INVESTIGATING THE CONVERGENCE OF CARDIAC AND BEHAVIOURAL INDICATORS OF DISTRESS REGULATION IN TODDLERHOOD

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ABSTRACT

The ability to respond and regulate from distress is critical to everyday functioning for children and adults. Profiles of distress responding have been linked with individual differences in psychological and cognitive outcomes across development. However, no studies to date have examined the longitudinal patterns of both physiological and behavioural distress responding within a high distress context (i.e., pain) in toddlerhood. This dissertation consists of three studies examining the development of behavioural and physiological distress regulation in the second and third years of life as well as the convergence between these distress indicators over time. Study 1 is a published systematic review (Waxman, DiLorenzo, & Pillai Riddell, 2020) that synthesizes the direction and magnitude of the relation between behavioural and cardiac indicators of distress in toddlerhood (12 to 47 months of age). Study 1 revealed that the magnitude of the association between behavioural and *most* cardiac indicators of distress might be smaller than previously expected (i.e., Cohen's $d < 0.2$). However, methodological differences may also be responsible for study heterogeneity. Study 2 (Waxman et al., 2020) and Study 3 (Waxman et al., in press) were based on an ongoing longitudinal cohort of caregiver-infant dyads observed during vaccination appointments during the second year of life (12-month vaccination [N=158], 18-month vaccination [N=122]). Study 2 used path analyses to investigate the predictive and concurrent relations between toddlers' pain-related behavioural distress and cardiac (i.e., heart rate [HR], respiratory sinus arrhythmia) responses during 12- and 18-month vaccinations. Study 2 demonstrated that behavioural and cardiac indicators of pain-related distress during 12- and 18-month vaccinations reflect unique aspects of the nociceptive response.

Study 3 utilized parallel-process growth mixture modeling to describe patterns of distress responses to vaccinations as indexed by both pain-related behavioural distress and HR at 12 and 18 months. Study 3 revealed developmental differences and increasing variability in behavioural and cardiac distress responses across the second year of life. Clinical implications and suggestions for future research are discussed.

DEDICATION

This dissertation is dedicated to the children and families who participated in our research. Thank you for allowing us to be part of your lives and to observe your interactions for the last 5 years. Your openness and willingness to participate in our work has provided a richness of information and an understanding that we are eternally grateful for.

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PUBLICATION DISCLOSURE

The below work in this dissertation has been published under the following citations:

Waxman, J. A., DiLorenzo, M. G., & Pillai Riddell, R. P. (2020). Convergence of behavioral and cardiac indicators of distress in toddlerhood: A systematic review and narrative synthesis. *International Journal of Behavioral Development.* Advance online publication. doi: 10.1177/0165025420922618

Waxman, J. A., DiLorenzo, M. G., Pillai Riddell, R. P., Flora, D. B., Schmidt, L. A., Garfield, H., Flanders, D., Weinberg, E., & Savolv, D. (2020). An examination of the reciprocal and concurrent relations between behavioral and cardiac indicators of acute pain in toddlerhood. *PAIN, 161*(7), 1518-1531. doi: 10.1097/j.pain.0000000000001840

Waxman, J. A., DiLorenzo, M. G., Pillai Riddell, R. P., Flora, D. B., Schmidt, L. A., Garfield, H., Flanders, D., Weinberg, E., & Savolv, D. (in press). Investigating convergence of cardiac and behavioral indicators of distress during routine vaccinations over the second year of life. *Developmental Psychobiology.*

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DISSERTATION SYNOPSIS

A lack of clarity and consensus in conceptualizing the different components of distress has been a challenge in the pediatric pain and developmental psychophysiological literature (e.g., Cole et al., 2004; Pillai Riddell et al., 2016). Specifically, controversy exists regarding the degree to which expressed emotion behaviours, emotion regulatory behaviours, and physiological indicators coordinate across a distressing event (Cole et al., 2004). There appear to be no studies to date that have examined the longitudinal patterns of behavioural and physiological distress responding within a high distress context (i.e., pain) in toddlerhood. Response patterns developed in toddlerhood can be reliably linked to future developmental outcomes (e.g., Qu & Leerkes, 2018), highlighting the importance of studying distress regulation in toddlerhood. To address these issues, three broad research aims shaped the development of this dissertation: (1) Systematically review the extant literature on the direction and magnitude of the relation between behavioural and cardiac distress indicators in toddlerhood; (2) Informed by current gaps and methodological limitations in the literature, examine the concurrent and reciprocal relations between pain-related behavioural distress and cardiac indicators (heart rate [HR], respiratory sinus arrhythmia [RSA]) during 12- and 18-month vaccinations; and (3) Investigate the individual variability in patterns of regulatory trajectories (i.e., concurrent pain-related behavioural distress and HR) post-needle during the 12- and 18-month vaccinations. These research aims were addressed in three separate studies, all of which are published (Waxman, DiLorenzo, & Pillai Riddell, 2020; Waxman et al., 2020; Waxman et al., in press).

Appendix A provides a 2-page summary of the major statistical results across all three papers.

The first study was a systematic review that organized and synthesized the literature on the relation between behavioural and cardiac indicators measured during distress in toddlerhood. The only consistent finding within the narrative synthesis was that HR and expressed emotion behaviours were positively associated. The magnitude of the association between behavioural and most cardiac indicators of distress was generally small (i.e., Cohen's $d < 0.2$).

For the second and third studies, participants were part of an ongoing longitudinal cohort of caregiver-infant dyads observed during vaccination appointments during the second year of life. Data were obtained from the 12-month (N=158) and 18-month vaccination (N=122) appointments. Path modeling was used in Study 2 and parallel-process growth mixture modeling was used in Study 3.

Study 2 focused on examining the concurrent and reciprocal relations between toddlers' pain-related behavioural distress and cardiac responses during 12- and 18-month vaccinations. Results from Study 2 demonstrated that higher levels of pain-related behavioural distress immediately post-needle were related to higher HR and lower RSA 30-seconds post-needle. HR had larger and more consistent concurrent relations with behaviour than RSA. The results also highlighted the need to account for baseline behavioural and physiological distress when assessing pain in toddlerhood. In Study 3, profiles of toddler pain-related behavioural distress and HR during vaccinations at 12- and 18-months were discerned. Results produced two distinct profiles at 12 months and

three distinct profiles of at 18 months. All profiles had comparable behavioural distress and HR responses post-vaccination, with most participants displaying high arousal and regulation to baseline levels following the vaccination. However, at 18 months, an important minority had a blunted response or did not regulate to a low level of distress by 3 minutes post-needle. Post-hoc analyses revealed that higher baseline behavioural distress predicted membership in the majority groups at 12 and 18 months.

This three-study work makes an important contribution to the literature on distress regulation. The systematic review provided concrete suggestions for improving the quality and consistency of research on distress regulation in toddlerhood. Results from Study 2 and 3 suggest that HR and behaviour are complementary indicators of high distress in toddlerhood, which should be used *in conjunction* with expressed pain behaviours and other physiological and cortical indicators to properly encapsulate the nociceptive response. The importance of baseline levels of behavioural and physiological distress in predicting optimal regulation from distress highlights that these are essential factors in the assessment of distress, and the need to support parents to regulate their toddlers prior to distressing events to potentially prevent maladaptive distress regulation.

Chapter 1: Introduction

Distress Regulation

The concept of *distress regulation* has been discussed for decades (Kopp, 1989), and over the years there have been numerous definitions posed within the child and adult literatures. In a seminal review, Cole and colleagues (2004) challenged the field to adopt a more scientifically rigorous approach to studying distress regulation. This call to action has led to a more explicit and fully encapsulating definition of distress regulation. Indeed, a recent definition states that distress regulation can be defined as "those behaviours, skills and strategies, whether conscious or unconscious, automatic or effortful, that serve to modulate, inhibit and enhance emotional experiences and expressions." (Calkins & Perry, 2016, p. 395) The ability to regulate distress through implementation of these behaviours, skills, and strategies is critical to everyday functioning for children and adults and this ability has been demonstrated to emerge starting in early postnatal life (Calkins & Perry, 2016). There is a shift from passive (e.g., caregiver providing physical soothing when the infant is distressed) to active (e.g., child distracting themselves with a toy instead of throwing a tantrum) distress regulation that occurs through the caregiver using specific strategies and behaviours within dyadic interactions (Calkins & Perry, 2016). These behaviours become integrated into the child's repertoire of emotion regulation skills and can be observed at both the behavioural and biological level (Calkins & Dedmon, 2000; Calkins & Hill, 2007). Over a series of three papers, this dissertation will examine the literature and subsequently fills in some gaps in the literature regarding the development of both behavioural and cardiac indicators of distress during early childhood.

Profiles of distress responding have been linked with individual differences in a range of behaviours, such as risk taking, learning, self-regulation, and attachment (Del Giudice et al., 2011). Del Giudice and colleagues posit that the stress response system gathers information from the environment and translates it into broadband individual differences in behaviour and physiology. Specifically, certain response patterns (i.e., high physiological responding and low behavioural distress) have been linked to high inhibitory control, executive function, and delay of gratification, which are all traits that promote optimal learning and development (e.g., Beauchaine, 2001; Calkins, 1997; Stifter & Corey, 2001). Alternatively, other response patterns (i.e., low physiological and behavioural responding) have been linked to less optimal developmental outcomes, such as increased impulsivity, risk taking, and callous and unemotional traits (Enebrink et al., 2005; Frick et al., 2003; Shirtcliff et al., 2009). Despite the stability of these distress responses across development and associations with developmental and psychological outcomes, an integrated understanding of the development of behavioural and physiological distress regulation in early childhood is lacking.

The current dissertation focuses on attaining a better understanding of the development of distress responses and includes a systematic review and narrative synthesis on the relation between behavioural and cardiac indicators used to measure distress in infancy and toddlerhood (Chapter 2). In Chapter 2, relevant literature is presented that provides a rationale for focusing on toddlerhood and the development of behavioural and physiological (i.e., cardiac indicators) distress responding, as well as

associations between distress regulation patterns and developmental outcomes; challenges with assessment of distress regulation in toddlerhood are also discussed.

Pain as a Context to Study Distress Responding

In North America, healthy children are exposed to a significant number of painful medical procedures throughout childhood, such as vaccinations and blood draws (e.g., upwards of 20 vaccine needles over the first 5 years of life; https://www.cdc.gov/vaccines/hcp/vis/vis-statements/multi.html). Despite these procedures being part of routine and preventative healthcare, research from our group has found that the majority of infants do not regulate to low levels of behavioural pain-related distress following vaccination across the first year of life (Pillai Riddell et al., 2013). Additionally, approximately one quarter of pre-schoolers (i.e., ages 4 to 6) have difficulty regulating their pain-related distress following vaccination (Waxman et al., 2017). These challenges may be related to the fact that adequate parental pain management strategies are seldom used during routine vaccinations (Lisi, Campbell, Pillai Riddell, Garfield, & Greenberg, 2013). Complicating this picture further, because pain is a subjective experience, a young child's lack of declarative abilities make pain assessment much more complex (Anand 2007). It is well-established that infant responses to significant numbers of painful experiences in early infancy (such as experienced by hospitalized infants) are associated with long-term developmental outcomes (e.g., Schneider et al., 2018; Valeri et al., 2016). Long-lasting consequences include delays in motor and brain development as well as deficits in cognition and emotion regulation (Brummelte et a., 2012; Ranger et al., 2013; Valeri, Holsti, & Linhares, 2015; Vinall et al., 2013; Vinall et al., 2014). The negative impact of unmanaged pain on developmental outcomes behooves parents and

clinicians to adequately understand non-verbal pain responding in infants and young children.

Defining Pain

Pain has been defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (International Association for the Study of Pain, 2017). It is important to emphasize that pain has both a sensory and an emotional component, with pain encompassing the actual physical phenomena, the emotional state of the person, as well as the context or situation associated with the pain (McGrath & Finley, 2003). Skepticism towards infant pain characterized much of the 20th-century research and clinical practice, with infant surgery routinely conducted with no or minimal anesthesia well into the 1980s (Schecter, Allan, & Hanson, 1986). It is now well established that pain transmission pathways in the brain are fully developed by 22 to 24 weeks gestation (Byers & Thornley, 2004). Conversely, pain inhibitory systems are not fully developed in infants, suggesting that infants may feel even more pain than older children (Byers & Thornley, 2004). Consequently, infant pain has now been addressed in the International Association for the Study of Pain's definition of pain, as it is emphasized that, "the inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment" (International Association for the Study of Pain, 2017).

Pain is substantially more than mere sensory experience. Indeed, theoretical models have broadened their scope to include a multitude of factors implicated in pain

experience (i.e. biological substrates, internal cognitive/affective/biological schema, social and environmental factors) (Craig, Lilley, & Gilbert, 1996). For example, both internal factors (e.g., temperament, previous pain experiences) and external factors (e.g., social context, responsivity of others) impact the sensory and affective components of pain (Pillai Riddell & Chambers, 2007). Relatedly, Williams and Craig (2016) proposed defining pain as a "distressing experience associated with actual or potential tissue damage with sensory, emotional, cognitive and social components." This new definition revealed challenges with the current definition of pain, including the exclusion of cognitive and social components of pain and the priority of subjectivity and self-report at the expense of nonverbal behaviors in pain assessment. The latter challenge is particularly troubling, given that nonverbal behaviours are prominent sources of information about pain in those whose subjective experience of pain cannot be communicated (Williams & Craig, 2016). Therefore, it is important to use a biopsychosocial framework when understanding the development of pain. In the following section, the original and revised versions of the Development of Infant Actions in Pain Responding (DIAPR; Pillai Riddell et al., 2013; Goubert Pillai Riddell, Simons, & Borsook, in press) model are discussed as a biopsychosocial theory of pain.

The Development of Infant Actions in Pain Responding (DIAPR model)

The DIAPR model (Pillai Riddell et al., 2013) built upon the sociocommunication model of pain (Craig & Pillai Riddell, 2003), which posited that pain is influenced by the dyadic interactions between the child and caregiver as well as the broader social ecological systems within which the child's pain is suffered. Limitations of the sociocommunication model are that it did not consider the unique context of early

childhood, when the child is wholly dependent on their caregiver for regulation from pain, and it also did not address the different phases of pain. Rothbart and Derryberry (1981) provided an important distinction between distress reactivity and regulation in relation to infant temperament, which had not been considered in the sociocommunication model of pain. Specifically, Rothbart and Derryberry (1981) posited that *reactivity* reflects the individual's behavioural and biological initial reaction to distress, whereas the behaviours and biological processes used to modulate distress (e.g., attention, behavioural approach/avoidance, self-soothing) reflect *regulation.* While reactivity responses tend to be highly dependent on antecedent circumstances (e.g., genetics, sensory thresholds, caregiver factors), the development of distress regulation responses seems to emerge during the second year of life and coincides with the development of certain cognitive capacities (Kopp, 1988; Posner & Rothbart, 1980).

The DIAPR model (Pillai Riddell et al., 2013) addressed these limitations by focusing on the development of behavioural pain responses following acute pain over the first years of life. This DIAPR model emphasized the need to understand the different influences at play when trying to predict an infant's immediate behavioural pain *reactivity* compared to the infant's behavioural pain *regulation* (Din Osmun et al., 2014; Lisi et al. 2013). Specifically, the model focuses on the primary role of the dynamics between the caregiver and child and includes separate contextual and individual factors affecting pain reactivity and regulation, an indirect influence of larger social contexts, and three transactional systems involving the infant, caregiver, or the infant-caregiver dyad (Pillai Riddell, 2013).

Building on this foundation, the revised-DIAPR model (DIAPR-R; Pillai Riddell, as cited in Goubert et al., in press) begins with an acutely painful incident, which triggers biological processes (i.e., changes in the peripheral and central nervous system) related to one's pain threshold. These biological processes, referred to as *nociception,* are then transduced into an initial pain reaction (i.e., the initial milliseconds of the infant's pain-related responses that do not have to be centrally mediated; Verriostis et al., 2016). This phase involves initial somatic behaviours and autonomic nervous system (ANS) physiology. After the initial pain reaction, the infant is able to *perceive* the painful stimulus and mount behaviours that are more centrally controlled. These behaviours interact with the infant's ongoing ANS reactivity and pain-related behaviours. Once the peak pain-related reactivity has passed, the infant's observable somatic behaviours and ANS physiology reflect the infant's return to baseline. These somatic behaviours and ANS physiology are considered measurable indicators of *pain-related regulation*. The DIAPR-R model posits that the infant begins the process of pain-related regulation to return to homeostasis. The acute pain sequence, from reactivity to regulation, is embedded within a larger caregiver context (e.g., the parent's pain schemas and stress physiology).

Taken together, the process of transducing noxious stimulation into the infant's pain experience is dynamic. Indeed, an infant's pain experience depends on individual physiology, personal experience (e.g., previous pain experiences), and social context (e.g., caregiver behaviour and physiology, culture, family norms). As well, the experience of pain is hypothesized to change dramatically across the first years of life, given the child's steep trajectory of cortical, biochemical, physiological, behavioural, and social

development (Fitzgerald, 2015; Pillai Riddell et al., 2013). The current dissertation focuses on the development of behavioural and cardiac indicators of early childhood pain reactivity and regulation within the vaccination context.

Understanding Behavioural and Cardiac Indicators

Behavioural Indicators

Common behavioural indicators of infant pain include observations of facial expressions (e.g., brow bulge, eye squeeze, nasolabial furrow, and open mouth), cry features (e.g., frequency and intensity), and motor activity (e.g., arm and leg activity, posture, muscle tone) (Craig, Whitfield, Grunau, Linton & Hadjistavropoulos, 1993; Grunau & Craig, 1990; Stevens et al., 2007). An additional indicator utilized by health professionals is observation of consolability. Although this indicator is considered subjective and vague, given that there is no standard method to console a crying or distressed infant, it continues to be used as a measure of discomfort (Hummel & van Dijk, 2006).

Challenges with behavioural pain assessment include the specificity of behavioural pain measures (Ahola Kohut & Pillai Riddell, 2009). Given that cognitive capacities develop across infancy and early childhood, it is difficult to discern and express pain differently than other negative affect states (e.g., agitation, hunger) (Ahola Kohut, Pillai Riddell, Flora, & Oster, 2012; Belleini 2012; Hadjistavropoulos, Craig et al., 1997; Oberlander & Saul, 2002). Additional challenges with behavioural indicators of pain are that physician, nurse, and parent judgments have been identified to have biases stemming from intrinsic factors in the raters (e.g., age, sex, culture, profession) (Pillai

Riddell & Craig, 2007). Finally, factors such as behavioural state (i.e., awake, sleeping) are known to impact an infant's behavioural response to pain. As such, behavioural pain scales that do not include modifying factors such as behavioural state may adequately capture the infant's pain response. Despite these challenges, behavioural indicators are still recognized as the most valid indicators of pain for infants (Välitalo et al., 2016). However, physiological indicators are often utilized to obtain a more objective measure of an infant's pain experience.

Physiological Indicators

The most frequently utilized physiological indicators of infant pain include heart rate, blood pressure, oxygen saturation, and breathing patterns (Sweet & McGrath, 1998). These physiological responses provide important information about the infant's capacity to react and regulate in the face of a painful event (Stevens et al., 2007). One of the most commonly utilized autonomic biomarkers is heart rate, given the ease of data collection and the additional indicators of ANS function that can be derived from its measurement (Stevens et al., 2007). In addition to heart rate being considered the "window" into the central nervous system, it is also closely linked to systems that modulate pain reactivity (Randich & Maixner, 1984). As such, ANS indicators of pain, such as heart rate, are pervasive in the hospital setting (Grunau, Holsti, & Peters, 2006). However, there are a number of challenges related to infant pain assessment when utilizing physiological indicators.

Similar to challenges with behavioural pain indicators, physiological indicators are regarded as a proxy measures of reactivity or response following a painful event, and

are not considered direct measures of infant pain (Stevens et al., 2007). Indeed, physiological indicators always lack specificity to pain because physiological responses do not only change as a direct response to noxious stimulation (Pillai Riddell et al., 2016). Additionally, physiological indicators of pain are also impacted by contextual factors (e.g., gestational and postnatal age, medication use, health status, number of painful procedures, time since last painful procedure, sleep/wake state) (Ranger, Johnston, & Anand, 2007). Moreover, the Law of Initial Values (Bernston, Uchino, & Cacioppo, 1994) states that the size of the psychophysiological response depends on the initial baseline level of measure. However, many studies and assessment tools do not consider these contextual factors or baseline level of physiological variables when assessing infant pain using physiological indicators.

Methodological challenges related to ANS indicators of pain have also been delineated in a systematic review completed by our group (Waxman et al., 2016). Specifically, the literature suggested that in the first year of life, there is natural variability in heart rate responding following acutely painful procedures in both later preterm and full-term infants. Thus, it is possible that more naturally physiologically reactive infants, and not infants with higher subjective pain, receive higher scores on physiological pain assessment tools. Adding to this challenge, infant ANS responses to noxious stimuli have not been properly examined longitudinally to provide valid norms for comparison. Further methodological challenges have been noted and relate to variation in heart rate indicators depending on the length of measurement epoch (Stevens et al., 2007), with cardiac indicators naturally increasing with longer recording times (Saul et al., 1988). Relatedly, variation in epoch length also impacts indicators and

studies have not been consistent in their epoch length (e.g., 30 seconds to 5-minute recordings; Stevens et al., 2007).

Taken together, although ANS indicators are commonly used to assess pain in infancy, there are a number of challenges to interpreting these indicators. Specifically, future research is needed to understand how typically developing infants' ANS physiology responds to noxious stimulation across development and standardized measurement epochs are needed to compare results across ages and painful procedures. The complex nature of pain in infancy and the challenges with unidimensional behavioural and physiological indicators suggests that composite measures of pain (e.g., behavioural and cardiac indicators) may be warranted (Stevens et al., 2007). In the following section, common challenges with multidimensional indicators are discussed.

