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Is autonomic functioning distinctly associated with anxiety and unsociability in preschoolers?

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ABSTRACT

There are many benefits of peer interactions for children's social, emotional, and cognitive development, and isolation from peers may have negative consequences for children. Although biological processes may underlie social withdrawal broadly, distinct patterns may be associated with withdrawal behaviors depending on their underlying motivation (e.g., shy versus disinterested). This study investigated the role of autonomic nervous system activity, as assessed via skin conductance level (SCL) and respiratory sinus arrhythmia (RSA) in predicting changes in unsociability (e.g., lack of interest in peers) and anxious-fearfulness (e.g., discomfort among peers). Data were collected using a community sample of 92 US preschool children (45.7% female; $M_{age} = 45.51$ months, $SD_{age} = 3.81$ months) at two time points one year apart. Gender differences were also explored. Baseline physiology was assessed while viewing a neutral video clip, and reactivity was assessed while viewing social exclusion and post-aggression discussion videos. For all children, coinhibition (i.e., SCL inhibition accompanied by RSA inhibition) to the post-aggression discussion video and blunted SCL activation to the exclusion video were prospectively associated with higher levels of anxious-fearfulness one year later. For boys only, baseline reciprocal sympathetic activation (i.e., SCL activation and RSA inhibition) was prospectively related to higher levels of unsociability one year later. For girls only, RSA inhibition in response to the post-aggression discussion video was prospectively related to higher levels of unsociability one year later. Findings contribute to a growing literature on autonomic reactivity in preschoolers' adjustment and suggest possible differences in the physiological processes underlying unsociability and anxious-fearfulness.

1. Introduction

There are many benefits of peer interactions for children's social, emotional, and cognitive development (Rubin et al., 2006). Social withdrawal, when children remove themselves from the peer group to engage in solitary behaviors, may hinder children from meeting their fundamental need for social connection (Coplan et al., 2018, 2019; Coplan and Weeks, 2010a). Moreover, children who are comparatively more withdrawn than their peers often remain so over time (Mills and Rubin, 2013), increasing their risk for other socioemotional difficulties (e.g., anxiety, low self-esteem, depressive symptoms; Rubin et al., 2009). Two common expressions of social withdrawal are unsociability, defined as voluntary isolation from peers due to lack of interest, and anxious-fearfulness, defined as negative affect in social situations (Coplan et al., 2019; Rubin et al., 2010).

There may be underlying biological processes that are related to social reticence (Rubin et al., 2009), such as autonomic nervous system (ANS) functioning (El-Sheikh and Erath, 2011). In fact, ANS activity, at rest and in response to a stressor, has been linked with internalizing behavior problems, including anxiety and social reticence (e.g., Dieleman et al., 2015; Graziano and Derefinko, 2013; Henderson et al., 2004). However, despite research indicating that the two branches of the ANS (i.e., sympathetic and parasympathetic) work together to support adaptive functioning, these branches have tended to be explored in isolation (El-Sheikh and Erath, 2011). It is also not clear whether ANS activity is uniquely associated with anxious-fearfulness versus unsociability. Further, children's gender may have implications for the physiological processes associated with risk (e.g., El-Sheikh et al., 2013).

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Thus, the purpose of the present study was to investigate associations between physiological processes and unsociability and anxious-fearfulness across two time points in early childhood, as well as potential gender differences in these effects.

1.1. Anxious-fearfulness versus unsociability

In early childhood, both anxious-fearfulness and unsociability may limit social interactions with peers (Coplan et al., 2019; Rubin et al., 2010). However, the motivations (e.g., shy, disinterested; Coplan et al., 2018) underlying these limited social interactions differ. Specifically, anxious-fearful children desire interaction but feel emotionally uncomfortable (e.g., display negative affect) among peers (Ladd et al., 2009). Research suggests that children's early anxious-fearfulness, particularly in social situations, may indicate risk for later social and mental health challenges (e.g., depression, anxiety, externalizing problems; Mian et al., 2011; see Coplan and Armer, 2007) and early mortality (Jokela et al., 2009).

In contrast, unsociability is a form of asocial or withdrawn behaviors (e.g., Ladd and Profilet, 1996; Ladd et al., 2009) characterized by a preference for solitary activities without anxious motivations (e.g., Coplan et al., 2013). Some researchers have argued that unsociability is relatively benign in early childhood (Coplan et al., 2019). In fact, in some studies, early childhood unsociability was not associated with peer problems, social anxiety, or depression, when also controlling for shyness and social avoidance (Coplan et al., 2018). These findings may stem from the fact that unsociable children are less interested in initiating peer interaction than their non-withdrawn peers but are no less capable of engaging with them when necessary (Coplan et al., 2013). However, the implications of unsociability may vary across development (e.g., Wang et al., 2013). Unsociability may be increasingly associated with poor developmental outcomes across the transition to adolescence, as peers view solitude and withdrawal negatively during this developmental period (Barstead et al., 2018; Coplan et al., 2019; Ribeiro et al., 2022; Wang et al., 2013). Further, even in early childhood, unsociability may be related to some indices of peer challenges (e.g., lower prosocial behavior, social exclusion; Coplan et al., 2004; Ooi et al., 2018), and may have significant developmental implications (e.g., later anxiety symptoms; Kopala-Sibley and Klein, 2017).

1.2. ANS activity

Given the distinct motivational underpinnings of anxious-fearfulness and unsociability, an important research question is whether biological factors, including ANS functioning, are uniquely associated with unsociability and anxious-fearfulness. The sympathetic nervous system (SNS), one of the two branches of the ANS, orchestrates the "fight or flight" stress response to real or perceived threats (Dawson et al., 2007). SNS activity is often indexed through skin conductance level (SCL), which reflects electrodermal activity of the sweat glands (Dawson et al., 2007), and can be assessed at baseline or in response to a stressor or stimulus (i.e., reactivity, SCLR). Higher levels of baseline SNS activity are thought to reflect a fearful and inhibited temperament, as well as a tendency to withdraw from novel situations, and may represent broad risk for internalizing problems (e.g., Fowles et al., 2000; Kagan et al., 1987).

In terms of reactivity, both chronic SNS activation and activation that is greater in intensity or duration than would be expected given the stressor may represent risk for negative outcomes (Porges, 2007). Indeed, in middle childhood, higher levels of anxiety, broadly, and social anxiety, specifically, have been associated with greater SNS arousal (i.e., SCL and heart rate) at baseline and in response to a stressor (e.g., Dieleman et al., 2015; Schmitz et al., 2011), although findings are somewhat mixed among older children (e.g., Schmitz et al., 2013). In a sample of 4-year-olds, indices of SCLR (i.e., skin conductance response amplitudes, overall reactivity) were associated with higher fearful temperament (Fowles et al., 2000). These findings suggest that greater SCL at rest and SCLR to stress or challenge may be related to anxious-fearfulness in young children.

The other branch of the ANS is the parasympathetic nervous system (PNS), often described as the "rest and digest" system, which is a fast-acting system that dampens the effect of other stress response systems (i.e., SNS, hypothalamic-pituitary-adrenal axis). One common index of PNS arousal is respiratory sinus arrhythmia (RSA), which reflects the rhythmic fluctuation of heart rate during spontaneous breathing (Porges, 2007). Low baseline RSA is hypothesized to reflect a more limited emotion regulation capacity (Stone et al., 2020) and is correlated with symptoms of internalizing psychopathology, including social anxiety (Alkozei et al., 2015; Beauchaine, 2015). These findings suggest that lower PNS activity at rest may be related to anxious-fearfulness during early childhood.

With respect to reactivity, decreased PNS activity (also called PNS inhibition) facilitates the mobilization of metabolic resources for coping with stressors (Porges, 2007). Therefore, RSA inhibition in the context of a stressor may be an adaptive response that reflects greater emotion regulation abilities (Porges, 2007; see Graziano and Derefinko, 2013, for meta-analytic findings with children). However, theorists have debated the meaning of RSA inhibition, and excessive RSA inhibition to negative emotion evocation may serve as an indicator of poor self-regulation (Beauchaine and Thayer, 2015; Beauchaine et al., 2019). Patterns of "adaptive" PNS responding likely depend on the requirements of the social context (Hastings and Kahle, 2019). In safe contexts, RSA activation may be adaptive because it facilitates social engagement. In contrast, during challenge, mild RSA inhibition may be adaptive because it facilitates coping, and, in the context of threat, greater RSA inhibition may be adaptive because it facilitates contextually-appropriate fight-or-flight responses (Hastings and Kahle, 2019). Additionally, the autonomic flexibility model suggests that anxiety may be tied to rigid physiological responses (Friedman, 2007). In other words, the capacity to physiologically respond to challenges in contextually appropriate ways may be indicative of better adjustment, whereas rigid or inflexible physiological responding may be indicative of maladjustment. Indeed, some research indicates that, among young children high in behavioral inhibition, anxiety is related to inflexible RSA responding to social stress (Wagner et al., 2023). Thus, theory and research underscore the importance of context in understanding the significance of physiological reactivity.

