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Abstract

Behavioral Inhibition (BI) is a temperamental trait that refers to the avoidance and withdrawal from novel situations, objects, and people. It is considered a risk marker for later anxiety disorders. However, for BI to be a true risk marker it must predate the disorder onset and it should be associated with other well-known risk markers, such as early emerging symptoms. The majority of research exploring BI as a risk marker has largely utilized parent- or self- report measures of BI and has focused on middle to late childhood and adolescence. Further, BI has been linked with social anxiety specifically as well as depression, suggesting that BI subtypes may exist that differentially relate to disorder onset. The present study expanded on the current literature by examining BI and its association with early emerging anxiety symptoms in a community sample of 409 three-year old children. In addition, child sex was explored as a potential moderator of these relationships. Observational ratings of child BI taken from three episodes (stranger task, jumping spider, and risk room) in the Laboratory Temperament Assessment Battery (Lab-TAB) were used for analyses. Early emerging symptoms were indexed via the Child Behavior Checklist completed by the child's primary caregiver. Results revealed an association between high levels of social BI and anxiety related symptoms. High BI expressed during novel situations was associated with higher depressive symptoms in boys only, while high BI expressed in threat related tasks was associated with higher anxious-depressed symptoms in girls. Findings suggest that BI may be a multivariate construct that is differentially related to the development of psychopathology in boys and girls.

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Behavioral Inhibition and Parent Reported Anxiety Symptoms in Early Childhood:

Moderation by Child Sex

Anxiety disorders are the most common mental health issues in Canada (Byron, Campbell, Dunbar, Ogllvie, Sales, & Stewart, 2002). They are categorized into six groups in the Diagnostic Statistics Manual of Mental Disorders (American Psychiatric Association, 2013); however, all categories share a group of main characteristics that include; intense fear, anxious arousal, irrational thoughts, and avoidance (Anxiety Disorders Association of Canada, 2004). The prevalence rate in Canada for all the anxiety classifications for one year is approximately 15% (Government of Canada, 2013). Approximately one in four Canadians will meet criteria for an anxiety disorder in their lifetime (Anxiety Disorders Association of Canada, 2003).

With prevalence being so high, the socioeconomic burden for anxiety disorders in Canada is large. Costs related to anxiety disorders have yet to be examined directly in Canada, but it has been suggested that Canada may be similar to the United States for economic costs (Koerner, Dugas, Savard, Gaudet, Turcotte, & Marchand, 2004). In 2003, direct and indirect costs associated with all mental health disorders totaled approximately $51 billion (Lim, Jacobs, Ohinmaa, Schopflocher, & Dewa, 2008). This burden resulted from impact on the health-care system, persons suffering, and impact on society as a whole (Koerner et al., 2004). Direct costs include treatment and hospital visits while indirect costs include unemployment and disability insurance, lost productivity, and absenteeism to name a few (Lim et al., 2008). According Greenberg et al., (as cited by Roberge, Fournier, Duhoux, Nguyen, & Smolders, 2008) anxiety disorders account for approximately 31% of the annual mental health costs in the United States. Not only are anxiety disorders the most common of mental health issues for adults but they are also the most common disorder in childhood (Nevo & Manassis, 2009). According to Costello,

Mustillo, Erkianli, Keeler, & Angold (2003), the cumulative predicted prevalence rate of any anxiety disorder in children by the age of 16 would be approximately 10%. Like adults, impairments from anxiety can have debilitating effects on children including but not limited to relationship, school, and sleep problems, and increased suicide risk (Chase & Pincus, 2011; Fox, Barrett, & Shortt, 2002; Gould et al., 1998; Grills-Taqueshel, Fletcher, Vaughn & Stuebing, 2012). These sequelae of childhood anxiety disorders can have lasting effects especially if left untreated. Anxiety disorders can have a profound impact on social functioning that can persist over time due in part to the chronic nature of these disorders and their strong homotypic and heterotypic continuity (Ferdinand, Dieleman, Ormel, & Verhulst, 2007; Costello et al., 2003). Treatments for anxiety disorders often address only presenting symptoms and high rates of comorbidity can make them challenging to treat, resulting in high rates of recurrence across development. As such, it is important to intervene at the earliest possible time.

Early prevention and intervention programs can directly reduce suffering and economic costs associated with anxiety disorders. However, according to Rapee, Kennedy, Ingram, Edwards, & Sweeny (2005), most of the focus in anxiety disorder treatment has been on tertiary care programs. They suggest that the focus for anxiety disorders should be moved to early prevention programs because a portion of individuals with anxiety do not access the help that is available, and the individuals who do seek treatment have often suffered for many years prior. Intervening prior to the onset of a full-blown anxiety disorder is extremely important as individuals who go for many years without diagnosis or treatment may suffer many life interferences that can influence the course and severity of their disorder (Rapee et al., 2005).

In order to develop and implement a beneficial prevention or early intervention program, researchers must seek to identify putative risk markers for the development of anxiety. A 'risk

marker' refers to an attribute that is associated with increased probability of a disorder (Mash & Wolfe, 2013). As such, risk markers predate the onset of an anxiety disorder and help to identify individuals at risk, thus providing a focal point for targeted prevention and early intervention programs. It is imperative to identify well established risk markers for anxiety so that preventative action can be taken as early as possible. One risk marker receiving considerable research attention thus far is behavioral inhibition or BI.

Behavioral Inhibition

BI is a temperament construct that refers to the avoidance of and withdrawal from novel situations, situations, and people (Kagan, Reznick, Clarke, Snidman, & Garcia-Coll, 1984; Rosenbaum et al., 1993). Copious research has demonstrated strong associations between BI and anxiety disorders and symptoms in adolescence and adulthood (Hirshfeld et al., 1992; Kagan, & Snidman, 1999; Rosenbaum et al., 1993). This literature is very important to the understanding of anxiety disorders, but fails to establish BI as a *risk marker*, as BI is typically measured concurrently with threshold anxiety symptoms; as such, BI may be merely a symptom of the disorder. Van Brakel, Muris, Bogels, & Thomassen (2006) examined the association between BI and anxiety symptoms in children (mean age of 12.7 years) using the Behavioral Inhibition Scale (BIS) and the Screen for Child Anxiety Related Emotional Disorders (SCARED). They concluded that BI was positively correlated with anxiety ratings; however, it is unclear whether BI may be associated with subthreshold symptoms earlier in development.

Other research on BI and its association with anxiety disorders has also tended to focus on middle childhood, adolescence, and adulthood (van Brakel et al., 2006; Viana & Gratz, 2012). These studies have most commonly used self-report or parent-report measures which can have an inherent bias as individuals may over or under-report their own or their child's symptoms,

especially if mental health issues are present in the individual reporting symptoms (Hughes & Gullone, 2010). Also, if researchers are using self-report or parent-reported measures to determine BI and anxiety symptoms, associations may be inflated due to shared method variance (van Brakel et al., 2006; Hopkins, Lavigne, Gouze, LeBailly, & Bryant, 2013).

For example, Neal, Edelman, & Glachan (2002) conducted a study of individuals that had a mean age of 42.7. The participants were administered five self-report measures that included a battery of tests that indicted current internalizing psychopathology and behavioral inhibition that was recalled from childhood. They found that individuals who reported having high BI as a child also presented with a current anxiety disorder. This is important, as it shows that there is an association between behavioral inhibition and anxiety, but the results are contingent on individuals' long-term memory and it is difficult to disentangle whether BI actually predated anxiety disorder onset. Thus, not only are they subject to negative recall biases that have been well established in depression and anxiety (Beck, 2008; Dozois & Dobson, 2001) but shared method variance may also be a confounding factor.

A less common means of measuring behavioral inhibition as a construct is through observational methods. These methods typically require individuals to complete a battery of laboratory tasks designed to elicit BI that involve exposure to novel or ambiguous stimuli, situations, and people. These observational laboratory methods take time and are more expensive than questionnaires (Dougherty et al., 2011) which may explain why researchers favour easily administered and cost-effective questionnaire reports. However, observational methods of BI offer the opportunity to reduce shared method variance as symptoms of psychopathology are typically measured via self or informant report.

An important gap in the literature exploring the association between BI and anxiety is the paucity of this research in early childhood. This may be because it has been suggested that a child's temperamental traits at that age may be affected by normal developmental changes and, as mentioned earlier, that parent reports of temperament may influence the stability estimates (Pitzer, Esser, Schmidt, & Laucht, 2007). Children go through different stages where they commonly display fears that disappear as they move through to the next developmental stage (Mash & Wolfe, 2013). Common fears of children include; wariness of strangers, separation from parents, specific fears (thunder, fire, water), animals, and school to name a few. As the child grows, their fears usually dissipate and no issues develop; however, it is well recognized that it is not just "fear" but its severity, chronicity, and impact on functioning that is important when diagnosing any mental disorder (Mash & Wolfe, 2013) making the study of temperament traits such as BI at this age important for the prediction of anxiety disorders.

There has been an ongoing debate in the developmental psychopathology literature regarding the stability of temperamental traits across development (Novosad & Thomon, 1999). Durbin, Hayden, Klein, & Olino (2007) examined temperamental traits in a longitudinal study of children assessed at ages three, five, and seven. They evaluated two superfactors of temperament, namely, positive emotionality and negative emotionality. These temperament factors are broad in concept and have subordinate traits within the categories. For example, fear, sadness, and anger fall under negative emotionality. They assessed children using laboratory measures (Lab-TAB), home observation measures and parent report-measures to assess across and within method variance.   The results showed considerable stability of temperament even from this early age. Kagan et al. (1984) examined a small sample of children with and without high behavioral inhibition at 21 months and then again at four years of age. The children

participated in a laboratory observational assessment on two occasions. They found the children

at 21 months who were inhibited were still inhibited at four suggesting that BI was moderately

stable over the preschool years. Although the debate remains, it appears that multiple research

studies have found temperament to remain moderately stable over time. Specifically, *behavioral*

inhibition (BI) has shown to be one of the most stable temperament traits across development

(Fox, Henderson, Marshall, Nichols, & Ghera, 2005). This suggest that any associations

established between BI and other anxiety risk markers in early childhood are likely to persist

over time and be developmentally meaningful making research within this age group worth

pursuing.

Approximately 15% of children are considered to have an inhibited temperament (Fox et

al., 2005). It is important to note that not all children who have an inhibited temperament will

develop later psychopathology (Muris, van Brakel, Arntz, & Schouten, 2011). Although

research has found BI to be moderately stable over time, individual differences do occur and

children who exhibit BI at one point, may not continue to display it at a later time.

One area of research that has received little attention in establishing BI as a risk factor for

anxiety is its association with other well-established risk markers. BI is considered one of the

most well-studied and well-established temperament constructs associated with anxiety risk;

however, studies fail to associate with other known risk markers early in life (Grant et al., 2009).

As noted previously, if BI is a true risk marker for later internalizing psychopathology,

specifically, anxiety disorders, then it should predate the onset of an anxiety disorder; however, it

should also be associated with other well-established risk markers.

Early Emerging Symptoms

Another well-researched risk marker of anxiety disorders is early emerging symptoms. These are sub-threshold symptoms that do not meet the requirements for a diagnosis in the Diagnostic and Statistical Manual of Mental Disorders. Sub-threshold anxiety symptoms demonstrate good predictive validity for the onset of later anxiety disorders and show some degree of homotypic continuity (Ferdinand et al., 2007). Despite the fact that BI and early-emerging symptoms are both considered well established risk markers for anxiety disorders, to this authors' knowledge, only two studies have directly compared these two factors. Muris et al. (2011) investigated BI and anxiety related symptoms in a cohort of children aged five to eight during a three year period. They conducted structured interviews with the children using the DOMINIC questionnaire and had caregivers fill out two questionnaires; the Behavioral Inhibition Instrument (BII) and the Screen for Child Anxiety Related Emotional disorders (SCARED). The researchers found that BI was associated with early emerging symptoms of social anxiety from the DOMINIC questionnaire interview ($r = .71$) from occasion one to occasion two, but had a much lower correlation with other anxiety disorders ($r = .28$). From occasion two to three, BI was associated with SCARED social anxiety ($r = .72$), and also showed lower correlations with SCARED other anxiety disorders ($r = .34$).