The Relationship between Behavioural and Physiological Indicators of Pain

Studies have confirmed that early childhood pain assessment tools that use both behaviour and physiology are more reliable than pain assessments based on only one (Belleini, 2012). However, there is clear consensus among pediatric pain researchers and clinicians that physiological and behavioural measures of pain do not always converge following noxious stimulation (Pillai Riddell et al., 2016). Indeed, past research on the relation between behavioural and ANS measures during acute pain has been limited to early infancy (i.e., first four months of life; Waxman et al., 2016), with results revealing small to moderate positive correlations that were generally less than $r = 0.3$ (Grunau et al., 2010; Johnston et al., 1995; Lewis & Ramsay, 1995; Owens & Todt, 1984; Ranger et al., 2007; Ranger et al., 2015; Stevens, Johnston, Petryshen, & Taddio, 2010), or negative

correlations post-acute pain (Craig, Whitfield, Grunau, Linton, & Hadjistavropoulos, 1993; Gibbins et al., 2008; Johnston & Strada, 1986). This research suggests that behavioural and physiological measures of pain represent important, unique information about the complex nociceptive response and that more research should be done to understand their interrelationships.

However, large intra-individual variability in the relation between physiology and behaviour suggests that each individual has their own unique way of manifesting pain (Anand & Craig, 1996). As well, while behavioural measures such as facial actions are more likely to respond selectively to pain (Craig et al., 1993), physiological indicators are found to change in response to painful stimuli but also for numerous other reasons that are not specific to pain (Johnston et al., 1999; Sweet & McGrath, 1998). Based on these individual- and indicator-based differences, some researchers and clinicians have suggested that assessment tools should distinguish between behavioural and physiological indicators. Certainly, it is unclear how strongly these indicators are correlated across the timeline of noxious events (Hummel & van Dijk, 2006). Additionally, age-based differences are expected as cognitive and physical development are characterized by more differentiated behavioural and physiological responses with increasing age (Anand & Craig, 1996; Davis, 1990; McGrath & Unruh, 1994). Future research is needed that accounts for individual variability within multidimensional infant pain-responding patterns (i.e., behavioural and cardiac responses) and relevant covariates that are known to affect these measures (Waxman et al., 2016). Development of a clinically useful pain assessment tool depends on our understanding of how nociceptive stimulation alters activity across all levels of the peripheral and central nervous system

and how this activity is linked to overt behaviours seen in the clinical setting (Pillai Riddell et al., 2016). However, given the lack of information that is currently available on the normative behavioural and cardiac responses to acute pain in infancy and toddlerhood, it is first important to provide normative data regarding how infants and toddlers respond to acute procedural pain. Given the frequency of vaccinations over the first years of life, this pain context can provide valuable insight for understanding how behaviour and physiology develop with age.

Current Dissertation

Although behavioural and cardiac indicators of distress in infancy and toddlerhood are commonly utilized in psychophysiological and pediatric pain literature, and are posited to encapsulate the same underlying process of distress regulation, a comprehensive and systematic review of the direction and magnitude of the relation between these indicators in response to differing levels of distress has yet to be conducted. Additionally, there is currently little research on the longitudinal patterns of convergence and divergence between cardiac and behavioural responding within a high-distress context in toddlerhood. Moreover, research has not implemented sophisticated analyses to take into account contextual factors (i.e., baseline responses, time since last feeding or nap), phase of distress (i.e., reactivity vs. regulation from distress), and the dynamic and potentially reciprocal nature of distress.

This dissertation is the compilation of three studies: The first is a systematic review and narrative synthesis on the convergence between behavioural and cardiac indicators of distress in toddlerhood (Chapter 2; Waxman, DiLorenzo, & Pillai Riddell, 2020); the second study utilizes variable-centered analyses of data collected during

routine vaccinations at 12 and 18 months to better understand the predictive, reciprocal, and concurrent relations among behavioural and cardiac indicators (i.e., heart rate [HR], respiratory sinus arrhythmia [RSA]) (Chapter 4; Waxman et al., 2020); and the third study utilizes person-centered analyses to describe the variability in distress regulation post-needle at 12 and 18 months, as indexed by both behavioural and cardiac responses (Chapter 6; Waxman et al., in press). There are bridging chapters (Chapters 3 and 5) between each of the chapters dedicated to these studies. To facilitate an overall understanding of the dissertation, all the research questions, analyses, and results across the three studies are summarized in a two-page outline created for dissertation readers (see Appendix A).

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Chapter 2: Convergence of behavioral and cardiac indicators of distress in toddlerhood: A systematic review and narrative synthesis [1]

Measurement of responses during distress involves examining one's behavioral and biological initial reaction to changes in the environment (i.e., reactivity), or the behaviors and biologic processes (e.g., attention, behavioral approach/avoidance, self-soothing) used to recover from emotional challenges and return to homeostasis (i.e., regulation) (Kopp, 1982; Rothbart & Derrberry, 1981). While distress reactivity tends to be highly dependent on antecedent circumstances (e.g., genetics, sensory thresholds, caregiver factors), the process of distress regulation is more complex and dynamic, as it involves the conscious and unconscious sequencing of emotion, behavioral regulatory strategies, and biology (Calkins & Leerkes, 2004; Ekas, Braungart-Rieker, & Messinger, 2018). These are considered distinct yet interrelated facets of distress (Barrett, 2013; Cole, Marin, & Dennis, 2004; Thompson, Lewis, & Calkins, 2008).

It is believed that reactivity and regulatory responses go through an especially malleable period early in life (Campos, Campos, & Barrett, 1989; Cole et al., 2004), with behavioral, physiological, attentional, emotional, cognitive and interpersonal or social processes having unique yet interrelated developmental trajectories that emerge across infancy, toddlerhood, and early childhood (Calkins & Fox, 2002). Calkins (2010) suggested that research investigating distress reactivity and regulation utilize a biopsychosocial theoretical framework, which considers how the child's biology,

[1] This is the author's version of the published manuscript:
Waxman, J. A., DiLorenzo, M. G., & Pillai Riddell, R. P. (2020). Convergence of behavioral and cardiac indicators of distress in toddlerhood: A systematic review and narrative synthesis. *International Journal of Behavioral Development.* Advance online publication. doi: 10.1177/0165025420922618

behavior, and social context fundamentally change one another continuously over the course of time. Indeed, systematic research using a behavioral indicator of distress suggest that there is significant variability in how infants regulate from distress across the first year of life (Pillai Riddell et al., 2013) As such, it is important for research on the development of distress regulation to not collapse across developmental stages (i.e., infancy, toddlerhood, early childhood).

The focus of the current systematic review will be on distress reactivity and regulation in toddlerhood (i.e., 2nd and 3rd years of life). Toddlerhood is a critical developmental phase to examine distress reactivity and regulation, as it coincides with developments in motor, language, and representation skills, and encompasses the transition from passive caregiver-directed regulation to more active and purposeful self-regulation (Kopp, 1982; Rothbart et al., 1992). Infants initially depend on their primary caregiver to understand and scaffold adaptive regulation from distressing events (Pillai Riddell & Racine, 2009; Ekas 2018). However, by the end of the first year of post-natal life, the child is able to deploy strategies aimed at modifying several aspects of the emotion, including the intensity and duration of the emotions, and also impact caregivers' behaviors when they need help regulating their emotions (Ekas et al., 2018). The purpose of the current review is to provide a more nuanced understanding of the patterning of cardiac and behavioral responses in toddlerhood during negatively-valenced events. Foundational to a review of this kind requires an understanding of the development of behavioral and biological reactivity and regulatory responses to distress and its associations with developmental outcomes.

Development of behavioral and biological regulatory responses

Behavioral regulation skills are developing from birth, with a shift from utilizing primitive mechanisms of self-soothing and signaling (e.g., sucking, moving away, crying) to more voluntary, simple actions to modify arousal levels (e.g., self-initiated distraction) (Kopp, 1982; Rothbart et al., 1992). Maturation of different biological support systems is implicated in the increasingly sophisticated emotions and behavioral regulation strategies observed across infancy and early childhood, as physiological changes are hypothesized to prepare the individual for actions associated with emotional events. (Calkins & Hill, 2007). The majority of empirical work that focuses on underlying physiological components highlights the maturation of the autonomic nervous system as playing a fundamental role in emotion reactivity and regulation (Santucci et al., 2008). Both the parasympathetic (PNS) and sympathetic nervous system (SNS) have been implicated in the development of distress responses (both reactivity and regulatory). Indeed, the Polyvagal Theory (Porges, 1995) specified these two branches as serving different evolutionary stress responses in mammals. The vegetative vagus (i.e., SNS activity) represents the more primitive branch, which mediates the coping strategies of reptiles. Higher levels of SNS activity tend to be associated with physiological arousal, metabolic output and fight or flight responding during distress (Porges, 2007). In contrast, the smart vagus (i.e., PNS activity) is more evolved and mediates cardiac activity when demands require more sophisticated coping. Specifically, decreasing levels of PNS activity tend to be associated with orienting, self-soothing behaviors, responding to challenges and increased metabolic output (Porges, 2007).

Associations between behavioral and cardiac reactivity/regulation and developmental outcomes

The limited research available suggests that there is moderate stability of behavioral and cardiac reactivity and regulatory responses across the lifespan (Gunnar & Vasquez, 2006; Matthews & Phillips, 2010; Seifer, Schiller, Sameroff, Resnick, & Riordan, 1996; Stifter & Fox, 1990), and that response patterns developed in toddlerhood can be reliably linked to future developmental outcomes (Beauchaine, 2001; Gedron & Feldman, 2009; Qu & Leerkes, 2018). Indeed, certain reactivity and regulation patterns after a distressing event (e.g., partial vagal withdrawal leading to SNS activation, more effective behavioral strategies employed) early in life have been linked to traits that promote optimal learning and development, such as high inhibitory control, executive function, and delay of gratification (e.g., Obradović & Finch, 2017; Santucci et al., 2008). Alternatively, certain reactivity and regulation patterns after a distressing event (e.g., excessive vagal withdrawal, ineffective regulatory strategies employed) are associated with negative emotional traits (e.g., depression, anxiety and aggression) and states (e.g., panic and anger; Beauchaine, 2001; Enebrink et al., 2005; Frick et al., 2003; Shirtcliff et al., 2009). As such, an understanding of how these distress-related behavioral and cardiac responses are associated has important implications for child development.

Challenges with assessment of distress regulation in toddlerhood

Developmental scientists have utilized a variety of paradigms to observe the intensity and temporal features (e.g., speed of onset or recovery from distress) of negative emotions. Although there is agreement on the multifaceted nature of emotions,

considerable controversy exists regarding the degree to which different aspects of emotion (i.e., emotion expression, emotion regulatory behaviors, biology) coordinate across a distressing event (Cole et al., 2004). This debate is complicated further in infancy and toddlerhood, as unlike behavioral responses to distress, individuals mount a variety of physiological responses that are not directly observable. Indeed, responses during distress responding is a dynamic process that involves the coordination of cognitive, cortical, cardiac, and endocrine systems, in addition to emotion and behavioral strategies (Ekas et al., 2018). Understanding how these different systems coordinate is a critical area of study. However, in order to better understand how these systems coordinate, challenges with behavioral observation systems and cardiac indicators and methodologies need to be addressed.

Phases of distress responding. As mentioned previously, seminal research by Rothbart and Derryberry (1981) provided an important distinction between distress reactivity and regulation in relation to infant temperament, which could fruitfully be applied to behavioral and cardiac indicators of distress. While reactivity tends to be highly dependent on innate, biological factors, the development of distress regulation seems to emerge during the second year of life and coincides with the development of certain cognitive capacities (Rothbart & Derryberry, 1981). Thus, behavioral and physiological responses underlying distress reactivity and regulation may not be identically associated across time. Despite the importance of considering phases of distress responding when attempting to capture toddlers' distress, it is unclear how the available literature has examined phases of distress responding. As such, the length (in seconds) and timing (concurrent [behavior and cardiac data acquired simultaneously

within the same distress paradigm] or predictive [behavior and cardiac data acquired at different times within the same appointment]) of measurement epochs[2] will be important considerations in the review.

Behavioral distress responding. Various methodologies have been used to measure behavioral responses to distress across development. Researchers tend to observe the latency, intensity, frequency, and duration of emotional behaviors, the attentional and behavioral strategies used to manage such reactions, or a combination of these emotional reactions and regulatory strategies. Complicating the picture further, researchers have measured behavioral outcomes differently, using either continuous composite scores or extremes of a dimension. Cole and colleagues (2004) have described this debate regarding how to best distinguish emotion expression behaviors from emotion regulation behaviors themselves, and suggest that independent measurement of the activated emotion (e.g., observed facial expressions and behaviors) and the resultant regulatory strategy (e.g., soothing, visual engagement) is necessary. It is posited that global measures that encapsulate both expressed emotions and emotion regulatory strategies fail to capture the dynamic and temporal nature of expressed emotion and emotion regulation. In order to continue pushing developmental literature forward, it is important for behavioral measures to be separated based on whether they measured expressed emotion behaviors (i.e., activated emotion) or emotion regulation behaviors (i.e. behaviors that serve to bring the organism back to homeostasis). This distinction will be addressed in the following review.

[2] "Measurement epoch" refers to the time interval (in seconds) that behavioral and cardiac data were analyzed/coded across a distress task. The measurement epoch is the final time interval utilized in order to measure the association between behavioral and cardiac indicators during distress. For example, for a given study, heart rate variability may have been edited in 30 second epochs and averaged across a two-minute distress paradigm, but the final "measurement epoch" length for that study would be 120 seconds.

Cardiac distress responding. The development of behavioral distress responses has been reliably linked to continuous changes across biological systems, with the majority of empirical work highlighting the integral role of the autonomic nervous system in the regulation of emotions (Santucci et al., 2008). Specifically, brain regions associated with emotions and cognitions can influence brainstem cardio-respiratory control centers and thus affect cardiac indicators (Berntson, Cacioppo, & Quigley, 1994). Perry and Calkins (2018) posited that physiological indicators help identify underlying mechanisms that are imperative in understanding expressed emotion responses and emotion regulation responses that cannot be communicated through behavioral observation alone. As mentioned above, both branches (i.e., PNS/SNS) have been implicated in the development of distress responses; however, the convergence (i.e., strength) and direction of the relationship between behavioral and cardiac indicators during distress has not been reliably established.

Common cardiac indicators used in the literature include heart rate (HR), heart period (HP), and heart rate variability (HRV). HR measures the number of contractions of the heart per minute and reflects both sympathetic and parasympathetic activity. Specifically, increases in sympathetic activity lead to increased HR and the focusing of attention, which is essential for generating emotional responses that are effective in modulating arousal (Bornstein & Seuss, 2000). HP refers to the interval between heartbeats (in milliseconds) and is inversely related to HR (i.e., low HP is equivalent to high HR) (Burgess et al., 2003). Regarding HRV, one of the most common indexes is respiratory sinus arrhythmia (RSA). RSA is thought to reflect the variability in the time between heartbeats that occurs at the frequency of respiration, with higher values of RSA

reflecting greater levels of parasympathetic influence (Berntson, Quigley, & Lozano, 2007). During times of distress, vagal influence is withdrawn which results in increased sympathetic activation (i.e., fight or flight response) (Gatzke-Kopp & Ram, 2018). Seminal research has found that decreasing levels of PNS activity during distress, as indexed by decreasing RSA, tend to be associated with orienting, responding to challenge, and increased metabolic output (Porges, 2007). Two other related HRV indicators that are used in conjunction with behavior are RSA change score (decreases in RSA from baseline to distress) and baseline RSA. RSA change score is posited to be related to physiological regulation of emotions or coping efforts, and reflective of vagal withdrawal. On the other hand, baseline or tonic RSA is thought to reflect an individual's temperamental reactivity and emotionality (Beuchaine, 2001; Porges, 1995). Pre-ejection period (PEP) is validated as an index of sympathetic nervous system functioning (Sherwood et al., 1990), and reflects the time interval (in milliseconds) between the onset of ventricular depolarization and the onset of left ventricular ejection (i.e., ejection of blood into the aorta) (Bernston et al., 2007). Shorter PEP suggests greater sympathetic influence, which is functionally related to an individual's mobilization of resources to meet environmental demands associated with stress (Beauchaine, 2001). Sherwood and colleagues' (1990) guideline for impedance cardiography spurred much research utilizing PEP in children, adolescents and adults (e.g., Beauchaine, 2001; Beauchaine, Gatzke-Kopp & Mead, 2007; Crowell, Beauchaine, Gatzke-Kopp, Sylvers, & Mead, 2004; Brenner, Beauchaine, & Sylvers, 2005). However, scientists have less frequently investigated the increase in sympathetic influence during distress in toddlerhood (e.g., Buss & Goldsmith, & Davidson, 2005; Kahle et al., 2018). Thus, it is unclear in early

childhood how sympathetic (versus parasympathetic) measures compare to traditional quantifications of reactivity and regulation responses.

Current review

Developmental research on toddler distress-related reactivity and regulatory responses has largely focused on direct behavioral observations and parent reports of distress rather than multidimensional approaches incorporating both behavioral and physiological indicators. A review by Barrett (2015) highlighted that there may be inter- and intra-individual variability in behavioral and physiological responses to emotions within and across time. Thus, there may not be a single, consistent pattern of relations in the PNS, facial movements, or other behaviors for each emotion category. Without proper systematic reviews that examine how multiple dimensions of distress-related responses react together (such as behavior and cardiac) that takes into account the types of behavior (expressed emotion versus emotion regulation behaviors) and cardiac responses (e.g. HR, HP, RSA, PEP) across developmental stages, it is difficult to assess the current state of the field.

The main goal of the present study was to systematically review the findings on the concurrent (i.e., within-session) relation between behavioral and cardiac indicators measured during distress in toddlerhood. Based on the methodological and conceptual issues outlined above, our original aim was to conduct a meta-analysis, in order to quantitatively estimate the overall relation between behavioral and cardiac indexes of distress in toddlerhood and investigate how the effect varied according to certain study characteristics (e.g., timing of measurement for behavioral and cardiac indicators, length

of measurement epochs, inclusion of covariates and moderators). However, based on the guidelines outlined by Higgins & Green (2011), it was concluded that a meta-analysis would not be appropriate. Specifically, many studies utilized multiple, divergent cardiac indicators (i.e., baseline, task and/or change scores for HR, HP, RSA, and PEP) and behavioral coding schemes. Given that the studies are clinically diverse, a meta-analysis may have obscured genuine differences in effects (Higgins & Green, 2011). Therefore, we undertook a narrative synthesis, which is an approach to the systematic review and synthesis of findings from multiple studies that relies primarily on the use of words and text to summarize and explain the findings of the synthesis (Popay et al., 2006). A narrative synthesis was deemed a more appropriate analytic approach, as it involves the interpretation of statistical data, with the ability to couch the synthesis based on differences in methodology, methodological rigor and overall study quality (Popay et al., 2006).

The aim of our narrative synthesis was to examine the direction and magnitude of the relation between behavioral and cardiac indicators measured during distress in toddlerhood, taking into account the methodological limitations based on study characteristics that may influence the relation between indicators. In accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009), the quality of the research articles included in the synthesis was assessed. As well, the methodological rigor of physiological data collection was examined, as this has been shown to be an important factor in recent meta-analyses and empirical work involving cardiac indicators (e.g., Beauchaine et al., 2019; Shader et al., 2017). Previous meta-analyses in infants, children, and adults revealed a significant

association (r = 0.09 to 0.15) between cardiac indicators and self-regulation (Holzman & Bridgett, 2017; Zahn et al., 2016) that was impacted by publication bias. As such, we hypothesized a similar degree of association between behavioral and cardiac indexes measured during distress in toddlerhood. However, we expected the direction of association to vary according to cardiac indicator (i.e., positive associations with SNS indicators [i.e., HR, HP, PEP], negative associations with PNS indicators [i.e., RSA]).

There are a number of variables that are hypothesized to impact the relation between behavioral and cardiac indicators of distress in toddlerhood. A plethora of research exists that highlights the impact of sex, age, and attachment on the development of emotion regulation (see Perry & Calkins, 2018 for a review of the development of emotion regulation processes in childhood). Studies examining sex differences in emotional responding have reported mixed results. These differences in results are posited to be due to differences in methodology (e.g., level of emotional responding under study [e.g., behavior, psychophysiology], stimuli utilized [positive vs. negative emotion]) (Domes et al., 2009). With regards to age, across early development there is a dramatic growth in the acquisition and display of emotion regulation skills and abilities (Calkins, 2010). These changes in emotion regulation skills across time have been linked to differences in caregiver-infant attachment, as caregivers assist in the regulation of arousal in infancy and early childhood (Perry & Calkins, 2018). Infants with secure attachment relationships with their primary caregivers will seek and receive caregiver support during times of distress. However, infants with insecure attachment will develop ineffective strategies to heighten their call for support or suppress their emotions (Zimmer-Gembeck et al., 2017). Additionally, baseline responses (i.e., cardiac and

behavior prior to task) are known to impact the size of a psychophysiological response to distress (Bernston, Uchino, & Cacioppo, 1994; Oberlander & Saul, 2002; Waxman et al., 2016). No a priori hypotheses were made regarding the impact of behavioral coding scheme, study characteristics, or methodological considerations on the association between indexes of distress in toddlerhood, given the dearth of research in this area.