1.3. Interactions between the SNS and PNS

Accumulating research indicates that the SNS and PNS work together to promote adjustment outcomes (e.g., El-Sheikh and Erath, 2011). Within an organ system, the SNS and PNS have opposing effects on arousal, such that increases in SNS activity function to increase arousal (e.g., increase heart rate), whereas increases in PNS activity function to decrease arousal (e.g., decrease heart rate). Reciprocal activation (i.e., activation of one branch accompanied by inhibition of the other branch) results in a directional effect on arousal (either an overall increase or decrease; El-Sheikh et al., 2009; see Table 1). Importantly, however, the two branches of the ANS do not always respond to stimuli in a coordinated, reciprocal manner; coactivation (i.e., activation of both systems) and coinhibition (i.e., inhibition of both systems) may also occur. These patterns have ambiguous net effects on peripheral measures of arousal, such as heart rate (El-Sheikh and Erath, 2011).

Some researchers have hypothesized that reciprocal patterns promote adaptive functioning through active responses to stressors or self-soothing (El-Sheikh et al., 2009). In contrast, nonreciprocal patterns may be maladaptive, reflecting a dysregulated or ambiguous response to stress (El-Sheikh et al., 2009). Although several researchers have provided support for this perspective with respect to internalizing symptoms broadly, effects depended on contextual/environmental risk (e.g., Philbrook et al., 2018) or contextual/environmental risk and gender (e.

Table 1
Autonomic nervous system profiles.

Profile	SNS activity	PNS activity	Net effect on ANS arousal
Reciprocal sympathetic	Activation (high SCLR)	Inhibition (low RSAR)	Increase
Reciprocal parasympathetic	Inhibition (low SCLR)	Activation (high RSAR)	Decrease
Coactivation	Activation (high SCLR)	Activation (high RSAR)	Ambiguous
Coinhibition	Inhibition (low SCLR)	Inhibition (low RSAR)	Ambiguous

Note. Adapted from "Marital conflict and children's externalizing behavior: Interactions between parasympathetic and sympathetic nervous system activity," by El-Sheikh et al., 2009, Monographs of the Society for Research in Child Development, 74(1), p. 17. Copyright 2009 by John Wiley and Sons. Adapted with permission.

g., El-Sheikh et al., 2013). Moreover, other researchers have found that reciprocal patterns were linked to internalizing symptoms (Benito-Gomez et al., 2019), particularly in the context of contextual risk (e.g., Abaied et al., 2018; El-Sheikh et al., 2013).

As with studies investigating physiological reactivity of the branches of the ANS in isolation, the implications of autonomic coordination across the SNS and PNS likely depend on the context in which it is elicited. In fact, in one recent study, children's trajectories of aggression were associated with distinct patterns of ANS coordination to fear, happiness, and sadness (e.g., Murray-Close et al., 2023). Gatzke-Kopp and Ram (2018) demonstrated that, among young children (5–8 years), coordination across the SNS and PNS was typical during exposure to approach stimuli (happy and anger), whereas a lack of coordination was more typical during exposure to avoidance stimuli (fear and sadness). Cooperative, reciprocal coordination may be adaptive in approach-oriented contexts that require the mobilization of metabolic resources to support behavioral responses (Gatzke-Kopp and Ram, 2018). However, avoidance may be more closely tied to non-reciprocal responses, which may better support behaviors reflecting both vigilance and inhibition (Gatzke-Kopp et al., 2020). Further, in response to the same task, coordinated ANS responses may reflect a tendency to remain engaged whereas uncoordinated ANS responses may reflect a tendency to disengage from the task (Gatzke-Kopp et al., 2020). These findings raise the possibility that anxious-fearfulness may be tied to physiological reactions that underlie avoidance and disengagement, such as nonreciprocal ANS responses, to social challenge.

To date, studies of ANS coordination have tended to focus on reactivity, although some researchers have assessed baseline levels. On the one hand, uncoordinated patterns of ANS activity at baseline appeared to be related to poor developmental outcomes, particularly given contextual risk (e.g., Chong et al., 2022; El-Sheikh et al., 2013; Suurland et al., 2018), perhaps because these uncoordinated patterns reflect a poor state of readiness to cope with challenges (El-Sheikh et al., 2013). However, in other research, sympathetic dominance (indexed by greater SNS as compared to PNS activity) at rest was associated with heightened symptoms of depression and anxiety in a sample of women (Stone et al., 2020). Similarly, in a sample of adults, sympathetic dominance at rest was associated with major depressive disorder (Brush et al., 2019). High levels of SNS activity combined with low PNS activity at rest may reflect an ineffective or maladaptive use of metabolic resources thought to increase risk for symptoms of depression and anxiety (Stone et al., 2020). This perspective is consistent with research demonstrating that both heightened SNS activity and lower PNS activity at rest are related to anxiety (Friedman, 2007). Given the mixed findings to date, additional research is needed to investigate the interactive profiles in the context of baseline activity.

1.4. Present study

In the present study, we investigated short-term longitudinal associations between activity of the PNS and SNS, as well as their interactions, to predict anxious-fearfulness and unsociability in a sample of preschool children across one calendar year. We specifically focused on the early childhood period given the significant debate in the field regarding the implications of unsociability for adjustment during this developmental period (e.g., Coplan et al., 2013; Ooi et al., 2018; Wang et al., 2013). For instance, it is possible that despite differences in the outward appearance of anxiety or fear when interacting with peers, children exhibiting both anxious-fearfulness and unsociability may exhibit dysregulated physiological reactions to peer stress. Thus, findings have important implications for theoretical models and research studies on the significance of subtypes of social withdrawal in early childhood. In addition, we adopted a short-term longitudinal design to better understand how patterns of ANS activity are related to *changes* in subtypes of social withdrawal behaviors over time. Importantly, the physiological correlates associated with outcomes may depend on whether analytic approaches reflect concurrent or longitudinal associations (e.g., Obradović et al., 2011). Further, concurrent associations raise questions regarding directionality of effects; for instance, it is plausible that behaviors such as social withdrawal are related to changes in stress system functioning over time. Indeed, theorists have argued that social isolation may serve to alter stress system functioning (e.g., Cacioppo et al., 2011). Thus, the short-term longitudinal design adopted in the present is important in testing the directionality of effects hypothesized in the present study (i.e., physiological activity preceding social behavior).

Given accumulating evidence highlighting the importance of stimuli used to assess physiological reactivity and research indicating that responses to social stressors may be particularly relevant for predicting social behavior (e.g., Obradović et al., 2011), reactivity was assessed in response to a depiction of social exclusion and reactivity to a post-aggression discussion. Although both represent social challenges, the post-aggression discussion focuses on navigating the aftermath of aggression, and includes a resolution to the exclusion experience as well as supportive social conversations that include efforts to address experiences of aggression and to help the victim feel better. Thus, in contrast to the social exclusion video, the post-aggression discussion may serve as a more ambiguous social challenge with potential interpretations of threat as well as support (see Perry et al., 2022). Further, some prior research has highlighted the significance of ANS responses when dealing with the aftermath of social stressors (Hill-Soderlund et al., 2008).

At baseline, we hypothesized that anxious-fearfulness would be uniquely associated with main effects of higher SCL and lower RSA (Beauchaine, 2015; Fowles et al., 2000; Kagan et al., 1987; Stone et al., 2020); we also investigated interactions across ANS branches, but given the mixed findings regarding the implications of ANS coordination and developmental outcomes, these were exploratory in nature. With respect to reactivity, we predicted that anxious-fearfulness would be associated with similar patterns of ANS reactivity to both the exclusion and post-aggression discussion videos, as the autonomic flexibility model suggests that anxiety is tied to rigid physiological responses (Friedman, 2007). Further, even social challenges that have potentially supportive elements (i.e., post-aggression discussion) may be threatening to anxious-fearful youth as anxious individuals tend to appraise even positive social experiences negatively, fear social evaluation, and are intolerant of uncertainty (Nishikawa et al., 2022). Thus, we anticipated that anxious-fearful children would exhibit main effects of greater SCL activation, as SCLR may be indicative of a higher fearful temperament (Fowles et al., 2000), and greater RSA activation in response to videos, as this likely reflects a maladaptive response to challenge or threat (Hastings and Kahle, 2019). With respect to ANS coordination, we anticipated that anxious-fearful children would exhibit a lack of coordination across branches of the ANS to videos depicting social exclusion and post-aggression discussion, potentially indicative of avoidance (Gatzke-Kopp and Ram, 2018) and disengagement (Gatzke-Kopp et al., 2020).