The second study was completed by Grant et al., (2009) who utilized data from the National Survey of Children and Youth which included measures tapping temperament traits and anxiety related symptoms. This study consisted of five cycles of parent-reported measures and during cycle five, child-reported data was collected. The researchers used the fussy-difficult temperament trait and an unadaptable temperament trait of fearful-distress that they suggested was related to behavioral inhibition in children at an early age (two to three year olds). They found that only fussy-difficult temperament predicted anxiety symptoms at ages six to seven

years old and only unadaptable temperament significantly predicted anxiety symptomatology at

ages eight and nine. Importantly, the unadaptable temperament scale is defined by rigidity and

resistance to change and does not map well onto any well recognized definition of BI making

replication of these results with more valid BI measures warranted.

To this author's knowledge, no study has examined the association BI and anxiety

symptoms in early childhood or utilizing observational measures of BI.

Sex differences

A second gap in the literature lies in the concept of sex differences related to anxiety in

early childhood. Anxiety represents a disorder with a high female preponderance in adulthood.

As such, investigation into the etiological mechanisms through which this occurs is important

(McLean, Asnaani, Litz, & Hoffmann, 2011).   There has been well documented research about

sex differences of anxiety disorders in adolescence and adulthood (Mackinaw-Koons & Vasey,

2000). Indeed, females generally score higher on the "Big Five" personality traits of

neuroticism, a construct that is related to anxiety (Lynn & Martin, 1997). Adult females also

tend to score higher on measures of worry than their male counterparts (McCann, Stewin, &

Short, 1991). However, when looking at major temperament traits for children, specifically

positive and negative affectivity, sex differences tend to be smaller than in samples of

adolescents and adults (Olino et al., 2013).   There has been little research on this but what has

been done has highlighted notable differences on subordinate constructs that fall under positive

or negative affectivity. Fear, for one, has been found to be higher in girls than in boys in as

young as three and a half years of age (Dougherty et al., 2011). This suggests that sex

differences in fearfulness are evident very early in development. Gagne, Miller, & Goldsmith

(2013) also found that shyness was higher in girls at the age of 36 months noting that this sex

difference seemed to emerge between toddlerhood and preschool age around the age of three.

Fear and shyness are both broad components of BI and this research suggests that sex should be

considered when examining associations between BI and anxiety symptoms even at an early age.

Social Anxiety and Depression

Lastly, but crucial to a review of previous literature and important to the study of BI as a

risk marker, multiple studies have found that BI is more strongly associated with social anxiety

disorder than other types of anxiety, such as generalized anxiety disorder (Essex, Klein, Slattery,

Goldsmith, & Kalin, 2010; Gladstone, Parker, Mitchell, Wilhem, & Malhi, 2005; Schwartz,

Snidman, & Kagan, 1999; Biederman et al. 2001).   Even though many researchers have found a

stronger correlation with BI and social anxiety, it should be noted that some researchers have

found BI to be associated with any anxiety disorder (Rosenbaum et al., 1993). As mentioned

earlier, Muris et al. (2011) found that BI was significantly and positively correlated with social

anxiety ($r = 0.71$) but they also found a correlation between BI and other anxiety disorders ($r =$

0.28) albeit not as strong as the association social anxiety.

Anxiety disorders are commonly comorbid with other anxiety disorders and also

depression (Brady & Kendall, 1992; Costello et al., 2003). After a review by Brady & Kendall

(1992), they found that comorbidity rates for anxiety and depression have not been consistent,

ranging from 15.9% to 61.9%.   With the well documented research about the comorbidity of

anxiety and depression, it should not come as a surprise that some research has found behavioral

inhibition to be associated with depression (Gladstone & Parker, 2006). This may be because of

the overlap with anxiety.

At face value, this research suggests that BI is related to many different forms of

internalizing psychopathology. An alternative hypothesis is that BI may be a multi-faceted

construct with BI expressed in social versus non-social situations having differential predictive validity for social versus other anxiety or mood disorders. This is important to examine because if this is the case, then both laboratory and questionnaire based BI assessment should tap both social and non-social BI constructs when examining BI as a risk marker. In addition, BI expressed across social and non-social situations should be examined separately as a predictor of psychopathology. Unfortunately this is not current practice in the developmental psychopathology literature.

Dyson, Klein, Olino, Dougherty, & Durbin (2011) examined this further by observing a sample of 559 pre-school aged children using a laboratory assessment followed by a diagnostic interview, and lastly a parent-report questionnaire. They hypothesized that social and non-social BI would not be associated. They also believed that parent-report measures of shyness, social phobia symptoms, and social BI would be correlated with laboratory assessed social BI and that parent-reports of fearfulness, specific phobia symptoms, and non-social BI would be correlated with non-social laboratory assessed BI. Three tasks from the Laboratory Temperament Assessment Battery (Lab-TAB) were used in this study (Goldsmith, Reilly, Lemery, Longley, & Prescott, 1995). Dyson and colleagues (2011) found that laboratory assessments of social and non-social BI were not significantly associated. They also found a significant association between parent-report measures of shyness, social phobia symptoms, and social BI and BI expressed during social tasks from the laboratory assessment. Fearfulness, specific phobia symptoms and non-social BI was related to the non-social tasks from the laboratory assessment. Importantly, these authors did not examine associations with other types of internalizing psychopathology but their findings reveal the extreme importance of examining BI as a multi-faceted construct.

Aim of Study

The aim of the current research was to explore associations between BI and another known risk marker of anxiety, namely, early emerging symptoms. This association is currently not well-established in early childhood, and delineates a gap in our understanding of BI as a risk marker for anxiety. Further, potential effects of child sex as a moderator of this relationship were explored as there is currently little research on sex differences in early childhood regarding anxiety related differences. Next, this study examined if there were differences in the above described relationships when BI expressed during social and non-social laboratory tasks is examined separately as an independent variable in predicting anxiety (dependant variable; both social and generalized). Lastly, the current study explored depression and Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms in association with BI to examine the specificity of relationship between behavioral inhibition and anxiety. To avoid confounds created by shared method variance, the primary independent variable of interest was BI (within-subjects) which was measured by observation methods using three episodes from the Laboratory Assessment Battery (Lab-TAB; Goldsmith et al., 1995) while child symptoms were measured using the Child Behavior Checklist (CBCL; Achenbach, 1991) completed by the child's primary caregiver.

Based on reviews of previous literature, the following hypotheses were derived. It was first hypothesized that there would be a positive association between BI and anxiety related symptomatology. Secondly, it was hypothesized that the association between BI and anxiety related symptomatology would be moderated by child sex. There were no strong expectations as to whether boys or girls would show a stronger association between BI and anxiety symptoms. Girls may show a stronger association between BI and anxiety related symptoms suggesting a

pathway between BI and a female preponderance of anxiety later in life. On the other hand,

there is the possibility that the association may be more marked for boys. As boys are socialized

to be more risk-taking and less fearful, high BI in boys may be more "atypical" and indicative of

greater risk. The third hypothesis was that social and non-social constructs would be

differentially associated to different types of internalizing psychopathology (anxiety versus

depression symptoms). The fourth and final hypothesis was that BI would be specific to

internalizing disorders and therefore unrelated to an externalizing control (ADHD symptoms).

Method

Participants

Participants were an unselected community sample of 409 children (201 boys; 49.1%)

between three and four years of age ($M = 3.43$, $SD = 0.30$) and their caregivers recruited for a

study of child emotional development. Participants were recruited through a developmental

database (14%), flyers posted in local preschools (18%), advertisements posted on community

websites (21%), friend referrals (40%), and other sources (7%). Families were compensated for

their participation. Children who had been previously diagnosed with a major psychological or

medical condition were excluded from participation after a preliminary telephone screening

process. Child participants were predominately Caucasian (90%), and of average cognitive

ability ($M = 112.00$, $SD = 14.05$) as approximated by the Peabody Picture Vocabulary Test-

Fourth Edition, a measure of receptive vocabulary (PPVT; Dunn &Dunn, 2007). Primary

caregivers were predominantly the child's mothers ($N = 380$; 93%) and were an average of 33.53

years old ($SD = 5.07$). Family income was distributed broadly, with 16 participants (3.90%)

reporting an income less than $20,000, 44 participants (10.70%) reporting an income between

$20,000 and $40,000; 92 participants (22.40%) reporting an income between $40,001 and

$70,000; 115 participants (28.00%) reporting an income between $70,001 and $100,000; and 122

participants (29.80%) reporting an income greater than $100,001. The family demographic data

for this sample closely resembles the most recent London, Ontario census data available

(Statistics Canada, 2006).

Procedure

Laboratory assessment of BI. Children and their primary caregivers took part in a

laboratory visit approximately one and a half to two hours in duration. Primary caregivers were

first asked by a female research assistant to read the letter of information and then asked to sign

an informed consent for themselves and one for the child participating (See Appendix A), while

the main experimenter conducted the PPVT with the child. The child proband then took part in

episodes from the standardized Laboratory Temperament Assessment Battery (Lab-TAB;

Goldsmith, Reilly, Lemery, Longley & Prescott, 1995) that was used to assess child

temperament. All 409 laboratory visits were conducted by one of three female experimenters

(two graduate students and one laboratory coordinator) with the same experimenter working with

an individual child for the duration of the visit. Children completed 12 tasks designed to elicit

different emotional and behavioural responses and to measure multiple domains of child

temperament. Order effects were controlled by placing a positive affectivity task between the

episodes that were meant to elicit a negative affect. This may alleviate any carry over effects

that may occur from back to back episodes of tasks used to induce negative affect.   The tasks

were performed in the following order; Risk Room, Tower of Patience, Puzzle with Parents,

Stranger Approach, Make that Car Go, Transparent Box, Pop-Up Snakes, Jumping Spider, Snack

Delay, Green Circles, Popping Bubbles, and Box Empty. The child was given a break outside of

the testing room between tasks while stimuli were changed over. During the break, the child and

the experimenter played quietly with coloring books, puzzles, and play-dough. While necessary, this break also served to help avoid fatigue.

Three of the 12 tasks were used to measure BI; risk room, stranger, and jumping spider. The three tasks are described below; the other tasks in the battery will not be discussed here as they were designed to elicit other aspects of temperament not relevant to this study. Although some tasks were used to elicit mildly negative responses, all tasks ended positively. If a child ever reacted with an extreme negative response (high negative affect lasting 20 seconds or more) the task was ended immediately. Lab-TAB scripts used for the BI tasks can be found in Appendix B.

Risk room. The experimenter brought the child and caregiver into a room filled with novel and ambiguous stimuli. Items included; a gorilla mask, a cloth tunnel, a painted cardboard box designed to look like a monster, a balance beam, and a set of stairs that could be jumped off onto a mattress. The experimenter explained to the child that she forgot something and had to leave the room to go get it. The child was then told by the experimenter that they could play with the items in the room however they wanted. The caregiver was asked to work unobtrusively on a questionnaire in the corner of the room. The experimenter then left the room for 5 minutes. Upon her return, she asked the child to engage briefly with each of the stimuli.

Stranger approach. The primary caregiver was not present in the room during this task. After bringing the child into the room, the experimenter told the child that she forgot the toy and had to go get it. The experimenter instructed the child to wait against the wall and then left the room. Upon her exit, a friendly male stranger (research assistant) entered the room and approached the child. The male tried to engage the child in conversation using a script for approximately one minute. After one minute, the male stranger left the room and the female

experimenter returned. The experimenter asked the child if a man came into the room while she

was gone and then explained that the man was her friend and he was just looking for some

papers. At this point the experimenter opened the hallway door allowing the stranger to stand in

the doorway. The experimenter spoke to the stranger, and gave him the papers. The stranger then

said goodbye to the child and told them to have fun today.

Jumping spider. The female experimenter brought the child and caregiver into a room.

The caregiver was asked to work unobtrusively on questionnaires in the corner of the room. The

experimenter asked the child to sit at a table and wait for her friend who was going to bring in

the toy for the activity. A female research assistant entered the room with a toy spider attached

to a wire in an aquarium. The wire could be manipulated inconspicuously by the experimenter to

make the spider jump. The aquarium was brought to the table where the child and experimenter

sat. The female experimenter proceeded to tell the child that it was a pet spider, that was nice,

fuzzy, did not bite, and everyone liked it. The experimenter then asked the child to pet the

spider. When the child reached to touch the spider, the experimenter made the spider jump.