Method

Search strategy

A systematic search was conducted using Medline, Embase, PsycINFO, and CINAHL in July 2017 for English-language references. An updated search was conducted in Embase in March 2019 to include any relevant articles published between July 2017 and March 2019. The original search was not limited by year published to encompass both historical and contemporary articles and reviews. Search terms related to distress-inducing contexts, behavioral and cardiac measures, and infancy or toddlerhood were systematically paired (Appendix B). Hand-searching reference lists of relevant studies and systematic reviews on the convergence between behavioral and cardiac measures of distress were also conducted. Our review followed an *a priori* protocol according to the PRISMA guidelines (Moher et al., 2009). The review protocol was registered on the PROSPERO website (Booth, 2013) before data extraction (registration no. CRD42017077288).

Inclusion/exclusion criteria and study selection

English-language, prospective-observational or descriptive studies on humans who were 12 months to less than four years of age, undergoing an acutely distressing

task, monitored using video recording and electrocardiography, were included. Our definition of observational studies included cohort studies in which participants were prospectively identified and followed up during distress tasks using behavioral and cardiac indicators as well as cross-sectional studies that observed participants during a distress task using both a behavioral and cardiac measure.

Studies were excluded if they described non-human animal models of distress, did not include a distressing event (e.g., pain, fear, frustration), did not include both a cardiac and behavioral measure of distress in toddlerhood (i.e., 12 months to less than four years of age), did not measure behavioral and cardiac outcomes during the same time period (i.e., within the same age and appointment), or did not report quantitative or qualitative information regarding the relation between the behavioral and cardiac measures. Review articles, case studies, and conference abstracts were also excluded.

Two authors designed the abstract selection criteria with an initial selection of 500 abstracts. Covidence software (www.covidence.org) was used to independently read and select among all the retrieved references and abstracts. All abstracts were double-coded for reliability purposes. Percentage agreement between the reviewers before consensus coding was 97.9%. Disagreements between the two reviewers were flagged and resolved through discussion. Full texts of potentially eligible studies were retrieved (see Figure 1 for PRISMA flowchart).

Data extraction

A database was created recording a description of the distress paradigm (e.g., fear, frustration), behavioral coding system, cardiac indicator (i.e., HR, RSA [specifying if

computed through time-domain, frequency-domain, and non-linear analysis], PEP), results, and any additional variables that were included when analyzing the relation between behavioral and cardiac measures during distress (i.e., covariates, moderators). It was important to investigate covariates and moderators included in the studies because there are many variables that have been posited to affect the cardiac system or behavioral response to distress (e.g., baseline physiology, sex, age, attachment). Although previous meta-analyses (Holzman & Bridgett, 2017; Zahn et al., 2016) have found significant associations between behavioral self-regulation and heart rate variability measured through time-domain analyses, there was not enough variability in the studies to discuss these differences in methodology. The strength of the relation between behavioral and cardiac indicators was extracted from the available analysis (e.g., *r, t*-score, r^2) and converted to a standardized mean-difference effect size (i.e., Cohen's D; Cohen, 1988). When data were not provided in the articles, the authors were contacted via e-mail on two separate occasions. All studies were extracted by two reviewers. Discrepancies were minimal and resolved through consensus.

Quality assessment and methodological rigor

To evaluate the overall quality of evidence in our systematic review, a modification of the checklists designed by the National Heart, Blood, and Lungs Institute (2014), Downs and Black (1998), and Crombie and McQuay (1998) was used. The National Heart, Blood, and Lungs Institute has provided a checklist for assessing the quality of observational cohort and cross-sectional studies, and the Downs and Black (1998) and Crombie (1996) measures were chosen based on a multidisciplinary collaborative review discussing quality in case-control, cohort, and cross-sectional

studies (Sanderson, Tatt, & Higgins, 2007). Checklist items were scored as *yes* (1), *no* (0), or *unable to determine*. All extractions were consensus-coded for quality scores to ensure reliability. Disagreements were minimal (reliability between authors was 90%) and resolved through discussion. The National Heart, Lung, and Blood institute stated that their checklist is not intended to create a cut-off that allows one to arrive at a categorical judgment of quality. Instead, it suggests thinking about the items in the checklist and how each communicates something about the potential for bias in a study. These items were chosen through a consensus with the authors of the current paper, who agreed that those selected were most reflective of study quality (i.e., sample size justification, predictor and outcome variables being clearly defined, reliable, valid, and implemented consistently, blinding, and key potential confounding variables measured and adjusted for their impact). A proportion score of the criteria met was also calculated. Higher proportions reflect studies that have taken greater precaution against bias and can be considered relatively higher in study quality. The lead author coded every article and one of two other authors double-coded each article with overall reliability being 94% agreement. All discrepancies were discussed and consensus was used for the final value.

In addition to assessing the overall quality of the studies, we approximated the rigor of the physiological data collection. In order to investigate methodological rigor, we coded whether authors indicated: electrocardiography sampling rate, high frequency band used to define RSA (only for studies that used frequency-domain quantification), percentage of missing data, whether 1996 Task Force guidelines (as an index that authors adhered to psychophysiological standards) were referenced, and whether a "true"

baseline condition was utilized (i.e., stimulus free) in cardiac data collection. Checklist items were scored as *yes* (1), *no* (0), or *unable to determine*.

Narrative Synthesis Structure

The narrative synthesis included an examination of the general direction (positive, negative) and magnitude (Cohen's D*;* Cohen, 1988) of the findings. Note that the magnitude of the effect sizes is not categorized (i.e., small, medium, large), given that this categorization is meaningless in the absence of a frame of reference, which is the goal of the current study (see Funder & Ozer, 2019 for an overview of the problematic nature of effect size categorizations). Based on the articles found, studies were first organized according to the cardiac indicator (HR [baseline, task, change score], HP [baseline, standard deviation], RSA [baseline, task, change score], PEP [task]). In Beauchaine's (2001) seminal work describing an integrated model of ANS functioning and psychopathology, the author asserts that a source of confusion in the literature describing behavioral and cardiac indicators of emotion regulation is the definition of different cardiac indicators. As such, for the purposes of the current paper, cardiac measures labeled "task" are reflective of when cardiac indicators are collected across the distressing procedure, while "change score" is utilized when cardiac indicators were measured during a baseline period and distress paradigm, with one score being subtracted from the other score (e.g., baseline RSA minus RSA during the distress paradigm). A large proportion of the studies included fell under the "task" category, yet did not account for participants' baseline responses. The *Law of Initial Value* asserts that the size of a psychophysiological response depends on the initial baseline level of the measure (Bernston, Uchino, & Cacioppo, 1994; Oberlander & Saul, 2002; Waxman et al., 2016).

As such, it is difficult to distinguish whether associations between behavioral and cardiac indicators are related to individual physiological differences in baseline or distress reactivity. Given the limited number of studies, we chose to include "task" measures in order to draw conclusions from the current state of the field. Related to RSA change scores, this term was originally conceptualized in the psychophysiology literature as task (e.g., distress paradigm) minus baseline, with negative scores indicating reduced RSA following distress (Beauchaine et al., 2019). However, a recent meta-analysis by Beauchaine and colleagues (2019) found that authors were computing RSA in differing ways (i.e., baseline minus task, regressing baseline RSA onto task RSA), which impacted the overall relations with other indicators/outcome measures. As such, we have noted how the "change score" was calculated for each study in order to account for this in our narrative synthesis (Table 1).

The type of behavioral coding scheme (expressed emotion behaviors, emotion regulatory behaviors) utilized in each study was the next level of organization. Given the wide variety of behavioral coding schemes used, the behavioral coding systems were categorized based on Cole and colleagues (2004) assertion to separate behaviors reflecting expressed emotions versus behaviors enacted to regulate emotions. Two authors investigated the behavioral coding schemes used in each study, and made a forced-choice decision to categorize the coding scheme as representing either expressed emotion behaviors (e.g., facial fear, vocal distress, bodily fear) or emotion regulatory behaviors (e.g., self comforting, mother-orientation, distraction, aggression/venting, constructive coping). If coding systems were mixed (i.e., included expressed emotion behaviors and emotion regulatory behaviors), the study was categorized based on what

the majority of items represented in the coding scheme. Disagreements between coders were minimal and resolved through consensus coding.

After grouping studies in conceptual categories (i.e., Cardiac Response Type [HR: baseline, task, change score; HP: baseline, standard deviation; RSA: baseline, task, change score; PEP: task] by Behavioral Response Type [emotion expression behavior, emotion regulation behavior), findings were then further contextualized by methodological differences (*timing of measurement* as either concurrent or predictive, *epoch length* [length of both behavioral and cardiac measurement epochs in seconds], and *covariates/moderators* [were additional measures controlled for or investigated in analysis]). We required at least two studies in a given category (e.g., distress HR by expressed emotion) to perform a narrative synthesis. During our initial extraction, effect sizes were not found to qualitatively differ across distress paradigms (i.e., fear, frustration). As such, results were collapsed across distress paradigms to maximize the number of studies in each category of the narrative synthesis. There were no other types of distress paradigms.

Results

Studies included

Through the electronic searches, 2424 unique articles were identified. These articles were then reviewed by title and abstract and were included or excluded based on the *a priori* selection criteria. A total of 61 articles were then examined in a full-text review and of these, 22 (involving 2504 participants) fulfilled the inclusion criteria. These studies were included in the final review.

Study characteristics

Table 1 provides an overview of the studies included, including country of origin, sample size, study design, distress paradigm, behavioral coding scheme, definition of the baseline measurement paradigm, cardiac indicator, methodological details (i.e., timing of measurement, epoch length), and quality score. Fear and frustration were the only types of distress paradigms found in the literature search. Please see Table 1 for details.

Quality assessment and methodological rigor

Quality assessment. The lowest quality proportion score was .43 (Provost et al., 1979), the median quality score for the papers was .55, and the highest score was .81 (Hay et al., 2017). Only one study was given an overall quality judgment of *higher* (see Figure 2 for breakdown of items on quality checklist). We decided that a more extensive description study quality was necessary, as the quality of these studies would contextualize the overall findings from the narrative synthesis. Only six of 22 studies provided a sample size justification, and approximately half of the studies relied on small sample sizes ($N < 100$) for their statistical analyses. Additionally, several studies did not include clearly defined, reliable, valid, and consistently implemented predictor (6/22) or outcome (9/22) variables. Specifically, validation studies for behavioral indicators of distress were not commonly cited and inter-rater reliability estimates were not consistently reported. As well, for cardiac indicators, the number of coders and reliability estimates was only included for one study. Most studies did not describe blinding their coders to the study hypotheses (19/22). Finally, only 12 studies statistically accounted for

at least one key potential confounding variable that is known to impact physiological variables (e.g., age, sex, baseline characteristics, time of day, activity level, respiration).

Methodological rigor. Large inconsistencies in reporting were noted when investigating the methodological rigor for physiological data collection (See Table 2 and Figure 3 for breakdown of items coded for methodological rigor).

Relation between behavioral and cardiac measures of distress

Table 3 provides a descriptive catalogue of all behavioral distress measures investigated. The coding systems have been organized by whether they were coding emotion expression or emotion regulation behaviors. Tables 4a, 5a, and 6a summarize effect sizes for each study in the narrative synthesis. Adjoining tables have been created (i.e., 4b, 5b, 6b) to provide the patterns of results from each of the cells of the summary tables (Cardiac Indicator by Behavior Type). As noted above, narrative syntheses were only completed if there were at least two studies in a cell (e.g. expressed emotion behavior by task HR cell). However, summaries of findings for single studies are still indicated in Tables 4a, 5a, and 6a.

Heart rate

Heart rate and expressed emotion behaviors

Baseline HR (See Table 4a and 4b: Column 2, Row 2): The calculated Cohen's D was .14 for the relation between baseline HR and expressed emotion behaviors (Paret et al., 2015; D= .14). One study (Calkins et al., 1998) did not provide data on the relation between indicators. These studies acquired baseline HR data prior to the distressing task

(predictive measurement), utilized validated distress paradigms (i.e., Interesting but Scary paradigm, Toy Removal), and had varying epoch lengths for analyzing behavioral (i.e., 120 s) and cardiac (i.e., > 120 and 300 s) indicators. Calkins and colleagues (1998) controlled for sex in their analysis and did not find differences in the strength of relation between distress indicators.

Task HR (See Table 4a and 4b: Column 3, Row 2): Across the four studies included (Baker et al., 2012; Buss et al., 2005; Hay et al, 2017; Provost et al., 1979), there was a positive relation between task HR and expressed emotion behaviors. Effect sizes that ranged from a D = .05 to D = .54. Regarding methodological considerations, all studies acquired behavioral and cardiac data concurrently during the distress paradigm, had varying behavioral and cardiac (i.e., 180 to 600 s) measurement epochs, the majority of studies utilized validated distress paradigms (i.e., Strange Situation procedure, Toy Removal) (Baker et al., 2012; Buss et al., 2005; Provost et al., 1979), and included covariates or moderators in the analyses (Baker et al., 2012; Buss et al., 2005; Hay et al., 2017). Sex was a covariate in three studies (Baker et al., 2012; Buss et al., 2005; Hay et al., 2017). Only one study found a significant effect of sex on the relation between behavioral and cardiac indicators of distress (Hay et al., 2017), with male sex increasing the strength of the relationship between distress indicators. Hay and colleagues (2017) also controlled for baseline physiology (i.e., HR and activity) and childhood adversity in their analyses, and found that holding these variables constant increased the strength of the relationship between distress indicators.

Heart period

Heart period and expressed emotion behaviors

Baseline HP (See Table 4a and 4b: Column 5, Row 2): Results were mixed regarding the relation between baseline HP and expressed emotion behaviors, with two studies not reporting the relation between expressed emotion and baseline HP (Calkins et al., 1998; Garcia Coll et al., 1984), and one study (Stifter et al., 1996) finding a positive relation between distress indicators (i.e., D = .3). Studies included in the synthesis collected baseline cardiac data (Calkins et al., 1998; Stifter et al., 1996) or cardiac data in response to auditory and visual stimuli (Garcia Coll et al., 1984), reflecting predictive measurements. Two studies included validated distress paradigms (i.e., Behavioral Inhibition task, Toy Removal); however, Stifter and colleagues (1996) incorporated multiple distress paradigms (e.g., Toy Removal and Still Face Paradigm). Additionally, most studies utilized inconsistent measurement epochs for behavior (i.e., 120 to 180 s) and cardiac (i.e., 300 s or analyzed by group) data collection, and did not include covariates or moderators in their analyses (Calkins et al., 1998; Stifter et al., 1996). Garcia Coll and colleagues (1984) investigated sex as a moderator in their analyses, and found that sex did not impact the relation between distress indicators.

Heart period and emotion regulation behaviors

Baseline HP (See Table 4a and 4b: Column 5, Row 3): A Cohen's D of .12 was found (i.e., Burgess et al., 2003) between baseline HP and emotion regulation behaviors (Burgess et al., 2003). Data was not reported for one study (Calkins et al., 1992). These studies collected baseline cardiac data (Calkins et al., 1992) or cardiac data in response to

auditory and visual stimuli (Burgess et al., 2003) (predictive measurement), and both studies utilized a validated distress paradigm (i.e., Behavioral Inhibition task). Both studies used varying measurement lengths for behavioral (i.e., 420 s or not reported) and cardiac (i.e., 180 to 300 s) measurement epochs, and there were no covariates or moderators included in the analyses.

Respiratory sinus arrhythmia

RSA and expressed emotion behaviors

Baseline RSA (See Table 5a and 5b: Column 2, Row 2): Most studies found near-zero relationships (i.e., D = -.00 to D = .01) between baseline RSA and expressed emotion behaviors (Bockneck et al., 2018; Calkins et al., 1998; Paret et al., 2015; Voegtline, 2010). However, two studies (Calkins et al., 1998; Stifter et al., 1996) found a positive relation among distress indicators (i.e., D = .29 to .34). Most studies utilized validated distress paradigms and collected baseline cardiac data prior to the distress paradigm (predictive measurement). Behavior (i.e., 120 to 270 s) and cardiac (i.e., 120 to 900 s) measurement epochs were variable, and only two studies included analyses with covariates or moderators (Calkins et al., 1998; Paret et al., 2015). Although sex did not moderate the relationship between distress indicators (Calkins et al., 1998), attachment status did moderate the relationship between baseline RSA and expressed emotion (Paret et al., 2015).

Task RSA (See Table 5a and 5b: Column 3, Row 2): Across the three studies included (Brooker et al., 2010; Buss et al., 2005; Kahle et al., 2018), calculated Cohen's D ranged from -.15 to .00 for the relation between task RSA and expressed emotion

behaviors. All studies utilized validated distress induction tasks and measured emotion expression behaviors and cardiac indicators concurrently during the distress task. The studies varied greatly in their behavioral and cardiac (i.e., 12 to 600 s) measurement epochs. No studies investigated relevant covariates.

RSA change score (See Table 5a and 5b: Column 4, Row 2): Findings were mixed regarding the relationship between RSA change score and expressed emotion behaviors (D = -.15 to .20; Brooker et al., 2010, Voegtline, 2010). However, differences in how RSA change score was calculated (Brooker et al., 2010: Task-Baseline; Voegtline, 2010: Baseline-Task) were noted. Both studies utilized well-validated distress paradigms (i.e., Stranger Approach, Toy Removal). One study (Brooker et al., 2010) collected behavioral and cardiac data concurrently, while one study investigated RSA suppression in response to a different task (Voegtline, 2010). The studies included utilized varying measurement epochs for behavior (i.e., 120 to 150 s) and cardiac (i.e., 30 to 900 s) indicators, and only one study (Brooker et al., 2010) utilized covariates in their analysis. Specifically, movement was controlled for in the analysis, and decreased the relationship between distress indicators.

RSA and emotion regulation behaviors

Baseline RSA (See Table 5a and 5b: Column 2, Row 3): Calculated Cohen's D was between .002 and .15 for studies investigating the association between baseline RSA and emotion regulation behaviors (Burgess et al., 2003; Calkins et al., 1992; Liew et al., 2011). All studies utilized validated distress paradigms but did not collect baseline cardiac data consistently. Specifically, cardiac indicators were collected at baseline

(Calkins et al., 1992) while receiving visual and auditory stimuli (Burgess et al., 2003) or while watching an empathy inducing video (Liew et al., 2011). Cardiac (i.e., 42 to 300 s) and behavioral (i.e., 120 to 420 s) epochs varied in length. Liew and colleagues (2011) were the only study to utilize covariates, and they found that although sex did not affect the relationship between distress indicators, older children had a stronger relationship between baseline RSA and emotion regulation behaviors.

Task RSA (See Table 5a and 5b: Column 3, Row 3): The results were inconsistent across the three studies that investigated the relationship between task RSA and emotion regulation behaviors (Fox et al., 1989; Rubin et al., 1997; Kahle et al., 2018). While one study (Kahle et al., 2018) found near-zero associations between indicators (Cohen's D = -.00 to 01), two studies found a negative relation (D = -.07, Rubin et al., 1997; D = -1.61 to -.23, Fox et al., 1989). Distress paradigms were validated and all studies used concurrent measurement of the behavioral and cardiac indicators. Only one study analyzed the behavioral (i.e., 60 to 180 s) and cardiac (i.e., 12 to 25 seconds) data using distinct measurement epochs (Kahle et al., 2018). The other two studies (Fox et al., 1989; Rubin et al., 1997) used variable measurement epochs and did not describe the range in length of behavioral or cardiac measurement epochs. One study (Rubin et al., 1997) investigated maternal over-solicitousness and separation/reunion distress as moderators and found that the relationship between indicators did not differ depending on these variables. One study (Fox et al., 1989) investigated sex as a moderator, and found that the relationship between indicators did not depend on sex.

RSA change score (See Table 5a and 5b: Column 4, Row 3): The results were inconsistent from the four analyses (Augustine et al., 2018; Calkins et al., 2000; Liew et

al., 2011 had two age groups representing two different analyses) investigating the relationships between RSA change score and emotion regulation behaviors. For two studies, data on the relation between indicators was not reported or findings were near-zero (Calkins et al., 2000, null findings but data not provided; Liew et al., 2011, D = .04). Two studies found negative, albeit sometimes near-zero, relationships between RSA suppression and emotion regulation behaviors (compliance [D = -.04], comfort seeking [D = -.19], proximity to mother [D = -.93]). Two of the studies conceptualized RSA change score similarly (Baseline-Task; Augustine et al., 2018, Calkins et al., 2000), yet one study (Liew et al., 2011) utilized a unique method to calculate RSA change score (i.e., regression of baseline RSA onto task RSA). Only one study had a distress paradigm that was not validated (Augustine et al., 2018) and two of the three studies used concurrent measurement of the behavioral and cardiac indicators (Augustine et al., 2018; Calkins et al., 2000). Studies included ranged in length of behavioral (i.e., 120 to 300 s) and cardiac (i.e., 42 to 300 s) measurement epochs. One study (Liew et al., 2011) investigated sex as a covariate, and found that the relationship between indicators did not depend on sex.

Pre-ejection period (PEP)

PEP and expressed emotion behaviors

Task PEP (See Table 7a and 7b: Column 2, Row 2): Both studies (Buss et al., 2005; Kahle et al., 2018) found near-zero relations (Buss et al., 2005, D = .04; Kahle et al., 2018, D = -.005-.001) between task PEP and expressed emotion behaviors. Distress paradigms were validated and behavioral and cardiac measures were collected

concurrently during the distress paradigm. Behavioral (i.e., 60 to 120 s vs. 60 to 600 s) and cardiac (i.e., 12 to 25 s vs. 60 to 600 s) measurement epochs varied between the two studies included. Only one study had covariates in their analysis (Buss et al., 2005), which was described in a previous section.