For unsociability, we held two competing hypotheses. First, unsociability may be unrelated to patterns of ANS functioning indicative of fear or withdrawal. In fact, Henderson et al. (2004) reported that socially reticent (similar to anxious-fearfulness) preschoolers had lower baseline RSA than children who exhibited constructive solitude (similar to unsociability). Alternatively, unsociable youth may exhibit patterns of ANS activity to peer challenge indicative of avoidance and disengagement, even in the absence of outward behavioral indices of distress. Indeed, in one recent study, a preference for solitude at ages 4 and 5 years was predicted by a combination of a failure to exhibit RSA inhibition to unfamiliar challenge (e.g., viewing toy mobile) at 5 months and negative affect to unfamiliar events (e.g., viewing a clown) at 19 months (Morneau-Vaillancourt et al., 2022). These findings suggest that poor physiological regulation of emotion, as indicated by impaired RSA inhibition to challenge, may play a role in preference for solitude (Morneau-Vaillancourt et al., 2022). Further, these findings are reminiscent of research indicating that, despite appearing well-regulated, infants with an insecure-avoidant attachment style showed greater SNS activity and greater PNS response to social stress than securely attached infants (Hill-Soderlund et al., 2008).

Finally, the physiological profiles associated with risk sometimes differ by gender (see El-Sheikh and Erath, 2011), although the direction of effects has varied. In fact, gender differences have been found for the physiological correlates of other social behaviors in early childhood, including with the present sample (Lent et al., 2022), and for internalizing problems in adolescence. Regarding SNS reactivity, some researchers have reported gender differences in associations between higher SCLR and risk for internalizing problems, although effects often depended on contextual risk (El-Sheikh, 2005; Fletcher et al., 2019). Regarding PNS activity, some work indicates that, especially among boys, low resting RSA increases risk for symptoms of anxiety/depression in the context of contextual stressors (McLaughlin et al. (2015). In contrast, other studies indicate that RSA reactivity is more strongly related to internalizing problems or social anxiety among girls than boys (e.g., Ho et al., 2020; Shanahan et al., 2014). Limited research has investigated gender differences in associations between ANS coordination and internalizing problems such as anxiety. In one notable exception, in the context of high marital conflict, coinhibition at baseline was longitudinally related to high levels of depressive and anxiety symptoms over time for girls but not boys (El-Sheikh et al., 2013). In contrast, in a sample of emerging adults, sympathetic dominance in response to stress tasks was related to increases in depressive symptoms across a 6-week follow-up for men but not women (Choi et al., 2021). Thus, although several studies have suggested that distinct patterns of ANS activity may be associated with internalizing problems across gender, the pattern of effects has differed across studies; further, to our knowledge, no research has investigated gender differences in the role of ANS activity in the development of facets of social withdrawal. As a result, we investigated gender differences in the unique physiological correlates of anxious-fearfulness and unsociability.

Findings from the present study have the potential to provide theorists and researchers with additional insights regarding similarities and differences in the processes that underlie distinct subtypes of social withdrawal. These insights, in turn, may help practitioners tailor interventions that support young children in developing skills for increasing engagement and interactions with peers, which serve as critical experiences for positive youth development (Rubin et al., 2006). For instance, if subtypes of social withdrawal are related to rigid physiological responses or patterns indicative of avoidance (Gatzke-Kopp and Ram, 2018) and disengagement (Gatzke-Kopp et al., 2020), practitioners may benefit from integrating strategies that help young children constructively engage with peer stressors.

2. Materials and methods

2.1. Participants

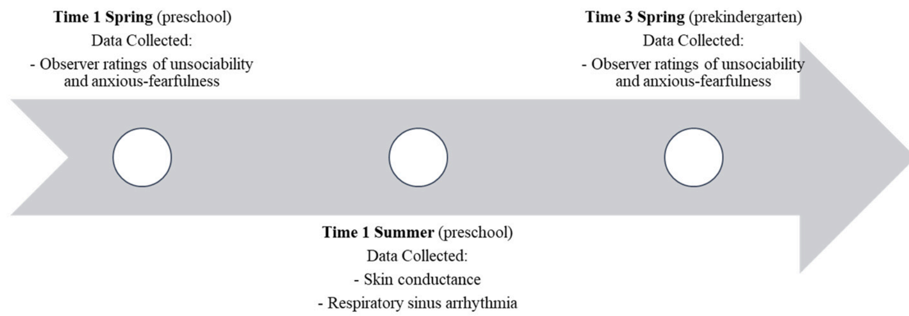
Participants were part of a larger longitudinal study investigating temperament and aggressive behaviors in a typically developing sample (Ostrov et al., 2023). Four cohorts were recruited over a four-year period (2015–2019) from ten National Association for the Education of Young Children (NAEYC) accredited or recently accredited early childhood education centers in a large northeastern city and surrounding suburbs of the United States. Of the 300 fully consented participants, 94 child participants participated in a summer physiology assessment and were considered for this study. Ultimately 92 participants were included (45.7% female; M_{age} = 45.51 months, SD = 3.81 months) and two participants were excluded from analyses (for one, physiology was measured at Time 3 instead of Time 1 summer; the other had missing data on all variables of interest). Participants were 69.9% White, 9.7% Asian/Pacific Islander, 3.2% African American/Black, 1.1% Hispanic/Latinx, 15.1% multiracial, and 1% missing information on race/ethnicity. Based on Hollingshead's (1975) 9-point scoring system (i.e., 9 = executives and professionals, 1 = service workers) of parents' occupation, the sample was, on average, middle to upper-middle class (M = 7.88, SD = 1.33). Within the larger sample, parent occupation was unavailable for 24% of participants; as a proxy for SES, each school was rank ordered based on the proportion of high vs. low SES participants (see Perhamus and Ostrov, 2021). The rank order code was available for all participants and indicated that, on average, schools served middle class families (M = 5.47, SD = 2.29). To be consistent with other publications using this dataset (e.g., Ostrov et al., 2023), school code was tested as a possible covariate.

2.2. Procedure

This study is a secondary data analysis of a larger study (Ostrov et al., 2023). All procedures from the larger study were approved by the local institutional review board (IRB). Every child in participating classrooms was invited to participate, and parents provided written consent for their children's participation both prior to the beginning of the study and again prior to the physiology assessment. Approximately 56% of eligible families returned consent forms to participate in the study.

In-school data were collected starting in the spring of children's 3-year-old year, and participants were followed through a lab session in the summer (T1 Spring/Summer), and in-school data collection the following spring (T3; see Fig. 1). A second timepoint of data was collected in the fall, but is not used in the present manuscript. Short-term longitudinal analyses were conducted between T1 and T3. During T1 and T3, trained research assistants (RAs) spent approximately 2 months in the children's classrooms conducting naturalistic observations of aggression, victimization, and other social behaviors using the Early Childhood Observation System (e.g., Ostrov and Keating, 2004). After conducting these observations over a 2–3 month period, observers completed a rating scale of children's anxious-fearfulness and unsociability. If there were multiple observers who observed a child in each room, one RA per room was randomly selected to complete a questionnaire packet on that child's behavior.

The summer after children turned 4 years old (T1 Summer), consented families from the larger project were invited to participate in a lab session, which included physiological data collection (31% of the larger sample participated in the summer session). Parents provided written consent and children provided assent for this summer lab session. As compensation, parents received $30–$40 in gift cards and children were given a small educational toy.



Note. Time 2 data were collected in the fall/winter of the prekindergarten year but is not used in the present study.

Fig. 1. Timeline of data collection.

