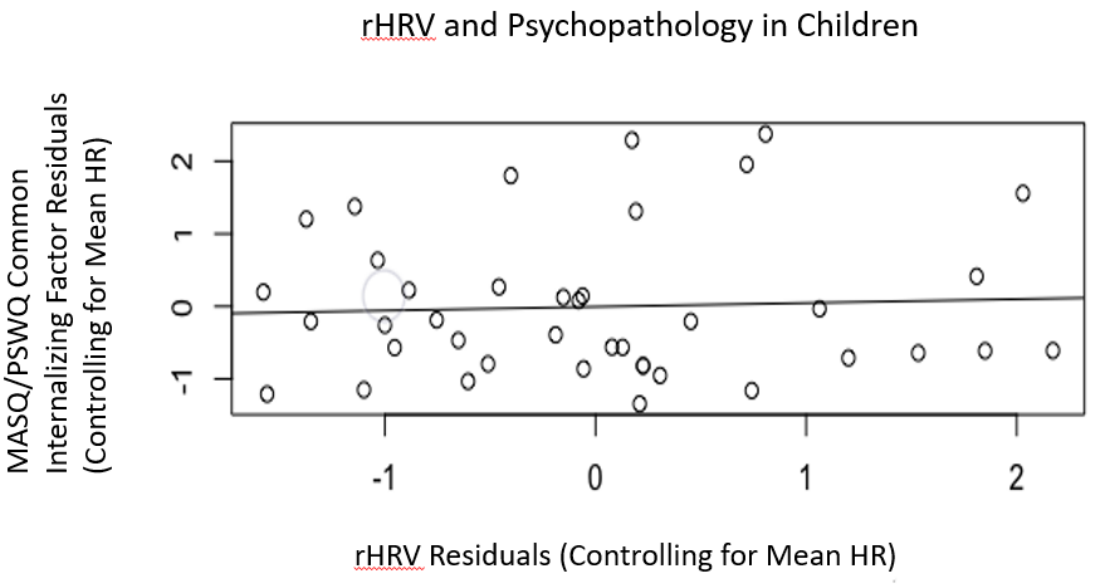

Figure 11: Association of rHRV residuals with Common Internalizing Measure residuals scores in children; the effect of mean heart rate removed. DF = 37, R^2 = 0.0526, P = 0.7473

rHRV values were analyzed with TP1 psychopathology factor scores of: Anxious Arousal, Low Positive Affect, Worry, and the Common Internalizing Factor, each a separate in a separate linear model; for parents only. No significant correlation was found between rHRV and AA, LPA, Worry, or CIF in parents, as seen in Figures 10 and 11, and Tables 4 and 5. Psychopathology Factor scores have been shown to be stable across time (Hatoum 2018). The reason behind not having TP2 factor score data for the MASQ and PSWQ is the same as the reason there is no GABA data for TP2; the experts working on processing the data still must finish their analyses and quality control.

DISCUSSION

Unfortunately, none of the proposed relationships were observed. No significant relationships were discovered between TP2 rHRV and a) TP1 PFC levels of GABA, b) measures of emotional control or c) symptom severity on measures related to psychopathology. One notable result of practical importance that has not been systematically looked at in the literature came from the rHRV measurement. That showed that cutting off the first one minute of data versus cutting of the first five minutes of data does not impact rHRV values significantly, showing that 1 minute is a reasonable time for scanner acclimation for participants, which allows for a longer and richer data set to process rHRV. It is likely that MRI procedure setup, including the technician setting up the participant, and aligning brain images in the scanner to be useful to analyze (which takes at least two to five minutes), may help acclimate participants to the scanner. Scanner setup and image alignment (shimming) protocols are common across almost all

neuroimaging studies, giving more strength to the conclusion that it is not necessary to cut off more than one minute of data.

While no significant relationships were observed with the current data set, the null results must be interpreted with caution. The study is on-going and will involve a larger sample size for Time Point 2, roughly 160-200 total participants rather than the current 80, and more than the current 21 total adult-only participants for the key GABA-rHRV analyses, and other key TP2 data including TP2 GABA concentrations and brain anatomy data will be required to make further conclusions. GABA data is not stable over time in children, as their brains are developing and have changing neurotransmitter levels, so their data cannot be reliably used in this study until TP2 data is acquired and processed (Sturman 2011).