Discussion

To our knowledge, the current study is the first to systematically review the available literature on the relation between behavioral and cardiac indicators measured during distress in toddlerhood, taking into account behavioral coding scheme (i.e., emotion expression behaviors, emotion regulation behaviors) was used, which type of cardiac measure was used (i.e., HR, HP, RSA, PEP), and various methodological factors (i.e., timing of measurement [concurrent versus predictive], length of behavioral and cardiac measurement epochs, and covariates/moderators). The narrative synthesis suggested that much of the literature is mixed regarding the association between cardiac and behavioral indicators measured during distress in toddlerhood. The only consistent relationship between cardiac and behavioral indicators was that task HR and expressed emotion behaviors are positively associated with standardized effect sizes in the range of Cohen's D = .05 to D = .54. Relationships were variable between baseline HR and expressed emotion behaviors (Cohen's D not reported to .14), baseline HP and emotion regulation behaviors (Cohen's D not reported to .12), task RSA and expressed emotion behaviors (Cohen's D = -.15 to .00), and baseline RSA and emotion regulation behaviors (Cohen's D = .002 to .15). Overall effect sizes across cardiac indicators were similar to those reported in previous meta-analyses on the association between RSA and behavior across the lifespan (Holzman & Bridgett, 2017; Zahn et al., 2016). As such, it is possible

that the magnitude of the association between behavioral and *most* cardiac indicators of distress may in fact be smaller than previously expected (i.e., Cohen's $D < 0.2$).

Importantly, the conclusions need to be couched within the lower quality ratings that the majority (21/22) of the studies received. Indeed, studies did not use consistent, valid, and reliable behavioral coding schemes, report reliability coefficients for heart rate indicators, or use analytic techniques that accounted for variables known to impact toddler physiology. Additionally, there was substantial variability in methods of physiological data collection. Of note, studies that fell under the "task" HR, RSA and PEP did not account for baseline physiology, and as such we cannot conclude if the lack of or consistent associations between indicators in this category is related to differences in baseline or task physiology. Additionally, many studies were not using "true" baseline conditions, which may have increased the literature-wide noise in both resting RSA and RSA change scores. Additionally, many of the sampling rates were below that which is preferred (i.e., 1024 Hz; Beauchaine et al., 2019), and high frequency bands were often not specified when RSA was measured. It is possible that studies not reporting frequency bands may be using bands appropriate for older children and adults, which will create large over- and under-estimates of resting RSA and RSA change scores, respectively (Shader et al., 2017). Missing data was not reported in almost one third of the studies, which may have impacted the overall relation between indicators. Finally, the 1996 Task Force guidelines were not cited in any studies published after 1996, which may suggest that noise could have been introduced into the data by not following rigorous methodological standards. Next, we review our narrative synthesis findings and contextualize them based on past literature and methodological challenges within the

studies included. We then discuss limitations of our research and implications for future research and clinical practice.

Associations between behavioral and cardiac indicators during distress

Heart rate

The narrative synthesis suggests that task HR is consistently associated with behavioral indicators of expressed emotion behavior. Specifically, the indicators are positively associated with an overall effect size with standardized mean differences in the D = .05 to .54 range. This result suggests that physiological systems might be implicated in affective behavior (Davidson, Jackson, & Kalin, 2000) and suggests that cardiac-behavior associations are strongest when the eliciting events are the same for the cardiac and behavioral measures (i.e., concurrent instead of predictive associations). Indeed, baseline HR was not related to indicators of expressed emotion across studies (i.e., D= .14, other study did not provide effect size data). No research has investigated the relation between HR indicators and emotion regulation behaviors, which may be linked given that emotion regulation may affect the intensive and temporal features of emotion (Thompson, 1994).

Heart period

Regarding HP, research has only investigated the relation between baseline HP and expressed emotions and emotion regulation behaviors. Although findings were mixed regarding the relation between baseline HP and expressed emotion behaviors, the relations between baseline HP and emotion regulation behaviors were consistently near zero (i.e., D= .15 or effect size data not provided). The results suggest that although HR

and HP are often considered inversely related, there may be distinct, albeit weaker, associations between HP and behavioral indicators. It is noteworthy that we found no studies from the last 15 years that utilized HP, likely bolstering the proposition that it has not been a promising indicator in terms of relationships with behavior.

Respiratory sinus arrhythmia

In regards to expressed emotion behaviors, the relations with baseline RSA and RSA change score were mixed (i.e., positive and negative), while relationships with task RSA were consistently near zero (i.e., D = -.15 to .00). Baseline RSA was weakly associated with emotion regulation behaviors (D = .002 to .15), while results were mixed (i.e., near-zero or negative relations) for task RSA and RSA change score. These results are consistent with classical theories suggesting that the SNS is most associated with fear and frustration (Fowles, 1988). As such, toddlers' responses to fear and frustration inductions may be more relevant to sympathetic responses such as HR whereas RSA may be more related to regulatory strategies that are aligned with the parasympathetic nervous system (e.g., self-soothing) (Kahle et al., 2018). Indeed, preliminary research by Kahle and colleagues (2018) provides credence for this assertion, but there is currently not enough research investigating the relation between task RSA and emotion regulation behaviors to confirm these hypotheses.

Pre-ejection period

Scientists have only begun assessing the increase in sympathetic influence, as indexed by PEP, during emotional challenge in toddlers. Two studies investigating task PEP and emotion expression behaviors found near-zero associations (i.e., D = -.005 to

.04) between these variables. PEP may be more related to emotion dysregulation or emotion expression behaviors that are poorly matched to context (Cole & Hall, 2008; Davidson, Jackson, & Kalin, 2000). Alternatively, Kahle and colleagues (2018) suggest that attempts to regulate emotions are more strongly linked with physiology than expressions of anger. Unfortunately, there was a lack of research investigating the association between PEP and emotion expression or regulatory behaviors to make any definitive claims.

Methodological challenges

Despite some consistent patterns emerging in the literature, many associations between cardiac and behavioral indicators were inconsistent and no conclusions could be drawn outside the relation between task HR and emotion expression behaviors. Relatedly, most studies were rated as lower quality and did not report a justification for their sample size, used predictor and outcome variables with questionable reliability and validity, and did not use proper blinding procedures or covariates. Given that 60% of the studies were published before guidelines were provided for reporting observational studies (von Elm et al., 2007), these limitations are not unexpected. It may be that bias-reducing procedures were in fact undertaken, but not reported. However, to clarify the mixed relations between behavioral and physiological indicators during distress, a more in-depth discussion of the methodological differences (i.e., behavioral and cardiac measures, timing and length of measurement epochs, additional analyses) among studies is discussed below.

Behavioral indices measured during distress. Behavioral indicators of emotion expression have been integral in understanding the development of emotion regulation. However, our synthesis revealed that there are not many valid or reliable measures of emotion expression behaviors being used, and the same higher quality behavioral coding systems are not implemented consistently across studies. Indeed, only nine of 22 studies provided citations regarding the validity and reliability of their behavioral coding system. This finding is concerning because most studies included coding systems that involve a high degree of subjectivity and, as such, could introduce a substantial amount of measurement error. Additionally, despite Cole and colleagues (2004) assertion that we need independent measurement of the activated emotion and the putative regulatory strategies to assess the dynamic nature of emotion properly, behavioral indicators are not commonly coded or categorized as such. Thus, we organized the behavioral coding schemes as either emotion expression behaviors or emotion regulation behaviors, but at times this dichotomization was challenging due to the inclusion of behaviors that were from both expression and regulatory categories. Despite these challenges, our decision to dichotomize studies based on this distinction led to a more nuanced understanding of behavioral responses during distress, as expressed emotions were most consistently related to task HR. This finding is consistent with past research that has suggested that measures of discrete negative emotions (e.g., fear, sadness) are more sensitive than global ratings of negative affect or distress (Buss & Goldsmith, 2007). Yet, emotion regulation behaviors were inconsistently related to cardiac indexes during distress. Many studies investigated behavioral inhibition, a dispositional construct defined by an inborn bias to respond to unfamiliar events by showing anxiety (Kagan, 1994). Behavioral inhibition is

often reflected in regulatory behaviors (e.g., latency to approach the stranger or unfamiliar objects, time spent proximal to the mother, or physical distance from the mother), and past work has found little evidence that indexes of behavioral inhibition are consistent across situations (Rubin et al., 1997). Additionally, recent work (e.g., Kahle et al., 2018; Perry, Calkins & Bell, 2016) has suggested that emotion regulation behaviors should be further categorized by strategy (e.g., distraction, orienting toward mother, verbalizations), as each strategy can be differentially related to the SNS and PNS. Given that most studies included in the review collapsed across behavioral regulation strategies, it was not possible to further dichotomize emotion regulation strategies. However, this may account for the inconsistent relations between emotion regulation behaviors and cardiac indicators. Moreover, initially we had wanted to more specifically distinguish between the peak reactivity phase to a stressor and the less proximal regulation phase. However, the majority of studies (16/21) did not distinguish between initial post-stressor (reactivity) and less-proximal (regulation) measurement epochs (i.e. researchers collapsed coding systems over the toddler's immediate reaction to stimuli and the regulation from the stimuli), which is an important distinction that should be made in the future.

Cardiac indices measured during distress. Our narrative synthesis suggests that task HR is positively correlated with expressed emotion behaviors. However, baseline HR and HP, baseline and task RSA, and task PEP are not consistently related to expressed emotion or emotion regulation behaviors. Past research has suggested that there are important developmental changes in the sympathetic and parasympathetic nervous system across toddlerhood. Thus, in the second year of life, autonomic measures such as RSA may be more reflective of physiological regulation than simply emotional

reactivity or arousal (Eisenberg et al., 1995). Furthermore, few studies have investigated the stability of cardiac measures across the first years of life, which limits our ability to state that these measures represent stable individual differences in distress responses. Finally, baseline and task RSA reflect different aspects of self-regulatory functioning (Porges, 2007). While baseline RSA reflects an individual's ability to regulate internal bodily processes and temperamental characteristics (Beauchaine, 2001; Porges, 1996), task RSA (following a distressing event) is intended to reflect an individual's ability to attend and respond to environmental demands (Beauchaine, Gatzke-Kopp, & Mead, 2007). As such, null or mixed findings may result from researchers choosing cardiac measures that are not aligned theoretically with their behavioral outcomes. Again, the lack of disambiguation in studies between reactivity and regulation measurement epochs would likely have an important impact.

Timing and length of measurement. As mentioned above, key methodological factors that we hypothesized to affect the relation between behavioral and cardiac indexes were the timing and length of measurement (i.e., epoch length) for behavioral and cardiac indicators. Indeed, distress reactivity and regulation have been clearly delineated in psychophysiological research (Rothbart & Derryberry, 1981) and researchers have suggested that distress may be more accurately measured through temporally sensitive techniques that capture dynamic change (Thompson, Lewis, & Calkins, 2008). Regarding the timing of measurement, most of the stronger relations were characterized by concurrent measurement of behavioral and cardiac indexes during distress. As such, studies that use predictive analyses (e.g., baseline cardiac variables) may not capture salient aspects of toddlers' distress within these specific paradigms, which helps explain

the weaker relations between indicators. Relatedly, the operationalization of baseline cardiac indicators differed across studies, with some studies investigating physiology at rest whereas others collected physiological data during presentation of auditory or visual stimuli. It can be posited that the degree of association may be affected by the operationalization of baseline physiology. As well, although most studies collapsed their behavioral and cardiac measures across the entire distress paradigm, studies that differentiated between phases of distress (i.e., reactivity vs. regulation measurement epochs) had stronger effects.

Additional analyses. Only approximately half of the studies (12/22) included in our review measured and adjusted for key potential confounding variables that are known to impact physiology (i.e., age, sex, baseline characteristics, time of day, activity level, and respiration; Oberlander & Saul, 2002; Waxman et al., 2016). Moreover, the covariates and moderators used were not consistent across studies, and included child-level (i.e., sex, age, baseline heart rate, and activity) and dyad-level (i.e., attachment, early adversity) factors. Sex was the most widely examined variable across studies, and results suggest that there are not sex differences in the relation between behavioral and cardiac indicators following distress during this specific developmental stage. These results counter past research that has suggested that the association between behavioral and cardiac indexes of emotion may differ for boys and girls (Buss et al., 2005). As well, although previous research has noted that there are rapid physiological changes in infancy and toddlerhood which make it difficult to detect the relationship between behavior and biology (Stifter & Jain, 1996), little research has controlled for or examined the effect of age in its analyses. Other child-level (i.e., baseline heart rate/activity) and dyad factors

(i.e., attachment, early adversity) were significant covariates or moderators in the relation between behavioral and cardiac indexes. However, conclusions cannot be made given that these variables were tested in only one study each.

Limitations

Despite our detailed search strategy, it is possible that we omitted relevant studies. We specifically excluded non-English publications, which may have eliminated relevant studies. Moreover, we collapsed across tasks (i.e., fear vs. frustration paradigms) given a lack of *qualitative* differences in effect sizes. However, it is possible that if quantitative techniques were utilized (i.e., meta-analysis), there may have been task-related differences in the association between emotion expression/regulatory behaviors and cardiac indicators of distress. Indeed, research in preschool- and school-aged children (e.g., Quigley & Stifter, 2006; Zeytinoglu, Calkins, & Leerkes, 2019) has found task-related (e.g., cognitive vs. negatively-valenced emotional challenges) differences in cardiac reactivity and the magnitude of physiological responses. It is also important to consider that almost all studies (21/22) were rated as *lower* quality. However, this result may be partly due to reporting standards for papers that reflect journal requirements and decisions by journal reviewers and editors instead of the quality of the original study. Using quality checklists that delineate clear standards of reporting are critical. The EQUATOR network provides an excellent resource to ensure high quality reporting scores (http://www.equator-network.org/). Relatedly, many studies did not report data on the relation between indicators when results were null. As such, the synthesis may under-estimate the relation between indicators given that null results may have been a result of small sample sizes and not due to a lack of relation. Additionally, most studies were from

North-American researchers, which may limit the generalizability of our findings. Finally, the study focused on research that specifically analyzed the concurrent relation (i.e., within the same appointment) between behavioral and cardiac indexes measured during distress and did not include studies that investigated the longitudinal relation between these measures. As such, not all studies with informative findings pertaining to predictive relations between these measures were included.

4.4 Conclusions and Future Directions

The current study is one of the first to review the concurrent relation between behavioral and cardiac indicators measured during distress during the second and third years of life. The narrative synthesis suggests that currently only HR during the post-stimuli phase is consistently related to expressed emotion behaviors (i.e., standardized mean difference, D = .05 to .54). These results suggest that research using *distress* as a key construct could obtain divergent results depending on whether distress is measured with cardiac or behavioral indicators. Significant gaps in the literature were highlighted, with a lack of studies investigating the relation between HR indicators and emotion regulation behaviors, HP responding and behavioral indicators, and PEP and emotion regulation behaviors. Furthermore, it is noteworthy that there are currently no studies on the relation between behavioral and physiological indicators in severe distress (e.g., pain) in toddlerhood. Given the particular salience of high distress experiences in development and increased dependency on parents during high distress, this will be an important avenue to investigate.

Mixed or near-zero associations may suggest that context-appropriate physiological and expressed emotion behaviors or emotion regulation behaviors can co-occur without being closely linked (Hastings et al., 2009). Relatedly, a recent review by Barrett and colleagues (2019) found that there is not yet sufficient evidence to conclude that facial movements have the sensitivity or specificity to express the instance of any emotion category. As such, it is possible that the reliability of facial expressions relating to distressing emotions may not emerge until later childhood or adolescence.

However, it is possible that the lack of relations between behavioral and cardiac indicators of distress may differ due to methodological inconsistences. Thus, another important contribution of this work is concrete suggestions for improving the quality and consistency of research in this area. Based on our review, we recommend the following guidelines for future work examining behavioral and cardiac measures of distress:

1. Improve study methodology (e.g. psychometrically sound baseline conditions and behavioral measures, appropriate frequency bands for measuring RSA in toddlerhood (i.e., 0.24 to 1.04 hZ), coder blinding to hypotheses, distinguish between phases of the post-stimuli responses [e.g. immediate responses versus more distal responses]) and/or reporting practices (e.g. reporting on standardized data collection [1996 Task Force], ECG sampling rates/high frequency HRV bands, sufficient statistics, recruitment rates/missing data, participation rates, study population characteristics).
2. Behavioral coding schemes are needed that clearly distinguish specific types of behavior being measured (i.e., *emotion expression behavior* vs. *emotion regulation behavior*) and have established psychometrics.

3. Reliability coefficients for cardiac data reduction need to be reported. A review by Laborde and colleagues (2017) noted that the ease of access to cardiac indicators in psychophysiological research has been overshadowed by the difficulty of editing and interpreting cardiac findings. Our results confirm this assertion and we suggest that future research mirror reporting standards for behavioral observation tools. Specifically, authors should report number of coders editing cardiac data and report reliability coding (e.g., double coding 10-20% of the sample, reporting reliability coefficients).
4. The relative contributions of parasympathetic and sympathetic reactivity and regulation need to be accounted for in future research (e.g. collection of HR, RSA, pre-ejection period, galvanic skin response, cortisol, cortical responses).
5. Temporal measurement epochs need to be defined in a similar manner across studies to facilitate comparisons (e.g., utilizing concurrent 30 second measurement epochs for both behavioral coding and cardiac data reduction) of the impact of phase of distress (i.e., reactivity phase vs. regulation phase) on the relation between behavioral and cardiac indicators of distress.
6. Statistical approaches that capture dynamic aspects of distress reactivity and regulation (e.g., latent growth curve models) should be utilized, given that levels of parasympathetic and sympathetic activity are likely to fluctuate over the course of a distressing event. As well, a consistent set of covariates should be included for all studies investigating the relation between behavioral and cardiac indexes of distress in toddlerhood. These covariates should include but are not limited to age, sex, time since last feeding and nap, baseline physiological variables, activity, and respiration rate.

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Figure 1. Systematic Review PRISMA Flowchart.

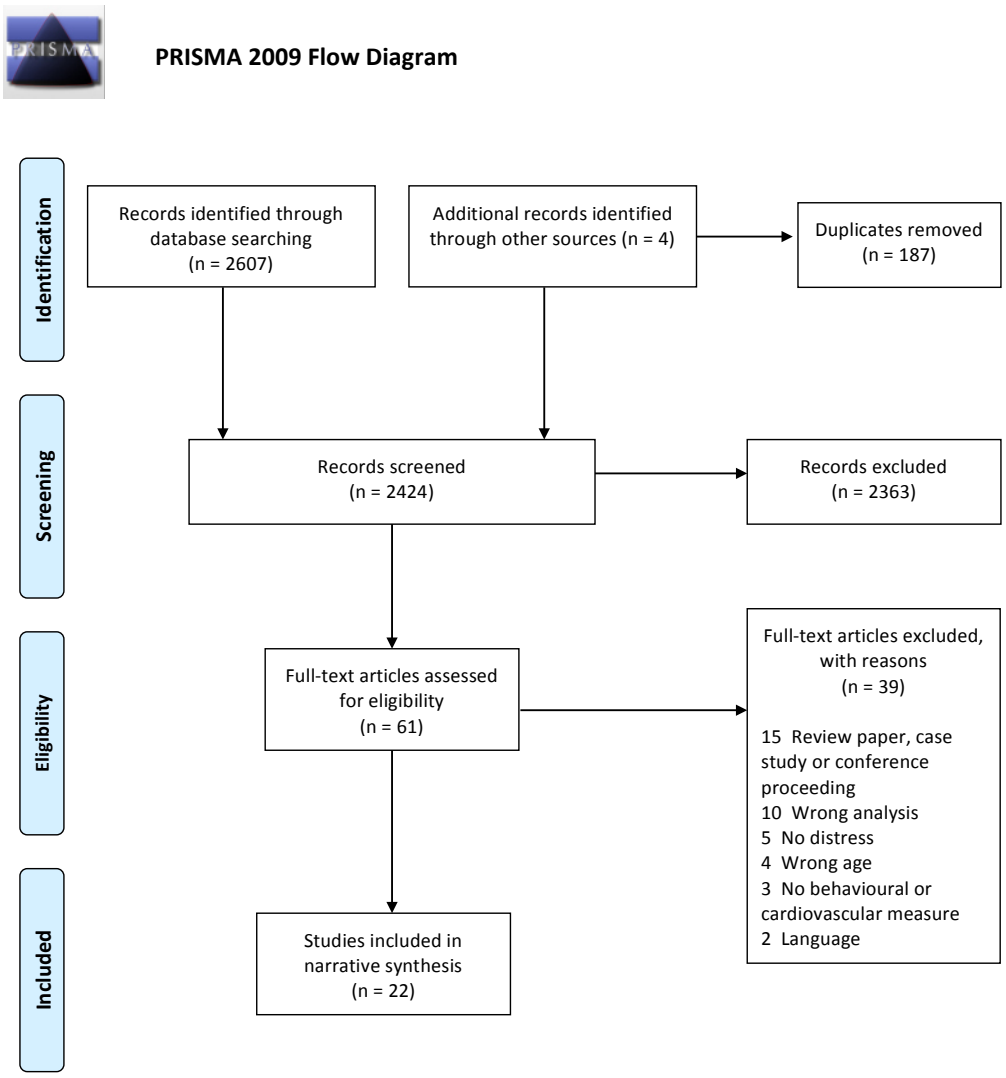

Figure 2. Bar graph illustrating the number of studies (out of 22) that fulfilled each item on the quality assessment.

