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The impact of self-reported levels of anxiety on respiratory sinus arrhythmia levels in adults who stutter

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ABSTRACT

Purpose: This study investigated whether subjective levels of anxiety predict respiratory sinus arrythmia (RSA) levels in adults who stutter (AWS) compared to (ANS) during baseline and social stress situations.

Methods: Participants were eight AWS and 10 ANS who performed a modified version of the Trier Social Stress Test (TSST-M). For this, participants were required to prepare and deliver a 5-minute speech and perform a nonword reading task in front of what was perceived as a group of professionals trained in public speaking. Measures of respiratory sinus arrhythmia (RSA) were calculated for baseline and TSST-M conditions. Participants also completed the State-Trait Anxiety Inventory (STAI), both the trait (STAI-T) and state (STAI-S) portion, which served as subjective anxiety ratings. Univariate analyses of variances (UNIANOVA) were used to assess the effects of the STAI-T and STAI-S anxiety on respiratory sinus arrhythmia (RSA) levels at pre-stress and TSST-M conditions. RSA, an index of parasympathetic nervous system activity, is considered to be a measure of emotional regulation. The strength of the effects of STAI-T and STAI-S on RSA levels was evaluated with the unstandardized coefficients for each group separately.

Results: Results showed a significant difference between groups for the effects of STAI-T on RSA values for the pre-stress nonword reading task. No other significant differences were found between groups for the pre-stress or TSST-M conditions. Slope estimates showed that STAI-T was a significant predictor of RSA values for pre-stress speaking conditions for the AWS but not ANS. No significant fixed effects or interaction effects were found for the STAI-S and RSA levels in the AWS or ANS. Nor were there significant effects of STAI-T on RSA levels in the AWS or ANS for TSST-M conditions. Descriptive analysis revealed the effects found in the AWS during pre-stress conditions were attributed to a subgroup of AWS who reported low self-reports of anxiety (i.e. STAI-T) and high levels of emotional regulation (i.e. RSA) across social stress conditions.

Discussion: Low self-reported STAI-T scores simultaneous with high RSA levels in some AWS may reflect a self-regulatory strategy adapted in response to chronic, daily stress associated with stuttering.

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1. Introduction

There is overwhelming evidence to suggest that people who stutter are at risk for developing symptoms of anxiety (Craig & Tran, 2014; Iverach & Rapee, 2014; Iverach et al., 2009; Menzies, Onslow, & Packman, 1999; Mulcahy, Hennessey, Beilby, & Byrnes, 2008). Negative emotional reactions to speaking including feelings of frustration, embarrassment, rejection, and humiliation are thought to lead to high levels of anxiety in adults who stutter (AWS) (Craig & Tran, 2014; Guitar, 2019). Several studies report high levels of state anxiety in AWS (Blumgart, Tran, & Craig, 2010; Iverach et al., 2009), which refers to the current state of feeling, at the time of the self-report. Others report anxiety in AWS is more specific to social situations, which has bene proposed to emerge from living with stuttering for many years (Blumgart et al., 2010; Iverach et al., 2009). For instance, Blumgart et al. (2010) found that 40 % of AWS met criteria for a Social Anxiety Disorder and Iverach et al. (2009) found that out of 27 AWS undergoing treatment, 30 % exhibited a diagnosis of social anxiety. Importantly, not all studies report differences in social anxiety in AWS (Blood, Blood, Bennett, Simpson, & Susman, 1994; Blumgart et al., 2010; Manning & Beck, 2013), suggesting that individual differences exist within the stuttering population. While a recent meta- analysis by Craig and Tran (2014) revealed that AWS were almost one standard deviation above controls for social anxiety, they also reported that AWS were over one-half a standard deviation above controls for levels of trait anxiety (Craig & Tran, 2014). Trait anxiety refers to the stable tendency of an individual to experience chronic, negative emotions such as worry and fear (Kraaimaat, Vanryckeghem, & Van Dam-Baggen, 2002). Based on this research, it can be suspected that the chronic, daily social stressors experienced by AWS can lead to elevated levels of trait anxiety. Little is known, however, as to how these elevated levels of self-reported anxiety relate to emotional reactivity and regulatory processes in AWS during social situations.

1.1. *Autonomic nervous system*

The autonomic nervous system is responsible for controlling physiological activity associated with emotional reactivity and regulatory processes such as heart rate, respiratory control and pupil dilation, allowing a person to respond to day-to-day environmental stimuli. Two main branches of the autonomic nervous system, the sympathetic and parasympathetic branches, work complementary to each other, forming what is considered an autonomic balance (Bernston, Cacioppo, & Grossman, 2007; Berntson, Cacioppo, Quigley, & Karen, 1991; Porges, 2007) that is continuously changing throughout day-to-day interactions and environmental events. The sympathetic nervous system plays a large role in preparing the body to react to emotional situations. The commonly known term “fight or flight response”, refers to the physiological changes governed by the sympathetic nervous system that drive the body to prepare for action, resulting in increases in heart rate, blood pressure, sweat production and cortisol levels (Bernston et al., 2007; Porges, 2007). Increases in sympathetic nervous system activity occur when input from the vagus nerve is inhibited. The vagus nerve sends autonomic fibers to the heart, lungs and digestive organs via the parasympathetic nervous system and plays a role in emotional regulation. When the vagus nerve is inhibited, sympathetic nervous system increases which lead to elevated levels of heart rate, sweat production, and blood pressure (Porges, 2007). When the vagus nerve is disinhibited, parasympathetic influences dominate, resulting in decreases in heart rate. The parasympathetic system works on a time scale of milliseconds and compared to the more slowly acting sympathetic branch, is considered to be responsible for the instantaneous cardiac responses that are required when responding to environmental demands.