In addition to neurotransmitter levels, it will be interesting to see how brain anatomy, particularly cortical thickness, is associated with rHRV in this sample. It is known that rHRV is associated with decreased cortical thickness in healthy adolescents, which could indicate a relationship between rHRV and cortical pruning, a process which optimizes brain network connections to be more efficient for cognitive function and brain communication in development (Sturman 2011). Greater cortical thickness is associated with reduced rHRV in female adolescents, which could have implications in gender differences of development. Lateral orbitofrontal cortex and anterior cingulate cortex, both involved in emotional regulation, have a significant association between cortical thickness of the orbitofrontal cortex and anterior cingulate cortex and rHRV, showing another connection to emotional regulation and rHRV (Koenig 2017). Greater levels of depression severity have been associated with lower cortical thickness of the right insula, meaning the insula could have less impact affecting the body to reflect emotional states, which could lead to decreased parasympathetic control over rHRV in

adolescents. Additionally, greater cortical thickness of frontal brain regions in adolescents with MDD may serve as a compensatory mechanism, beneficial to maintain autonomic balance, according to some studies. These pieces of evidence show that studies that measure brain anatomy as well as rHRV can show important relationships between emotional regulation and autonomic control.

CONCERNS

The main concern with rHRV measurements in a neuroimaging study using PRV as a proxy is that motion can change the level of blood moving to the fingertip, therefore disrupting PPG measurements. Thus, rPRV will always have limitations as a proxy for rHRV. Though corrections can be made to motion artifacts, which can smooth motion artifacts by referencing peaks around that data (Kubios 2018), it is unclear whether moving disrupted this samples' PRV values and gave corrupted rHRV data. However, in parents, rHRV was significantly negatively correlated with age, which is supported by literature, and gives the data credibility when using it as a proxy to ECG measured rHRV (Shaffer 2017). While rPRV is not a perfect measure, it is more time efficient to use in the scanner and it is highly likely that the most of the data is relatively uncorrupted, provided that data above 125ms is cutoff, as this was likely due to finger motion rather than normal rHRV.

Correlations with all of the pre-scan questionnaire measures and rHRV were performed, however, it may be the case that exercise was poorly indexed. There was no differentiation between moderate to vigorous exercise in hours per week, which could give poor readings of whether participants were athletes or not, which is known to have an effect on rHRV (May, McBerty, Zaky, & Gianotti, 2017). Exercise was also self-reported, which could vary widely.

However, this measure was not significantly associated with rHRV, so there may not be an issue with the current data, but this should be noted when analyzing the full TP2 data set.

TP1 GABA (for parents) and psychopathology data (for parents and children) was not significantly correlated with current rHRV measures, but TP2 data is more likely to have stronger associations (or at least higher reliability), due to closer times of measurements to each other. However, ERQ TP2 data did not correlate with rHRV measures, which was unexpected. Cognitive control usually positively correlates with rHRV (Gross 1998, 2003, 2011). This could potentially be due to low power, as the number of subjects in each group of parents and children is relatively small at this point. Still, it is worth more investigation into how cognitive control is associated with rHRV, and how these survey measures relate to physiological data.

FUTURE DIRECTIONS

Findings from this study suggest that waiting one minute or five minutes for starting rHRV collection has no effect on scanner acclimation. In addition, calculations of rHRV from PPG measurements are relatively easy to set up and process. Critically, they do not add much time to MRI scanner protocol. This is important because scanner time is extremely costly, and most studies maximize time to acquire brain data. PPG is a quick and inexpensive way to measure physiological data as well. rHRV measurements from the MRI scanner environment can offer physiological data that can reference to autonomic activity in addition to whatever MRI (or MRS) data is being collected. With MRS technology, studies can go a step further in a multi-level analysis, going from molecules, (e.g. neurotransmitter concentration) to circuitry, to function, behavior and cognitive and emotional state, and add physiological factors that relate to any other given level of analysis.