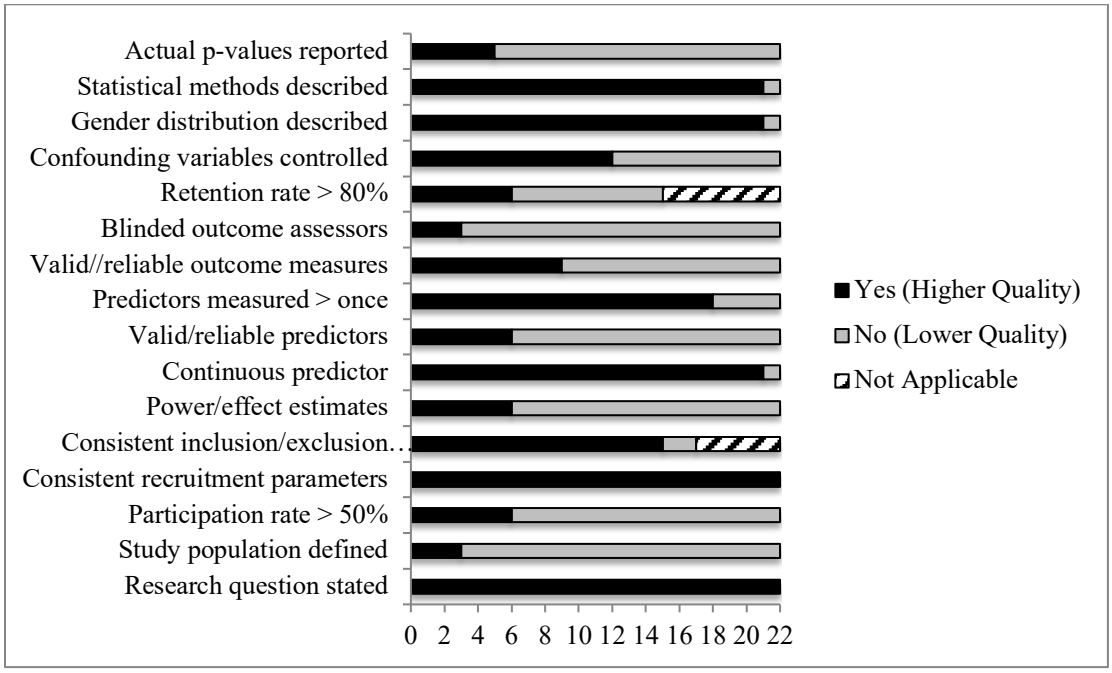

Figure 3. Bar graph illustrating the number of studies (out of 22) that fulfilled each item on the methodological rigor assessment.

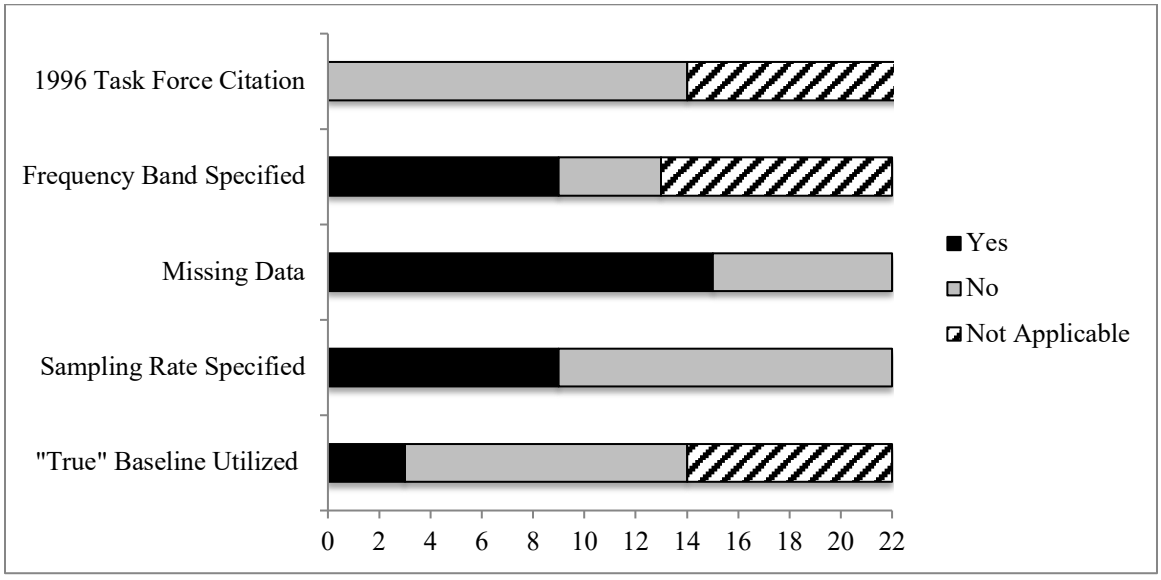

Table 1. Study characteristics.

Study	Country	N	Year	Type of Study	Distress Paradigm	Definition of Cardiac Baseline	Behavioral Coding Scheme	Cardiac Metric	Timing of Measurement	Measurement Epochs	Quality score
Augustine (2018)	United States	138	2	B	Clean-up Task	Free play with caregiver	Emotion Regulation Behaviors*	RSA Change Score (Baseline-Task)	Concurrent	Behavioral and Cardiac Metric: < 300 s	.56 (Lower)
Baker (2012)	United Kingdom	70	2, 3	B	LabTAB Fear Paradigm (Goldsmith & Rothbart, 1999)	Sitting	Emotion Expression Behaviors	HR Responding	Concurrent	Behavioral and Cardiac Metric: 210 s	.53 (Lower)
Bocknek (2018)	United States	130	2	CS	*Behavioral Paradigm:* Lab-TAB Locomotor Version (Goldsmith & Rothbart, 1991) *Cardiac paradigm:* Baseline	No/low-stress family reading activity	Emotion Expression Behaviors	Baseline RSA	Predictive	Behavioral: 270 s Cardiac Metric: 120 s	.8 (Higher)

Bohlin (1993)	Sweden	31	2	B	Stranger-Wariness Situation (Skarin, 1997; Waters et al., 1975)	5 s recording prior to Stranger-Wariness Situation	Emotion Expression Behaviors	HR Change Score (Baseline-Task)	Concurrent	Behavioral and Cardiac Metric: 5 s	.50 (Lower)
Brooker (2010)	United States	88	2	CS	Stranger Approach Episode (Buss & Goldsmith, 2000)	Reading or coloring	Emotion Expression Behaviors	RSA Responding, RSA Change Score (Task-Baseline)	Concurrent	Behavioral: 150 s Cardiac Metric: 30 s	.47 (Lower)
Burgess (2003)	United States	172	2	B	*Behavioral Paradigm:* Inhibition task (unfamiliar room, adult stranger, novel toy/object, adult stranger dressed in a clown costume, inflatable tunnel) *Cardiac paradigm:* Baseline	Presentation of auditory and visual stimuli	Emotion Regulation Behaviors	Baseline HP, Baseline RSA	Predictive	Behavioral: approx. 420 s Cardiac Metric: 180 s	.50 (Lower)

Buss (2005)	United States	68	2	CS	Stranger Approach (Buss & Goldsmith, 2000) Toy Removal Bayley Scale of Infant Development (BSID-II; Baley, 1993)	Presentation of auditory and visual stimuli	Emotion Expression Behaviors*	HR Responding, RSA Responding, PEP Responding	Concurrent	Behavioral and Cardiac Metric: *Cognitive*- 600 s; *Fear*- 150 s; *Frustration*- 60 s	.67 (Lower)
Calkins (1992)	United States	52	2	B	*Behavioral Paradigm:* Inhibition task (unfamiliar room, adult stranger, novel toy/object, adult stranger dressed in a clown costume, inflatable tunnel) *Cardiac paradigm:* Baseline	Sitting	Emotion Regulation Behaviors*	Baseline HP, Baseline RSA	Predictive	Behavioral: Not described (variable) Cardiac Metric: 300 s	.44 (Lower)

Calkins (1998)	United States	73	2	CS	*Behavioral paradigm*: Toy removal (plastic barrier, plexiglas barrier) *Cardiac paradigm*: Baseline	Presentation of auditory and visual stimuli	Emotion Expression Behaviors	Baseline RSA	Predictive	Behavioral: 120 s Cardiac metric: 300 s	.73 (Lower)
Calkins (2000)	United States	99	2	CS	*Behavioral paradigms:* Toy removal (plastic barrier; frustration), toy spider (fear) *Cardiac paradigm*: Baseline	Presentation of auditory and visual stimuli	Emotion Regulation Behaviors*	RSA Change Score (Baseline-Task)	Concurrent	Behavioral: 120-300 s Cardiac metric: 300 s	.6 (Lower)
Fox (1989)	United States	52	2	B	*Behavioral paradigm:* Three situations (interaction with an unfamiliar adult, presentation of a novel object (robot), strange situation procedure (Ainsworth & Bell, 1970))	*Baseline 1:* Sitting; *Baseline 2:* Presentation of auditory and visual stimuli	Emotion Regulation Behaviors	RSA Change Score (High vs. Low RSA)	Concurrent	Behavioral: Not described Cardiac metric: Group membership (High vs. Low RSA)	.56 (Lower)

					Cardiac paradigm: Four situations: (free play, interaction with an unfamiliar adult, presentation of a novel object (robot), strange situation procedure (Ainsworth & Bell, 1970))						
Garcia Coll (1984)	United States	117	2,3	B	*Behavioral paradigm:* Six situations (warm-up, free play, reaction to modelling, reaction to unfamiliar adult, reaction to unfamiliar object (robot), and separation from mother) *Cardiac paradigm:* Baseline	Presentation of auditory and visual stimuli	Emotion Expression Behaviors*	Baseline HP, Baseline HP standard deviation	Predictive	Behavioral: Group Membership (Inhibited vs. Uninhibited) Cardiac Metric: 120-180 s	.69 (Lower)

Hay (2017)	United Stated	332	2	B	Teddy Bear Picnic Paradigm	Not described	Emotion Expression Behaviors	HR Responding	Concurrent	Behavioral: Not described (variable) Cardiac metric: Not described (variable)	.81 (Lower)
Kagan (1989)	United States	100	2	B	Inhibition task (mask, reaction to unfamiliar adult, reaction to alarm, reaction to unfamiliar object (robot))	Sitting	Emotion Regulation Behaviors	HR Responding	Concurrent	Behavioral: Not described (variable) Cardiac metric: Not described (variable)	.60 (Lower)
Kahle (2018)	United States	83	3	C	Impossibly Perfect Circles Task	Not described	Emotion Expression Behaviors; Emotion Regulation Behaviors	RSA Responding, PEP Responding	Concurrent	Behavioral: *Reactivity*- Approx. 180 s; *Recovery*- Approx 60 s Cardiac metric: *Reactivity*	.63 (Lower)

										and Recovery-12-25 s	
Liew (2011)	United States	247	2, 3	B	*Behavioral paradigm:* LabTAB (Jumping Spider), Reactions to stranger (Zahn-Waxler & Radke-Yarrow, 1990) *Cardiac paradigm:* Baseline Empathy-eliciting films (distressed, crying babies)	Presentation of auditory and visual stimuli	Emotion Regulation Behaviors	RSA Change Score (regression of task RSA on baseline RSA)	Predictive	Behavioral: 120 s Cardiac metric: 42-180 s	.56 (Lower)
Paret (2015)	Canada	33	3	Both	Interesting but Scary Paradigm (Forbes, Evans, Moran, & Pederson, 2007)	Presentation of auditory and visual stimuli	Emotion Expression Behaviors	Baseline HR, Baseline RSA	Predictive	Behavioral: 120 s Cardiac metric: > 120 s	.44 (Lower)
Provost (1979)	Canada	40	2	CS	Stranger Approach (Ainsworth & Bell, 1970; Provost &	Between Task HR	Emotion Expression Behaviors	HR Responding	Concurrent	Behavioral: 180 s Cardiac	.43 (Lower)

					Decarie, 1974) Toy Removal (Goulet, 1968)					metric: 180 s	
Rubin (1997)	United States	108	2	CS	Three situations (interaction with an unfamiliar adult, presentation of a novel object (robot/tunnel), strange situation procedure (Ainsworth & Bell, 1970)) *Cardiac paradigm:* Unfamiliar video stimuli and stranger presence combined	Presentation of auditory and visual stimuli	Emotion Regulation Behaviors	RSA Responding	Concurrent	Behavioral: Not described (variable) Cardiac metric: Not described (variable)	.69 (Lower)
Spangler (1993)	Germany	41	2	CS	Ainsworth's Strange Situation Procedure (Ainsworth & Wittig, 1969)	Between Task HR	Emotion Regulation Behaviors	HR Change Score (Baseline-Task)	Concurrent	Behavioral: Not described (variable) Cardiac metric: Not described	.47 (Lower)

										(variable)	
Stifter (1996)	United States	100	2	B	*Behavioral paradigm*: Toy removal (while mother held neutral posture, not interaction with child) *Cardiac paradigm*: Baseline	Sitting	Emotion Expression Behaviors	Baseline HP, Baseline RSA	Predictive	Behavioral: 120 s Cardiac metric: 600 s	.50 (Lower)
Voegtline (2010)	United States	330	2	B	*Behavioral paradigms:* Children were presented with four unusual masks in succession (a long-nosed woman, a Frankenstein, a goofy vampire, and a bald conehead) Toy removal (while mother held neutral posture, not interacting with child) *Cardiac paradigm*: Baseline and	Sitting	Emotion Expression Behaviors	Baseline RSA; RSA Change Score (Baseline-Task)	Predictive	Behavioral: 120 s Cardiac metric: *Baseline*-300 s *; Task*-900 s	.56 (Lower)

	Mental development test.	

Note. **Year:** 2= 12-36 months, 3= 36-48 months; **Type of Study**: Longitudinal (L), Cross-Sectional (CS), Both (B); **Behavioral Coding Scheme**: * = both emotion expression and emotion regulation behaviors included in coding scheme, forced choice made; **Cardiac Measure**: Heart Period (HP), Heart Rate (HR), Pre-ejection Period (PEP), Respiratory Sinus Arrhythmia (RSA); **Timing of measurement**: Concurrent- heart rate and behavior measured concurrently, Predictive- heart rate or behavior collected before the other measure; **Epochs**: length of measurement epochs (in seconds) for both behavioural coding scheme and cardiac metric. **Quality Score**: proportion score based on number of items endorsed divided by the total number of applicable questions, in brackets is the overall quality judgment.

Table 2. Description of results from methodological rigor assessment.

	ECG Sampling Rate (Hz)	High Frequency Band (Hz)	Missing Data (%)	1996 Task Force Mention
Augustine (2018)	1000	0.24-1.04	23	N
Baker (2012)	50-60	N/A	0-13	N
Bockneck (2018)	Not described	0.24-1.04	Not described	N
Bohlin (1993)	Not described	N/A	Not described	N/A
Brooker (2009)	42-250	0.24-1.04	0-5	N
Burgess (2003)	512	0.2-1.00	30	N
Buss (2005)	500	0.24-1.04	25	N
Calkins (1992)	Not described	N/A	Not described	N/A
Calkins (1998)	Not described	0.24-1.04	7	N
Calkins (2000)	Not described	Not described	8	N
Fox (1989)	Not described	Not described	38	N/A
Garcia Coll (1984)	200	N/A	6-9	N/A
Hay (2017)	30	N/A	6	N
Kagan (1989)	Not described	N/A	31	N/A
Kahle (2018)	500	0.24-1.04	PEP: 1-36 RSA:1-34	N
Liew (2011)	Not described	N/A	1	N

Paret (2015)	500	N/A	Not described	N
Provost (1979)	Not described	N/A	Not described	N/A
Rubin (1997)	Not described	Not described	9	N
Spangler (1993)	Not described	N/A	Not described	N/A
Stifter (1996)	Not described	0.24-1.04	Not described	N/A
Voegtline (2010)	Not described	0.24-1.04	40	N

Note. Hz = hertz; Not described= information was not provided in paper; N/A= not applicable to study; N= no citation.

Table 3. Description of behavioral measures utilized in analysis with cardiac indicators for included studies.

Study	Emotion Expression Behaviors	Emotion Regulation Behaviors
Augustine (2018)	**Child Affect:** continuously coded throughout the clean-up task based on the 7-point scale.	**Compliance:** proportion of time children engaged in committed compliance, defined by immediate and sustained clean-up behavior upon initial request, or situational compliance, defined by clean-up behavior with repeated maternal prompts. **Defiance:** proportion of time children engaged in any behavior that was resistant, confrontational, or rebellious. A composite compliance score was calculated (observed affect reversed, compliance, and defiance reversed) as the mean of standardized scores for each measure. Higher scores represent well-regulated behavior.
Baker (2012)	LabTAB Behavioural Coding (Facial Fear, Vocal Distress, Bodily Fear).	
Bocknek (2018)	Behavioral coding yielded four frustration scores (Latency to Anger, Struggle, Distress Vocalizations, Facial Anger). A composite was created by standardizing each individual code and summing the scores.	
Bohlin (1993)	Overt facial, cry and body behaviours (Waters et al., 1975); Global rating of the overall response to the stranger was coded on a 6-point scale from very positive (1) to very	

	negative (6).	
Brooker (2009)	Global ratings of negative affect (ratings were assigned that ranged from (1) absence of behaviour to (5) behaviour of highest intensity and duration).	
Burgess (2003)		Low, medium and high behavioural inhibition (composite measure of inhibition [i.e., latency to vocalization, proportion of time spent proximal to mother, latency to approach/touch novel stimuli]).
Buss (2005)	**Baseline/Cognitive Paradigm:** Frequency of crying/fussing, negative facial expressions, and neutral and positive expressions; **Fear Paradigm:** Frequency of facial fear, facial sadness, bodily fear, bodily sadness, vocal distress; **Frustration Paradigm:** Frequency of crying, facial anger, bodily anger, facial sadness, bodily sadness.	**Fear Paradigm:** Frequency of escape/avoidance behaviors.
Calkins (1992)		**Standardized sum of the following variables**: Proximity to mother during free play, latency to vocalize during free play, proximity to mother during stranger approach, clown, robot and tunnel, latency to approach stranger, tunnel, robot and clown, latency to cry to the approach of stranger, latency to vocalize to the stranger and clown.
Calkins (1998)	**Frustration distress:** latency to cry (in sec), the intensity of distress (scored every 10 sec on a scale of 0 [no distress] to 5 [full blown scream or cry]), the frequency of fussing (scored as the number of 10-sec intervals in which a fuss or	

	fret was present), the duration of crying in sec. Measures of reactivity were computed for each frustration task and a summary score was created by summarizing three of the four tasks.	
Calkins (2000)	**Negative affect:** instances of sadness, anger, tension, worry, and vocal expression of fussing (negative verbalization associated with the emotions of anger, sadness, tension, and worry)	**Orienting to task object:** looking at, touching, or manipulating task object; **Distraction:** attending to or manipulating an object other than the task object; **Aggression/venting:** banging, kicking, throwing, hitting the task object, or aggression directed toward mother or experimenter. A **composite score** was calculated through factor analysis (negative affect, orienting to task object, distraction and aggression/venting).
Fox (1989)		Latency to cry, latency to approach the stranger and proximity to mother during stranger presence, latency to approach and latency to touch robot.
Garcia Coll (1984)	**Inhibition index:** inhibition or apprehension behaviours (crying, fretting, distress vocalizations,), display of distress to the model.	**Inhibition index:** inhibition or apprehension behaviours (withdrawals, and absence of spontaneous interactions with the experimenter), latencies to interact with toys or with the adult, inhibition of play.
Hay (2017)	**Distress Observation System** (Demetriou & Hay, 2004): infant's mild [whimpering, whining, fussing] and strong [crying, weeping, screaming] distress).	
Kagan (1989)		**Average of standardized scores for variables within an episode and then averaged across episodes:** Latency to leave the parent, latency to touch a first object, percent of time the child was within arm's reach of the parent (before and during paradigm), noting when child retreats to parent, latency to

		interact with the unfamiliar object.
Kahle (2018)	**Expressed emotion:** observed anger was coded on a scale from 1 to 4, ranging from "none" to "strong or persistent," respectively, for the duration of each phase (reactivity and recovery). Anger was coded as facial signs of anger and frustration (e.g., square-shaped mouth, showing teeth, furrowed brow); harsh, impatient, or callous vocal intonation; and aggressive, frustrated, or annoyed behaviors (e.g., slapping hand down on the paper; eye rolls).	**Emotion regulation behaviours:** *self-soothing* included physical behaviors such as repetitive arm stroking, sucking fingers, or repetitive touching of the lips or mouth; *attention diversion* included behaviors such as fixating their gaze elsewhere in the room, playing, or scribbling; *verbalizations* included statements that reflected regulatory attempts.
Liew (2011)		**Overall fearfulness composite:** children's approach to the spider and proximity to mother; Child empathic concern: concerned attention & hypothesis testing, personal distress (self-soothing and comfort seeking), helping.
Paret (2015)	**Behavioural Inhibition Scales** (Marshall & Stevenson-Hinde, 1998; Stevenson-Hinde & Shouldice, 1995): non-verbal anxiety and low verbal responsiveness measured on a scale from 1 (relaxed and responsive) to 9 (high tension/no verbal response). Global ratings of behavioural inhibition were made on a scale from 1 (no signs of inhibition) to 9 (extreme inhibition).	
Provost (1979)	Action of the child (body actions, gestures, postures, locomotion), hedonic tone of facial expressions and vocalizations. Coders made a forced choice of emotion (interest, joy, fear, anger, distress, other).	