Parasympathetic nervous system activity can be indexed by measuring respiratory sinus arrhythmia (RSA), which is a metric of high frequency HR variability. (i.e., beat-to-beat variability). An increase in RSA (i.e., increase in inhibitory control on the sympathetic branch) is associated with decreases in heart rate and increases in HR variability (Porges, 2007). Studies have found a positive association between RSA levels and emotional regulation. For instance, research has shown that increases in RSA reflect positive affect (Calkins, 1997), better social awareness (Seuss, Porges, & Plude, 1994), as well as improved behavioral regulation (Stifter & Fox, 1990), social engagement (Balzarotti, Biassoni, Colombo, & Ciceri, 2017; Mauss, Wilhelm, & Gross, 2010; Thayer and Lane, 2000) and effortful control (i.e., regulation of appetitive or aversive stimuli) (Sulik, Eisenberg, Silva, Spinard, & Kupfer, 2013; Sulik, Eisenberg, Spinrad, & Silva, 2015). For instance, by tracking facial expressions and RSA levels when viewing negative stimuli, Pu, Schmeichel, and Demaree (2009) reported that individuals with high RSA levels were better able to suppress negative emotion in a nonclinical population. Others report high RSA in individuals who are more effective at regulating stress through the use of attentional processes (Balle et al., 2013; Spangler & Friedman, 2015) and self-control strategies (Geisler & Kubiak, 2009; Pu et al., 2009) such as cognitive reappraisal or suppression (Fabes & Eisenberg, 1997; Volokhov & Demaree, 2010). From this perspective, increased RSA (i.e. vagal input) may help prevent or reduce daily responses to stress (Fabes & Eisenberg, 1997) and improve the ability to socially engage in a flexible, adaptive manner (Porges, 1992). Conversely, lower RSA levels or decreased heart rate variability, has been found in high anxious individuals (Park, Van Bavel, Vasey, & Thayer, 2012; Park, Vasey, Van Bavel, & Thayer, 2013; Park, Vasey, Van Bavel, & Thayer, 2014) and has been suggested to contribute to poor inhibitory control (Park, Moon, Kim, & Lee, 2012; Park, Van Bavel et al., 2012; Park et al., 2013) and reduced attentional regulation (e.g., Park, Moon et al., 2012).

1.2. *Autonomic nervous system activity and anxiety*

The relationship between sympathetic and parasympathetic nervous system activity in response to stress has been studied extensively in individuals with high anxiety (Jezova, Makatsori, Duncko, Moncek, & Jakubek, 2004; Jonsson, 2007). Although the nature of this relationship is unclear (Davies et al., 2002; Knyazev, Slobodskaya, & Wilson, 2002; Watkins, Grossman, Krishnan, & Blumenthal, 1999), several studies show high versus low anxious individuals exhibit elevated levels of sympathetic activity and subsequent lower parasympathetic control at rest and during challenge (Balzarotti et al., 2017; Brosschot, VanDijk, & Thayer, 2007).

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Low parasympathetic levels have also been found in individuals with higher levels of trait anxiety (Fuller, 1992; Piccirillo et al., 1997; Watkins, Grossman, Krishnan, & Sherwood, 1998) and increased daily worry (Brosschot et al., 2007) as well as in populations diagnosed with General Anxiety Disorders (Lyonfields, Borkovec, & Thayer, 1995; Thayer, Friedman, & Borkovec, 1996).

Not all studies report increased sympathetic and decreased parasympathetic activity in individual with high levels of anxiety, however. Some studies report lower cortisol levels (Vingerhoets et al., 1996) and sweat production (Wilken, Smith, Tola, & Mann, 2000) as well as an overall reduced stress responsiveness (Anegg et al., 2002) in high anxious individuals. Jezova et al. (2004) found that adults with high trait anxiety showed an inability to release adequate levels of stress-induced hormones (e.g., HPA axis) during a modified version of the Trier Social Stress test (Kirschbaum, Pirke, & Hellhammer, 1993). Jonsson (2007) found high baseline levels of RSA in high versus low state-anxious adults and suspected it to be associated with the need to increase their attention to their surroundings (i.e., hypervigiliance), which is a common characteristic found in individuals with anxiety (Eysenck, 1997; Thayer, Friedman, Borkovec, Johnsen, & Molina, 2000).

In summary, many studies report individuals with high levels of anxiety exhibit decreased parasympathetic activity and subsequent increased sympathetic activity, which is thought to reflect poor attentional (Park, Moon et al., 2012; Park, Van Bavel et al., 2012) and emotional regulation (Lyonfields et al., 1995; Thayer et al., 1996). On the contrary, other studies report that high anxious individuals exhibit what appears to be a defensive response, characteristic of increased attention and hypervigilance that is associated with reduced heart rate (Thayer et al., 2000) and increased levels of RSA (Jonsson (2007). This later disposition renders the autonomic system less able to respond effectively to changing or novel environmental stimuli.

1.3. Autonomic nervous system activity in adults who stutter

Given the nature of stuttering and its association with anxiety (Alm, 2014; Craig & Tran, 2014; Iverach & Rapee, 2014), a number of studies have explored the effects of stress on the autonomic nervous system in AWS. Peters and Hulstijn (1984) assessed skin conductance, heart rate and pulse volume in AWS compared to ANS before and during the performance of a number of speech (e.g., reading and conversation task) and nonspeech (e.g., mirror writing and intelligence test) tasks. While both AWS and ANS showed comparable rises in skin conductance in response to study tasks, AWS did not show increases in HR to the same extent as the ANS just prior to the spontaneous speaking task. Similar results were found in a study by Weber and Smith (1990) where the AWS and ANS showed comparable increases in skin conductance levels (SCL) when performing spontaneous speaking and reading tasks; however, just prior to the spontaneous speaking task, when preparing to speak, the AWS' HR decreased while the ANS showed an increase. As AWS transitioned into the speaking task, similar to Peters and Hultijn (1984), both groups showed increases in HR, however the AWS' were much less pronounced. Reduced HR prior to stimulus onset in AWS have been reported in other studies (e.g., Caruso, Chodzko-Zajki, Bidinger, & Sommers, 1994) and taken together suggest an autonomic coactivation where parasympathetic influences are working concurrently with sympathetic influences. From this perspective, it can be hypothesized that the anticipation of speaking resulted in an increase in parasympathetic input that suppressed sympathetic influences, leading to the temporary slowing of the heart (i.e., bradycardia), a suggestion made by Alm (2014).

More recent investigations have examined autonomic activity in AWS when speaking under anxiety -inducing conditions that are specific to feelings of social stress. Dietrich and Roaman (2001) required AWS to rate on a 7-point scale how they would feel speaking in 20 different anxiety-provoking speaking situations (e.g., speaking on the phone). They were then asked to enact four of the 20 speaking situations while their SCL were monitored. Situations ranged from calling a local business to discussing their experiences with stuttering while being video recorded with the expectation that it would be shown to a classroom of students. Results showed no relationship between sympathetic nervous system activity (i.e., SCL) and self-ratings of anxiety promoting situations. The authors speculated that one reason for the lack of correlation may be that the reenacted speaking situations did not elicit as much anxiety as was originally predicted. Alternatively, based on previous evidence of decreased heart rate during stress (Caruso et al., 1994; Peters & Hultijn, 1984; Weber & Smith, 1990), an increase in parasympathetic input may have led to increases in inhibitory activity on the sympathetic branch, resulting in suppressed skin conductance levels. However, this is speculative as HR and RSA measures were not included in the assessment.