2.3. Measures

2.3.1. Observer ratings of unsociability and anxious-fearfulness

Unsociability and anxious-fearfulness were measured using observer report on the Child Behavior Scale (CBS; Ladd and Profilet, 1996; Ladd et al., 2009). Four items assessed anxious-fearfulness (e.g., "Tends to be fearful or afraid of new things or new situations") and six items assessed unsociability (e.g., "Withdraws from peer activities"). Publications using the CBS have also called the unsociability scale asocial withdrawal. Items were rated on a 1 (*Doesn't apply*) to 3 (*Certainly applies*) scale and averaged within subscale. RAs have been found to be valid and reliable informants of children's social behavior after spending considerable time in the classroom (e.g., Blakely-McClure and Ostrov, 2018; Perry and Ostrov, 2018). The CBS has demonstrated strong psychometric properties in prior work (e.g., Ladd and Profilet, 1996; Ladd et al., 2009), and both subscales demonstrated acceptable internal consistency at T1 (Cronbach's αs = 0.61–0.95) and T3 (Cronbach's αs = 0.67–0.94).

2.3.2. Psychophysiology: Skin conductance and respiratory sinus arrhythmia

Skin conductance, heart rate, and interbeat interval were assessed using equipment developed by Biolog (UFI 3991; see Sijtsema et al., 2011). Prior to the session, to facilitate assent, participants were able to apply sham electrodes on a stuffed bear and have any questions answered by research staff. Skin conductance and electrocardiogram (ECG) leads were positioned on participants by trained graduate assistants in the presence of their parent, followed by a 5-minute accommodation period. During the application and removal of electrodes, children received a sticker for each sensor and placed it on a coloring page depicting the stuffed bear. Disposable ECG electrodes were affixed to the participant's right and left rib in axial formation, and to their sternum. SCL was assessed with two skin conductance electrodes attached to the distal phalanges of the first and second fingers of the child's nondominant hand and secured with adhesive collars. Participants were encouraged to wash and dry their hands prior to the session. Finally, a respiration belt (i.e., Biolog Pneumotrace respiration transducer) was placed around the participant's diaphragm to measure respiration as a possible covariate of RSA (Berntson et al., 2017). Parents then left the room and viewed the remainder of the session via camera from an adjacent room to reduce parental interference. Research assistants noted the room temperature as a possible covariate of SCLR and RSAR.

Measuring psychophysiological responses to social exclusion present some unique challenges for this period in development. It would likely be unethical to directly manipulate social exclusion among young children as compared to later developmental periods when common approaches include computer simulated social exclusion in middle childhood (e.g., Cyberball; Crowley et al., 2010) and lab-based (e.g., Seddon et al., 2020) or online versions (e.g., Gunnar et al., 2021) of evaluative stress tasks (e.g., Trier Social Stress Test) in adolescence. Instead, classic developmental theory suggests young children have some perspective taking challenges and have been described as having egocentric tendencies and biases (Flavell, 1968) that may allow the indirect exposure to social exclusion to provide a valid but safe indication of their own reactivity to social exclusion. With that said, as mentioned above, careful steps were taken to obtain child assent using developmentally appropriate and concrete demonstrations of the physiology task and children were able to provide their verbal assent/dissent prior to initiating and during the protocol. Additional challenges of using physiology assessments with preschoolers include reducing movement which was supported by having an RA seated next to participants narrating the tasks and encouraging them to sit still until the videos were over. Failure to attend to the videos may reduce the validity of the task, which is a second key challenge that was addressed by having the RA provide developmentally appropriate labelled positive praise for attending to the videos and redirect children back to the tasks if needed.

Participants viewed a 3-minute developmentally appropriate baseline video (i.e., a clip of a cartoon dog exploring his neighborhood) to allow recording of resting autonomic arousal without the child getting bored and restless (see Calkins and Keane, 2004). Children then watched two additional 3-minute videos. The first video (exclusion) depicted a relational conflict in which a *Sesame Street* character refuses to let Big Bird join a club while using verbal insults as justification for the social exclusion. The second video (post-aggression discussion) depicted Big Bird, two adults, and characters from the exclusion video navigating the aftermath of exclusion, including discussing the exclusion experience, helping the victim feel better, and highlighting how to address bullying (see Perry et al., 2022).

RSA was assessed using a sampling rate of 1000 Hz. Cardiac interbeat intervals (IBI) were measured as time in milliseconds between successive R waves of the electrocardiogram. Trained research assistants manually edited IBI artifacts due to movement or digitizing error in CardioEdit (Brain-Body Center, 2007). Next, CardioBatch, which uses a time-series moving 21-point detrending polynomial algorithm, was used to calculate RSA (see Porges, 1985). The frequency band-pass parameters used in the present study were 0.24 to 1.04 Hz to be consistent with spontaneous respiration in children. The mean RSA for each video segment was computed to yield a measure of RSA for each participant. RSA is reported in units of $\ln(ms)^2$. The respiration belt sampled respiration at a rate of 10 Hz. SCL was assessed using Ag/AgCl electrodes and was measured in microsiemens. A thin layer of an isotonic NaCl electrolyte gel was used on the electrodes to increase conduction and adhesive collars were used to limit the gel to a 1 cm diameter circle on the participants' fingers.

SCLR and RSAR to exclusion and post-aggression discussion were calculated by subtracting the mean arousal during the baseline video from mean arousal during the exclusion clip and post-aggression discussion clip, respectively. This is consistent with prior work examining

SCLR and RSAR (e.g., Beauchaine et al., 2013; El-Sheikh et al., 2011; Kalvin et al., 2016). For SCLR, positive values indicated SNS activation to the stimulus, whereas negative values indicated SNS inhibition. For RSAR, positive values indicated RSA activation and negative values indicated RSA inhibition.

2.4. Data analytic plan

Prior to running the primary analyses, descriptive statistics were gathered, and all continuous variables were winsorized to within three standard deviations of the mean to decrease the influence of outliers (Kline, 2015). Predictors were also mean-centered to facilitate interpretation of the interactions. In all path analyses, maximum likelihood estimation with robust standard errors (MLR) was used in Mplus version 8.3 (Muthén & Muthén, 1998–2022) to accommodate variable skew. Several fit indices were examined including a likelihood ratio χ^2 test where $p > .05$ indicates good model fit, comparative fit index (CFI) where values >0.95 suggest good fit, standardized root mean-square residual (SRMR) where values <0.08 represent mediocre fit, and values <0.05 indicate close fit, and root mean square error of approximation (RMSEA), where values <0.08 suggest mediocre fit, and values <0.05 indicate close fit (Hu and Bentler, 1999).

We conducted a series of multigroup models with gender as the grouping variable to investigate gender differences in associations. Initially, all paths were constrained across gender. We used modification indices and theory to guide the releasing of pathways that differed at $p < .05$. In addition, omnibus Wald Chi-square tests of parameter equalities (Muthén & Muthén, 1998–2022) simultaneously tested for gender differences in the main effects and the interaction between RSA and SCL in the prediction of each outcome, respectively. We conducted these tests in this fashion because the RSA and SCL interaction was dependent upon the main effects. When Wald Chi-Square tests of gender moderation were significant ($p < .05$) the entire block (i.e., both main effects and the interaction to the outcome) was allowed to vary freely across groups; when associations were not moderated by gender, the block was constrained across groups. T1 anxious-fearfulness and unsociability were included in all models as covariates. Several additional covariates were considered, including demographic variables (child age and race and school SES), as well as variables that may be related to RSAR and SCLR (respiration, body mass index [BMI], and room temperature). Within the path models, we regressed correlated T3 anxious-fearfulness and unsociability on the pertinent RSA/RSAR, SCL/SCLR, and interaction terms, T1 anxious-fearfulness and unsociability, and the covariates. Separate models were run for the baseline, exclusion, and post-aggression discussion videos. If interactions were significant, they were probed at one standard deviation above and below the mean of the moderator, to determine whether the effect of SCL or SCLR (predictor) was significant at various levels of RSA or RSAR (moderator). Given the complexity of the study models relative to the sample size, no adjustments were made for multiple comparisons. To help address Type 1 error concerns, findings that approach significance ($p < .10$) are not interpreted.

3. Results

Descriptive statistics, including the number of participants who contributed data for each measure, are presented in Table 2. Observers completed questionnaires on anxious-fearfulness and unsociability after spending considerable time in the classroom (i.e., an average of 7.82 ten-minute observations [$SD = 0.59$] at T1 and an average of 7.55 ten-minute observations [$SD = 1.08$] at T3). Descriptive statistics indicated generally low levels of anxious-fearfulness and unsociability at both time points with some degree of overlap in the constructs (see Tables 2 and 3). For participants who had complete data at both time points, there was no mean level change in anxious-fearfulness [$t\,(68) = 0.48$, $p = .64$, Cohen's d = 0.06] or unsociability [$t\,(68) = -0.32$, $p = .75$, Cohen's d = −0.04]. Previous research with this sample indicated that children tend to exhibit an increase in SCL and a decrease in RSA from baseline to watching the exclusion and post-aggression discussion videos (Lent et al., 2022).