Rubin (1997)		**Non-social inhibition:** amount of physical contact with his or her mother in the first and second free play episodes; **Adult-social inhibition:** maintenance of contact with mother in the truck, robot, and tunnel episodes, the child's latency to approach the stranger and/or touch the truck, and robot, and latency to pass through the tunnel (all of which required approaching, to within touching distance, the stranger); **Traditional inhibition:** adult-social and non-social scores combined.
Spangler (1993)		Duration of Object Orientation: looking to mother, looking to specific objects, object manipulation and unspecific looking for every 1-sec interval
Stifter (1996)	Negative vocalizations coded every 10s on a 5-point scale of 0 (no negative vocalizations) to 4 (shrieking, hysterical crying) (Fish et al, 1991). The score was summed and divided by the length of the frustration period.	
Voegtline (2010)	Three levels of negative emotional reactivity were coded second by second from video recordings for the total duration of the task: **low reactivity** (fussing, whining, frowning, furrowed brow, crinkled nose, and slightly open or pressed lips), **medium reactivity** (crying, wide squared mouth, and eyes open or partially opened), and **high reactivity** (screams, wails, eyes partially or completely closed, and wide open mouth). A composite score for negative reactivity was created by summing the seconds of low, medium, and high negative reactivity.	

Note. Certain studies included emotion expression behaviours and emotion regulatory behaviors, but only analysed the relation with cardiac indicators for one behavioral coding scheme. Only relevant coding schemes were included in the table. Where no relevant data is available, grey shading is used.

Table 4a. Summary of study findings (effect sizes) for heart rate (HR) and heart period (HP) with Behaviors (Emotion Expression, Emotion Regulation).

Behavioural Coding Scheme	Baseline HR	Task HR	HR Change Score	Baseline HP	Baseline HP Standard Deviation
Emotion **Expression** **Behaviors**	**Calkins 1998** (18 months- null [data not provided]; P, N) **Paret 2015** (24 months- null [.14]; P, N)	**Baker 2012** (12 months- positive [.54]; 24 months- positive[.46]; 36 months- positive [.46]; C, N) **Buss 2005** (24 months- positive [.31-.46]; C, N) **Hay 2017** (12 months- positive [.05]; C, N) **Provost, 1979** (12 months- positive [.45-.54]; C, Y)	**Bohlin 1993** (12 months- positive [.37-.64]; C, Y)	**Calkins 1998** (18 months- null [data not provided]; P, N) **Garcia Coll 1984** (21 months- null [data not provided]; P, Y) **Stifter 1996** (18 months- positive [.30]; P, N)	**Garcia Coll 1984** (21 mo- null [data not provided]; P, Y)

Emotion Regulation Behaviors		**Kagan 1989** (14 and 20 months- null [data not provided]; C, N)	**Spangler 1993** (12 months- null [data not provided]; C, Y)	**Burgess 2003** (24 months- null [.12]; P, N) **Calkins 1992** (24 months- null [data not provided]; P, N)	

Note. Cohen's D was calculated for the results available, where no data is available, grey shading is used. Results displayed as (*Participant Age*, *Direction of association* (positive, negative) [effect size]); *Time of measurement*: Concurrent (C)- heart rate and behaviour measured concurrently, Predictive (P)- heart rate or behaviour collected before the other measure, *Epochs*: Yes (Y)- behaviour or cardiovascular measure analysed in reactivity and/or regulation phases; No (N)- behaviour and cardiovascular not analysed in reactivity and/or regulation phases (collapsed across baseline or reactivity and regulation phases).

Table 4b. Summary of study findings (patterns of results) for heart rate (HR) and heart period (HP) with Behaviors (Emotion Expression, Emotion Regulation).

Behavioural Coding Scheme	Baseline HR	Task HR	HR Change Score	Baseline HP	Baseline HP Standard Deviation
Emotion Expression Behaviors	**Weak** (2 studies; N= 106; 18-24 months)	**Positive** (6 studies; n = 620; 12, 18, 24-36 months)	**Positive** (1 study; n = 31; 12 months)	**Unclear Direction and Effect Size** (3 studies; N = 290; 18-21 months)	**Weak** (1 study; n= 117; 21 months)
Emotion Regulation Behaviors		**Weak** (1 study; n= 100; 14 and 20 months)	**Weak** (1 study; n= 41; 12 months)	**Weak** (2 studies; N = 224; 24 months)	

Note. Results displayed as: *Overall Pattern Found*: Positive/Negative, Weak= no studies with significant findings; Unclear Direction and Effect Size= studies had differing directionality and magnitude of relationships (*Number of studies, N/n=* number of participants in studies/study, respectively*, Participant Age*). Where no data is available, grey shading is used.

Table 5a. Summary of study findings (effect sizes) for respiratory sinus arrhythmia (RSA) with Behaviors (Emotion Expression, Emotion Regulation).

Behavioural Coding Scheme	Baseline RSA	Task RSA	RSA Change Score
Emotion Expression Behaviors	**Bocknek 2018** (24-31 months- null [-0.0]; N, P)	**Brooker 2010** (24 months- null [data not provided]; N, C)	**Brooker 2010** (24 months- null [.2]; N, C)
	Calkins 1998 (18 months- *distress task*: positive [.29]; *composite distress measure*: null [data not provided]; N, P)	**Buss 2005** (24 months- null [-.15]; N, C)	**Voegtline 2010** (24 months- *fear*: null [-.05]; *frustration:* negative [-.15]; N, P)
	Paret, 2015 (36 months- null [.013]; N, P)	**Kahle 2018** (42 months- *reactivity:* null [-.00- -.02], *recovery:* null [-.08-.00]; Y, C)	
	Stifter 1996 (18 months- positive [.34]; N, P)		
	Voegtline 2010 (24 months- null [-.14]; N, P)		

Emotion Regulation Behaviors			
	Burgess 2003 (24 months- null [.15]; N, P)	**Fox 1989** (14 months- negative [-1.61- -.23]; N, C)	**Augustine 2018** (24 months- negative [-.04]; N, C)
	Calkins 1992 (24 months- null [data not provided]; N, P)	**Rubin 1997** (24 months- negative [-.07]; N, C)	**Calkins 2000** (30 months- null [data not provided]; N, C)
	Liew 2011 (18 months- null [.002], 30 months- null [.002]; Y, P)	**Kahle 2018** (42 months- null [-.00-.01]; Y, C)	**Liew 2011** (18 months- null [.04]; 30 months- negative [-.19]; Y, P)

Note. Cohen's D was calculated for the results available. Results displayed as (*Participant Age*, *Direction of association* (positive, negative), *Magnitude of association* (null, small, medium, large) [effect size]); *Time of measurement*: Concurrent (C)- heart rate and behaviour measured concurrently, Predictive (P)- heart rate or behaviour collected before the other measure, *Epochs*: Yes (Y)- behaviour or cardiovascular measure analysed in reactivity and/or regulation phases; No (N)- behaviour and cardiovascular not analysed in reactivity and/or regulation phases (collapsed across baseline or reactivity and regulation phases).

Table 5b. Summary of study findings (patterns of results) for respiratory sinus arrhythmia (RSA) with Behaviors (Emotion Expression, Emotion Regulation).

Behavioural Coding Scheme	Baseline RSA	Task RSA	RSA Change Score
Emotion Expression Behaviors	**Weak** (4 studies; N= 566; 18-36 months)	**Weak** (3 studies; N= 239; 24-42 months)	**Weak** (2 studies; N= 418; 24 months)
	Positive (2 studies, n= 173; 18 months)		**Negative** (1 study; n= 330; 24 months)
Emotion Regulation Behaviors	**Weak** (3 studies; N= 471; 18-24 months)	**Weak** (1 study, N = 83; 24-42 months)	**Weak** (2 studies; N= 346; 18-30 months)
		Negative (2 studies, N = 243; 14-42 months)	**Negative** (1 study; n= 138; 18 months-30 months)

Note. Results displayed as: *Overall Pattern Found*: Positive/Negative/Weak (near-zero associations); Unclear Direction and Effect Size= studies had differing directionality and magnitude of relationships (*Number of studies, N/n=* number of participants in studies/study, respectively*, Participant Age*). Where no data is available, grey shading is used.

Table 6a. Summary of study findings (effect sizes) for pre-ejection period (PEP) with Behaviors (Emotion Expression, Emotion Regulation)

Behavioural Coding Scheme	Task PEP
Emotion Expression Behaviors	**Buss 2005** (24 months- null [.04]; N, C)
	Kahle 2018 (42 months- *reactivity:* null [-.00], *recovery:* null [-.005-.001]; Y, C)
Emotion Regulatory Behaviors	**Kahle 2018** (42 months- *reactivity:* null/negative [-.00-.005], *recovery:* null [-.00-.001]; Y, C)

Note. Cohen's D was calculated for the results available. Results displayed as (*Participant Age*, *Direction of association* (positive, negative), *Magnitude of association* (null, small, medium, large) [effect size]); *Time of measurement*: Concurrent (C)- heart rate and behaviour measured concurrently, Predictive (P)- heart rate or behaviour collected before the other measure, *Epochs*: Yes (Y)- behaviour or cardiovascular measure analysed in reactivity and/or regulation phases; No (N)- behaviour and cardiovascular not analysed in reactivity and/or regulation phases (collapsed across baseline or reactivity and regulation phases).

Table 6b. Summary of study findings (patterns of results) for pre-ejection period (PEP) with Behaviors (Emotion Expression, Regulation)

Behavioural Coding Scheme	**Task PEP**
Emotion Expression Behaviors	**Weak** (2 studies; N= 151; 24-42 months)
Emotion Regulatory Behaviors	**Unclear Direction and Effect Size** (1 study; n= 83; 42 months)

Note. Results displayed as: *Overall Pattern Found*: Positive/Negative, Small/Medium/Large, No Effect= no studies with significant findings; Unclear Direction and Effect Size= studies had differing directionality and magnitude of relationships (*Number of studies, N/n*= number of participants in studies/study, respectively*, Participant Age*). Where no data is available, grey shading is used.

Chapter 3: Understanding and Filling Gaps in The Literature

The primary aim of the systematic review and narrative synthesis that comprised Study 1 (Chapter 2) was to summarize and evaluate the literature examining the direction and magnitude of the relation between behavioural and cardiac indicators of distress in toddlerhood in different contexts (e.g., fear, frustration, pain). However, upon reviewing the available literature, several challenges were noted in the consistency of the methodology of the studies reviewed. A major concern elucidated in the review was that studies utilized a variety of cardiac indicators (i.e., heart rate [HR], heart period [HP], respiratory sinus arrhythmia [RSA], and pre-ejection period [PEP]) to measure distress in toddlerhood. Additionally, despite Cole and colleagues' (2004) call for the independent measurement of the activated emotion (e.g., observed facial expressions and behaviours) and the resultant regulatory strategy (e.g., soothing, distraction), behavioural coding schemes did not often distinguish between toddlers' behavioural expressions of distress and the behavioural strategies used to regulate from distress.

To make sense of the available literature (i.e., 22 studies, $N = 2504$), the review was organized into cardiac and behavioural categories (i.e., cardiac response type [i.e., HR, HP, RSA, PEP] by behavioural response type [i.e., emotion expression behaviour vs. emotion regulation behaviour]). The results syntheses in Tables 4 to 6 of Chapter 2 revealed that relations were small (Cohen's $D < 0.2$) between emotion expression/regulatory behaviours and baseline HR/HP/RSA, task RSA, and task PEP. However, task HR and emotion expression behaviours were related when concurrently measured during times of distress (standardized effect sizes in the range of $D = .05$ to $D = .54$). As well, relations among these variables were only available for fear and frustration

paradigms, with no research being found in high-distress contexts. We posited that the lack of relation among certain cardiac and behavioural distress indicators might be due to methodological inconsistences. Indeed, the quality of the studies included in the synthesis was generally rated as Low and thus we made a number of concrete suggestions for improving the quality and consistency of research investigating behavioural and cardiac measures of distress in toddlerhood in Chapter 2.

Given the limitations of the studies included in the Chapter 2 (Systematic Review), and the lack of research within high-distress contexts, the goal of the next chapter (Chapter 4, Dissertation Study 2) was to examine the concurrent and reciprocal relations between healthy toddlers' pain-related behavioural distress (i.e., emotion expression behaviours) and cardiac indicators (i.e., HR, RSA) during routine vaccinations at 12 and 18 months, while taking into account the methodological recommendations outlined in Chapter 2. It is only through rigorous methodological standards that we can establish the magnitude and directionality of the relation between distress indicators in toddlerhood. This study used a subsample of a longitudinal cohort in which caregivers and their children were observed during routine well-baby visits across the second year of life (12, 18, and 24 months). To date, 158 and 122 caregiver-toddler dyads have been successfully recruited at the 12-month and 18-month vaccinations, respectively. Chapter 4 provides an integrative understanding of the development of early childhood distress regulation and clarifies the overall direction and magnitude of the relation between emotion expression behaviours and different cardiac indicators of distress during a high-distress situation.

References

Cole, P. M., Martin, S. E., & Dennis, T. A. (2004). Emotion regulation as a scientific construct: Methodological challenges and directions for child development research. *Child Development, 75*(2), 317-333. doi: 10.1111/j.1467-8624.2004.00673.x

Chapter 4: An Examination of the Reciprocal and Concurrent Relations Between Behavioral and Cardiac Indicators of Acute Pain in Toddlerhood [3]

1. Introduction

It is well established that very young children can experience pain, with pain transmission pathways in the brain being fully developed by 22 to 24 weeks of gestation [49]. Exposure to repeated painful experiences in infancy has been reliably linked to altered brain development and pain intensity as well as poor early neurodevelopment and quality of cognitive and motor development [e.g., 48, 54]. A challenge with pain management in young children is that, despite knowing that early painful experiences impact a child's physical and neuropsychological development, there is currently no gold-standard pain indicator because reliable self-report does not occur until approximately 6 to 7 years of age [56].

When infants and young children are hospitalized, in the absence of self-report measures, current clinical pain scoring systems rely on multiple indicators that incorporate behavioral (e.g., facial expression, body movements) and physiological responses (e.g., heart rate, oxymetry) [45]. Although some indicators have been validated in clinical samples [10, 20, 22, 25, 38], scores derived from these indicators have not consistently converged with pain-specific cortical activity [50]. This discrepancy reflects the possibility that behavioral and physiological measures of pain-related distress each

[3] This is the author's version of the published manuscript:
Waxman, J. A., DiLorenzo, M. G., Pillai Riddell, R. P., Flora, D. B., Schmidt, L. A., Garfield, H., Flanders, D., Weinberg, E., & Savolv, D. (2020). An examination of the reciprocal and concurrent relations between behavioral and cardiac indicators of acute pain in toddlerhood. *PAIN, 161(7),* 1518-1531. doi: 10.1097/j.pain.0000000000001840

represent important, unique information about the nociceptive response in infancy and toddlerhood [11].

In most empirical work focusing on underlying physiological components of distress, maturation of the autonomic nervous system is highlighted as fundamental for emotion regulation [46]. Indeed, pain scales that include both behavioral and cardiac indicators are pervasive in the hospital setting. However, little research has examined how behavioral and cardiac responses to acutely painful procedures converge after the first four months of life [58]. Results from the little available research are equivocal with studies finding small-to-moderate positive correlations [e.g., 34, 44] or describing non-significant or divergent responses post-acute pain [e.g., 16, 45]. Longitudinal research examining behavioral pain-related distress responses in infancy found differences in behavioral pain-related distress by 12 months of age, which were posited to be due to trait-like differences in negative affect regulation, distress or pain responding [41]. The sensitivity of cardiac indicators in response to acute pain in later infancy and toddlerhood is unknown. This association is the focus of the current analyses.

Roué and colleagues [45] have called for more research on improving measurement of behavioral and physiological responses to best encompass an infant's pain-related distress. However, the convergence of different indicators first needs to be established in healthy samples to provide a knowledge base [45]. The current study examines the predictive and concurrent within- and between-measure and contextual (i.e., baseline responses, time since last feeding and nap) relations between toddlers' expressed pain behaviors and cardiac responses (i.e., HR, RSA) during 12- and 18-month vaccinations. Predictive and concurrent relations were examined, as recent research has

found that individual differences in expressed emotion are associated with differences in cardiac reactivity and recovery across distressing events (i.e., anger induction) in toddlerhood [26]. The 12- and 18-month ages were investigated separately because cognitive and physical development has been characterized by more differentiated behavioral and physiological responses at 18 months than at 12 months [1, 31]. We hypothesized that preceding expressed pain-related responses (behaviors or cardiac responses) would predict subsequent expressed pain-related responses (behaviors or cardiac responses) within an indicator (e.g. behavior predicting behavior), given that previous research in typically developing infants has found that earlier infant pain behavior is a strong predictor of subsequent infant pain behavior within the immunization context [9]. Small associations have been found between expressed emotion and cardiac indicators of distress (i.e., fear, frustration) measured concurrently (i.e., during the same visit) in toddlerhood [57]. As such, we hypothesized that expressed pain-related responses (behaviors or cardiac responses) would predict subsequent expressed pain-related responses between indicators (e.g. behavior predicting HR). As HR and RSA reflect largely sympathetic and parasympathetic functioning, respectively [4, 6], we hypothesized positive relations between behavior and HR and negative relations between behavior and RSA.

2. Method

2.1 Participants

Ethical approval was obtained through the research ethics review board at the participating university. After agreeing to speak to a researcher about the study,

caregivers were approached by a research assistant who explained the study and then asked them to sign informed consent forms.

The data are part of an on-going longitudinal study in which caregiver-toddler dyads were recruited from two pediatric clinics in the greater Toronto area and observed with a cohort-sequential design during vaccinations over the second year of life (12, 18, and 24 months). Toddlers were recruited at 12 or 18 months of age. Of the 374 families approached for recruitment at 12 or 18 months, 41 were ineligible based on exclusion criteria (i.e., child was hospitalized in a neonatal intensive care unit, was more than three weeks premature, suspected of a developmental delay, had a known heart condition, or the caregiver was not fluent in English). In total, 158 and 122 caregiver-toddler dyads were successfully recruited at the 12-month and 18-month vaccinations, respectively. A total of 72 participants were observed at both the 12- and 18- month vaccinations. Table 1 lists demographic characteristics of the participants included. Overall, participants were healthy, from middle-class families, and had well-educated caregivers.

Participants had diverse cultural backgrounds. Many of the primary caregivers were born in Canada (59%), yet a substantial percentage were born outside of Canada (Asia [23%], Europe [9%], South America [6%], or Australia [2%], United States [1%]). In addition, their rating of acculturation suggested an integrated cultural background, with strong identification with both their heritage culture (a culture that influenced generations of their family) and mainstream Canadian culture (the culture in which they currently live).

2.2 Procedure

Caregivers filled out a short demographic questionnaire before each vaccination appointment. During each vaccination, caregiver-toddler dyads were simultaneously videotaped and connected to equipment to measure their heart rate before and after the child's vaccinations. Based on methodology from a previous longitudinal infant cohort followed during their well-baby visits [41], at both the 12-and 18-month vaccinations, toddlers were observed 1 minute before, immediately after the final needle, 1 minute after the final needle, and 2 minutes after the final needle. Noldus and MindWare technologies were utilized to synchronize acquisition and analysis of the physiological data and video recordings. The dyads were observed with minimal interference from the research team aside from videotaping and the cardiac monitoring procedures. At both the 12- and 18-month vaccinations, caregivers were given a sheet outlining evidence-based pain management strategies (3 P's of Helping your Child during Vaccinations A Parent's Guide: Children over 1 year old [52]).

2.3 Measures

2.3.1 Caregiver demographic information

Caregivers were asked to complete a short demographic questionnaire that asked for caregiver age, relation to the child, self-reported heritage culture, and child age and sex. Caregivers were also asked to report important infant factors that are known to impact physiological indicators [36, 52], such as time since last feeding and since last nap (parent report in minutes).

2.3.2 Pain behaviors

The Face, Legs, Activity, Cry, Consolability coding system (FLACC; [32]) was used to assess the degree of behavioral pain across the vaccination appointments. The degree of behavioral pain was measured with five types of pain behaviors (face, legs, activity, cry, consolability) during seven different epochs (60 to 1 seconds prior to the first needle [FLACCB]; 0 to 29 seconds immediately after the last needle [FLACC0]; 30 to 59 seconds after the last needle [FLACC1]; 60 to 89 seconds after the last needle [FLACC2]; 90 to 119 seconds after the last needle [FLACC3]; 120 to 149 seconds after the last needle [FLACC4]; and finally, 150 to 179 seconds after the last needle [FLACC5]). Each behavior was scored with a 0 to 2 scale (e.g., on the Face scale, no expression or smile is scored 0, occasional observations of certain facial expressions (i.e., grimace, frown), or the child being withdrawn is scored 1, and constant frown, clenched jaw, or quivering chin is scored 2), resulting in possible total scores between 0 and 10 for each epoch. There were no significant differences between the two 30-second baseline epochs, and so they were averaged to provide a more robust baseline indicator. Moderate to high concurrent validity as well as item-total and inter-rater reliability have been demonstrated for FLACC scores in the acute pain context [33]. To ensure high reliability, coders were trained by a primary FLACC coder. A total of 20% of the sample was reliability-coded throughout the coding process, with unreliable codes (i.e., intraclass correlation for a given epoch below 0.8) being consensus coded with the primary and reliability coders present. This is a rare occurrence as inter-rater reliability between the coders was high (intraclass correlations between 0.9 and 0.93). The coders were blind to the study hypotheses.

2.3.3 Cardiac indicators: Heart rate and respiratory sinus arrhythmia

Cardiac data were collected continuously using MindWare ambulatory monitors (MW 1000A) at a sampling rate of 500 Hz. Three adhesive electrodes collected electrocardiography (ECG), with one electrode placed above the right shoulder blade, one electrode placed on the bottom-most left rib, and a ground electrode placed on the bottom-most right rib. Using MindWare Biolab 3.3, ECG signals were continuously acquired. ECG data were edited in MindWare HRV 3.1.5, with heart rate (HR) computed through identification of R-waves, and spectral analysis of the ECG data being used to compute respiratory sinus arrhythmia (RSA) [4]. We used a frequency band of 0.24 to 1.04 Hz to quantify RSA within the range of spontaneous respiration in young children [22]. To ensure high reliability, coders were trained by an experienced primary coder. A total of 20% of the sample was reliability coded throughout the coding process, with unreliable codes (i.e., intraclass correlation for a given epoch below 0.9) being recoded after consultation with the primary coder. The coders were blind to the study hypotheses and inter-rater reliability between the coders was high (intraclass correlations between 0.95 and 0.99).