After four trials, the participant was shown that the spider was just a toy.

Questionnaire Data. A set of questionnaire measures were given to the primary

caregiver at the beginning of the laboratory visit. The complete questionnaire packet included

many measures tapping multiple child and caregiver related constructs. Caregivers were

instructed to work on the questionnaires during the visit and to complete them at home if they

remained unfinished when the visit ended. Caregivers were provided with an envelope and paid

postage with which to return the data. Two of the measures included in the questionnaire packet

will be used for the purposes of this study: a child symptom measure (Child Behavior Checklist;

Achenbach, 1991) which will be described in more detail in the measures section of this paper, and a basic demographic information form (see Appendix C).

Measures

 Coding procedures for BI. All tasks were video recorded behind a two-way mirror and later coded by trained graduate and undergraduate raters who were unaware of the parenting measures. BI was coded using an approach that was similar to most previous studies (e.g., Kagan, 1989; Olino, Klein, Dyson, Rose, & Durbin, 2010; Pfeifer, Goldsmith, Davidson, & Rickman, 2002). An intraclass correlation of .80 was reached between raters and a graduate level master rater before training was completed. Inter-rater reliability was then assessed on 10 - 15% of the videos [ICCs = .92 (risk room); .91 (jumping spider); and .87 (stranger approach)]. Coders met periodically throughout the coding process to ask questions and to avoid observer drift.

 For coding purposes, the episodes were separated into time-intervals of 20 to 30 seconds, known as "epochs." Each epoch was rated for behavioral and affective codes (Goldsmith et al., 1995). See Appendix D for full descriptions of coding procedure for each task. In each task, the time of the first definite fear response was recorded as well as the peak intensity rating of facial, bodily, and vocal fear for each epoch. In risk room and stranger approach epochs were coded from 0 (absent) to 3 (highly present and salient) for facial, bodily, and vocal fear. For the jumping spider task, epochs were coded from 0 (absent) to 3 (highly present and salient) for facial and vocal, and 0 (absent) to 2 (highly present and salient) for body fear.

 Risk room was sectioned into two phases for coding. In addition to the fearful affect codes described above, the epochs for risk room in the first phase were also coded for objects touched, tentative play, referencing of the parent prior to engaging with an object, proximity to the parent, fearful/wary questions/comments, amount of time spent talking, amount of time spent

playing, and sad affect. In the second phase, besides the above mentioned, coding was also

completed for time to comply with the experimenters request to touch an object, noncompliance

with the experimenters request, and to the referencing the experimenter prior to touching an

object.

In the stranger approach epochs, the time of the child's first vocalization was recorded

along with the time of the first fear response. Each epoch was also coded for still/freezing,

approach behavior (orienting/moving towards the stranger), avoidance of the stranger

(orienting/moving away from the stranger), gaze aversion, verbal/nonverbal interaction with the

stranger, and angry and sad affect.

In jumping spider, there were a total of four epochs defined by the period of time

between prompts to touch the spider. In addition to the fearful affect coding described

previously, each epoch was coded for approach behavior, withdrawal behavior, gaze aversion,

and startle response. The task was also coded for whether or not the child chose to play with the

spider after they were told it was a toy, and whether or not they verbalized throughout the

episode.

BI was computed as the average standardized ratings of these variables. The following

aggregates were used in the analyses: risk room BI (α = .93), spider BI (α = .86), stranger BI (α

= .61), overall BI (all three tasks; α = .93), and non-social BI (risk room and spider only; α =

.93).

Child internalizing symptoms. The child's primary caregiver completed the Child

Behavior Checklist (CBCL; Achenbach, 1991). The CBCL is a standardized parent report

measure that is used to assess emotional and behavioral problems in children (Ferdinand, 2008).

Internal consistency reliability estimates for internalizing, externalizing, and total problem scores

range from 0.89 to 0.96 and on the anxious/depressed scale range from 0.86 to 0.88 (Achenbach, 1991). The checklist contains 113 questions that parents answer using a 3-point likert scale; 0 (not true), 1 (somewhat true), or 2 (very true). To thoroughly test the hypotheses of this study, the DSM-oriented sub-scales were examined. The DSM oriented subscales were derived to be consistent with the DSM-IV diagnostic criteria (Achenbach & Dumenci, 2001). They are used as a supplement to the syndrome scales of the CBCL since the CBCL syndrome scales do not separate internalizing problems such as anxiety and depression. The DSM-oriented scales consist of six scales; Affective problems (related to depressive or mood disorders), anxiety problems, Attention Deficit/Hyperactivity problems, Conduct problems, Oppositional Defiant problems, and Somatic Problems (Ebesutani et al., 2008). Anxiety problems ($\alpha = .62$), affective problems ($\alpha = .56$), and ADHD ($\alpha = .62$) problems were used in analyses. ADHD problems were included in the analyses to establish specificity of behavioral inhibition in relation to internalizing symptoms. The CBCL also offers eight cross-informant syndrome scales; Withdrawn, Somatic, Anxious/Depressed, Social, Thought, Attention, Delinquent (Rule-Breaking), and Aggressive. The anxious-depressed syndrome scale was also included in analyses ($\alpha = .68$) to examine broad internalizing risk.

Results

All analyses were conducted in IBM SPSS Statistics (v.21). Table 1 shows the bivariate correlations between independent variables and possible covariates. Significant negative correlations were found between child age and all BI variables with the exception of BI expressed during the Jumping Spider task, suggesting that older children exhibit lower BI.

Table 1

Correlations between Independent Variables and Possible Covariates

	1	2	3	4	5	6	7	8	9
1. Child age in years	-								
2. Child Sex	.06	-							
3. Family income	.06	-.02	-						
4. PPVT standard score[1]	.05	.07	.11*	-					
5. Stranger BI aggregate	-.10*	.09	.06	-.02	-				
6. Spider BI aggregate	.02	.03	.06	.04	.03	-			
7. Risk room BI aggregate	-.14**	.07	.05	-.01	.25**	.10**	-		
8. Non-social BI aggregate	-.13**	.07	.06	.01	.24**	.40**	.95**	-	
9. All tasks BI aggregate	-.15**	.07	.07	.00	.37**	.41**	.93**	.99*	-
Mean	3.43	.51	3.73	112.00	.00	.00	-.00	-.00	-.00
(*SD*)	.30	.50	1.14	14.05	.30	.44	.30	.27	.25

* p < .05 level (2-tailed); ** *p* < .01 (2-tailed). *Note*: Child sex coded as males = 0, females = 1; interactions with child sex are point-biserial

[1] PPVT = Peabody Picture Vocabulary Test – Fourth Edition

Despite the magnitude of these correlations being small, age was controlled for in all analyses as a covariate. PPVT scores and family income were unrelated to BI. As expected, significant positive correlations were found between the independent BI aggregates of risk room BI, spider BI, stranger BI, non-social BI, and overall BI. These correlations ranged from low to moderate in magnitude (Cohen, 1988). Strong positive correlations were found between risk room BI, non-social BI, and overall BI. All symptom measures were positively correlated (see Table 2). The CBCL anxious-depressed symptoms scale was highly correlated with DSM anxiety problems scale as expected as they both measure anxiety issues. All other symptom scales were moderately correlated.

Multiple linear regression was used to explore main effects and interactions between all predictors and the four dependent variables of interest: CBCL anxious-depressed symptoms, DSM anxiety problems, DSM affective problems, and DSM ADHD problems. Child age, child sex, and the separate independent variables of the stranger BI aggregate, risk room BI aggregate, and spider BI aggregate were entered in step 1. In order to investigate sex by behavioral inhibition interactions, interaction terms created from child sex and centered BI variables were entered in step 2. For these analyses, interaction terms were made by first centering continuous variables, then multiplying two terms to create a term reflecting the product of both predictors (Aiken and West, 1991). As individual task BI variables (spider, stranger, and risk room), non-social BI (aggregate of risk room and spider), and overall BI (aggregate of all three tasks) represent overlapping constructs, separate models were run to test the main effects and sex interactions for overall BI first, followed by non-social versus social (stranger) BI, and lastly, BI during the three independent tasks.

Main Effects

Table 2

Correlations between Outcome Variables

	1	2	3	4
1. CBCL anxious-depressed	-			
2. DSM anxiety problems	.86**	-		
3. DSM affective problems	.50**	.43**	-	
4. DSM ADHD problems	.26**	.29**	.33**	-
Mean	2.06	1.69	1.82	2.23
(*SD*)	2.24	1.69	1.94	1.96

* $p < .05$ level (2-tailed); ** $p < .01$ (2-tailed).

There were no main effects found of BI variables on DSM affective problem scales

(*ps* > .79). A significant main effect of stranger BI on DSM anxiety problems was found, such

that high levels of social BI was associated with higher levels of anxiety symptoms

[*b* = 0.63, *t*(398) = 2.19,*p* = .03]. There was also a significant main effect of stranger BI on the

DSM ADHD problem scale, [*b* = -.66, *t*(398) = -1.96, *p* = .05] indicating that children with

higher BI during stranger have fewer ADHD symptoms. Significant main effects of child sex on

DSM anxiety problems [*b* = 0.61, *t*(402) = 2.28, *p* = .02] and CBCL anxious-depressed

symptoms [*b* = 0.65, *t*(402) = 2.94, *p* = .00] were revealed, with girls displaying higher anxiety

symptoms than boys.

Interactions

Several significant interactions were found between child sex and BI variables in

association with DSM affective problems and CBCL anxious-depressed symptoms. Significant

interactions were probed using procedures described by Aiken and West (1991) in which the

models were rerun examining the effect of the independent variable at different levels of the

moderator (tests of simple slopes). The Johnson-Neyman technique was then used to obtain

regions of significance (ROS) for each interaction (Johnson & Fay, 1950). The region of

significance can be defined as the level of behavioral inhibition at which males and females

differed significantly. One significant interaction and two trend level (*p* < .10) interactions were

found and will be described in sequence.

Child sex moderated the association between risk room BI and DSM affective problems

[*b* = -1.49, *t*(395) = -2.20, *p* = .03; see table 3]. Girls had lower levels of affective symptoms

with increasing risk room BI [*b* = -.73, *t*(395) = -1.55, *ps* = .12] whereas boys exhibited the

opposite effects [*b* = .76, *t*(395) = 1.56, *ps* = .12] though neither simple slope was significant (see

Table 3

Interaction between child sex and Risk Room BI in association with DSM Affective Problems

Predictor	DSM Affective problems		
	Unstandardized Coefficient (b)	*SE*	*t*
Child age in years	-.38	.33	-1.14
Child sex	.25	.20	1.29
Stranger BI aggregate	-.26	.43	-.60
Spider BI aggregate	-.12	.30	-.38
Risk room BI aggregate	.76	.49	1.56
Sex x Stranger	.78	.68	1.14
Sex x Spider	.42	.44	.94
Sex x Risk room	-1.49	.68	-2.20*

*$p < .05$

Figure 1). Boys and girls differed significantly in their levels of affective symptoms only at low (1 *SD* below the mean) levels of risk room BI [*b* = 0.70, *t*(395) = 2.52, *p* < .05]. The Johnson-Neyman region of significance was defined as values of risk room BI -.10 and lower.

A trend level interaction was found between sex and non-social BI in association with DSM affective problems [*b* = -1.35, *t*(397) = -1.82, *p* = .07; see table 4; see Figure 2]. Probing this interaction revealed the same pattern of findings as those in Figure 1 and suggests that BI expressed during the risk room task (rather than jumping spider) is responsible for this effect. Girls had lower levels of affective symptoms with increasing risk room BI, [*b* = -.60, *t*(397) = -1.17, *ps* = .24] whereas boys exhibited the opposite effects [*b* = .75, *t*(397) = 1.39, *ps* = .17]. Boys and girls differed significantly in their levels of affective symptoms only at low (1 *SD* below the mean) levels of risk room BI [*b* = .62, *t*(397) = 2.24, *p* < .05]. The Johnson-Neyman region of significance was defined as values of non-social BI -.12 and lower.