TP2 data, which will be completed far past the deadline of this thesis, will give crucial measures of neurotransmitter levels in the DLPFC and VLPFC. With successful rHRV measurements, associations between rHRV and neurotransmitters can be made with more confidence and related to the neurovisceral integration model and autonomic circuitry (Thayer 2009, Colzato 2018). In addition, brain anatomy, particularly cortical thickness, can be investigated regarding rHRV, as previous studies have shown that higher rHRV is associated with lower levels of depression and lower cortical thickness (Yoo 2017). This could be a method to investigate susceptibility to depression based on physiological signs (e.g. rHRV) and show how autonomic activity can affect the cortex (Yoo 2017). Relationships between rHRV, brain neurotransmitters and cortical thickness could help show how development affects cognition, autonomic activity and organization of brain networks. With the completion of TP2, it will be investigated whether higher GABA is positively associated with higher rHRV, and if cortical thickness and psychopathology modify these relationships. Investigating how a physiological measure of rHRV can predict measures of the brain and emotional regulation is important to studies that seek to gain a multi-level approach to analysis of the brain and its influence on cognition, emotion and behavior.

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APPENDICES

APPENDIX A1

fMRI acquisition. Images will be acquired on a Siemens Prisma (3T) MRI scanner with 32-channel parallel imaging located at the University of Colorado, Boulder. Functional imaging will use a T2*-weighted gradient-echo, echo-planar imaging (repetition time [TR] = 460 ms, echo time [TE] = 27.2 ms, flip angle = 44°, 56 slices parallel to the orbitofrontal cortex, thickness = 3 mm, zero gap, 82x82 in-plane resolution, in-plane FOV = 24.8cm, multi-band acceleration factor = 8). A high-resolution T1-weighted anatomical scan will be collected for each participant to localize functional activity. Resting state connectivity will be collected using parameters identical to prior functional task runs (i.e. TR TE, FOV, etc.) while participants fixate on a central crosshair. DTI data will be acquired using a sequence of 4 diffusion encoded spin echo EPI sequences acquiring a total of 173 diffusion directions (TR= 4000ms, GRAPPA off,

TE=112, FOV=224mm, 72 interleaved 2mm slices, zero gap, in-plane resolution 112x112, b-value 3000, multi-band acceleration factor = 3) (INC, CU Boulder 2017).

APPENDIX A2

To analyze HRV properly by measuring beat to beat intervals, all beats measured must be accurate and be due to the heart's contraction rather than movement of the participant wearing an HRV monitoring device, misplaced beats, or other artifacts. The output or reading of HRV is time variant, which means that it depends on exactly which time it is measured. Thus, a time varying threshold is calculated to determine the difference between normal and artifact heartbeat measurements. The threshold for each beat is calculated to be 5.2 times as much as the quartile deviation of beats in the dRR series, which typically accounts for 99.5% of all heartbeats, assuming normal distribution of the heart rate data (Kubios 2018). However, when a dRR series is not normally distributed, as is common for such a varying measure, some normal beats exceed this threshold, and a decision algorithm is used to further differentiate normal beats from artifacts (Kubios 2018). Non-normal beats that corrupt the dRR series measured form specific patterns, which helps in recognizing what sort of artifact the non-normal beat is, whether it be abnormal or ectopic, long, short, or a missed beat (Kubios 2018). Missed or extra beats are identified by comparing where the beat should have been in time compared to the surrounding 10 beats to where it lies in the dRR series. Correction of ectopic beats or artifacts happens once ectopic beats are identified. These beats are then replaced with interpolated RR values from beats surrounding the ectopic beat, placed according to the time at which the beat should have occurred (Kubios 2018). Missed beats are added by adding a new R-wave occurrence time according to surrounding beats, and extra beats are corrected by removing the extra R-wave detected and recalculating the RR interval at that time point of measurement. Detrending (e.g. remove motion, drift) is based on Smoothing Priors Approach (SPA), which preserves frequency components while acting like a high pass filter (editing out motion). The trend can be adjusted (Lambda) to be smoother or less smooth with more or less filtering. 500 Lambda is common across physiological literature (Kubios 2018, Colzato 2017, Thayer 2011).

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