Table 2
Descriptive statistics.

	N	Mean	SD	Min - Max	Skew
1. Age (months)	93	45.49	3.79	36.13–61.97	0.74
2. Gender	93	0.54	–	–	−0.15
3. Race/ethnicity	92	0.29	–	–	0.92
4. SES	92	5.47	2.29	1.00–10.00	−0.05
5. BMI	89	16.07	1.47	12.09–20.99	0.35
6. SCL baseline	82	14.02	7.86	2.30–37.99	0.90
7. RSA baseline	81	6.93	1.27	2.78–11.09	−0.06
8. SCLR-Excl.	80	2.13	3.67	−7.95-13.16	0.67
9. RSAR-Excl.	78	−0.13	0.40	−1.16-1.23	0.30
10. SCLR-PAD	80	3.90	4.21	−3.77-17.15	0.93
11. RSAR-PAD	78	−0.24	0.53	−1.90-1.42	−0.32
12. Anx-Fear T1	92	1.25	0.34	1.00–2.25	1.17
13. Unsoc T1	92	1.53	0.63	1.00–3.00	0.94
14. Anx-Fear T3	69	1.20	0.33	1.00–2.14	1.77
15. Unsoc T3	69	1.44	0.54	1.00–3.00	1.21

Note. Gender is coded: 0 = girls, 1 = boys; race/ethnicity is coded: 0 = White, non-Hispanic, 1 = people of color; SES = socioeconomic status; BMI = body mass index; SCLR = skin conductance level reactivity; RSAR = respiratory sinus arrhythmia reactivity; Excl. = Exclusion; PAD = post-aggression discussion; Anx-Fear = anxious-fearful, Unsoc = unsociability; T1 = Time 1; T3 = Time 3. Values represent post-winsorized data.

RSA/RSAR and respiration were not correlated, so respiration was not controlled for in analyses. However, room temperature was included as a covariate of SCLR, RSAR and the SCLR x RSAR interaction term as it was correlated with the SCLR variables ($rs = 0.29$–0.60, $ps < 0.01$) and, unexpectedly, with girls' RSAR during the post-aggression discussion ($r = -0.43$, $p = .009$). Room temperature did not correlate with baseline SCL or RSA and, thus, was not included in the baseline model. BMI did not correlate with any of the physiological variables; however, it unexpectedly correlated with T3 unsociability ($r = 0.29$, $p = .018$) and was included in all final models. Anxious-fearfulness demonstrated stability from T1 to T3 ($r = 0.25$, $p = .04$) whereas unsociability did not ($r = 0.19$, $p = .13$). Age was also included in all final models due to associations with key study variables.

Given that data collection occurred across two academic years, missing data was expected. In particular, children may have transitioned to universal pre-kindergarten classes for free or reduced cost within their neighborhoods. The overall percentage of missing data was 25 % from T1 to T3. Maximum likelihood missing data mechanisms assume the data are Missing at Random (MAR), which indicates that missingness is related to other variables within the model (Baraldi and Enders, 2010). We used logistic regressions to identify variables related to T3 missingness. These analyses demonstrated that missing data at T3 were associated with higher levels of unsociability at T1 (OR = 3.42, $p = .002$). Therefore, there is evidence that the data are MAR since higher levels of unsociability at T1 are associated with greater odds of missing data at T3. Full information maximum likelihood was used to account for missing data.

3.1. Primary analyses

3.1.1. Baseline model

A multigroup path analysis with gender as the grouping variable was conducted to test associations between SCL-baseline, RSA-baseline, and their interaction in the prediction of T3 anxious-fearfulness and unsociability. Wald tests demonstrated that the association between SCL-baseline, RSA-baseline, their interaction, and T3 anxious-fearfulness did not differ by gender [Wald $\Delta\chi^2(3) = 0.95$, $p = .814$] but these associations with T3 unsociability did differ by gender [Wald $\Delta\chi^2(3) = 8.26$, $p = .041$]. A model with the non-equivalent paths freed across

Table 3
Correlations of study variables.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Age (Months)	–														
2. Gender	−0.14	–													
3. Race/Ethnicity	−0.07	−0.16	–												
4. SES	−0.14	0.05	−0.12	–											
5. BMI	0.12	0.21^{+}	−0.29**	0.06	–										
6. SCL Baseline	0.02	0.15	−0.13	−0.04	0.18	–									
7. RSA Baseline	−0.07	−0.08	0.14	0.17	−0.19^{+}	−0.12	–								
8. SCLR-Excl.	0.00	−0.03	0.01	−0.07	−0.07	−0.12	−0.18	–							
9. RSAR-Excl.	0.07	−0.17	−0.10	0.14	0.08	−0.03	−0.22^{+}	−0.05	–						
10. SCLR-PAD	−0.04	−0.03	−0.01	0.09	0.11	0.08	−0.26*	0.60***	0.14	–					
11. RSAR-PAD	0.07	−0.03	−0.12	0.08	0.15	0.14	−0.17	−0.10	0.64***	0.01	–				
12. Anx-Fear T1	−0.04	−0.05	0.12	−0.04	−0.18^{+}	0.06	−0.08	−0.09	−0.04	−0.04	0.00	–			
13. Unsoc T1	−0.24*	0.19^{+}	0.07	−0.03	0.05	−0.16	−0.21^{+}	0.02	−0.09	0.05	−0.17	0.30**	–		
14. Anx-Fear T3	−0.16	0.04	−0.15	−0.02	0.04	0.02	0.04	−0.21	−0.17	−0.23^{+}	−0.26*	0.25*	0.20	–	
15. Unsoc T3	−0.02	−0.00	−0.09	0.06	0.29*	0.10	−0.21	−0.16	−0.01	0.02	−0.06	−0.04	0.19	0.31*	–

Note. Gender is coded: 0 = girls, 1 = boys; race/ethnicity is coded: 0 = White, non-Hispanic, 1 = people of color; SES = socioeconomic status; BMI = body mass index; SCLR = skin conductance level reactivity; RSAR = respiratory sinus arrhythmia reactivity; Excl. = Exclusion; PAD = post-aggression discussion; Anx-Fear = anxious-fearful, Unsoc = unsociability; T1 = Time 1; T3 = Time 3; +$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$; values represent post-winsorized data.

Table 4
Regression parameters for predicting growth in anxious-fearfulness and unsociability.