Lastly, a trend level interaction was found between sex and spider BI in association with the CBCL anxious-depressed symptoms [*b* = .88, *t*(395) = 1.74, *p* = .08; see table 5]. Girls have significantly higher CBCL symptoms with increasing spider BI [*b* = .89, *t*(395) = 2.39, *p* < .05]; however, there was no effect for boys [*b* = .01, *t*(395) = .03, *ps* = .98; see Figure 3]. Boys and girls differ significantly in their levels of anxious-depressed symptoms at moderate, (mean levels; *b* = 0.64, *t*(395) = 2.87, *p* < .01) to high levels of BI (1 *SD* above the mean; *b* = 1.03, *t*(395) = 3.25, *p* < .01). The Johnson-Neyman region of significance was defined as values of spider BI -.19 and higher.
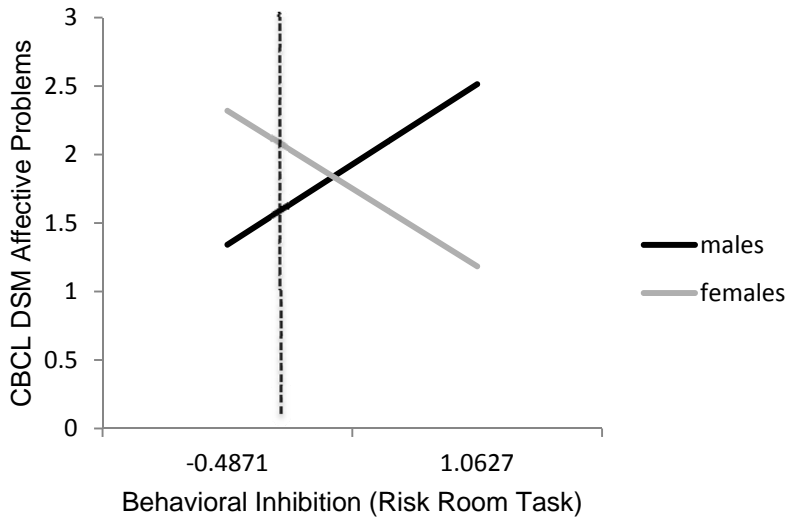
Figure 1. Relation between Affective Problems and Behavioral Inhibition as a Function of Child Sex. ROS = Region of Significance

Table 4

Trend level interaction between Non-social BI and child sex in association with DSM Affective Problems

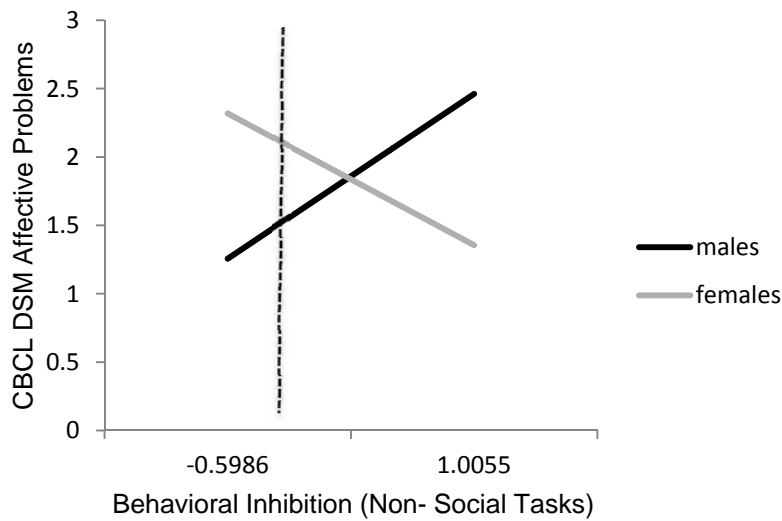| Predictor	DSM Affective problems		
	Unstandardized coefficient (b)	*SE*	*t*
Child age in years	-.40	.33	-1.20
Child sex	.25	.20	1.30
Stranger BI aggregate	-.23	.43	-.54
Non-social BI aggregate	.75	.54	1.39
Sex x Stranger BI	.73	.68	1.07
Sex x Non-social BI	-1.35	.74	-1.82[†]

[†] < .10 (trend level)

Figure 2. Relation between Affective Problems and Non-social Behavioral Inhibition as a Function of Child Sex. ROS = Region of Significance

Table 5

Trend level interaction between Spider BI and child sex in association with CBCL Anxious-Depressed Symptoms

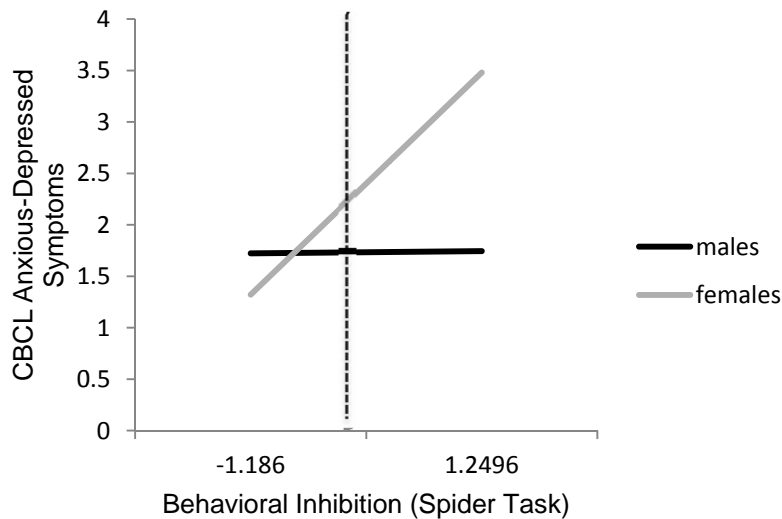| Predictor	CBCL Anxious-depressed symptoms		
	Unstandardized coefficient (b)	*SE*	*t*
Child age in years	.15	.38	.39
Child sex	.64	.22	2.87*
Stranger BI aggregate	.70	.50	1.41
Spider BI aggregate	.01	.34	.03
Risk room BI aggregate	.22	.56	.41
Sex x Stranger	-.46	.78	-.59
Sex x Spider	.88	.51	1.74[†]
Sex x Risk room	-.05	.77	-.07

[†] < .10 (trend level); *p < .05

Figure 3. Relation between Anxious-Depressed Symptoms and Behavioral Inhibition as a Function of Child Sex. ROS = Region of Significance

Discussion

Previous research has shown that BI is strongly associated with anxiety disorders (Kagan et al., 1984; Rosenbaum et al., 1993) and has thus been considered a risk marker for anxiety (Kagan & Snidman, 1999; Lahat, Hong, & Fox, 2011; Muris et al., 2011). However, there has been little research on BI in early childhood or in community samples which are needed to establish BI as a marker of *risk* for anxiety. To establish BI as a risk marker, it must predate disorder onset and should be associated with other known markers of disorder risk. The current study aimed to establish an association between BI and a well-known risk marker for anxiety development, subthreshold symptoms, in a community sample of preschool-aged children. Findings. This study found that stranger (social) BI was positively associated with anxiety symptoms in early childhood but was negatively associated with ADHD problems. In addition, girls at the age of three clearly displayed higher levels of anxiety-related symptoms than boys. Interestingly, it was also discovered that child sex moderated the association between BI and anxiety-related symptoms as well as affective problems.

Interestingly, while there was a positive association found between the construct of stranger BI, which is a social construct, and DSM anxiety problems there was no relationship between overall BI and anxiety. This finding adds to the growing amount of literature that suggests BI is more strongly associated with social anxiety rather than generalized anxiety (Essex et al., 2010; Gladstoneetal., 2005; Schwartz et al., 1999; Biederman et al. 2001; Hirshfeld-Becker et al., 2007). The current findings suggest that BI expressed during social situations may be a particularly salient risk marker for anxiety; whereas BI expressed in non-social situations, like when encountering novel but non-social objects are tasks, is not as important for anxiety disorder development. Interestingly, there was a negative association

between behavioral inhibition and DSM ADHD problems. Children who displayed lower levels

of stranger BI had higher ADHD problems. This coincides with literature from Kerr, Tremblay,

Pagani, & Vitaro (1997) who found similar results regarding disruptive problems (externalizing

problems) in boys. They found that boys who were inhibited were more likely to display higher

levels of disruptive behavior. This was initially the same outcome that Biederman and

colleagues (2001) found when they investigated the associations between BI and internalizing

and externalizing disorders in a sample of children aged 21 months to seven and a half years old.

They reported that children who exhibited low levels of BI had significantly higher levels of

disruptive problems. In 2007, Hirshfeld-Becker and colleagues conducted another study

examining BI in children and did not find any significant associations with disruptive problems

and behavioral inhibition. The difference in findings may be a result of issues with the power to

detect effects as the sample size varied widely from 99 (Hirshfeld et al., 2001) to 778 (Kerr et al.,

1997). In summary, although behavioral inhibition is positively associated with anxiety

symptoms, it also has a negative relationship with externalizing problems and may be useful in

identifying children at risk for externalizing disorders, although future research is warranted.

This study found significant associations between risk room BI and DSM affective

problems that were moderated by child sex. Child sex also moderated the association between

non-social BI and DSM affective problems at a trend level, but risk room BI was likely driving

this effect as there was not interaction between spider BI and child sex in predicting child

affective symptoms. McLean & Anderson (2009) suggested that socialization of children starts at

a very young age and reinforcement guides children as to whether they should continue to

display the behavior. For example, it is acceptable to girls to show fear and anxiety but not boys.

Boys are taught to problem solve and have control over their emotions. An interesting discovery

in this sample was that boys who displayed higher levels of non-social BI also displayed greater

affective problems whereas the opposite effects were shown for girls, despite the fact that the

simple slopes were non-significant. Further inspection revealed that girls and boys differed

significantly in their levels of affective problems only at low levels of behavioral inhibition.

Overall, these findings suggest that high BI expressed in novel situations may be indicative of

greater risk for depressive disorders for boys. It also suggests that girls display low levels of BI

in novel situations are at greater risk for depressive disorders. Of note, as depressive disorders

display a large degree of heterotypic continuity across development (Luby et al., 2003) it is

likely that high BI during novel situations represents a more broad *internalizing disorder* risk

than solely depression risk, with the opposite being true for females. Lastly, while it appears in

Figure 1 and 2 that girls and boys would differ significantly when BI levels were high, this was

not the case and could be due to the restricted range of this community sample. Not many

children displayed extremely high levels of BI so the power to detect associations at that level of

the analysis was reduced.

       This research found support for the exploratory hypothesis that child sex would moderate

the relationship between BI and anxiety-related symptoms. The association between CBCL

anxious-depressed symptoms and BI during the spider task was significantly moderated by child

sex, such that girls had significantly higher anxious-depressed symptoms with increasing BI

while boys did not. Between the two non-social tasks; the spider task is more of a pure threat

task, whereas risk room involves reactions to and exploration of novel and ambiguous stimuli.

Since no interaction was found between child sex and the less threatening *risk room* BI task in

predicting anxiety-related symptoms, these data suggest that high levels fear/BI expressed during

a threat related task such as the jumping spider task may be more problematic for girls than boys.

This may partially account for the preponderance of females with anxious and depressive disorders later in adolescence and adulthood as the CBCL anxious-depressed syndrome scale includes a broad array of internalizing symptoms relevant to both anxiety and depression.

  This research also supported the hypothesis that different constructs of BI measures have differential predictive potential for varying forms of psychopathology. This concurs with literature that has suggested that BI tends to be a multivariate construct (Dyson et al., 2011). The three tasks employed in this study have meaningful differences. The stranger BI task is more of a social task in that a stranger approaches the child and tries to engage in conversation. The spider BI task is a threat related task and the risk room BI task is exploratory and involves the child engaging in novel toys at their own pace. The differences in these tasks are important because when the tasks where put together to create an overall BI variable, there were no significant associations found. However, when the tasks were separated significant main effects and interactions were found. Interestingly, the different BI constructs also had different effects on anxiety, depression, and ADHD symptomatology. Stranger BI was associated with DSM anxiety symptoms in children, whereas risk room BI (non-social) was associated with DSM affective (depressive) problems, suggesting that "social" BI may be pertinent to anxiety and "novel" BI may be particularly important for depression. Spider BI (threat related) was associated with anxious-depressed symptoms in girls rather than boys. This suggests that "threat" related BI may be more representative of broad internalizing risk for females only. Lastly, stranger BI also had a negative relationship with ADHD problems. Overall, findings suggest that BI is a multivariate construct with three potential sub-traits and this variation should be reflected in how behavioral inhibition is measured and utilized in future developmental psychopathology research.