Trained coders identified any misidentified R-waves from the raw physiological data. Editing issues (e.g., cutting segments of data, identifying R-waves on data with artifact) were addressed and corrected in consultation with the experienced primary coder. In the case of artifact, the decision to include the data was made on an epoch-by-epoch basis in consultation with the primary coder. The primary reason for excluding an epoch of HR/RSA data was serial missing R-waves (where a “midbeat” could not be

estimated). In all cases, the key decision rule was whether edited epochs were consistent with the individual's other portions of data. Table 2 provides a breakdown of the reasons toddlers' HR/RSA data were not used, that were not a result of editing challenges. The amount of artifact editing did not exceed 5% and did not systematically relate to any of the study measures.

Cardiac values (i.e., HR, RSA) were calculated during seven different epochs (60 to 0 seconds prior to the first needle [HRB, RSAB]; 0 to 30 seconds immediately after the last needle [HR0, RSA0]; 30 to 60 seconds after the last needle [HR1, RSA1]; 60 to 90 seconds after the last needle [HR2, RSA2]; 90 to 120 seconds after the last needle [HR3, RSA3]; 120 to 150 seconds after the last needle [HR4, RSA4]; and 150 to 180 seconds after the last needle [HR5, RSA5]). Because there were no significant differences between two 30-second baseline epochs, they were collapsed to provide a more robust baseline indicator. Inclusion of a 60-second baseline epoch is consistent with the Task Force standards of measurement, physiological interpretation, and clinical use of HRV data [35, 53], which states that approximately one minute of data is needed to assess the high frequency components of HRV (i.e., RSA). Heart rate indicators significantly differed across 30-second post-needle epochs, and thus no post-needle epochs were combined.

2.4 Analysis Plan

To examine reciprocal influences on toddler's expressed pain behaviors and cardiac responses (i.e., HR, RSA) in the 12- and 18-month vaccination contexts, four autoregressive cross-lagged path models [see 28 for review of the statistical approach]

(Figures 1-4) were estimated using structural equation modeling software using a robust full-information maximum likelihood estimator (FIML) to incorporate incomplete cases and account for the degree of non-normality in the data. These models were specified so that for both toddlers' behavioral pain and cardiac responses, three types of relations were examined simultaneously: (1) *Predictive Within-Measure:* the prediction of behavioral pain response (or cardiac response) from the behavioral pain response (or cardiac response) that directly preceded it (e.g., RSA immediately post-needle [RSA0] predicting RSA 30-seconds following the needle [RSA1]); (2) *Predictive Between-Measure*: the prediction of a behavioral pain response (or cardiac response) from the cardiac response (or behavioral pain response) that directly preceded it (e.g., HR immediately post-needle [HR0] predicting behavioral pain 30-seconds following the needle [FLACC1]); (3) *Concurrent (Residual) Between-Measure*: the concurrent residual relations between behavioral pain response and cardiac responses at baseline and each of the six post-needle epochs, after controlling for their predictors in the model (e.g., behavioral pain response immediately following the last needle [FLACC0] with RSA immediately following the last needle [RSA0], after accounting for baseline variables).

A final analysis examined *contextual factors* that may impact the relation between behavioral pain and cardiac responses. Specifically, baseline responses (i.e., baseline behavioral pain responses, baseline HR, and baseline RSA) were included in the initial autoregressive cross-lagged models as covariates, given that the *Law of Initial Value* asserts that the size of a psychophysiological response depends on the initial baseline level of the measure [5]. Additionally, level of arousal has recently been shown to be a determinant of pain-related brain activity [24]. Calculation of baseline responses was

outlined in sections 2.3.2 and 2.3.3. Time since last feeding and since last nap (in minutes) were also included in the initial model as covariates as these factors are known to impact infant physiology [36, 58].

4. Results

Tables 3 and 4 present the means and SDs of all variables in the 12- and 18-month models and Tables 5 to 8 present the correlations among all variables in the 12- and 18-month models. Because FIML was utilized, the models were fitted to 147 and 122 participants at 12 and 18 months, respectively.

The relations between toddlers' behavioral and cardiac responses during 12-month vaccinations

4.1 Relations between pain behaviors and heart rate

The autoregressive cross-lagged path model in Figure 1 fit the data adequately (CFI= .94; RMSEA = .08). Standardized estimates of significant paths are reported in Figure 1 and all standardized and unstandardized estimates are reported in Table 9.

Predictive within-measure. Across the vaccination period at 12 months, each post-needle behavioral response significantly positively predicted the subsequent behavioral response (standardized *B*s = 0.63 to 0.72), and each post-needle HR response significantly positively predicted the subsequent HR response (standardized *B*s = 0.70 to 0.91).

Predictive between-measure. Higher HR pre-needle (HRB) significantly predicted a lower behavioral pain response immediately following the vaccination (FLACC0) (standardized *B*= -0.19, *p* = .05). As well, higher HR 90-seconds following

the vaccination (HR3) significantly predicted a higher behavioral pain response 120-seconds following the vaccination (FLACC4) (standardized B= 0.23, p = .01). No other HR epoch significantly predicted behavioral pain scores across the vaccination period. Next, a higher behavioral pain response immediately following the vaccination (FLACC0) significantly predicted toddlers' HR 30-seconds following the vaccination (HR1) (standardized B= 0.25, p < .001). None of the other six behavioral pain response epochs significantly predicted subsequent HR responses.

Concurrent (residual) between-measure. HR and behavioral pain responses remained significantly positively related controlling for their predictors in the model at each epoch (residual rs = .42 to .58); however the concurrent residual relations were not significant immediately (residual r = .12, p = .19) or 60-seconds (residual r = .23, p = .09) following the vaccination.

Contextual factors. At 12 months, baseline pain behaviors (FLACCB) significantly predicted subsequent pain behaviors immediately (FLACC0) following the vaccination (standardized B = 0.40, p < .001). Time since last nap (standardized B = -0.06, p = .44) and last feeding (standardized B = 0.05, p = .46) did not significantly predict pain behaviors (FLACC0) immediately following the vaccination. Baseline HR (HRB) significantly predicted HR immediately (HR0) following the vaccination (standardized B= 0.47, p < .001). Time since last nap (standardized B = -0.00, p = .98) and last feeding (standardized B = -0.02, p = .78) did not significantly predict HR (HR0) immediately following the vaccination.

4.2 Relations between pain behaviors and respiratory sinus arrhythmia

The autoregressive cross-lagged path model in Figure 2 fit the data adequately

(CFI= .88; RMSEA = .08). Standardized estimates of significant paths are reported in Figure 2 and all standardized and unstandardized estimates are reported in Table 10.

Predictive within-measure. Across the vaccination period at 12 months, each post-needle behavioral response significantly positively predicted the subsequent behavioral response (standardized *B*s = 0.67 to 0.76), and each post-needle RSA response significantly positively predicted the subsequent RSA response (standardized *B*s = 0.30 to 0.57).

Predictive between-measure. Higher RSA 60-seconds following the vaccination (RSA2) significantly predicted a higher behavioral pain response 90-seconds following the vaccination (FLACC3) (*B*= 0.16, *p*= .02). No other RSA epoch significantly predicted behavioral pain scores across the vaccination period. Next, a higher behavioral pain response pre-needle (FLACCB) significantly predicted lower RSA immediately following the vaccination (RSA0) (standardized *B*= -0.22, *p* = .03). As well, a higher behavioral pain response immediately following the vaccination (FLACC0) significantly predicted lower RSA 30-seconds following the vaccination (RSA1) (standardized *B*= -0.23, *p* = .002). None of the other behavioral pain response epochs significantly predicted subsequent RSA responses.

Concurrent (residual) between-measure. RSA and behavioral pain responses remained significantly negatively related controlling for their predictors in the model pre-vaccination, as well as 30- and 120-seconds post-vaccination (residual *r*s = -.25 to -.41). The concurrent residual relations were not significant immediately (residual *r* = .09, *p* = .35), 60 (residual *r* = -.06, *p* = .49), 90 (residual *r* = -.18, *p* = .09), or 120 seconds (residual *r* = -.03, *p* = .77) following the vaccination.

Contextual factors. At 12 months, baseline pain behaviors significantly predicted subsequent pain behaviors immediately (FLACC0) following the vaccination (standardized $B = 0.27$, $p < .001$). Time since last nap (standardized $B = -0.05$, $p = .54$) and last feeding (standardized $B = 0.04$, $p = .46$) did not significantly predict pain behaviors (FLACC0) immediately following the vaccination. Baseline RSA did not significantly predict RSA immediately (RSA0) following the vaccination (standardized $B = -0.06$, $p = .47$). Time since last nap (standardized $B = -0.01$, $p = .95$) and last feeding (standardized $B = 0.04$, $p = .66$) did not significantly predict RSA (RSA0) immediately following the vaccination.

The relations between toddlers' behavioral and cardiac responses during 18-month vaccinations

4.3 Relations between pain behaviors and heart rate

The autoregressive cross-lagged path model in Figure 3 fit the data adequately (CFI= .96; RMSEA = .07). Standardized estimates of significant paths are reported in Figure 3 and all standardized and unstandardized estimates are reported in Table 11.

Predictive within-measure. Across the vaccination period at 18 months, each post-needle behavioral response significantly positively predicted the subsequent behavioral response (standardized Bs = 0.62 to 0.85) and each post-needle HR response significantly positively predicted the subsequent HR response (standardized Bs = 0.72 to 0.91).

Predictive between-measure. No HR epoch significantly predicted subsequent behavioral pain scores across the vaccination period. However, a higher behavioral pain response immediately following the vaccination (FLACC0) significantly predicted

toddlers' HR 30-seconds following the vaccination (HR1) (standardized B= 0.20, p = .001). No other behavioral pain response epochs significantly predicted subsequent HR responses.

Concurrent (residual) between-measure. HR and behavioral pain responses remained significantly positively related controlling for their predictors in the model at each epoch (residual rs = .37 to .66).

Contextual factors. At 18 months, baseline pain behaviors (FLACCB) significantly predicted subsequent pain behaviors immediately (FLACC0) following the vaccination (standardized B = 0.45, p < .001). Time since last nap (standardized B = -0.10, p = .34) and last feeding (standardized B = 0.06, p = .45) did not significantly predict pain behaviors (FLACC0) immediately following the vaccination. Baseline HR (HRB) significantly predicted HR immediately (HR0) following the vaccination (standardized B= 0.65, p < .001). Time since last nap (standardized B = -0.13, p = .07) and last feeding (standardized B = -0.03, p = .72) did not significantly predict HR (HR0) immediately following the vaccination.

4.4 Relations between pain behaviors and respiratory sinus arrhythmia

The autoregressive cross-lagged path model in Figure 4 fit the data adequately (CFI= .93; RMSEA = .08). Standardized estimates of significant paths are reported in Figure 3 and all standardized and unstandardized estimates are reported in Table 12.

Predictive within-measure. Across the vaccination period at 18 months, each post-needle behavioral response significantly positively predicted the subsequent behavioral response (standardized Bs = 0.67 to 0.84) and each post-needle RSA response

significantly positively predicted the subsequent RSA response (standardized *B*s = 0.40 to 0.81).

Predictive between-measure. No RSA epoch significantly predicted subsequent behavioral pain scores across the vaccination period. A higher behavioral pain response immediately following the vaccination (FLACC0) significantly predicted lower RSA 30-seconds following the vaccination (RSA1) (standardized B= -0.16, p = .02). None of the other behavioral pain response epochs significantly predicted subsequent RSA responses.

Concurrent (residual) between-measure. RSA and behavioral pain responses remained significantly negatively related controlling for their predictors in the model pre-vaccination and 60-seconds post-vaccination (residual *r*s = -.31 to -.37). The concurrent residual relations were not significant immediately (residual r = -.07, p = .38), 60 (residual r = -.26, p = .06), 90 (residual r = -.10, p = .39), 120 (residual r = -.04, p = .69), or 150 seconds (residual r = -.15, p = .18) following the vaccination.

Contextual factors. At 18 months, baseline pain behaviors significantly predicted subsequent pain behaviors immediately (FLACC0) following the vaccination (standardized B = 0.42, p < .001). Time since last nap (standardized B = -0.11, p = .30) and last feeding (standardized B = 0.08, p = .30) did not significantly predict pain behaviors (FLACC0) immediately following the vaccination. Baseline RSA significantly predicted RSA immediately (RSA0) following the vaccination (standardized B= 0.39, p < .001). Time since last nap (standardized B = -0.06, p = .50) and last feeding (standardized B = -0.05, p = .61) did not significantly predict RSA (RSA0) immediately following the vaccination.

5. Discussion

To our knowledge, this is the first longitudinal study of typically developing toddlers (i.e., 12 and 18 months) to examine the convergence of commonly utilized behavioral and cardiac indicators (i.e., HR, RSA) of acute pain-related distress. This study is novel in that the analyses examined predictive within-measure, predictive between-measure, and concurrent (residual) between-measure relations among behavioral and cardiac indicators of acute pain-related distress at 12 and 18 months. Additionally, contextual factors were investigated to determine whether baseline responses (i.e., baseline behavioral pain scores, HR, and RSA) or time since last feeding or nap predict behavior or physiology post-needle. The following discussion focuses on developmental trends based on changes in the strength of relation within- or between-measures from 12 to 18 months. Differences in the relation based on cardiac indicator (i.e., HR, RSA) and timing of measurement (i.e., pain reactivity versus regulation) are also discussed. In the following discussion, FLACC0, HR0, and RSA0 reflect the peak pain-related distress response that occurs immediately following the needle (reactivity), while subsequent FLACC, HR, and RSA epochs (i.e., 1-5) capture the process of pain-related distress regulation from the needle. This section ends with limitations of our research and implications for future research and clinical practice.

5.1 Within-measure relations

Within-measure relations across indicators (i.e., behavior, HR, and RSA) were positive, with each post-needle behavioral or cardiac response positively predicting the subsequent behavioral or cardiac response. These findings confirm past research suggesting that FLACC has high stability within the acute pain context in toddlerhood

[32], and cardiac indicators are stable within conditions [15]. Regarding developmental trends inferred by changes in the strength of relations, there were moderate to strong within-measure relations for behavioral and HR responses across ages. However, within-measure relations for RSA were stronger at 18 months than at 12 months of age. Previous research [42] has found age-related changes in RSA and noted that this reflects increased autonomic complexity across development. Indeed, weaker within-measure relations for RSA were found compared to behavioral pain scores and HR across ages, likely reflecting that RSA captures additional bio-psycho-social aspects of the toddler's pain experience [42]. Additionally, the strongest within-measure auto-correlations for RSA were found within the regulatory epochs (i.e., RSA1 to RSA5), which is consistent with theories suggesting that RSA is more reflective of parasympathetic versus sympathetic influence [4].

5.2 Predictive between-measure relations

Across ages and cardiac indicators, behavioral pain responses immediately post-needle positively predicted HR and negatively predicted RSA 30-seconds post-needle. These results suggest that expressed pain behaviors may have a regulating or dysregulating impact on toddler physiology in the initial reactivity period of the vaccination, which affects the entire regulatory phase through other within-measure and concurrent relations. This predictive relation between behavioral pain response and RSA represents vagal influence being withdrawn due to increased sympathetic (i.e., behavioral pain response) activation [6]. In addition to these consistent findings across 12 and 18 months, there were significant pathways at 12 months of age. Specifically, higher baseline HR predicted lower behavioral pain responses immediately post-needle, while

higher behavioral pain responses at baseline predicted lower RSA immediately post-needle. These divergent relations (i.e., HR predicting behavior, behavior predicting RSA) within the baseline and reactivity epochs are consistent with past research on toddler distress regulation, where one stress response compensates for another [30, 47, 55]. As well, HR and RSA responses 30 seconds post-needle positively predicted behavioral pain responses 60 seconds post-needle. These results suggest that toddler physiology may predict subsequent behavior, but only within the regulatory phase post-needle.

5.3 Concurrent (residual) between-measure relations

Overall, there were concurrent associations between behavioral pain responses and each of HR and RSA at 12 and 18 months, over and above their predictive autoregressive and cross-lagged effects. As expected, behavioral and HR responses were positively related and behavioral and RSA responses were negatively related. Regarding developmental differences, behavioral pain responses and HR were consistently related across the pre- and post-vaccination periods at 18 months, but not at 12 months. HR and behavioral pain responses were not significantly concurrently related immediately or 60 seconds following the vaccination at 12 months, over and above the contextual baseline factors. As such, it is possible that contextual baseline factors and previous behavioral pain responses and physiology more strongly predict certain post-vaccination pain-related distress responses than other indicators measured concurrently. Alternatively, behavioral pain responses and HR may reflect unique aspects of the nociceptive response at 12 months of age. Indeed, Roué and colleagues [45] found that some typically developing neonates presented with acute responses measured by physiological indicators whereas others presented with prolonged stressful responses characterized by expressed pain

behaviors.

Comparing the concurrent relation between behavioral pain responses and each of HR and RSA, HR had stronger and more consistent relations with behavior than RSA. These differences in magnitude of the relation between behavioral and cardiac pain-related distress indicators are consistent with studies investigating pain responses in younger preterm infants [29] and in non-pain contexts [2, 7, 8, 19, 21, 26, 43]. The strongest relations between behavioral pain responses and HR were within the reactivity phase, whereas the strongest associations between behavioral pain responses and RSA were within the regulation phases. These results confirm classical theories suggesting that the sympathetic nervous system is most associated with distress [14], whereas RSA may be more related to regulatory strategies that are aligned with the parasympathetic nervous system [25].

5.4 Contextual factors

Baseline responses consistently predicted future pain scores and physiology within the post-needle period at both 12 and 18 months, whereas time since last feeding and nap were not significantly related to behavioral pain scores and physiology at either age. Regarding developmental trends, baseline RSA only emerged as a significant predictor of future RSA scores within the post-vaccination period at 18 months. The emergence of baseline RSA as a significant predictor of future RSA scores at 18 months may reflect the many regulatory abilities and skills that emerge in toddlerhood [11]. Overall, these results are in line with the Law of Initial Value [5] but extend the theory to baseline behavioral pain scores in addition to physiology.

5.5 Limitations

Despite having sample size comparable to other studies of neonatal pain assessment [3, 12, 27, 37, 45, 50], generalizability of the current results is affected by the high education level of our participants. As well, our study included healthy toddlers born full-term who underwent a standardized acutely painful procedure, which limits generalizability to non-healthy neonates or premature infants who must undergo multiple acute painful or stressful procedures.

5.6 Conclusions: Clinical and Research Implications

In this study, normative data were provided regarding how commonly utilized behavioral and cardiac pain-related distress indicators are related within the acute pain context in toddlerhood. Our findings suggest that compared to RSA, HR is more strongly related and closely linked to behavioral pain indicators in toddlerhood. The shared yet unique variance between HR and pain behaviors suggests that these indicators would be complimentary measures of pain in toddlers, and confirms practice in neonatology where multimodal approaches to pain in those nonverbal children is the evidence-based bedside approach [e.g., 17]. Indeed, reliable differences in behavioral pain-related distress have been found in 12 month old infants [41], with environmental factors (i.e., attachment relationship with primary caregiver) leading some typically developing infants to exhibit less behavioral distress following vaccinations [18]. Based on the unique variance shared between HR and pain behaviors found in the current study, multimodal approaches that incorporate both cardiac and behavioral indicators are hypothesized to more accurately capture infant pain-related distress, especially for infants with dampened behavioral pain

responses. However, there are several challenges related to utility of current infant pain assessment tools (i.e. physiological, cortical, behavioral indicators) [39]. There is a lack of specificity to pain for physiological measures [39], behavioral indicators (i.e., facial actions, body movements, cry) are not reliably associated with parental judgments of infant pain [40], and interventions to alleviate pain have been shown to reduce behavioral pain scores without altering nociceptive brain and spinal cord activity [51]. Indeed, there is consensus among basic and clinical scientists that cortical, physiological, and behavioral measures of pain do not consistently converge [39]. Therefore, this research supports that use of HR *in conjunction* with expressed behavioral pain and other physiological and cortical indicators (e.g., oxygen saturation, electroencephalography, skin conductance, cortisol) to properly encapsulate the nociceptive response in toddlers. Although positive within-measure relations were found among behavioral and cardiac indicators across ages, behavioral and cardiac indicators of pain-related distress are not consistently concurrently related to each other after accounting for the within- and between-measure predictors as well as contextual factors (i.e., baseline responses). Given the inconsistent concurrent relations between indicators after accounting for predictive associations, pain scores may be misestimated if pain indicators are only utilized within the initial reactivity phase (e.g., 30-seconds post-needle) without accounting for these contextual factors (i.e., baseline responses). As mentioned previously, these inconsistent concurrent findings need to be also contextualized by the reciprocal relation between behavioral and cardiac indicators across the 12- and 18-month vaccinations. Specifically, across ages, behavioral pain responses immediately post-needle significantly predicted HR and RSA 30-seconds post-needle. It is important to consider the impact of toddlers'

initial behavioral response on their physiological and behavioral regulation from pain-related distress. Covariates included in the models may also account for weaker concurrent residual relations, as baseline behavioral pain scores and cardiac indicators significantly predicted subsequent pain scores and physiology within the post-vaccination period. Pain assessment tools should measure behavioral and physiological responses at baseline as well as across the initial reactivity and regulatory phases to provide a more holistic understanding of the toddler's pain experience because both indicators predict future pain scores.