	Baseline model			Social exclusion model			Post-aggression discussion model		
	b	β	p	b	β	p	b	β	p
Anxious-Fearful									
T1 Age ➔ T3 Anxious-Fearful	-0.01 / -0.01	-0.12 / -0.17	0.302 / 0.302	-0.02 / -0.02	-0.17 / -0.25	0.168 / 0.168	-0.02 / -0.02	-0.17 / -0.24	0.163 / 0.163
T1 BMI ➔ T3 Anxious-Fearful	0.04 / 0.04	0.16 / 0.16	0.245 / 0.245	0.02 / 0.02	0.08 / 0.08	0.480 / 0.480	0.05 / 0.05	0.21 / 0.21	0.069 / 0.069
T1 Anxious-Fearful ➔ T3 Anxious-Fearful	0.27 / 0.27	0.29 / 0.24	0.026 / 0.026	0.19 / 0.19	0.20 / 0.18	0.072 / 0.072	0.25 / 0.25	0.27 / 0.23	0.018 / 0.018
T1 Unsociability ➔ T3 Anxious-Fearful	0.08 / 0.08	0.13 / 0.15	0.464 / 0.464	0.08 / 0.08	0.13 / 0.15	0.472 / 0.472	0.05 / 0.05	0.08 / 0.09	0.664 / 0.664
SCL(R) ➔ T3 Anxious-Fearful	-0.00 / -0.00	-0.06 / -0.06	0.676 / 0.676	-0.02 / -0.02	-0.21 / -0.20	0.041 / 0.041	-0.02 / -0.02	-0.24 / -0.25	0.001 / 0.001
RSA(R) ➔ T3 Anxious-Fearful	0.03 / 0.03	0.10 / 0.11	0.407 / 0.407	-0.11 / -0.11	-0.14 / -0.11	0.282 / 0.282	-0.13 / -0.13	-0.23 / -0.20	0.043 / 0.043
SCL(R) x RSA(R) ➔ T3 Anxious-Fearful	-0.00 / -0.00	-0.07 / -0.08	0.579 / 0.579	0.01 / 0.01	0.03 / 0.02	0.796 / 0.796	0.03 / 0.03	0.15 / 0.18	0.037 / 0.037
Unsociability									
T1 Age ➔ T3 Unsociability	-0.02 / -0.02	-0.09 / -0.13	0.376 / 0.376	-0.01 / -0.01	-0.04 / -0.05	0.733 / 0.733	0.00 / 0.00	0.01 / 0.02	0.891 / 0.891
T1 BMI ➔ T3 Unsociability	**-0.01 / 0.20**	**-0.03 / 0.51**	**0.896 / 0.001**	**0.01 / 0.17**	**0.04 / 0.45**	**0.787 / 0.002**	**0.05 / 0.17**	**0.12 / 0.46**	**0.425 / 0.004**
T1 Anxious-Fearful ➔ T3 Unsociability	-0.25 / -0.25	-0.16 / -0.14	0.201 / 0.201	-0.23 / -0.23	-0.15 / -0.13	0.214 / 0.214	-0.20 / -0.19	-0.13 / -0.11	0.248 / 0.248
T1 Unsociability ➔ T3 Unsociability	0.13 / 0.13	0.13 / 0.15	0.347 / 0.347	0.21 / 0.21	0.22 / 0.25	0.143 / 0.143	0.27 / 0.27	0.27 / 0.33	0.079 / 0.079
SCL(R) ➔ T3 Unsociability	**0.01 / 0.01**	**0.07 / 0.17**	**0.675 / 0.189**	-0.02 / -0.02	-0.13 / -0.12	0.249 / 0.249	**-0.04 / 0.01**	**-0.27 / 0.09**	**0.296 / 0.343**
RSA(R) ➔ T3 Unsociability	**-0.00 / -0.18**	**-0.01 / -0.41**	**0.974 / 0.000**	-0.07 / -0.07	-0.06 / -0.05	0.661 / 0.661	**-0.28 / 0.22**	**-0.28 / 0.20**	**0.027 / 0.070**
SCL(R) x RSA(R) ➔ T3 Unsociability	**0.01 / -0.02**	**0.13 / -0.45**	**0.415 / 0.001**	0.01 / 0.01	0.03 / 0.02	0.869 / 0.869	**-0.03 / 0.04**	**-0.10 / 0.18**	**0.749 / 0.070**
	$\chi^2(20) = 12.42$, $p = .901$ CFI = 1.00 RMSEA = 0.00 SRMR = 0.07			χ^2 (45) = 38.48, $p = .743$ CFI = 1.00 RMSEA = 0.00 SRMR = 0.11			χ^2 (42) = 36.71, $p = .702$ CFI = 1.00 RMSEA = 0.00 SRMR = 0.11		

Note. Estimates show girls on left, boys on right. Bolded effects were significantly different across boys and girls ($p < .05$). When pathways are constrained across groups, unstandardized (b values) are constrained to be equal; however, standardized values (i.e., *β)* may vary across groups due to group differences in variances. T1 = time 1; T3 = time 3; RSAR = respiratory sinus arrhythmia reactivity; SCLR = skin conductance level reactivity.

gender and all others constrained provided an acceptable fit to the data (see Table 4). In this model, there was a significant main effect of RSA-baseline for boys but not girls, which was qualified by a significant interaction between SCL-baseline and RSA-baseline in predicting unsociability for boys only (see Fig. 2). At low levels of RSA-baseline, SCL-baseline was positively associated with boys' T3 unsociability ($b = 0.04$, $SE = 0.01$, $p = .002$); this pattern reflects reciprocal SNS activation. In contrast, at high levels of RSA-baseline, SCL-baseline was not significantly associated with boys' T3 unsociability ($b = -0.02$, $SE = 0.01$, $p = .08$).

3.1.2. Exclusion model

A multigroup path analysis with gender as the grouping variable was conducted to test whether associations between SCLR-exclusion, RSAR-exclusion, and their interaction in the prediction of T3 unsociability and anxious-fearfulness differed by gender. Wald tests demonstrated that there were no gender differences in the associations between SCLR-exclusion, RSAR-exclusion, or their interaction and T3 anxious-fearfulness [$\Delta\chi^2(3) = 2.62$, $p = .455$] or T3 unsociability [Wald $\Delta\chi^2(3) = 5.18$, $p = .159$]. A model with non-equivalent paths freed and all other paths constrained provided a good to acceptable fit to the data (see Table 4). For boys and girls, SCLR-exclusion predicted lower levels of T3 anxious-fearfulness at average levels of RSAR-exclusion. There was no relation between RSAR-exclusion and T3 anxious-fearfulness at average levels of SCL-R for boys or girls. Additionally, the interaction between SCLR-exclusion and RSAR-exclusion was not significant for boys or girls. No significant associations were found between SCLR-exclusion, RSAR-exclusion, or their interaction and T3 unsociability.

3.1.3. Post-aggression discussion model

A multigroup path analysis with gender as the grouping variable was conducted to test whether associations between SCLR-discussion, RSAR-discussion, and their interaction in the prediction of T3 unsociability and anxious-fearfulness differed by gender (see Table 4). Wald tests demonstrated that the association between SCLR-discussion, RSAR-discussion, their interaction, and T3 unsociability differed by gender [Wald $\Delta\chi^2(3) = 12.11$, $p = .007$], whereas associations with T3 anxious-fearfulness did not vary by gender [Wald $\Delta\chi^2(3) = 2.63$, $p = .452$]. A model with non-equivalent paths freed and all other paths constrained provided an acceptable fit to the data (see Table 4). For boys and girls there were significant main effects of SCLR-discussion and RSAR-discussion, which were qualified by a significant interaction between SCLR-discussion and RSAR-discussion in predicting T3 anxious-fearfulness (see Fig. 3). At low levels of RSAR-discussion, SCLR-discussion was negatively associated with T3 anxious-fearfulness ($b = -0.03$, $SE = 0.01$, $p < .001$); this pattern indicates that coinhibition was related to anxious-fearfulness. In contrast, at high levels of RSAR-discussion, SCLR-discussion was not significantly related to T3 anxious-fearfulness ($b = -0.005$, $SE = 0.01$, $p = .57$; see Fig. 3). Among girls only, there was a main effect of RSAR-discussion, such that lower RSAR-discussion (i.e., greater RSA inhibition) was associated with greater T3 unsociability, at average levels of SCLR-discussion.

4. Discussion

The purpose of the present study was to investigate the short-term longitudinal associations between SNS activity, PNS activity, and their interaction in the development of anxious-fearfulness and unsociability among young children. We also examined gender differences in effects. Interestingly, there was no mean level change in either anxious-fearfulness or unsociability, suggesting that overall levels of these social behaviors were relatively stable across the year; nevertheless, physiological processes may be associated with individual differences in children's relative change in these social behaviors over time. Descriptive findings from this sample indicate that, as expected, children exhibited an increase in SCL and a decrease in RSA from baseline to watching the exclusion and post-aggression discussion videos. This provides support for the validity of the peer exclusion and post-aggression discussion manipulations. Further, given that RSA withdrawal appears to support active coping in response challenge or threat, whereas RSA activation facilitates social engagement in safe contexts (Hastings and Kahle, 2019), these findings are consistent with the suggestion that youth may appraise both videos as socially challenging.

Primary findings indicated that, in response to the exclusion video, low SCLR was associated with the development of anxious-fearfulness

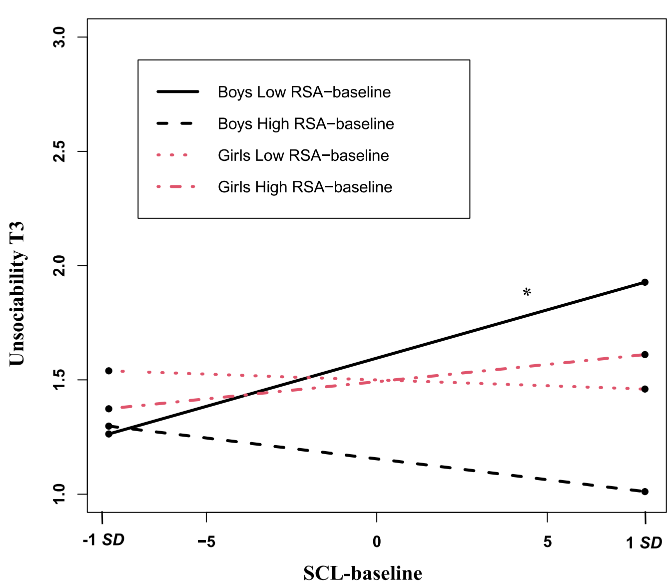


Fig. 2. Interaction between SCL-baseline and RSA-baseline predicting T3 unsociability for boys
Note. *$p < .05$. SCL = Skin Conductance Level, RSA = Respiratory Sinus Arrythmia, T3 = Time 3. Controlling for T1 and T3 anxious-fearfulness and T1 unsociability. The Y-axis reflects the minimum to maximum unsociability values in the sample.