This study has a number of strengths. First, it utilized a large community sample of healthy children and their primary caregivers. This allowed for true psychopathology *risk* to be assessed as children did not currently meet criteria for a disorder. This also improves the generalizability of the findings. Second, this study used observational measures of behavioral inhibition in a laboratory setting rather than self or informant reports, thereby reducing potential reporter bias as well as confounds introduced via shared method variance (Lavigne et al., 2013). In addition, BI was measured in three separate environments which not only allowed for the tasks to be clustered together, but also allowed for the examination of each BI construct separately as it related to early emerging symptoms. Most of the research that has used observational methods has used a cluster of constructs (Biederman et al., 2001; Hirshfeld-Becker et al., 2007; Kagan & Reznick, 1994; Kagan & Snidman, 1999). .

While this study is the first of its kind to examine associations between multiple BI constructs and child symptoms in a well-powered community sample, there are several limitations that should be addressed. First, these data are cross-sectional in nature and therefore directionality/causality cannot be inferred. Future longitudinal research is needed to determine if BI at age three predicts symptoms or full-blown disorder development over time. Second, developmental changes in behavioral constructs (such as BI) can occur over time, therefore temporal change in behavioral inhibition and its relation to environmental variables and symptoms development should be explored (Pitzer et al., 2007) although research has shown there is a degree of continuity in temperament traits such as BI over time (Caspi, Roberts, & Shiner, 2005). Future research should also explore potential moderators that serve to buffer or protect against the effect of BI on psychopathology outcomes. For example, Degnan & Fox (2007) found that, in infants and toddlers, factors such as supportive parenting and developing

attention and regulatory skills could reduce BI and/or ameliorate its effects. Lastly, although

using a community sample is considered a strength for this study, restricted range may have

affected the ability to detect effects, particularly at high levels of risk room BI. Future research

may benefit from selecting for samples that include more children with very high BI across tasks.

Overall, girls as young as three display significantly more anxiety-related symptoms than

boys which adds to the little current research suggesting sex differences in internalizing

symptoms at this age. BI during social situations or "social BI" is positively associated with

anxiety-related symptoms in children which may help to establish it as a risk marker for future

internalizing psychopathology as it is present before the onset of the disorders. As child sex was

found to moderate the associations between BI affective problems and anxious-depressed

symptoms, novel and threat related BI may represent sex specific risk markers for males and

females respectively. Lastly, these data provide further evidence that BI is a multivariate

construct (Dyson et al., 2011). This suggests that future research utilizing observed BI should

refrain from aggregating all BI tasks. In addition, questionnaire measures of BI should be

developed that account for variation in how and when BI is expressed.

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Appendix A: Letter of Information – Child and Parent Consent

Letter of Information—Parent Consent for Child

Project Title: Gene-Environment Interplay and the Development of Child Temperament

Investigators: Elizabeth P. Hayden, Ph.D., Shiva Singh, Ph.D., Kathleen Hill, Ph.D.,
          Xinyin Chen, Ph.D.

Throughout this document, the pronouns 'you' and 'your' should be read as referring to the participant rather than the parent/guardian/next of kin who is signing the consent form for the participant.

<u>**Introduction**</u>
You are being invited to participate in a research study. The purpose of this letter is to provide you with the information you need to make an informed decision about participating in this research.

<u>**Purpose of this study**</u>
The purpose of this study is to examine whether genes and environments influence the development of childhood emotions.

<u>**Procedures for the study**</u>
Your participation in this study involves multiple steps. The first step is participation in a laboratory visit. During this visit, you will participate in a series of standardized tasks designed to elicit emotional reactions. These tasks are designed to simulate situations that children encounter in everyday life, and will be videotaped for future coding of your behavior and emotional responses. To elicit frustration, some of these tasks involve deception (e.g., after being shown an exciting toy, it will be locked in a box. You will be asked to try to open the lock with a set of keys that don't work, before finally getting to play with the toy). Parents will participate in some of these tasks with you, and will also be videotaped. A complete description of all tasks will be given to you.

During this initial visit, we will obtain a sample of your DNA (genetic information). This procedure will entail you or a member of our staff gently rubbing the cheek inside your mouth with a cotton swab similar to those used to clean the ear. Two swabs will be used to ensure that usable cells are obtained.

During the second part of the study, you will participate in a set of tasks in your home. These tasks entail you and your parent interacting with toys and a book. The purpose is to examine how you and interact with one another in these everyday situations. Also during the home visit, you will be given a matching task to complete, but will not be given sufficient time to complete the task. To see how you respond to this task, we will use a kit to collect saliva samples for cortisol (stress hormone) analysis. This procedure is remarkably easy because cortisol can be

measured by obtaining small samples of saliva. To get a saliva sample, we have you eat a few grains of sugar sweetened KoolAid[tm]. This makes the saliva flow and makes the sampling pleasant. You then mouth a cotton dental roll (the kind your dentist uses) until it is wet with saliva. This usually takes approximately 1 minute. You will be asked to provide 6 samples total (one prior to, and the rest after, the matching task). So you will not feel bad about failing to complete the matching task, at the conclusion of the task, the experimenter will say that she accidentally brought a matching puzzle for older children. All these tasks will be videorecorded for future coding.

In approximately 30 months, we will contact you to request that you participate in another laboratory visit very similar to the first, during which you will again participate in a set of new tasks designed to measure emotional behavior. At that time, you will be given a complete description of all the new tasks.

Each of the lab visits will take approximately 2 hours to complete, and will take place in the Department of Psychology at the University of Western Ontario. The home visit will take approximately 2 hours to complete. Your participation in this study takes approximately six hours total.

Risks associated with this study

Some of the laboratory tasks are designed to elicit negative emotional responses, such as disappointment or frustration. These tasks are designed to resemble "real-life" situations that most children encounter routinely (e.g., having to wait to play with an exciting toy), and do not typically elicit extreme emotional reactions. If you were to become more than mildly upset, we would end the procedure immediately. The likelihood of this happening, in our experience, is very rare. Also, all tasks are designed to have a positive ending (e.g., children get to play with the exciting toy after a few minutes). You or your parent can also ask us to skip any task you think will cause more than temporary, slight distress.

Regarding the cortisol assessments, when children put anything in their mouths, there is always a risk of choking. We will ask you to remain seated during the saliva collection, and we will remove the cotton roll that you chew during sampling. This same procedure has been used in other laboratories with hundreds of children without any mishap.

Benefits

There are no known benefits to you from participating in this study. However, your participation may help us develop a better understanding of how specific genes and environments influence child emotional development.

Compensation and Costs

For your participation in the lab visits, you will be compensated with a payment of **$100** for each visit (**$200** total). You will receive a payment of **$90** for your participation in the home visit components of the study (i.e., the matching task and the parent-child interaction task). You will also be compensated for any expenses associated with study participation (e.g., parking, taxi cabs to the laboratory). You will receive a copy of the DVD of the laboratory visits, and you will receive several small toys and several stickers.

Participation

Participation in this study is voluntary. You have the right to be given all important information about the study and what you will be asked to do, and you should only agree to take part if you are satisfied that you know enough about the study procedures. You may refuse to participate or withdraw from the study at any time. However, withdrawal of your participation does not necessarily include withdrawal of any data compiled on you up to that point. You will not be eligible to participate if you have a medical or psychiatric condition that would prevent you from participating in our assessments, or if you do not speak English well enough to complete our assessment procedures.

Confidentiality

We will strive to ensure the confidentiality of your research-related records. Your research records will be stored in a locked cabinet in a secure office, and computer files containing study data will be stored on password protected computers. Videorecorded data will be viewed only by members of the research team. DVDs will be erased at your request at any time. We will not share data with any other researchers without first removing identifying markers. When we publish results of the study, your name will not be used. We will store your DNA indefinitely, unless you ask us to destroy it. Absolute confidentiality cannot be guaranteed as we may have to disclose certain information under certain laws.

Duration of the study and enrollment

Approximately 400 children will participate in this study. Data will be collected for this project over the next five years.

Contact information
If you have any questions about this study, contact Dr. Elizabeth Hayden via email at ehayden@uwo.ca or via telephone at (519) 661-3686.

SNUM_____4

If you have questions about the conduct of this study or your rights as a research subject, you may contact the Office of Research Ethics, The University of Western Ontario, by telephone at (519) 661-3036 or by e-mail at ethics@uwo.ca.

You do not waive any legal rights by signing the consent form.

Research Consent Form

Project Title: Gene-Environment Interplay and the Development of Child Temperament

Investigators: Elizabeth P. Hayden, Ph.D., Shiva Singh, Ph.D., Kathleen Hill, Ph.D.,
            Xinyin Chen, Ph.D.

I have read the Letter of Information, have had the nature of the study explained to me, and I agree to participate.

Name of Research Participant (child):_____

Name of Parent or Guardian:_____

Signature of Parent: _____

Date: _____

Person Obtaining Informed Consent:_____

Signature: _____

        Date: _____

Letter of Information—<u>Parent Consent for Self</u>

Project Title: Gene-Environment Interplay and the Development of Child Temperament

Investigators: Elizabeth P. Hayden, Ph.D., Shiva Singh, Ph.D., Kathleen Hill, Ph.D., Xinyin Chen, Ph.D.

Introduction

You are being invited to participate in a research study. The purpose of this letter is to provide you with the information you need to make an informed decision on participating in this research.

Purpose of this study

The purpose of this study is to examine whether genes and environments influence the development of childhood emotions.

Procedures for the study

The study involves several parts that occur at different time points. When you begin the study, you will be asked to complete paper-and-pencil measures of your child's behavior and moods. We will also ask you to complete paper-and-pencil measures about your personality and behavior, your home life, and your parenting behaviors. If appropriate, we will also ask you to complete paper-and-pencil measures about your child's coparent's personality and parenting behaviors.

Approximately 15 months later, an interviewer will interview you **or** your child's coparent about any life events occurring in your family since entering our study. At approximately 30 months after beginning the study, an interviewer will again interview you **or** your child's coparent about new life events experienced since the last interview, and will interview you about any problems you may have had with emotional, behavioral, or substance use problems. You will also be asked to complete another packet of questionnaires on your child at that time.

The report measures will take approximately 1 hour to complete, and will be collected either during your child's laboratory visit and/or on your own while at home. The life events interview takes about 45 minutes for most people to complete, although some people take more time and others take less. The interview about emotional, behavioral, and substance problems takes approximately 1 ½ hours for most people to complete, although this also varies. These interviews can be completed at our research lab, in your home, or over the telephone, depending on which is most convenient for you.

Risks associated with this study

While answering some of the questions on the self-report and interview measures, you may feel sad or upset. If this happens, the interviewer will discuss these feelings with you. We can also provide you with a referral to a local mental health care provider. However, in our experience, answering these questions does not distress most study participants. You can also decline to answer any questions you do not wish to answer.

Benefits

There are no known benefits to you from participating in this study. However, your participation may help us develop a better understanding of how specific genes and environments influence child emotional development.

Compensation and Costs

You will be compensated with a payment of **$55** for your completion of the baseline packet of questionnaires. This payment will be prorated if you do not complete all the questionnaires. Compensation for completing the two life events interviews at 15- and 30-month follow-up consists of a payment of **$45** for each (**$90** total to either you or your child's coparent). You will be compensated with a payment of **$60** for completion of the interview concerning past problems with emotional, behavioral, and substance use problems at 30-month follow-up. You will also be compensated for any expenses associated with study participation (e.g., parking, babysitting, taxi cabs to the laboratory). For the packet of questionnaires on your child's behavior which we will ask you to complete at 30-month follow-up, you will receive a payment of **$15**.

Participation

Participation in this study is voluntary. You have the right to be given all important information about the study and what you will be asked to do, and you should only agree to take part if you are satisfied that you know enough about the study procedures. You may refuse to participate, refuse to answer any questions, or withdraw from the study at any time. However, withdrawal of your participation does not necessarily include withdrawal of any data compiled on you up to that point. You will not be eligible to participate if you do not speak English well enough to complete our assessment procedures.