Bowers, Saltuklaroglu, and Kalinwoski (2012) assessed skin conductance reactivity and HR in AWS just prior to reading passages that included feared versus neutral phonemes under two separate conditions, solo reading and choral reading. Increases in skin conductance reactivity were found during the reading of feared versus neutral phonemes, while decreases occurred when choral versus solo reading. Measures of heart rate showed a triphasic response prior to speaking that represented an initial deceleration, followed by an acceleration and then another deceleration. A triphasic response has been reported to occur immediately prior to viewing negatively valenced stimuli in nonclinical populations of children (Berg & Byrd, 2002) as well as during situations that require increases in attention and monitoring in ANS (Gray & McNaughton, 2000) suggesting an autonomic response that is associated with the anticipation of negative stimuli and in the case of AWS in Bowers et al. (2012), suggestive to be due to the anticipation of stuttering. Another important finding from their study was that the final HR deceleration of the triphasic response was found to occur simultaneous with an increase in skin conductance response, thus providing further evidence for an autonomic coactivity where both sympathetic and parasympathetic influences are found prior to speaking.

Bauerly, Jones, and Miller (2019) assessed the autonomic, behavioral and acoustic effects of social stress in AWS compared to ANS using a modified version of the Trier Social Stress Test (TSST-M) (Kudielka, Hellhammer, & Kirschbaum, 1997). Results showed similar sympathetic nervous system activity between groups as both AWS and ANS showed significant increases in SCL and HR following social stress induction; however, differences in parasympathetic nervous system activity emerged in the AWS as they showed significantly greater RSA levels at rest. The authors suggested that the increase in RSA may have been associated with the significantly

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higher self-reports of trait anxiety and reflected the need to increase attention monitoring and adaptive regulatory strategies as they approached the novel laboratory situation (for related findings see(Balzarotti et al., 2017); Jonsson, 2007). Increased RSA levels in the AWS compared to the ANS were maintained during the speaking conditions, with increases found when having to speak in monologue during social stress induction.

Taken together, it is evident that autonomic nervous system activity is different in AWS compared to ANS when speaking under stressful conditions. More specifically, AWS do not appear to show the reciprocal relationship between sympathetic and parasympathetic branches found in controls and based on recent findings from Bauerly et al. (2019) suggest that this is due to an autonomic coactivity where parasympathetic influences remain high, despite sympathetic responses to stress. Factors that may play an influential role to this relationship require further investigation such as individual differences in anxiety levels.

1.4. Aims and hypothesis

The purpose of this study was to expand on the study from Bauerly et al. (2019) by considering the effects of self-reported levels of anxiety on RSA levels before and during social stress conditions in AWS and ANS. The relationship between self-reported anxiety and parasympathetic nervous system activity during social stress in AWS is unknown; however, investigations into this interaction may help explain some of the inconsistencies reported in the literature regarding sympathetic reactivity in AWS when speaking under social stress. Research assessing the relationship between anxiety and RSA in the nonclinical population is mixed with some reporting low RSA levels in high anxious individuals, while others reporting high RSA levels (Balzarotti et al., 2017; Jonsson, 2007). Given the high levels of RSA and subjective levels of state and trait anxiety observed in the AWS compared to ANS in Bauerly et al. (2019), further analysis on these variables were explored within and across groups. It was hypothesized that scores on Speilberger's State-Trait Anxiety Inventory (Speilberger, Gorsuch, Luschene, Vagg, & Jacobs, 1983) would be associated with RSA levels in both baseline and social stress conditions in both groups. Given that AWS, compared to ANS, showed higher levels of RSA, STAI-T (State-Trait Anxiety Inventory-Trait portion) and STAI-S (State-Trait Anxiety Inventory – State portion) in Bauerly et al. (2019), it was expected that this association would be stronger in the AWS.

2. Methods and procedures

2.1. Participants

Participants included eight AWS (4 males, 4 females) with an average age of 20.21 years (range 19–29) and 10 ANS (7 males, 3 females) with an average age of 25.8 years (range 18–48) (Bauerly et al., 2019). Participants' data included in the present study were also in Bauerly et al. (2019) study[1] with the exception of five of the participants whose ECG data had been corrupted. AWS were recruited from recruitment fliers and referrals from the Speech and Hearing Clinic at Plattsburgh State University. General subject inclusion criteria included the following: (1) English as the primary language; (2) self-reported negative medical history of neurological disorders or drug use affecting speech production; (3) self-reported negative history of psychiatric or developmental disorders; (4) self-reported negative history of cardiac arrhythmia or high blood pressure; (5) self-reported negative history of speech or language problems, other than stuttering for the AWS, (6) self-reported good ocular health and no history of visual or auditory pathologies, and (7) pure-tone conduction hearing thresholds within clinically normal limits (<20 dB HL from 1000 Hz – 3000 Hz).

Stuttering specific inclusion criteria for the AWS included the following: (1) no formal speech fluency treatment in the last year; (2) onset of stuttering in childhood (pre-puberty); (3) a minimum of 3% within-word disfluencies in at least one of the speaking conditions (reading, conversation); classified as either mild (n = 4), moderate (n = 2) or moderate-severe (n = 2) on the Stuttering Severity Index-4th Edition (Riley, 1994). All of the AWS reported that they had treatment that occured more than four years prior to participation in the study. All of the AWS reported that previous treatment included speech restructuring only (e.g., fluency shaping) and that no one had received strategies aimed at cognitive restructuring (Guitar, 2019).

All participants completed Speilberger et al.'s (1983) self-reported, State-Trait Anxiety Inventory (STAI) prior to performing the experimental tasks. The state portion of the STAI (STAI-S) is aimed at measuring an individual's emotional state at that moment while filling out the questionnaire. For this, participants responded to statements such as "I feel worried" with responses ranging from (1) not at all to (4) very much so. Some emotional qualities assessed by the STAI S-Anxiety Scale include feelings of tension, nervousness and worry (Speilberger et al., 1983) and high scores reflect increases in negative feelings towards everyday situations. The trait portion of the STAI (STAI-T) is aimed at assessing how a person generally feels overall and is not specific to a situation. For this participants responded to statements such as "I worry too much over something that really doesn't matter" with responses ranging from (1) almost never to (4) almost always. Similar to the state portion, high scores reflect increased negative emotional feelings associated with anxiety. The STAI-T has been a widely used measure of clinical anxiety (Speilberger, 1983). Similar to what was reported in a larger sample in Bauerly et al. (2019), AWS' STAI-T scores were significantly higher ($M = 36.88$, SD = 7.47) than ANS' ($M = 27.3$, SD = 4.16, $t(16) = 3.452$, $p = .003$ (two-tailed). However, different from what was reported in Bauerly et al. (2019) and due to the differences in sample size, STAI-S scores did not significantly differ between AWS ($M = 30.00$, SD = 5.78) and ANS ($M = 32.2$, SD = 6.37), $t(16) = .756$, $p = .460$. Please refer to Table 1 for a description of participant information for the AWS.