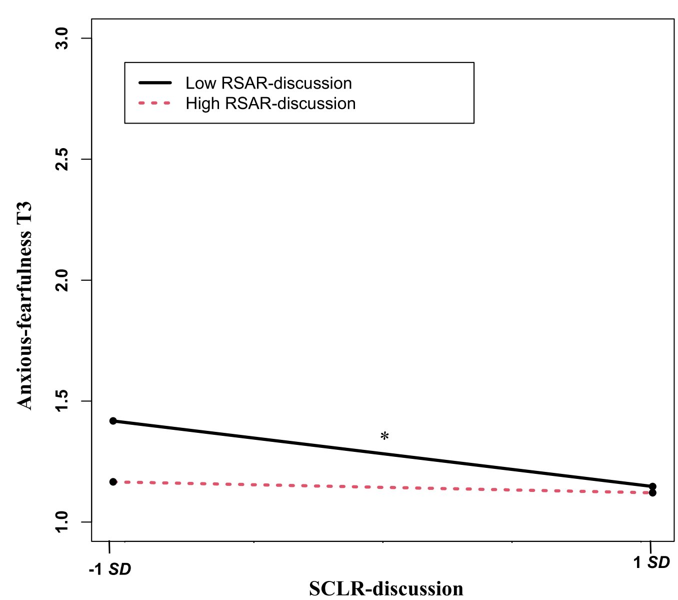


Fig. 3. Interaction between SCLR-discussion and RSAR-discussion predicting T3 anxious-fearfulness
Note. *$p < .05$. SCLR = Skin Conductance Level Reactivity, RSAR = Respiratory Sinus Arrythmia Reactivity, T3 = Time 3. Controlling for T1 and T3 anxious-fearfulness and T1 unsociability. The Y-axis reflects the minimum to maximum anxious-fearfulness values in the sample.

for boys and girls one year later. These findings were in contrast to our hypothesis that anxious-fearfulness would be associated with heightened SCLR. Although unexpected, it is notable that the research regarding associations between autonomic reactivity to social stress and anxiety in children is mixed (see Schmitz et al., 2013), with several studies with older children indicating blunted reactivity to stress in anxious youth. For instance, Schmitz et al. (2013) found that socially anxious 10–12-year-old children exhibited blunted heart rate reactivity to a social-evaluative stressor, with some evidence that this was sympathetically mediated (see also de la Torre-Luque et al., 2017). Similarly, Funke et al. (2017) found that 10–17-year-olds with comorbid general anxiety disorder and depression exhibited blunted sympathetic responses to stress. One potential interpretation of these findings is that anxiety in youth is associated with autonomic inflexibility, reflecting an inability to mount appropriate physiological responses to stress (Funke et al., 2017; Schmitz et al., 2013). However, given the mixed findings in this area, and the limited work investigating these effects in preschoolers exposed to exclusion, additional research is needed.

In the context of the post-aggression discussion video, low SCLR-discussion and low RSAR-discussion were associated with anxious-fearfulness one year later. However, these main effects were qualified by a significant interaction between SCLR-discussion and RSAR-discussion indicating that lower SCLR-discussion was associated with the development of anxious-fearfulness across the course of the study among children exhibiting low but not high levels of RSAR-discussion. This pattern reflects coinhibition (i.e., the interactive effect of co-occurring RSA inhibition and SCL inhibition), an uncoordinated ANS response. Previous research suggests that uncoordinated ANS responses to stress or challenge may be indicative of avoidance (Gatzke-Kopp and Ram, 2018) and disengagement (Gatzke-Kopp et al., 2020). In effect, anxious-fearful children may exhibit physiological responses to social challenges that facilitate avoiding interactions. Importantly, this avoidance appears to occur in efforts to manage the aftermath of peer stressors such as exclusion, even when this aftermath may include some potentially supportive interactions. These results are consistent with prior work indicating that anxious individuals tend to appraise even positive social experiences negatively, tend to fear social evaluation, and are intolerant of uncertainty (Nishikawa et al., 2022). Findings are also consistent with prior theory and work underscoring the role of avoidance in anxiety (Hofmann and Hay, 2018). Thus, coinhibition to the post-aggression discussion may reflect disengagement from and avoidance of opportunities to navigate the aftermath of social challenges, even when these experiences may include at least some positive and supportive social interactions. This tendency to avoid potentially supportive contexts may also be interpreted as indicating that anxiety is tied to rigid physiological responses (Friedman, 2007); in effect, avoidance may occur even when it is not contextually appropriate. Importantly, results are consistent with prior research documenting the importance of physiological reactivity after the acute experience of social stress has concluded in adaptive patterns of functioning (e.g., secure attachment; Hill-Soderlund et al., 2008). However, it is important to note that there were relatively low rates of anxious-fearfulness in our sample, warranting caution in interpreting our results especially in terms of the risk for clinically significant anxiety problems.

ANS activity was also associated with unsociability over time, although the pattern of effects depended on gender. For boys only, lower RSA-baseline was associated with the development of unsociability across the year. However, this main effect was qualified by a significant interaction between SCL-baseline and RSA-baseline, such that SCL-baseline was positively associated with unsociability at low but not high levels of RSA-baseline (i.e., reciprocal SNS activation). This finding is consistent with recent studies with adults indicating associations between sympathetic dominance (indexed by greater SNS as compared to PNS activity) and major depressive disorder (Brush et al., 2019), as well as symptoms of depression and anxiety in a sample of women (Stone et al., 2020). This sympathetic dominance may reflect an ineffective or maladaptive use of metabolic resources that increases risk for internalizing problems (Stone et al., 2020). Findings are also consistent with research demonstrating that both heightened SNS activity and lower PNS activity at rest are related to anxiety (Friedman, 2007), and indicate that coordination across these branches may play an important role in boys' unsociability. In effect, relatively more unsociable boys appear to exhibit a physiological pattern that increases metabolic resources for active coping in the absence of obvious threat. This heightened arousal may reflect hypervigilance and the inability to calm in neutral or nonthreatening contexts.

These findings raise the possibility that although boys exhibiting unsociable behavior may not appear to experience distress due to their lack of interactions with peers, this lack of visible distress may be misleading. The findings are consistent with other research areas in which individuals that appear well-regulated behaviorally nevertheless exhibit dysregulated patterns of physiological functioning (e.g., Hill-Soderlund et al., 2008). In effect, although not appearing visibly anxious, patterns of physiological activity indicative of hypervigilance or rigid and inflexible physiological responses (e.g., inability to calm in neutral contexts) may underlie unsociability in boys. This interpretation is speculative, however, given limited theory and research regarding the meaning of baseline ANS interactions.

In contrast, among girls only, lower RSAR-discussion (i.e., greater RSA inhibition) was associated with the development of unsociability one year later. In the context of SNS inhibition, RSA inhibition reflects an uncoordinated ANS response to stress or challenge which may be indicative of avoidance (Gatzke-Kopp and Ram, 2018) and disengagement (Gatzke-Kopp et al., 2020). However, in the absence of co-occurring SNS inhibition, RSA inhibition to challenge may be adaptive and facilitate coping (Hastings and Kahle, 2019). Interestingly, on average, children exhibited RSA inhibition to the post-aggression discussion task, suggesting that children may be appraising this task as challenging and requiring active coping. Thus, in contrast to findings for boys, results suggest that girls exhibiting more unsociable behaviors may effectively mobilize resources to engage with and cope during the aftermath of social challenge. These findings are consistent with the suggestion that youth exhibiting unsociable behavior may be capable of effectively engaging with peers when required to do so (Coplan et al., 2013), as well as with findings indicating that unsociability is not always associated with peer difficulties in girls (Coplan and Weeks, 2010b). Relatively more unsociable girls may find it easier to mount resources to cope with the aftermath of peer stress than their more socially engaged peers because they are less likely to focus on other people (see Coplan and Weeks, 2010b), perhaps making it easier to engage with potentially threatening discussions of events involving social conflict (e.g., exclusion). Further, relatively unsociable girls may be well-positioned to cope with peer difficulties because they are rarely the target of these behaviors. In fact, in the larger study of preschoolers from which the current physiology subsample was drawn, unsociability was associated with decreased experiences of peer victimization (Perry et al., 2021).