Confidentiality

We will strive to ensure the confidentiality of your research-related records. Your research records will be stored in a locked cabinet in a secure office, and computer files containing study data will be stored on password protected computers. We will not share data with any other researchers without first removing identifying markers. When we publish results of the study,

SNUM_____3

your name will not be used. Absolute confidentiality cannot be guaranteed as we may have to disclose certain information under certain laws.

Duration of the study and enrollment

Approximately 400 families will participate in this study. Data will be collected for this project over the next five years.

Contact information
If you have any questions about this study, contact Dr. Elizabeth Hayden via email at ehayden@uwo.ca or via telephone at (519) 661-3686.

If you have questions about the conduct of this study or your rights as a research subject, you may contact the Office of Research Ethics, The University of Western Ontario, by telephone at (519) 661-3036 or by e-mail at ethics@uwo.ca.

You do not waive any legal rights by signing the consent form.

SNUM_____4

Research Consent Form

Project Title: Gene-Environment Interplay and the Development of Child Temperament

Investigators: Elizabeth P. Hayden, Ph.D., Shiva Singh, Ph.D., Kathleen Hill, Ph.D.,
                Xinyin Chen, Ph.D.

I have read the Letter of Information, have had the nature of the study explained to me, and I agree to participate.

Research Participant:_____Signature: _____

Date: _____

Person Obtaining Informed Consent:_____

Signature: _____

Date: _____

Appendix B: Lab-TAB BI task scripts age 3

Episode # 1: Risk Room

Materials: - tunnel
                    - Halloween mask & table
                    - balance beam
                    - "monster" box
                    - stairs and mattress

Parent: In the room.

Setup: Follow attached diagram

Procedure: The experimenter leads the parent and child into the room and says:

> "I have something I need to go do, but while I'm gone, you can play with these toys however you like. I'll be back in a little bit."

To the parent: Before episode begins, remind parent that (s)he is to focus on the paperwork and avoid any attempts on the child to engage him/her in play. If the child tries to engage him/her, the parent should tell the child that (s)he has work on the paperwork.

> "I've left you some papers to work on, so X needs to play by himself/herself."

Child is left alone in the room for **5 mins**. The experimenter returns and says to the child:

> "Hi, I'm back. Did you look at all these things?"

Experimenter walks up to each object, then asks the child to touch/play with the object.

> "Can you show me how you crawl through that tunnel? Good."

> "Can you walk across this balance beam? I can help you if you want. Good."

> "Can you walk up those stairs, jump off the top, and land on that mattress? Good."

> "Can you put your hand inside that hole? Good job."

> "Can you touch that mask? Good job."

You may give **2 prompts** if the child fails to comply with the first request (1st prompt: "I'd really like to see you …"; 2nd prompt: repeat first command). Move on to the next object if child still fails to comply.

After the child has been asked to touch each object, the experimenter says:

> "Wow, you did a really good job exploring all those things. Do you want to go outside and play with some Play-doh until the next toys are ready?"

Episode #4: Stranger Approach

Materials: - adult size chair
                        - cue card & hat for stranger
                        - papers for experimenter

Setup: The adult size chair is placed against the back wall, just to the left of the wall's midpoint.

Parent: **Not in the room.**

Procedure: The experimenter leads the child to the back wall, and says:

> "You know what? It looks like [toy assistant name] forgot to get us a toy. I'm going to go get the toy so we can play with it. I'd like you to stand right here …

Experimenter positions child against the back wall.

> "You wait right here for me. I'll be right back with the toy."

Experimenter exits.

10 sec pause.

The stranger knocks on the door **3x**, and then enters the room.

During the following interaction, the stranger may respond to the child if s/he engages in conversation or verbalization. Responses should be short, and should not consist of questions. The stranger should maintain neutral affect (both facially and vocally) throughout.

Stranger says:
> "Hi."

2 sec pause.

> "Have you ever been here before?"

10 sec pause.

Stranger walks to the chair, stands in front of it, and says:
> "Are you having a good time here today?"

10 sec pause.

Stranger sits in the chair and says:
> "Are you playing with lots of toys?". **If** the child mentions a certain toy, the stranger says: "I like (toy name), too."

20 sec pause.

<u>"I came to pick up some papers. Was there a woman here?"</u>

After the child responds (or not), the stranger says:

<u>"I'll go look in the hall."</u>

Stranger exits and waits outside in the hallway.

15 sec pause.

Experimenter enters, holding several pieces of paper, and says:

<u>"Was there a man here?"</u>

After the child's response, the experimenter says:

<u>"Oh, that was my friend (name). He was here looking for these papers. Maybe he went into the hall."</u>

Experimenter opens the door and says to stranger:

<u>"Oh, hi (name). Here are the papers you wanted."</u>

Stranger enters the room, and says:

<u>"Thanks. Hi again – I found my papers. Goodbye – have fun today!"</u>

Stranger exits.

Episode #8: Pet Spider

Materials: -"spider" and spider house

Setup: The child-sized table and two chairs are in the main experimental area

Parent: In the room.

Procedure: The experimenter leads the child into the room and seats him/her at the table facing the camera.   As soon as they are seated, a research assistant knocks at the door and brings in the spider house, keeping the wire hidden from the child. The assistant hands the spider house to the experimenter (again, keeping the wire hidden from the child). The experimenter then says:

> "My friend XX just brought us my pet spider [sliding the top door open]. Look, he's a soft, fuzzy spider. Go ahead and pet him, he won't hurt you. He's a nice spider."

The experimenter waits until the child reaches into the house, coaxing him or her further if necessary. If the child refuses, repeat the previous statement up to a total of **four times**, pausing 5 sec in between each request to allow the child to comply.

Once the child's hand is about two inches from the spider, the experimenter makes the spider jump by using the wire. Always make the spider jump before the child reaches for it (i.e., you may need to make the spider jump almost immediately if the child reaches for it before you have asked him/her to). **Experimenter does not touch spider.** The experimenter should keep a neutral expression for five seconds, then says:

> "Oh, he jumped at you. But that's okay, he doesn't really bite. Go ahead and pet him."

Once again, the experimenter waits until the child reaches into the house, coaxing him or her further if necessary. Once the child's hand is about two inches from the spider, the experimenter makes the spider jump by using the wire. The experimenter should keep a neutral expression for five seconds. The request (and subsequent procedure, if the child actually touches the spider) is repeated twice for a total of four trials. After four trials, the experimenter says:

> "This is really just a toy. Let me show you how it works."

The experimenter shows the child the wire and how the spider works.

Appendix C: Basic Demographic Form

Family Information   SNUM_____

Today's Date: _____   Your Child's Age: _____

Your relationship to child   ☐ Mother   ☐ Father   ☐ Other

Child's Ethnicity:☐ White   ☐ African American   ☐ Asian   ☐ Other_____
                                                          (please specify)

With which adults does child currently live? (Check all that apply)

    ☐ biological mother   ☐ step-parent or father's companion

    ☐ biological father   ☐ step-parent or mother's companion

    ☐ adoptive mother   ☐ other relative(s)

    ☐ adoptive father   ☐ other non-relative(s)

Marital Status of child's biological parents

    ☐ married   ☐ separated

    ☐ living together   ☐ divorced

    ☐ mother deceased   ☐ never married

    ☐ father deceased   ☐ mother remarried

                                    ☐ father remarried

                                    ☐ other

SNUM_____

Please list the child's siblings in order of birth. (Please indicate first names)

First Name	Sex	Age	Living at Home
_____	☐ Male ☐ Female	_____	☐ Yes ☐ No
_____	☐ Male ☐ Female	_____	☐ Yes ☐ No
_____	☐ Male ☐ Female	_____	☐ Yes ☐ No
_____	☐ Male ☐ Female	_____	☐ Yes ☐ No
_____	☐ Male ☐ Female	_____	☐ Yes ☐ No
_____	☐ Male ☐ Female	_____	☐ Yes ☐ No

PARENT INFORMATION: (Please complete for biological parents if known)

Mother: Age _____   Present occupation _____

Father: Age _____   Present occupation _____

SNUM_____

Education Mother   Education Father

      ☐ 8th grade or less   ☐ 8th grade or less

      ☐ some high school   ☐ some high school

      ☐ high school graduate (or GED)   ☐ high school graduate (or GED)

      ☐ some college (or 2 year degree)   ☐ some college (or 2 year degree)

      ☐ 4 year college degree   ☐ 4 year college degree

      ☐ master's degree   ☐ master's degree

      ☐ doctoral degree   ☐ doctoral degree

Family Yearly Income

☐ <$20,000   ☐ $20,001 - $40,000   ☐ $40,001 - $70,000   ☐ $70,001 - $100,000   ☐ >$100,000

COMPLETE THIS SECTION ONLY IF THE ADULT(S) CURRENTLY CARING FOR CHILD IS/ARE NOT THE CHILD'S BIOLOGICAL PARENTS:

A. Relationship to child   ☐ adoptive parent   ☐ other relative   Age _____

                             ☐ step parent   ☐ other non-relative

B. Relationship to child   ☐ adoptive parent   ☐ other relative   Age _____

                             ☐ step parent   ☐ other non-relative

SNUM_____

Highest level of education for non biological guardian (above):

Caretaker A (above):   Caretaker B (above):

☐ 8th grade or less   ☐ 8th grade or less

☐ some high school   ☐ some high school

☐ high school graduate (or GED)   ☐ high school graduate (or GED)

☐ some college or 2 year degree   ☐ some college or 2 year degree

☐ 4 year college degree   ☐ 4 year college degree

☐ master's degree   ☐ master's degree

☐ doctoral degree   ☐ doctoral degree

Family yearly Income of non biological guardian:

☐ <$20,000   ☐ $20,001 - $40,000   ☐ $40,001 - $70,000   ☐ $70,001 - $100,000   ☐ > $100,000

CHILD'S MEDICAL HISTORY

Please mark the circle next to any medical conditions your child has ever had.

Epilepsy/seizures/convulsions	☐	Head injuries or lacerations leading to loss of consciousness	☐
Seizures with high temperatures	☐	Unconsciousness (other)	☐
Birth abnormalities	☐	Anemia	☐
Heart disease	☐	Lead poisoning	☐
Asthma	☐	Meningitis	☐

SNUM_____

Food sensitivities	☐	Encephalitis	☐
Allergies (describe)	☐	Mumps	☐

_____

Chicken pox	☐	Emergency room visit	☐
German measles	☐	Poisoning, medicines	☐
Whooping cough	☐	Poisoning, cleaning agents	☐
Problems with vision	☐	Poisoning, non-food item	☐
Problems with hearing	☐	Physical handicaps (describe below)	☐
Serious accident (describe below)	☐		

_____   Other diseases (describe below)   ☐

Fever over 104, unknown cause   ☐   _____

Is child taking medications for any conditions above?   ☐ Yes   ☐ No

Medication (specify) _____

Has your child ever been hospitalized for a medical problem? ☐ Yes   ☐ No

     If yes, number of times: _____

     If yes, reason: _____

CHILDHOOD HISTORY

How many pregnancies did mother have before the pregnancy with this child

(including those not carried to term)?   _____

Check any of the following occurring during the pregnancy with this child: (check as many as apply)

☐ severe nausea and vomiting   ☐ toxemia

☐ high blood pressure   ☐ rubella, mumps

☐ incompatible Rh factor   ☐ diabetes

☐ anemia

☐ bleeding 1st 3 mos   ☐ bleeding 2nd 3 mos   ☐ bleeding 3rd 3 mos

Medications during pregnancy:

_____

Check any of the following if they occurred at or following the delivery of the child: (check as many as apply)

☐ premature delivery   ☐ infant required oxygen

☐ caesarean section   ☐ infant required blood transfusion

☐ breech delivery (feet or buttocks first)   ☐ infant was placed in an incubator

☐ infant had cord around neck

☐ infant was blue at birth

☐ other problems (specify) _____

Child's weight at birth: _____   _____

                    (Pounds) (Ounces)

Did your child stay in the hospital after mother left?   ☐ Yes   ☐ No

SNUM_____

If yes, number of days _____

During the first year of life, did your child have difficulties in any of the following areas: (check all that apply)?