[1] The previous study by Bauerly, Jones, & Miller (2019) involved a larger sample number and a different hypotheses (none overlapping) from the present study.

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Table 1
Participant Information.

Subject	Group	Age	Gender	Length since treatment	SSI-4 standard scores (severity level)	STAI-S	STAI-T
1	AWS	21.0	F	>4 years	9 (mild)	32	28
2	AWS	24.9	F	>4 years	27 (moderate)	35	45
3	AWS	23.6	M	>10 years	30 (moderate-severe)	32	42
4	AWS	21.3	F	>10 years	9 (mild)	21	29
5	AWS	29.0	M	>10 years	32 (moderate-severe)	24	30
6	AWS	21.6	M	>10 years	10 (mild)	38	35
7	AWS	21.4	M	>5 years	22 (moderate)	32	47
9	AWS	19.1	F	>10 years	8 (mild)	26	39
1	ANS	25.0	M	n/a	n/a	30	30
2	ANS	25.3	M	n/a	n/a	32	44
3	ANS	19.1	F	n/a	n/a	27	36
4	ANS	21.2	F	n/a	n/a	23	26
5	ANS	48.1	F	n/a	n/a	22	29
6	ANS	27.8	M	n/a	n/a	31	37
7	ANS	23	M	n/a	n/a	33	37
8	ANS	21	M	n/a	n/a	25	33
9	ANS	18	F	n/a	n/a	22	23
10	ANS	30	F	n/a	n/a	28	27

Note: Participant numbers correspond to Bauerly et al. (2019).

2.2. Instrumentation

The physiological measures of skin conductance level (SCL) and heart rate (HR) were collected using Biopac MP 160 (Biopac Systems, Inc.) but only measures of HR are discussed here as this measure was used to obtain respiratory sinus arrhythmia (RSA). An electro-cardiogram (ECG) signal was used to derive HR measure. Two disposable Ag/AgCl electrodes were applied to the skin surface just below the right clavicle and the other at the 12th rib laterally on the left side and connected to an ECG 100C amplifier from Biopac MP 160 system (Biopac Systems, Inc.). Participants were also fitted with a head-mounted condenser microphone (AKG C410) for speech recordings that were used for measurements in a previous study (Bauerly et al., 2019).

2.3. Trier social stress test- modified

The Trier Social Stress Test (TSST) is a widely used laboratory procedure for inducing psychological stress (Kirschbaum et al., 1993; Kudielka et al., 1997). The TSST has been modified to meet the needs of a variety of research focuses but generally consists of a speech preparation, speech performance and verbal arithmetic condition followed by a recovery and debriefing period (Birkett, 2011). The TSST has been reliably shown to elicit elevated levels of heart rate, blood pressure, and skin conductance (Kirschbaum et al., 1993). For the purpose of assessing the relationship between self-reported anxiety and RSA levels during speaking (Bauerly et al., 2019), the TSST was modified by replacing a mental arithmetic task with a nonword reading task (TSST-M Nonword Reading). For all social stress conditions, participants were under the impression that their performance was video recorded for later viewing by a panel of judges trained in public speaking. A detailed description of the full TSST-M is outlined in Bauerly et al. (2019).

2.4. Procedures

This study consisted of one lab visit that included the following: (a) the consent process, (b) collection of pre-task measures including demographic information, stuttering severity and STAI questionnaire, (c) equipment set-up, (d) pre-stress, baseline conditions (Resting, Monologue, Reading), (e) stress conditions (TSST-M Preparation, TSST-M Monologue, TSST-M Nonword Reading), and (f) recovery (TSST-Recovery).

i *Resting Baseline.* Once participants were placed with physiological equipment for measuring HR, they were asked to sit quietly for five minutes. Baseline measures of heart rate were recorded at this time.
ii *Monologue Baseline*[2] . Participants were asked to describe, for five minutes, their dream job to the primary investigator. If they finished in less than five minutes, they were prompted to continue speaking. HR measures were recorded at this time.
iii *Reading Baseline*[3] . Following the five- minute monologue task, the participant was asked to perform a reading task where the ten CVC nonwords were in a different order across presentations. Sequences of 10 CVC nonwords were presented for three seconds each. The ten CVC nonwords were randomized and so the participant produced these ten CVC nonwords in a different order each time. Once the participant read the sequence out loud, the sequence disappeared, and a new sequence with the same words

[2] Monologue baseline was termed “Pre-stress Monologue” in Bauerly et al., 2019.
[3] Reading baseline was termed “Pre-stress Nonword” in Bauerly et al., 2019.

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but different order, was presented on the screen. This continued until 20 sequences were shown. HR measures were recorded during this task.

iv *TSST-M Preparation.* The speech preparation portion of the TSST (TSST-M Prep) required participants to prepare a speech as to why they would be a good candidate for their ideal job. They were informed that they would have five minutes to prepare and that their speech would be recorded for later viewing by a panel of judges trained in public speaking. The examiner provided the participant with a paper and pencil for note taking and then left the room for five minutes. HR measures were recorded at this time.

v *TSST-M Monologue.* After 5 min, the primary investigator returned to the room to begin the "social evaluative phase" of the TSST-M which included the TSST-M Monologue and Nonword Reading task conditions. The primary investigator positioned a video camera approximately four feet from the participant and began recording. The examiner then pulled out a paper and pen for note taking and gave the following instructions: "This is the speech portion of the task. You are to deliver a speech describing why you would be a good candidate for your ideal job. You should speak for the entire five-minute period. Your time begins now." If the participant stopped talking before five minutes was over, the investigator prompted: "you still have time remaining". At the end of the five-minute speech, the participant performed a nonword speaking task as described below. HR measures were recorded during this time.