However, an alternative explanation is that greater RSA inhibition to the post-aggression discussion among relatively unsociable girls may reflect hypervigilance to further social threat. Indeed, in contexts that are nonthreatening and that require calm social engagement, RSA activation, rather than RSA inhibition, is likely adaptive (Hastings et al., 2008; Porges, 2007). Although the post-aggression discussion depicts discussions that may require metabolic resources for active coping (facilitated by RSA inhibition), it also depicts a supportive social context that could benefit from calm physiological states (facilitated by RSA activation). Interestingly prior research with this sample found that RSA inhibition during the post-aggression discussion video was associated with lower concurrent social dominance (Lent et al., 2022); one interpretation of this finding is that dominant youth exhibit calm states during this social challenge that support dominance behaviors (which may include aggression as well as affiliative behaviors; Hawley, 1999; Pellegrini et al., 2007). However, it is notable that, overall, participants exhibited RSA inhibition to the post-aggression discussion video, suggesting that, on balance, youth tended to appraise this discussion as socially challenging and requiring active coping, rather than unambiguously supportive. Nevertheless, in the present study, we cannot rule out the possibility that RSA inhibition reflects hypervigilance among relatively unsociable girls. The varied outcomes associated with RSA inhibition to the post-aggression discussion highlight the need for future research to include stimuli that isolate components of active coping and challenges related to managing the fallout of peer stress from positive, socially supportive contexts. Further, as with anxious-fearfulness, there were relatively low rates of unsociability in our sample, warranting caution in interpreting our results especially in terms of the clinically meaningful unsociability.

Overall, findings indicated important gender differences in the associations between ANS activity and the development of unsociability. Interestingly, some researchers have found that unsociability is more strongly related to negative developmental outcomes for boys than for girls (Coplan and Weeks, 2010b; Doey et al., 2014). Further, social withdrawal broadly is often viewed more negatively when exhibited by males than females, perhaps because social withdrawal violates gender norms and expectations of male assertiveness across developmental periods (Bowker et al., 2020). In fact, in one recent study, emerging adults judged unsociability to be more wrong when enacted by men than women (Bowker et al., 2020). Findings from the present study extend this prior research and suggest that the underlying physiological processes of unsociability may also differ for boys versus girls, such that physiological indicators thought to reflect avoidance or disengagement (Morneau-Vaillancourt et al., 2022), particularly in the absence of threat or challenge (i.e., baseline), relate to unsociability in boys only. These boys may not have the skills to effectively engage in positive peer interactions, and thus may elect to withdraw from social interchanges.

In contrast, among girls, unsociable behavior may be more closely tied to internal preferences (e.g., desire for solitary play; focus on objects rather than people) given less pressure for social engagement due to gender norms (Bowker et al., 2020). For girls, then, unsociability in early childhood may be more closely tied to a preference for solitary play (Coplan et al., 2018). However, it is important to note that, at least in some studies, unsociable behavior was predictive of negative outcomes across genders in later developmental stages (e.g., adolescence, adulthood). Indeed, in one recent study, unsociability was related to loneliness, when controlling for shyness and avoidance, in a sample of US men and women (but not in the 9 other countries studied; Bowker et al., 2023). Similarly, in a study of US 5th–8th graders, unsociability was related to lower peer liking and higher peer exclusion when controlling for shyness and avoidance, and effects were not moderated by gender (Eggum et al., 2022). Further, even in early childhood, the finding that distinct physiological processes are related to unsociability in boys versus girls do not preclude the possibility that unsociability may be related to negative long-term outcomes across genders. Indeed, unsociability interferes with peer interactions, which play a critical role in supporting positive youth development (Rubin et al., 2006). It will be critical for future research to investigate gender differences in the correlates and consequences of unsociability in young children.

4.1. Strengths, limitations, and future directions

The present study has several strengths including the use of multiple methods (e.g., observer ratings and psychophysiological assessments) within the context of a two timepoint longitudinal design in an understudied developmental period. The longitudinal design adds to our confidence in the directionality of associations between ANS activity and changes in social withdrawal behaviors. Researchers have called for moving beyond the single biomarker approach and the present study adds to the small but growing literature on the individual and interactive effects across the branches of the ANS in youth adjustment; further, we investigated these associations in early childhood samples, which is an important contribution given that the vast majority of extant work in this area is conducted with adolescent or emerging adult samples (e.g., Abaied et al., 2018; Philbrook et al., 2018).

For ethical reasons, the social exclusion and post-exclusion discussion videos were passively viewed by children. Given the importance of context for parasympathetic regulation, future work may benefit from capturing children's ecologically valid physiological reactivity to in vivo peer interactions within the preschool classroom/playground using increasingly sophisticated wearable technology (e.g., Darling et al., 2022). This work could also examine a wider array of peer stressor contexts including physical, verbal, and relational victimization as well as bullying victimization subtypes (i.e., victimization in the context of a power imbalance with repetition; Perhamus et al., 2022). Naturalistic observations of children's behaviors may also be better able to distinguish between subtypes of social withdrawal rather than relying upon the global observer ratings used in the present study (Coplan et al., 2004).

Future work should also consider dynamic autonomic nervous system coordination using alternative physiological indices such as pre-ejection period (PEP); in fact, there are advantages to examining coordination within the same organ system over time (e.g., PEP and RSA; see Rudd and Yates, 2020). In addition, investigations of interactions with the ANS system and markers of the hypothalamic pituitary adrenal axis (e.g., salivary, hair, and nail cortisol) are warranted to understand coordination across multiple stress systems in young children.

Although the sample is similar in size to several other physiological studies with young children (e.g., Henderson et al., 2004; Wagner et al., 2023), a key limitation is that the sample size and power may not be sufficient for the complex multi-group comparisons, which await replication with a larger and more diverse sample. In fact, the sample size precluded our use of more advanced methods, such as a bifactor model, that evaluate the shared and unique features of anxious-fearfulness and unsociability, which is a limitation given significant associations between the two constructs. Future research should examine how sympathetic and parasympathetic functioning are associated with the shared and unique features of unsociability and anxious-fearfulness.

Finally, the participants in the current study consisted of a community sample recruited from ten early childhood education centers which were selected to represent the broader surrounding community. Thus, levels of unsociability and anxious-fearfulness were low in our sample and the results may not generalize to samples with higher rates of anxiety or unsociability. Indeed, the physiological correlates of levels of unsociability that are clinically significant may differ from those observed in the present study. Future research should aim to recruit children who exhibit clinical levels of these behaviors to test the generalizability of study findings.

4.2. Conclusion

In conclusion, the present study is one of the first to examine the role of autonomic nervous system coordination in the prediction of changes in subtypes of social withdrawal during early childhood. The findings add to the growing literature on autonomic coordination and the gender-linked pathways associated with various patterns of physiological reactivity. Specifically, we found that coinhibition (a nonreciprocal pattern of autonomic coordination) to the post-aggression discussion video predicted greater anxious-fearfulness for boys and girls. In addition, unsociability appeared to be related to physiological indicators of avoidance and disengagement among boys at baseline and indicators of greater parasympathetic mobilization of resources for coping (in response to post-aggression discussion) in girls. This finding highlights the possibility that although some children exhibiting unsociable behavior may not appear visibly distressed by their lack of interactions with peers, they may still exhibit indicators of physiological inflexibility. In addition, findings from the present study underscore the importance of physiological activity in the absence of stress and following peer stress in the development of social withdrawal behaviors. With replication, this research on the role of unique physiological precursors of anxious-fearfulness and unsociability provides novel insights regarding the distinct nature of these subtypes of withdrawal from peers and may have implications for the development of future intervention and prevention programs aimed at reducing the longer-term negative effects associated with these behavioral styles. Specifically, if current findings are replicated, future intervention efforts may target children's responses following a social stressor to reduce risk for the development of anxious withdrawal and unsociability. Additionally, targeting arousal during nonthreatening situations may be a viable avenue for reducing risk for developing unsociability for boys. Together, these efforts would have the potential to reduce the development of both anxious withdrawal and unsociability in early childhood, and increase opportunities for peer interactions, thus reducing potential adverse downstream effects at later ages.

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CRediT authorship contribution statement

Maria C. Lent: Writing – review & editing, Writing – original draft, Conceptualization. **Kristin J. Perry:** Writing – review & editing, Writing – original draft, Formal analysis. **Gretchen R. Perhamus:** Writing – review & editing, Writing – original draft, Project administration. **Casey Buck:** Writing – review & editing, Writing – original draft. **Dianna Murray-Close:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Jamie M. Ostrov:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

None.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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