    ☐ sleep problems   ☐ excessive crying

    ☐ feeding problems   ☐ difficult to comfort

    ☐ resisted being held   ☐ sluggish, nonresponsive

    ☐ overly active   ☐ fussy much of the time

    ☐ under active

Was child breast-fed?   ☐ Yes ☐ No   If yes, for how long? _____ (months)

Age child started walking without assistance: _____ (months)

Age child spoke first words: _____ (months)

Age child dressed without supervision: _____ (months)

Did your child have difficulties with the development of speech? (check all that apply)

    ☐ no difficulties   ☐ did not use "I" or "me"

    ☐ delayed speech   ☐ often repeated other's words

    ☐ stammering   ☐ talked excessively about one topic

    ☐ hard to understand   ☐ other

SNUM_____

Child's primary caregiver(s) are (check all that apply):

☐ Mother   ☐ Father   ☐ Grandparent   ☐ Live-in nanny/sitter   ☐ Other (specify)_____

How many hours per week does your child spend in the following:

preschool hours _____   daycare hours _____   other childcare setting hours _____

Does mother work outside of the home? ☐ Yes   ☐ No

If yes, how many hours per week? _____

Does father work outside of the home?   ☐ Yes   ☐ No

If yes, how many hours per week? _____

Appendix D: Lab-TAB coding manual and scoring sheets

LAB-TAB CODING MANUAL

1. Risk Room

Phase I (child alone):

Start time: Begin coding as soon as the child enters the room.
Stop time: Stop coding when the experimenter returns.

A. **Time of first <u>definite</u> fear response:** note the time (including secs) of the first DEFINITE fear response (definite = either a 1 or higher is coded for fearful affect or distress vocalization OR a 2 or higher is coded for postural/bodily fear).

B. Watch entire episode through once to record the time at which each object was first touched. **Objects touched:** record start time for the beginning of the episode, and the counter time when the object is <u>first</u> intentionally touched. Objects must be intentionally (not accidentally) touched, which can include exploration, rather than obvious playing.

C. Verbalizations
Time of first verbalization: record the counter time at which the child makes his/her first verbalization, which can take any tone of affect or content.

D. *Phase I scoring:*
Fearful Affect: rate the peak intensity of fearful/wary facial expression that occurs during the epoch
>   0 = no facial region shows codeable fear movement
>   1 = fear expression is ambiguous or is of low intensity; fear is evident in only one facial
>   region (i.e., brows raised in distress)
>   2 = fear expression is definitely present in at least 1 facial region (i.e., brows raised and
>   drawn together, upper eyelids raised)
>   3 = fear expression is definitely present in both facial regions (i.e., brows raised and
>   drawn together, upper eyelids raised to show whites of eyes, corners of mouth
>   opened and drawn back)

Bodily Fear: rate the peak intensity of fearful bodily expression that occurs during the epoch.
>   0 = child's body never reflects fear or weariness
>   1 = child's body reflects low intensity fear or weariness (e.g., cautious or wary gait;
>   slight tension; nervous twitching, hand tapping, foot swinging, etc.; diminished
>   activity level)
>   2 = child's body reflects moderate intensity fear or weariness or the display
>   lasts a majority of the epoch (e.g. slight defensive body posture; fearful tension)
>   3 = child's body reflects high intensity fear or weariness (e.g., definite defensive body
>   posture, jumping back in fear)

Tentative play: rate the peak intensity of hesitancy the child exhibits during the epoch; hesitancy is reflected by both wariness and physical cautiousness. Take into account the level of boldness vs. inhibition in the child's play, particularly the manner of their approach towards objects and the quality of their play with the objects

> 0 = no hesitancy; child readily engages in play with objects with <u>no</u> pauses to examine objects, <u>AND</u> expresses <u>no</u> wariness when in contact with objects -- child plays boldly
>
> 1 = slight hesitancy; child examines object or pauses briefly (i.e., 2-5 secs) <u>before</u> playing with it, but then does <u>not</u> express wariness while in contact with the object
>
> 2 = moderate hesitancy, as indicated by any of the following: child pauses 6 or more secs <u>before</u> playing with an object, or expresses wariness while in contact with the object, or clearly avoids an object
>
> 3 = extreme hesitancy; child does <u>not</u> explore or touch objects at all, but may look at or point to objects

References parent: the peak/max degree to which child references parent before engaging with a toy

> 0 = child does <u>NOT</u> comment to or glance toward the parent before engaging
>
> 1 = child looks to, or directs comment or question to parent <u>before</u> engaging with a toy
>
> 2 = child asks for permission or seeks reassurance from parent <u>before</u> engaging with a toy

Proximity to parent: <u>Closest</u> physical proximity of the child relative to the parent; this rating should reflect solely the child's physical distance from their mother, regardless of why the child is close to their mother.

> 0 = greater than one foot/arm's length from parent
>
> 1 = within one foot/arm's length from parent
>
> 2 = clinging to parent (clutching parent's body, sitting in parent's lap, burying head in parent's body).

Fearful/Wary Questions/Comments: any comments or questions that indicate fear (taking into account both tone of voice and content) , such as: "I don't like this", "That is scary".

> 0 = child did not make an utterance of this kind during the epoch
>
> 1 = child makes a low intensity fear or wariness verbalization
>
> 2 = child makes a moderate/high intensity fear or wariness verbalization

Amount of time talking: the overall amount/duration of verbalizations made by the child

> 0 = child does not speak
>
> 1 = child makes a brief utterance (e.g., "ooh"/"Ah", incomplete sentences)
>
> 2 = child makes an extended/complete utterance (e.g., child states a full sentence)

Time spent playing: degree to which the child engaged in purposeful manipulation, exploration, or symbolic interaction (e.g., talking to an object) with the objects
> 0 = child did not play with any toys during the epoch
> 1 = child played with toys for less than half of the epoch
> 2 = child played with toys for more than half of the epoch
> 3 = child played with the toys for the entire epoch

Sad affect: code the highest intensity sad affect that occurs during the epoch
> 0 = <u>NO</u> facial region shows codeable sadness movement
> 1 = droopy cheeks; slightly downturned mouth; slight raising of inner corners of
>   eyebrows; or, expression is fleeting
> 2 = definitely downturned mouth or definite raising of inner corners of eyebrows
> 3 = both definitely downturned mouth *and* definite raising of inner corners of eyebrows

Phase II (child & experimenter)

Start time: when experimenter returns
Stop time: after experimenter and child leave the room

> **A. Time to comply**: note the time, in seconds, at which the experimenter first asks the child to participate in an activity (time when request is completed), then note the time in seconds at which the child touches the object. If child fails to touch the object, record the time of next request.

> **B.** Phase 2 Scoring:
NOTE: do NOT code an epoch if less than 10 seconds in length.

Noncompliance: rate the peak intensity of noncompliant/oppositional behavior; include responses to the experimenter's requests to stand in a certain position, as well as to touch objects
> 0 = child complies readily with experimenter's requests, with <u>NO</u> signs of opposition
> 1 = child requires prompting (2 or more requests) from the experimenter to engage in the
>   requested activity, or exhibits <u>mild</u> opposition through facial, postural, or verbal
>   signs (i.e., ignores, shuffles feet, or says "no" in a neutral tone of voice); child
>   eventually complies
> 2 = child requires prompting (2 or more requests) to engage in the requested activity,
>   <u>AND</u> exhibits <u>moderate</u> opposition through facial, postural, or verbal signs (i.e.,
>   child grimaces strongly, crosses arms defiantly, or says "no" or some other
>   verbalization in an angry or whining tone of voice); child eventually complies
>   with the request, but compliance may not be complete.
> 3 = child requires prompting (more than 2 requests) to engage in the requested activity,
>   <u>AND</u> exhibits <u>strong</u> opposition through facial, postural, or verbal signs (i.e.,
>   child runs away, shakes head violently, refuses verbally to comply with task, or
>   may engage in other activities); child eventually complies with the request, but
>   compliance may not be complete
> 4 = child exhibits strong signs of opposition, AND <u>does NOT comply</u> with the request

References experimenter: the peak/max degree to which child references experimenter before complying with the request; should clearly reflect wariness/fear, rather than merely noncompliance

- 0 = child does <u>NOT</u> comment to or glance toward the experimenter in a timid manner before complying
- 1 = child questions the experimenter regarding the request before complying, or clearly looks again at the experimenter before complying (even though it is obvious they understand the request); child obviously seems timid about or is reluctant to engage in the requested behavior

Fearful/Wary Questions/Comments: note the peak intensity of any comments or questions that indicate fear (taking into account both tone of voice and content) , such as: "I don't like this", "That is scary".

- 0 = child did not make an utterance of this kind during the epoch
- 1 = child makes a low intensity fear or wariness verbalization
- 2 = child makes a moderate/high intensity fear or wariness verbalization

Fearful Affect: rate the peak intensity of fearful/wary facial expression that occurs during the epoch

- 0 = no facial region shows codeable fear movement
- 1 = fear expression is ambiguous or is of low intensity; fear is evident in only one facial region (i.e., brows raised in distress)
- 2 = fear expression is definitely present in at least 1 facial region (i.e., brows raised and drawn together, upper eyelids raised)
- 3 = fear expression is definitely present in both facial regions (i.e., brows raised and drawn together, upper eyelids raised to show whites of eyes, corners of mouth opened and drawn back)

Bodily Fear: rate the peak intensity of fearful bodily expression that occurs during the epoch.

- 0 = child's body never reflects fear or weariness
- 1 = child's body reflects low intensity fear or weariness (e.g., cautious or wary gait; slight tension; nervous twitching, hand tapping, foot swinging, etc.; diminished activity level)
- 2 = child's body reflects moderate intensity fear or weariness intensity or the display lasts a majority of the epoch (e.g. slight defensive body posture; fearful tension)
- 3 = child's body reflects high intensity fear or weariness (e.g., definite defensive body posture, jumping back in fear)

Tentative play: rate the peak intensity of hesitancy the child exhibits during the epoch; hesitancy is reflected by both wariness and physical cautiousness. Take into account the level of boldness vs. inhibition in the child's play, particularly the manner of their approach towards objects and the quality of their play with the objects

> 0 = no hesitancy; child readily engages in play with objects with <u>no</u> pauses to examine objects, <u>AND</u> expresses <u>no</u> wariness when in contact with objects -- child plays boldly
>
> 1 = slight hesitancy; child examines object or pauses briefly (i.e., 2-5 secs) <u>before</u> playing with it, but then does <u>not</u> express wariness while in contact with the object
>
> 2 = moderate hesitancy, as indicated by any of the following: child pauses 6 or more secs <u>before</u> playing with an object, expresses wariness while in contact with the object, or clearly avoids an object
>
> 3 = extreme hesitancy; child does <u>not</u> explore or touch objects at all, but may look at or point to objects

References parent: the peak/max degree to which child references parent before engaging with a toy

> 0 = child does <u>NOT</u> comment to or glance toward the parent before engaging
>
> 1 = child looks to, or directs comment or question to parent <u>before</u> engaging with a toy
>
> 2 = child asks for permission or seeks reassurance from parent <u>before</u> engaging with a toy

Proximity to parent: the <u>CLOSEST</u> physical proximity of the child relative to the parent; this rating should reflect solely the child's physical distance from their mother, regardless of why the child is close to their mother.

> 0 = greater than one foot/arm's length from parent
>
> 1 = within one foot/arm's length from parent
>
> 2 = clinging to parent (clutching parent's body, sitting in parent's lap, burying head in parent's body)

Sad affect: code the highest intensity sad affect that occurs during the epoch

> 0 = <u>NO</u> facial region shows codeable sadness movement
>
> 1 = droopy cheeks; slightly downturned mouth; slight raising of inner corners of eyebrows; or, expression is fleeting
>
> 2 = definitely downturned mouth or definite raising of inner corners of eyebrows
>
> 3 = both definitely downturned mouth *and* definite raising of inner corners of eyebrows

4. STRANGER APPROACH
Start time: begin coding when the experimenter and child enter the room
Stop time: end coding when the child leaves the room

    A. Time of first fear response: Note the time (including secs) from the time when the experimenter leaves the room to the first actual moment of a <u>definite</u> fear response (the first epoch is which a 1 or higher is coded for fearful affect or distress vocalizations, or a 2 or higher is coded for postural fear).

    B. Time of first vocalization: Note the time (including secs) from time when the stranger enters the room to the first vocalization, which can take any tone of affect or content.