vi *TSST-M Reading.* Following the five- minute speech, the participant was asked to perform the same reading task as described in the Reading baseline condition where they were required to read a ten, consonant-vowel-consonant (CVC) nonword sequence depicted on a computer screen at their normal speaking rate and loudness level. This condition was also being recorded and participants were told that this part of the task will also be viewed by a panel of judges trained in public speaking. Identical to the baseline condition, the ten CVC nonwords were in different order across presentations, presented for three seconds each, were randomized and counterbalanced across participants, totaling twenty sequences each. Following a sequence presentation, a new sequence was presented on the screen. HR measures were recorded during this task.

vii *TSST-M Recovery.* Participants were informed that the study was complete and they were asked to sit comfortably for five minutes while HR measures were collected. While there is still a level of stress to this condition as they were not yet debriefed on the nature of the social stress tasks, they were informed that there would be no other tasks to perform. The TSST-M Recovery period has traditionally assessed autonomic activity after the participant has been debriefed of the true nature of the study. However, several modified versions of the TSST have included debriefing following equipment removal (e.g. Mascret et al., 2016). Similar procedures were carried out in the current study where following the five-minute recovery period, equipment was removed and the participant was informed that their performance was not going to be evaluated.

2.5. Analysis and dependent variables

Biopac's Acknowledge software was used to collect ECG signals. Only measures of ECG are reported here as this was used to obtain RSA values. Please refer to Bauerly et al. (2019) for a complete description of measures used.

2.5.1. Measures of RSA

An ECG signal was collected for each participant using Biopac MP160 (Biopac Systems Inc.). The signal was digitized at 1250 Hz and band-pass filtered to remove high frequency noise and low frequency drift (.5 Hz: high pass cutoff; 35 Hz: low pass cutoff). Biopac's Acknowledge software processed the ECG signal by detecting the peak of the R-wave and timing the sequential inter-beat-intervals (IBI) in milliseconds (ms); this was done for each condition. The IBI time series was then processed by CardioEdit software (Brain-Body Center, University of Illinois at Chicago, 2007) to correct for artifacts due to ventricular arrhythmias and faulty detections due to movement. Only four participants required hand correction from artifact and these corrections occurred in less than 10 s of the data. The IBI time series was then used to derive measures of RSA. A band pass filter was then applied to each IBI time series to extract the variation of frequency of respiration associated with an adult (.12–.4 Hz) (Byrne et al., 1996). Estimates of variance derived from this procedure were natural log transformed (ln(ms)2). RSA was derived from sequential 30 s epochs in each condition and averaged to obtain a total of seven RSA values for each participant (Lewis et al., 2012).

RSA measures were then used to derive change scores from the baseline conditions of RSA Resting$_{baseline,}$ RSA Monologue$_{baseline,}$ and RSAReading$_{baseline}$ (Helman et al., 2008). Change scores allowed for a more accurate representation of individuals' response to stress as speaking without stress can introduce variability in HR (Tininenko, Measelle, Ablow, & High, 2012). For measuring change from the non-talking stress conditions, RSA Resting$_{baseline}$ was subtracted from the RSA Preparation and RSA Recovery conditions. For measuring change from the TSST-M talking condition, RSA Monologue$_{baseline}$ and RSA Reading$_{baseline}$ were subtracted from TSST-M Monologue and TSST-M Reading conditions, respectively. This yielded the following RSA measures: **Resting$_{baseline}$, Monologue$_{baseline}$, Reading$_{baseline}$, Prep$_{reactivity}$** (TSST-M Preparation- Resting$_{baseline}$**)**, **Monologue$_{reactivity}$** (TSST-M Monologue –Monologue$_{baseline}$), **Reading$_{reactivity}$** (TSST-M Reading – Reading$_{baseline}$), and **Recovery$_{reactivity}$** (TSST-M Recovery – Resting$_{baseline}$).

To assess inter-rater reliability for RSA, 25 % of the data was randomly selected and measured by a trained researcher (at the graduate level). Bivariate correlations for RSA Resting$_{baseline}$ (.89), RSA Monologue$_{baseline,}$ (.84) and RSAReading$_{baseline}$ (.92) values derived from the first author and trained researcher were significant ($p < .01$) (Everitt, 1996).

2.6. Statistical procedures

Statistical analysis were performed using SPSS 26.0. Statistical analysis is described below separately for baseline conditions and

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TSST-M conditions. Due to the exploratory nature of the study and the small sample sizes, we did not perform a Bonferroni correction and set alpha to 0.05.

2.6.1. *Effects of self-reported anxiety on baseline RSA levels in AWS and ANS*

In order to assess the effects of self-reported levels of anxiety on RSA measures, a univariate analysis of variance (UNIANOVA) was performed. A UNIANOVA provided a regression analysis and analysis of variance for the dependent variable, RSA, with the independent variables Group (AWS vs. ANS) and STAI-T for each condition (Resting Baseline, Monologue Baseline, Reading Baseline) separately. An additional set of UNIANOVAs were ran for the independent variable STAI-S. The strength of the effects of STAI-T and

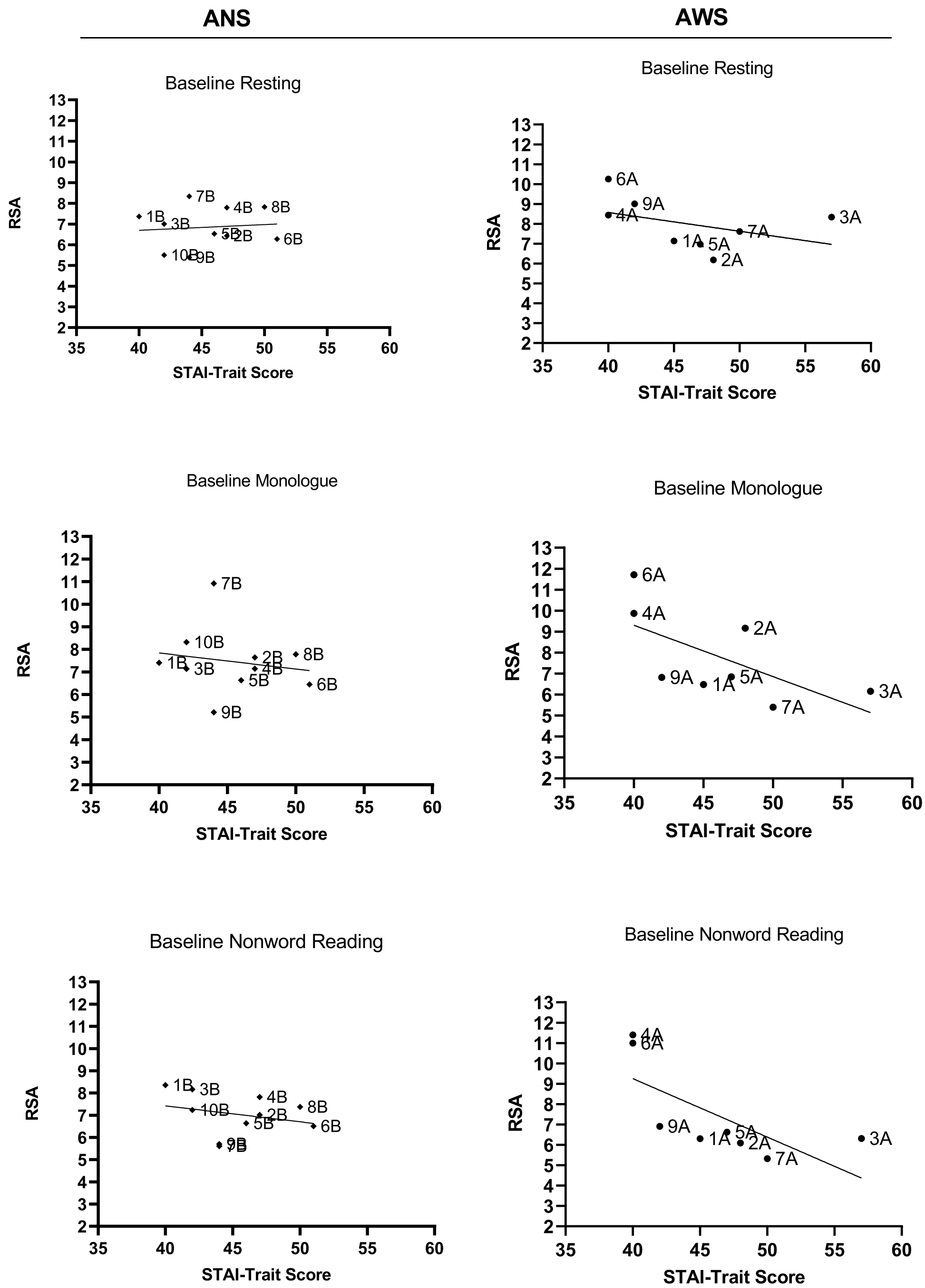

Fig. 1. Correlations between self-reported measures of STAI-T (Speilberger et al., 1983) and levels of respiratory sinus arrhythmia (RSA) for the adults who stutter (AWS) and adults who do not stutter (ANS) across pre-stress TSST-M conditions: Resting Baseline, Monologue Baseline, and Reading Baseline.

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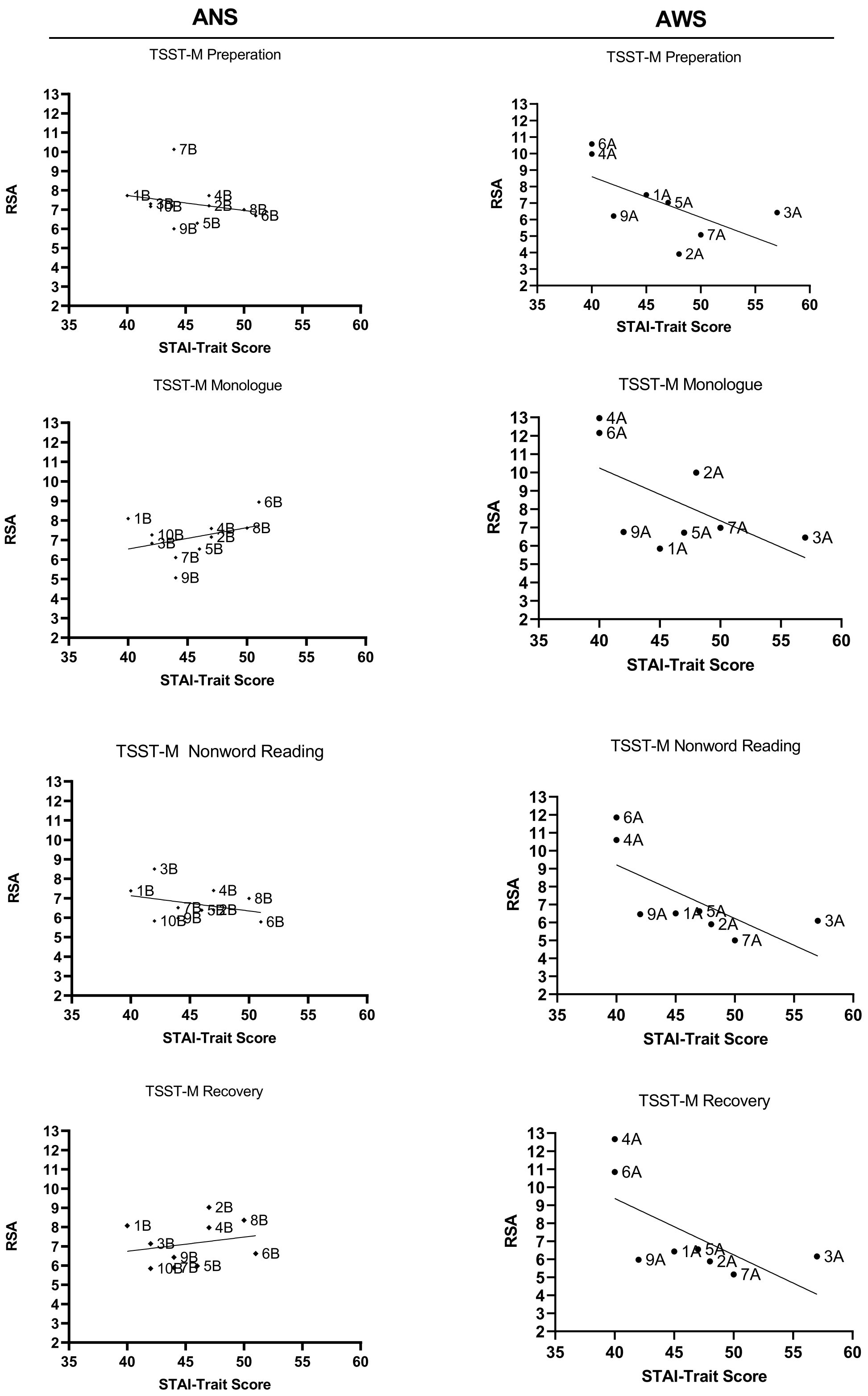

Fig. 2. Correlations between self-reported measures of STAI-T (Speilberger et al., 1983) and levels of respiratory sinus arrhythmia (RSA) for the adults who stutter (AWS) and adults who do not stutter (ANS) across TSST-M conditions: TSST-M Preparation, TSST-M Monologue, TSST-Nonword Reading, and TSST-M Recovery.