Fearful Affect: rate the peak intensity of fearful/wary facial expression that occurs during the epoch
    0 = no facial region shows codeable fear movement
    1 = fear expression is ambiguous or is of low intensity; fear is evident in only one facial
        region (i.e., brows raised in distress)
    2 = fear expression is definitely present in at least 1 facial region (i.e., brows raised and
        drawn together, upper eyelids raised)
    3 = fear expression is definitely present in both facial regions (i.e., brows raised and
        drawn together, upper eyelids raised to show whites of eyes, corners of mouth
        opened and drawn back)

Postural Fear: rate the peak intensity of fearful bodily expression that occurs during the epoch.
    0 = child's body never reflects fear or weariness
    1 = child's body reflects low intensity fear or weariness (e.g., cautious or wary gait;
        slight tension; nervous twitching, hand tapping, foot swinging, etc.; diminished
        activity level)
    2 = child's body reflects moderate intensity fear or weariness intensity or the display
        lasts a majority of the epoch (e.g. slight defensive body posture; fearful tension)
    3 = child's body reflects high intensity fear or weariness (e.g., definite defensive body
        posture, jumping back in fear)

Still/Freezing: total duration of Still/Freezing (in seconds). Duration of freezing is defined as a marked decrease in activity (>2 secs) with little or no movement, with or without any indication of muscular tension.

Distress vocalizations: rate the peak intensity of distress vocalizations that occur during the epoch
    0 = <u>NO</u> distress vocalizations
    1 = mild distress vocalizations that are ambiguous in nature
    2 = distress vocalizations that indicate some fear or sadness, either through the content or
        intonation, (e.g., "Who are you?", "Where's my mommy?", or nervous laughter)
    3 = vocalizations that indicate clearly fearful or sad overtones, either through content or
        intonation (e.g., "don't come closer", "I want my mommy")

Approach: rate the peak intensity of approach behaviors (any behavior initiated by the child to decrease the distance between himself and the stranger). If the child continues to face toward the stranger in subsequent epochs, s/he should continue to be coded a 1. Similarly, if the child stays within 3 feet of the stranger during subsequent epochs, s/he should continue to be coded a 3.

> 0 = <u>NO</u> approach behaviors
> 1 = child's body faces toward the stranger, or child goes hesitantly toward the door after the knock
> 2 = child takes 1or 2 <u>hesitant</u> steps toward the stranger, or goes boldly toward the door after the knock
> 3 = child takes 1 or 2 <u>non-hesitant</u> steps toward the stranger, <u>or</u> initiates some action to get within close proximity to the stranger (i.e., walks right up to the stranger)
> NA > code for epochs when the stranger is absent

Avoidance: rate the peak intensity of avoidance behaviors (behaviors initiated by the child to maintain or increase the distance between himself and the stranger). If the child is coded a 1 for one epoch, then continues to be turned away during the following epochs, s/he should continue to be coded a 1. Code similarly for 3 codes - if the child continues to stay at the far corner of the room, continue to code the child a 3.

> 0 = child exhibits <u>NO</u> avoidance -- child stands in place or approaches the stranger
> 1 = low avoidance -- child's body faces away from the stranger
> 2 = moderate avoidance -- child takes 1 or 2 steps away from the stranger
> 3 = high avoidance -- child takes more than 2 steps away from the stranger, possibly going to the far corner of the room, or moving to the parent or experimenter (when present)
> NA > coded for epochs when the stranger is absent

Gaze aversion: rate the peak intensity of gaze aversion that occurs during the epoch

> 0 = <u>NO</u> gaze aversion
> 1 = child glances down or away from the stranger in a deliberate attempt to avoid eye contact (i.e.,only darting glances toward stranger)
> 2 = child makes <u>NO</u> eye contact with the stranger at all during the epoch
> NA > coded for epochs when the stranger is absent

Verbal/nonverbal interaction: the peak quality of the child's verbal responses to the stranger

> 0 = child does <u>NOT</u> respond to questions or initiate conversation with stranger
> 1 = child makes neutral or eager responses to questions, either verbally or nonverbally (i.e., nodding in response to a question), but does <u>NOT</u> initiate conversation with stranger
> 2 =child initiates conversation with stranger, or elaborates on a response
> NA > coded for epochs when the stranger is absent

Angry affect: rate the peak intensity of angry facial affect that occurs during the epoch

    0 = <u>NO</u> facial region show codeable facial anger movement

    1 = anger expression is ambiguous or of low intensity; expression is present only in 1 facial region (i.e., furrowed brows, narrowed eyes, or tense/squarish mouth)

    2 = anger expression is definitely present in 1 facial region (i.e., furrowed brows, or tense/squarish mouth)

    3 = anger expression is definitely present in both facial regions (i.e., furrowed brows, narrowed eyes, and angular/tense mouth)

Sad affect: code the highest intensity sad affect that occurs during the epoch

    0 = <u>NO</u> facial region shows codeable sadness movement

    1 = droopy cheeks; slightly downturned mouth; slight raising of inner corners of eyebrows; or, expression is fleeting

    2 = definitely downturned mouth or definite raising of inner corners of eyebrows

    3 = both definitely downturned mouth *and* definite raising of inner corners of eyebrows

8. Jumping Spider

This episode is divided into four trials. Each trial begins as the experimenter <u>begins</u> to say "go ahead and pet the spider" or otherwise asks or explicitly prompts child to pet the spider. If child does not take his/her hand out of the cage before experimenter makes spider jump a second, third or fourth time, trials begin when experimenter makes spider jump. "After effects" are noted when the experimenter begins to request that child touch the spider, and lasts until child begins to operate the spider alone.

Variables to be scored:

a. Latency to fear response
b. Intensity of fear expression
c. Intensity of vocal distress
d. Intensity of bodily fear
e. Approach
f. Withdrawal
g. Gaze Aversion
h. Startle
i. Plays with spider
j. Verbalizations

a. **Time of fear response**: **Time of first <u>definite</u> fear response:** note the time (including secs) of the first DEFINITE fear response (definite = either a 1 or higher is coded for fearful affect or distress vocalization OR a 2 or higher is coded for bodily fear). Code as "9999" if no fear response occurs.

b. **Intensity of fear expression**: Peak intensity of fear or fear blends is noted in each epoch using affect descriptions and rated on the following scale:

    0 = No facial region show codeable fear movement.
    1 = Only one facial region shows codeable movement, identifying a low intensity fear, or expression is ambiguous.
    2 = Only 2 facial regions show codeable movement, or expression in one region (e.g., brows) is definite.
    3 = An appearance change occurs in all 3 facial regions, or coder otherwise has impression of strong facial fear.

c. **Intensity of vocal distress***: Peak intensity of vocal distress is noted in each epoch and rated on the following scale:
    0 = No distress vocalizations.
    1 = Mild vocalizations that may be difficult to identify as hedonically fearful.
    2 = Vocalizations that indicate some fear. For example, nervous laughter or fearful interjections such as "oh".
    3 = Scream or loud, fearful interjection. For example, "no!" or "whoa!"

*note that some vocalizations in the episode will not be fear related.

d. **Intensity of bodily fear**: Peak intensity of bodily fear (changes in body position or body movement) is noted in each epoch and rated on the following scale:
0 = Very low bodily fear, no sign of bodily fear.
1 = Low bodily fear. Decreased activity; an apparent or sudden decrease in the activity level of child. For example, child sitting still for a few seconds after petting spider.
2 = Medium bodily fear. Bodily tensing: visible tensing of muscles such as drawing back of shoulders, tensing chords in neck.

e. **Approach**: Presence of approach behaviors is noted in each epoch and rated on the following scale:
0 = Touches spider with no hesitation.
1 = Hesitates for one or two seconds before touching spider.
2 = Hesitates for three to five seconds before touching spider.
3 = Does not touch spider.

f. **Withdrawal**: Peak intensity of withdrawal behaviors is noted in each epoch and rated on the following scale:
0 = Very low withdrawal, child sits in place or makes minute movements away from spider.
1 = Low withdrawal, child pulls back in chair slowly, or makes some movement away from spider.
2 = Medium withdrawal, child turns/twists away from spider and/or pulls back from spider.
3 = High withdrawal, child moves away from table and/or jumps away from spider.

g. **Gaze Aversion**: Peak intensity of gaze avoidance is noted in each epoch and rated on the following scale:
0 = No aversion
1 = Briefly averts gaze.
2 = Averts gaze for two to three seconds or focuses on object other than spider for two or three seconds.
3 = Averts gaze for nearly all of the time between experimenter's requests to pet spider, or focuses on object other than spider for most of time between experimenter's request.

h. **Startle**: Presence of startle response is noted during each epoch. (1 = present, 0 = not present)

i. **Play with spider**: It is noted whether or not child plays with spider when given the opportunity to do so at end of 4th trial. (e.g., moves the spider or touches it) (yes = 1; no = 0)

j. **Verbalizations**: It is noted whether or not child vocalizes during episode (check box if child verbalizes).

Risk Room Scoring Sheet

Risk Room Episode #1

ID ☐☐☐

Coder Initials: ☐☐

Date ☐☐ / ☐☐ / ☐☐☐☐
     M   D   Y

Child's Sex ○ Male   ○ Female

Start time: ☐☐ : ☐☐ : ☐☐
            H   M   S

End time: ☐☐ : ☐☐ : ☐☐

Time of first definite fear response ☐☐ : ☐☐ : ☐☐

Objects touched

Object touched	counter time (hour:minute:sec)		
Tunnel	☐☐ : ☐☐ : ☐☐		
Stairs	☐☐ : ☐☐ : ☐☐		
Mattress	☐☐ : ☐☐ : ☐☐		
Balance Beam	☐☐ : ☐☐ : ☐☐		
Scary Box	☐☐ : ☐☐ : ☐☐		
Mask	☐☐ : ☐☐ : ☐☐		
Total # touched:	☐		

Verbalizations   **time at first verbalization** ☐☐ : ☐☐ : ☐☐

Latency to Comply	Time Asked	Time Touched
Tunnel	☐☐ : ☐☐ : ☐☐	☐☐ : ☐☐ : ☐☐
Balance Beam	☐☐ : ☐☐ : ☐☐	☐☐ : ☐☐ : ☐☐
Stairs	☐☐ : ☐☐ : ☐☐	☐☐ : ☐☐ : ☐☐
Box	☐☐ : ☐☐ : ☐☐	☐☐ : ☐☐ : ☐☐
Mask	☐☐ : ☐☐ : ☐☐	☐☐ : ☐☐ : ☐☐

Stranger Approach Scoring Sheet

Coder Initials: ☐☐

Stranger Approach Episode #4

ID ☐☐☐

Date ☐☐ / ☐☐ / ☐☐☐☐
     M   D   Y

Child's Sex   O Male   O Female

Start time: ☐☐ : ☐☐ : ☐☐
            H   M   S

Time when E says that she will leave the room: ☐☐ : ☐☐ : ☐☐

Time when S enters room: ☐☐ : ☐☐ : ☐☐

End time: ☐☐ : ☐☐ : ☐☐

Time of first definite fear response ☐☐ : ☐☐ : ☐☐

Time of first vocalization ☐☐ : ☐☐ : ☐☐

Epochs are 20 secs in duration

Time (min/sec)														
Fearful affect														
Postural fear														
Still/Freezing														
Vocal Fear														
Approach														
Avoidance														
Gaze Aversion														
Verbal/nonverbal interaction														
Angry affect														
Sad affect														

Jumping Spider Scoring Sheet

BI: Jumping Spider

Subject # _____
Coder: _____
Date: _____

Start time: _____

Time of first fear response: T1_____ T2_____ T3 _____ T4 _____

Scoring Intervals

Trial Number	1	2	3	4	After Effects
Time (begin/end)					
Peak Intensity of fear expression (0 -3)					
Peak Intensity of vocal distress (0-3)					
Peak intensity of bodily fear (0-2)					
Approach (0-3)					
Peak intensity of withdrawal (0-3)					
Gaze Aversion (0-3)					
Startle 1 = yes; 0 = no					
Spider jumped 1 = yes; 0 = no					

Child plays with spider when given the opportunity: YES   NO

Verbalizations: note whether child verbalizes or not during episode: YES   NO