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STAI-S on RSA levels was evaluated with the unstandardized coefficients for each group separately. 2.6.2 Effects of self-reported anxiety on RSA measures during TSST-M conditions in AWS and ANS In order to assess the effects of self-reported levels of anxiety on RSA measures for TSST-M condition, additional UNIANOVAs were performed on the dependent variable RSA with the independent variables Group (AWS vs. ANS) and STAI-T for each condition (TSST-M Preparation, TSST-M Monologue, TSST-M Nonword Reading, TSST-M Recovery) separately. An additional set of UNIANOVAs were ran for the independent variable STAI-S. The strength of the effects of STAI-T and STAI-S on RSA levels was evaluated with the unstandardized coefficients for each group separately.

3. Results

3.1. Effects of self-reported anxiety on baseline RSA levels in AWS and ANS

An ANOVA revealed no significant differences in the effects of STAI-T on RSA values between AWS and ANS for $\text{Resting}_{\text{Baseline}}$ ($p >$ .05). There was also no significant association between STAI-T and RSA for the AWS ($\beta = -.094$, $p = .23$) or ANS ($\beta = .028$, $p = .80$). Similarly, an ANOVA revealed no significant difference in the effects of STAI-T on RSA values between AWS and ANS for $\text{Monologue}_{\text{baseline}}$ ($p > .05$); however, RSA significantly decreased in AWS as a function of STAI-T ($\beta = -.245$, $p = .043$) but not the ANS ($\beta = -.071$, $p = .656$). In other words, RSA decreased with increasing STAI-T scores in the AWS but not the ANS. For $\text{Reading}_{\text{baseline}}$, there was a significant difference in the effects of STAI-T on RSA values between AWS and ANS, F(2,18), $p = .021$. Also, for $\text{Reading}_{\text{baseline}}$, RSA significantly decreased as a function of STAI-T ($\beta = -.280$, $p = .007$) in the AWS but not the ANS ($\beta = .072$, $p = .57$). No significant differences were found for STAI-S and RSA in any of the conditions for AWS or ANS ($p > .05$).

3.2. Effects of self-reported anxiety on RSA measures during TSST-M conditions in AWS and ANS

An ANOVAs revealed no significant difference in the effects of STAI-T on RSA between AWS and ANS for any of the TSST-M conditions ($p > .05$). Slope estimates in RSA as a function of STAI-T in AWS did not reach significance for $\text{Prep}_{\text{reactivity}}$, $\beta = -.152$, $p = .06$ or $\text{Recovery}_{\text{reactivity}}$, $\beta = -.218$, $p = .06$. Likewise, the ANS did not show a significant association between STAI-T and RSA for $\text{Prep}_{\text{reactivity}}$, $\beta = -.100$, $p = .37$ or $\text{Recovery}_{\text{reactivity}}$, $\beta = .046$, $p = .77$. For the talking stress conditions, STAI-T anxiety did not predict RSA levels in AWS for $\text{Monologue}_{\text{reactivity}}$, $\beta = 0.043$, $p = .69$ or Nonword $\text{Reading}_{\text{reactivity}}$, $\beta = .019$, $p = .68$ conditions. There were also no significant associations between STAI-T and RSA in the ANS for $\text{Monologue}_{\text{reactrivity}}$, $\beta = .181$, $p = .250$ or Nonword $\text{Reading}_{\text{reactivity}}$, $\beta = .007$, $p = .91$. There were also no differences found for STAI-S. (Figs. 1 and 2)

4. Discussion

The purpose of this study was to determine if self-reported measures of anxiety are predictive factors to baseline RSA and task-reactive RSA levels in AWS compared to ANS. Both AWS and ANS completed Speilberger et al.'s (1983) self-reported State-Trait Anxiety Inventory (STAI) prior to experimental tasks and participated in pre-stress ($\text{Rest}_{\text{Baseline}}$, $\text{Monologue}_{\text{Baseline}}$, $\text{Reading}_{\text{Baseline}}$) and stress ($\text{Prep}_{\text{Reactivity}}$, $\text{Monologue}_{\text{Reactrivity}}$, $\text{Reading}_{\text{Reactivity}}$, $\text{Recovery}_{\text{Reactivity}}$) conditions where their HR levels were collected. HR variability collected during these conditions was later assessed for RSA levels. Using this paradigm, we were able to determine whether self-reported anxiety is a predictive factor to baseline and task-reactive RSA measures.

A UNIANOVA indicated no significant differences in groups when assessing for the relationship between STAI-T and RSA; however, slope estimates indicated that self-reported trait anxiety (STAI-T) was a significant predictor of RSA levels in the AWS but not ANS during baseline conditions of speaking in monologue ($\text{Monologue}_{\text{Baseline}}$) and reading nonwords depicted on a screen ($\text{Reading}_{\text{Baseline}}$). STAI-T, however, was not shown to be a significant predictor of RSA levels in either AWS or ANS during any of the stress conditions. Likewise, STAI-S was not found to be a significant predictor of RSA levels for either group.

The lack of a relationship between self-reports of trait anxiety and RSA levels in the ANS was similar to previous literature reporting no association between trait anxiety and a stress response (Jezova et al., 2004). For instance, in a study by Mauss et al. (2010), those who perceived themselves as highly anxious did not exhibit the expected physiological responses that are sometimes associated with stress. This is in line with the current study as the ANS showed no relationship between the perceived levels of anxiety and the physiological responses associated with emotional regulation during task conditions. In this regard, the self- reports of anxiety used in the current study may not be as tightly coupled to parasympathetic activity, particularly for participants who do not exhibit high enough levels of anxiety. According to findings by Wilken et al. (2000), only those individuals who exhibit high self-reports of trait anxiety show a more tightly coupled physiological response, which is more representative of what was found in the present study as AWS' STAI-T scores were significantly higher than the ANS' (results also reported in Bauerly et al., 2019).

AWS reporting high trait anxiety exhibited lower RSA levels when speaking in a no- stress speaking situation. RSA is an index of parasympathetic activity or vagal input and is considered to play an inhibitory role on the sympathetic nervous system. High vagal input is suggested to be associated with better coping strategies (Brosschot et al., 2007; Demaree, Robinson, Everhart, & Schmeichel, 2004; Fabes & Eisenberg, 1997; Spangler & Friedman, 2015), improved attentional control (Balle et al., 2013; Mathewson et al., 2010; Park, Van Bavel et al., 2012) down-regulating of negative effect (Geisler & Kubiak, 2009), and reflect an adaptive regulatory strategy (Balzarotti et al., 2017). High RSA is therefore associated with the ability to regulate emotions and adapt to a changing environment (Thayer et al., 2000) and is frequently reported to be associated with low levels of anxiety (Mathewson et al., 2010; Watkins et al., 1998). When applying this information to the current study, we can speculate that those who exhibited low levels of RSA when speaking in pre-stress conditions were also the ones who reported feeling high levels trait anxiety, or in other words, higher stress on a

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general, day-to-day basis (Speilberger et al., 1983). From this perspective, low RSA reflects an increase in emotional reactivity when speaking, and based on self-reported STAI-T scores, this is a reflection of their day-to-day speaking interaction.

In Bauerly et al. (2019), group RSA levels were significantly higher in the AWS compared to the ANS across rest and speaking conditions. Results from the current project extend these findings and suggest that those AWS who exhibited high levels of RSA were the ones who reported low STAI-T scores. This suggests that those AWS with improved emotional regulation abilities are more likely to experience lower levels of anxiety during daily interactions. As an index of emotional regulation, higher RSA may improve the ability to regulate stress and may facilitate more effective engagement in social situations (Brosschot et al., 2007; Jezova et al., 2004). Results lend support for future research considering how individual differences in emotional regulation can effect treatment outcome.

The association between trait anxiety and RSA found during the baseline speaking conditions in AWS diminished during the stress conditions. This may be due to the trait portion of the STAI targeting individual's anxiety levels during everyday situations, situations similar to what was expected during the pre-stress conditions. The tasks involved in the TSST-M such as preparing and delivering a speech to a perceived panel of judges is more likely to elicit strong autonomic responses typical of social stress. These types of speaking situations are not specifically targeted in the STAI-T questionnaire and as a result it may not have adequately represented the perceived levels of anxiety experienced by the participant during the social stress conditions.

The lack of association between STAI-S and RSA levels was likely due to the nature of this sub-test. For the STAI-S, participants are required to respond to how they feel at that given moment. It is likely that participants were not experiencing feelings of stress, worry, or anxiety when completing the questionnaire. The only knowledge of the tasks ahead of them was that they were going to be required to perform a speaking task. Also, they completed in the questionnaire in a speech and language clinic, an environment in which they may have felt safe and free to 'let their guard down'. The STAI-T, on the other hand, is reflected in how they generally feel on a day-to-day basis. This score more closely relates to how they may have felt when performing the pre-stress Monologue and Reading tasks.

The inspection of individual differences was particularly important given the small size and exploratory nature of the study. Descriptive analysis lent support for individual differences within the AWS where self-reported trait anxiety was associated with RSA levels. Further research is needed into the investigation of potential subgroups who exhibit high versus low trait anxiety. The identification and classification of stuttering subtypes extends 50 years of research (for a reviewer see Yairi, 2007) and includes proposals based on a number of different stuttering characteristics such as internalizing versus externalizing stuttering (Douglas & Quarrington, 1952), adaptation effect (Newman, 1963) and stuttering severity level (Watson & Alfonso, 1987). Others have proposed stuttering subtypes based on gender (Silverman & Zimmer, 1982), handedness (Hinkle, 1971), genetics (Poulos & Webster, 1991; Seider, Gladstein, & Kidd, 1983; Suresh et al., 2006), and physiological differences such as oscillations of facial muscles associated with stuttering severity levels (Kelly, Smith, & Goffman, 1995). Studies assessing autonomic nervous system activity in AWS have yielded mixed results (Bauerly et al., 2019; Bowers et al., 2012; Brundage, Graap, Gibbons, Ferrer, & Brooks, 2006; Caruso et al., 1994; Weber & Smith, 1990) and to our knowledge, only Weber and Smith (1990) reported a correlation between increased levels of sympathetic nervous system activity and stuttering severity. The present study supports the need to investigate the potential of subtyping AWS based on physiological processes associated with self-reports of anxiety and the regulation of emotions. Studies assessing autonomic activity in children who stutter report greater emotional reactivity to negatively arousing stimuli (Jones et al., 2014) and this sensitivity may be the precursor to decreased tonic RSA levels later in life. This line of research has the potential for important clinical implications. For instance, those AWS who show increases in social anxiety may benefit from a cognitive behavioral approach aimed at strengthening emotional regulatory behavior whereas those who show adequate stress levels may benefit more from speech restructuring. Treatments catered to individual differences such as these would maximize clinic outcomes.

5. Limitations

One limitation to our study is the small sample size. Future studies including larger sample numbers will allow for stronger comparisons between anxiety and autonomic measures as well as stronger analysis of individual differences. While the STAI is a widely used measure of both state and trait anxiety, it is not specific to feelings of social anxiety which was the main focus of the experimental conditions. Also, this questionnaire was administered before the experimental tasks and so it failed to measure anxiety that may have been elicited in response to the social stress induction (i.e. TSST-M). As mentioned in Bauerly et al. (2019), not asking participants' their perceived levels of stress from the TSST-M conditions, prevented a direct comparison of perceived versus autonomic measures of anxiety. Future studies assessing the effects of social stress in AWS may benefit from administering standardized measures that are specific to social stress before and after stress induction. Also, by incorporating subjective questions following the social stress task such as "*How did that task make you feel?*" may be a useful way to gauge the participants general feelings and reflections when performing the task at hand. Finally, measuring behavioral changes during an actual moment of public speaking using tools such as the Virtual Reality Environments (Brundage & Hancock, 2015) may help in isolating specific behavioral characteristics that are unique to AWS with high levels of social anxiety.

6. Conclusion

In summary, findings from this study showed low trait anxiety predicted high RSA levels during pre-stress, talking conditions in AWS but not ANS. In other words, those who reported to have low anxiety levels in day-to-day interactions showed parasympathetic nervous system activity that represented better emotional regulatory strategies. Descriptive analysis revealed that this negative association was largely dependent on a subgroup of AWS, lending important implications for subtyping in the stuttering population. Results lend support for future research aimed at assessing anxiety and emotional processing in adults who stutter using a larger sample

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size.

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