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Figure 1. Autoregressive cross-lagged path model: relations between behavioural pain scores and heart rate during 12-month vaccination.

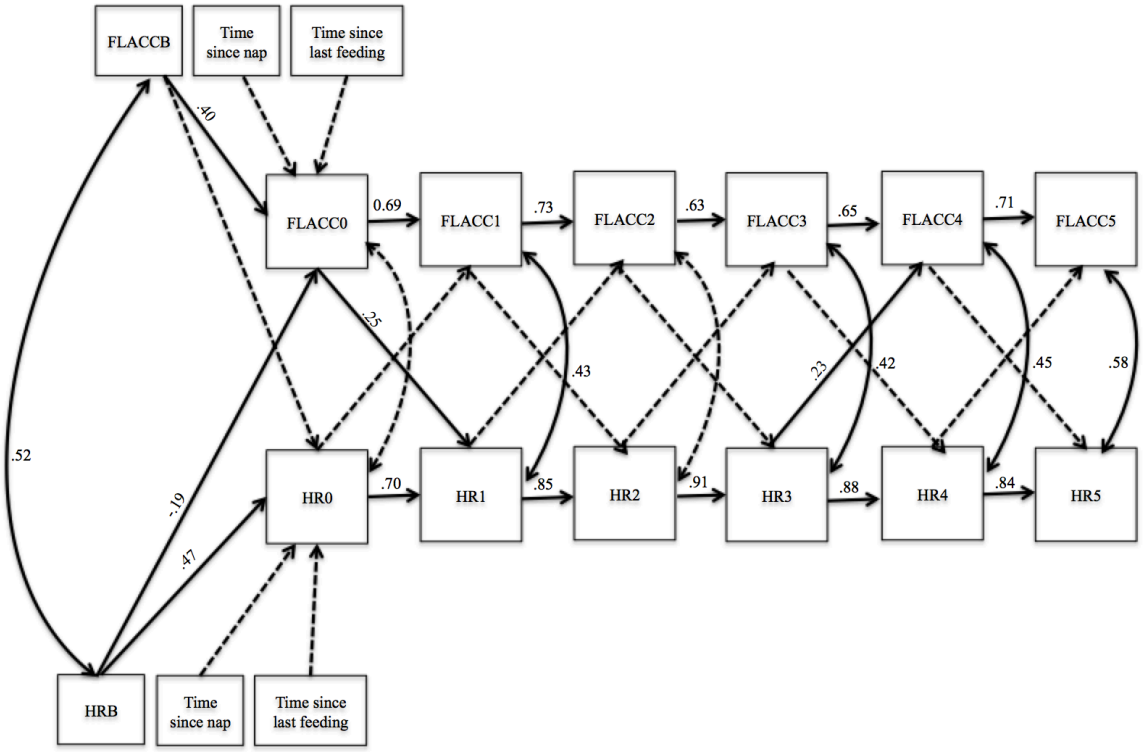

Figure 2. Autoregressive cross-lagged path model: relations between behavioural pain scores and respiratory sinus arrhythmia during 12-month vaccination.

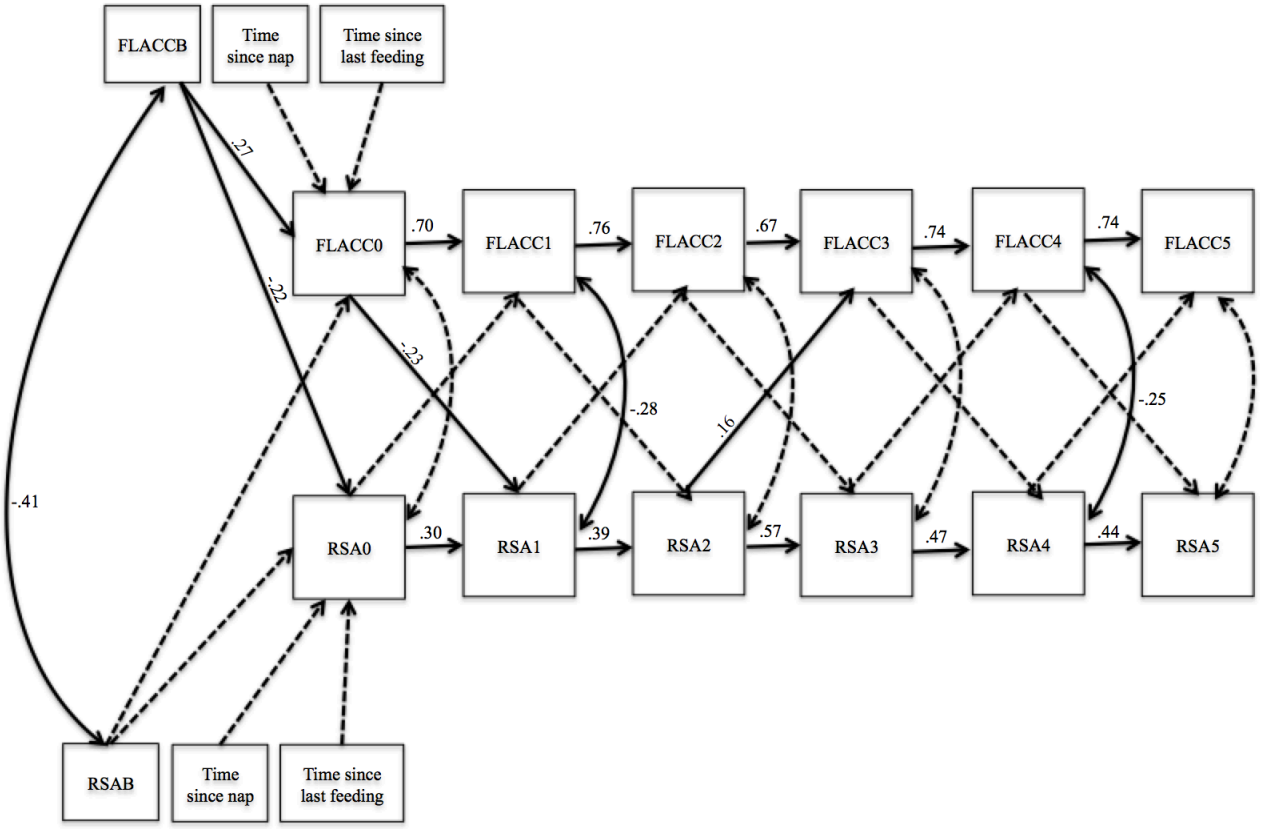

Figure 3. Autoregressive cross-lagged path model: relations between behavioural pain scores and heart rate during 18-month vaccination.

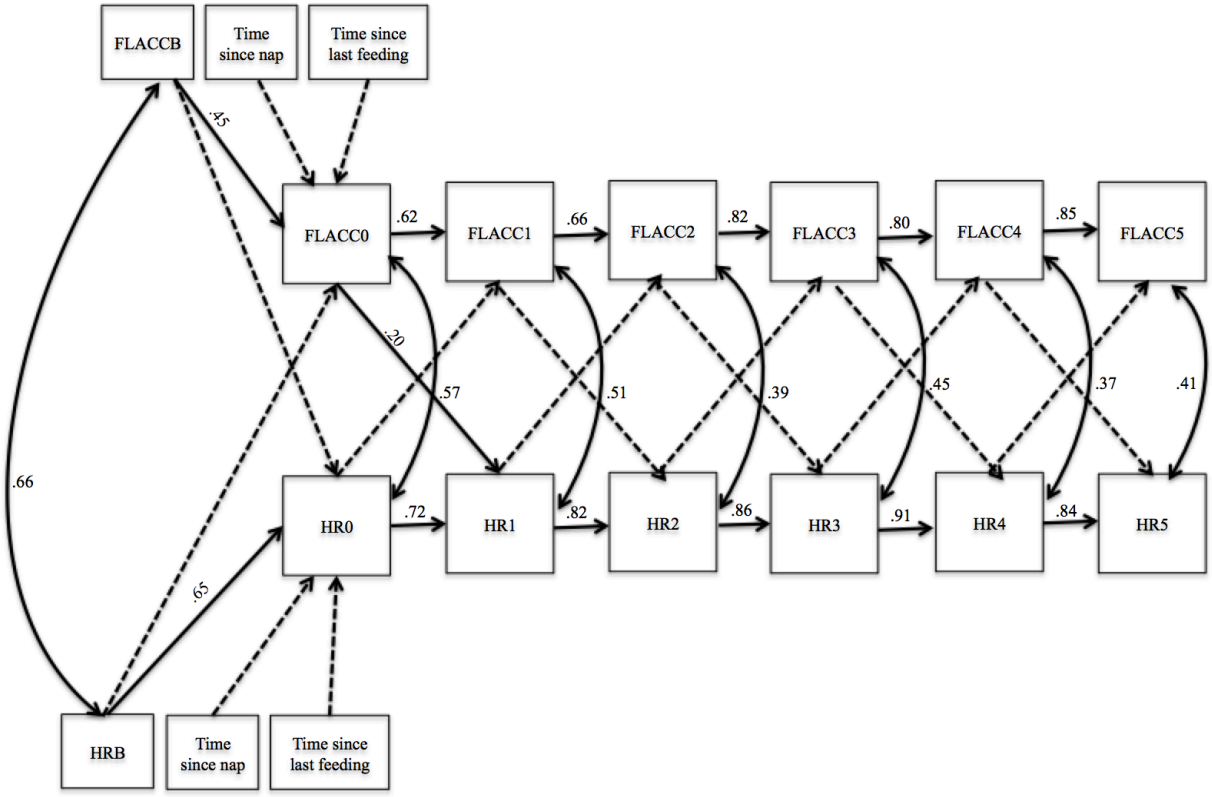

Figure 4. Autoregressive cross-lagged path model: relations between behavioural pain scores and respiratory sinus arrhythmia during 18-month vaccination.

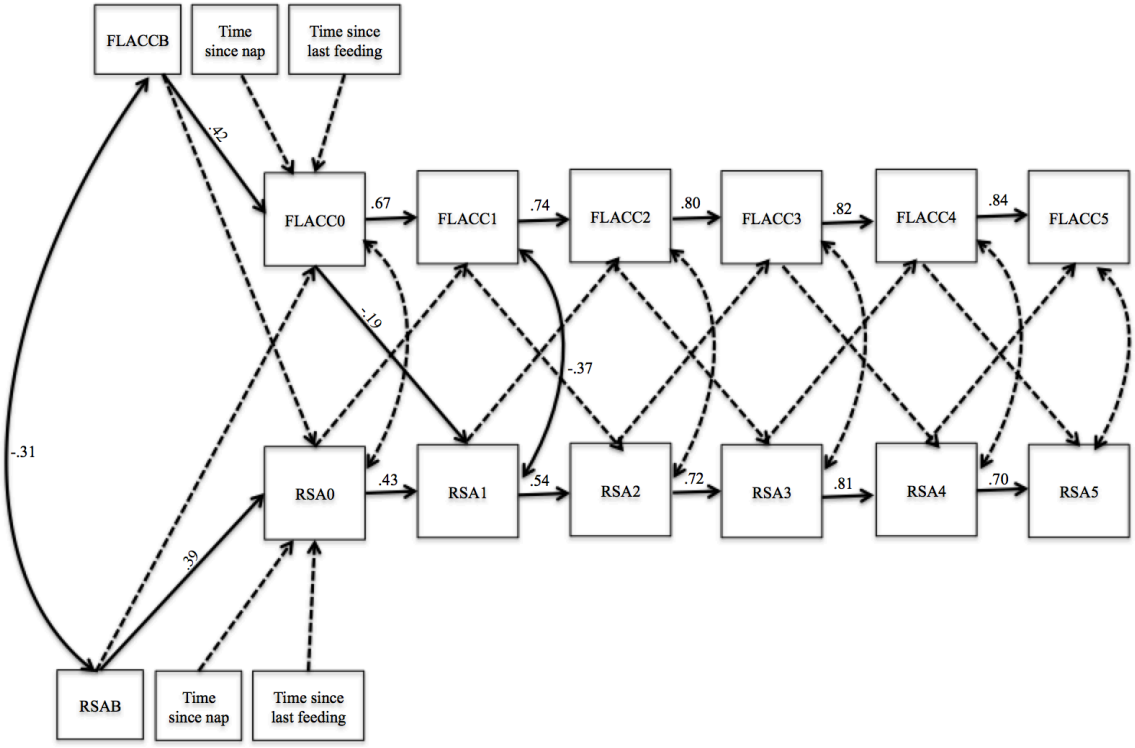

Table 1. Demographic information.

	Frequency (%)	
	12 months (n=158)	18 months (n= 122)
Sex of Infant		
Male	81 (54.4)	68 (58.1)
Female	68 (45.6)	49 (41.9)
Relationship to infant		
Mother	127	98
Father	12	17
Other	1	1
Education		
Graduate School/Professional Training	72	55
University Graduate (4 Years)	42	39
Partial University (at least 1 year)	4	3
Trade School/Community College	15	11
High School Graduate	2	1
Age	35.91 (5.19)	36.63 (6.19)
Acculturation status		
Way of life reflects heritage culture	6.63 (2.79)	5.66 (2.43)
Way of life reflects mainstream North American/Canadian culture	7.62 (2.28)	7.75 (1.66)

Note. Certain data points were missing, and as a result do not add to the total sample size at 12 and 18 months.

Table 2. Reasons for cardiac data not being usable.

	Frequency	
	12 months	18 months
Complete	129	101
Device Malfunction	19	15
Timing	5	4
No needle	2	2
Blocked	1	0
Electrode Removed	1	4
Declined Stickers	0	1
Lost to follow-up	0	23
Refused	0	14

Table 3. Means and standard deviations of variables used for 12-month models.

	Mean	SD	Scale Range
Time since last feeding (minutes)	103.97	74.94	0-420
Time since last nap (minutes)	110.40	76.71	0-390
FLACCB	1.68	1.83	0-8
FLACC0	7.30	1.55	1-10
FLACC1	6.28	2.5	0-10
FLACC2	5.21	2.86	0-10
FLACC3	4.2	2.85	0-9.5
FLACC4	3.39	2.84	0-9.5
FLACC5	3.03	2.85	0-9
HRB	129.92	13.94	81.17-180.48
HR0	150.82	21.40	84.54-207.41
HR1	154.59	22.90	77.45-191.58
HR2	146.06	20.90	85.89-195.91
HR3	140.36	18.66	84.12-193.89
HR4	136.38	17.53	80.03-183.94
HR5	134.07	17.08	77.54-191.58
RSAB	3.89	1.18	1.12-7.85
RSA0	4.23	2.23	0-9.69
RSA1	2.99	1.54	0-6.71
RSA2	3.43	1.32	0.11-6.81
RSA3	3.87	1.23	0.64-7.32
RSA4	3.81	1.13	0.88-6.36
RSA5	3.93	1.31	1.04-8.66

Note. FLACC= Face Legs Cry Consolability scale; HR= Heart Rate (beats per minute); RSA= Respiratory Sinus Arrhythmia.

Table 4. Means and standard deviations of variables observed at 18-month models.

	Mean	SD	Scale Range
Time since last feeding (minutes)	100.12	61.16	0-300
Time since last nap (minutes)	148.83	85.70	0-420
FLACCB	2.81	2.71	0-9.75
FLACC0	6.46	2.11	0-9
FLACC1	5.10	2.91	0-9.38
FLACC2	4.37	2.90	0-9
FLACC3	3.81	2.84	0-9.17
FLACC4	3.38	2.82	0-9.5
FLACC5	3.34	2.02	0-10
HRB	132.01	17.5	102.78-186.02
HR0	147.80	23.27	98.66-198.20
HR1	146.74	23.65	97.86-193.38
HR2	143.02	21.70	100.64-188.08
HR3	136.20	20.39	105.18-191.65
HR4	133.68	18.48	99.82-179.83
HR5	132.24	16.53	103.18-170.58
RSAB	4.00	1.26	1.22-7.08
RSA0	4.02	1.92	0-7.58
RSA1	3.45	1.75	0.14-8.15
RSA2	4.43	1.45	0-7.45
RSA3	4.02	1.52	0.18-7.91
RSA4	4.02	1.52	0.49-7.59
RSA5	4.12	1.38	1.12-6.90

Note. FLACC= Face Legs Cry Consolability scale; HR= Heart Rate (beats per minute); RSA= Respiratory Sinus Arrhythmia.

Table 5. Correlations among heart rate variables observed at 12 months.

	1.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Time since last feeding	-	0.11	0.02	0.01	-0.07	-0.15	-0.10	-0.86	-0.13	0.09	0.08	0.11	0.07	0.05	0.05	0.00
2. Time since last nap		-	0.02	0.00	0.02	0.07	0.02	0.06	-0.04	0.23*	0.02	0.25*	0.16	0.05	0.18	0.13
3. HRB			-	0.57*	0.49*	0.53*	0.60*	0.65*	0.60*	0.56*	0.03	0.11	0.14	0.14	0.25*	0.15
4. HR0				-	0.72*	0.61*	0.51*	0.47*	0.42*	0.40*	0.18	0.14	0.20*	0.12	0.15	0.14
5. HR1					-	0.85*	0.74*	0.65*	0.51*	0.39*	0.36*	0.45*	0.42*	0.31*	0.35*	0.30*
6. HR2						-	0.88*	0.75*	0.61*	0.35*	0.30*	0.40*	0.46*	0.32*	0.35*	0.30*
7. HR3							-	0.86*	0.72*	0.27*	0.25*	0.31*	0.37*	0.39*	0.45*	0.32*
8. HR4								-	0.77*	0.20*	0.09	0.22*	0.26*	0.34*	0.53*	0.34*
9. HR5									-	0.23*	0.14	0.15	0.20*	0.22*	0.39*	0.38*
10. FLACCB										-	0.29*	0.34*	0.23*	0.24*	0.27*	0.27*
11. FLACC0											-	0.70*	0.55*	0.38*	0.33*	0.35*
12. FLACC1												-	0.76*	0.54*	0.44*	0.42*
13. FLACC2													-	0.64*	0.49*	0.48*
14. FLACC3														-	0.74*	0.65*
15. FLACC4															-	0.73*
16. FLACC5																-

Note. FLACC= Face Legs Cry Consolability scale; HR= Heart Rate; * Correlation is significant at < 0.05 level (2-tailed).

Table 6. Correlations among respiratory sinus arrhythmia variables observed at 12 months.

	1.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Time since last feeding	-	0.11	0.02	0.01	-0.07	-0.15	-0.10	-0.09	-0.13	0.09	0.08	0.11	0.07	0.05	0.05	0.000
2. Time since last nap		-	-0.14	-0.06	-0.00	0.01	0.08	-0.07	-0.02	0.23*	0.02	0.25*	0.16	0.05	0.18	0.13
3. RSAB			-	0.29*	0.23*	0.40*	0.43*	0.42*	0.58*	-0.42*	-0.16	-0.14	-0.05	-0.04	-0.10	0.03
4. RSA0				-	0.29*	0.23*	0.16	0.28*	0.16	-0.32*	-0.04	0.02	0.03	0.05	0.07	0.01
5. RSA1					-	0.41*	0.43*	0.31*	0.21*	-0.33*	-0.23*	-0.33*	-0.24*	-0.16	-0.08	-0.09
6. RSA2						-	0.57*	0.44*	0.40*	-0.29*	-0.08	-0.16	-0.16	0.06	0.04	0.04
7. RSA3							-	0.47*	0.46*	-0.17	-0.21*	-0.23*	-0.12	-0.07	-0.06	0.00
8. RSA4								-	0.43*	-0.21*	0.00	-0.10	-0.02	0.00	-0.11	0.01
9. RSA5									-	-0.15	-0.01	0.08	0.11	0.07	0.00	0.04
10. FLACCB										-	0.29*	0.34*	0.23*	0.24*	0.27*	0.27*
11. FLACC0											-	0.70*	0.55*	0.38*	0.33*	0.35*
12. FLACC1												-	0.76*	0.54*	0.44*	0.42*
13. FLACC2													-	0.64*	0.49*	0.48*
14. FLACC3														-	0.74*	0.65*
15. FLACC4															-	0.73*
16. FLACC5																-

Table 7. Correlations among heart rate variables observed at 18 months.

	1.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Time since last feeding	-	0.11	0.09	0.06	0.14	0.13	0.06	0.06	0.08	-0.05	0.05	0.10	0.09	0.07	0.08	0.02
2. Time since last nap		-	-0.22	-0.24*	-0.11	0.01	0.07	0.02	-0.02	-0.11	-0.13	-0.07	-0.09	-0.08	-0.10	-0.10
3. HRB			-	0.75*	0.57*	0.46*	0.43*	0.42*	0.57*	0.67*	0.20	0.19	0.20	0.23*	0.18	0.31*
4. HR0				-	0.82*	0.66*	0.58*	0.48*	0.55*	0.59*	0.55*	0.41*	0.36*	0.32*	0.34*	0.36*
5. HR1					-	0.85*	0.66*	0.54*	0.52*	0.56*	0.60*	0.62*	0.56*	0.44*	0.36*	0.36*
6. HR2						-	0.83*	0.74*	0.65*	0.42*	0.48*	0.58*	0.63*	0.50*	0.40*	0.39*
7. HR3							-	0.88*	0.79*	0.26*	0.30*	0.35*	0.46*	0.49*	0.41*	0.39*
8. HR4								-	0.87*	0.21	0.25*	0.23*	0.36*	0.43*	0.46*	0.39*
9. HR5									-	0.31*	0.17	0.16	0.30*	0.41*	0.47*	0.50*
10. FLACCB										-	0.39*	0.40*	0.40*	0.42*	0.43*	0.52*
11. FLACC0											-	0.66*	0.59*	0.41*	0.41*	0.31*
12. FLACC1												-	0.74*	0.57*	0.51*	0.43*
13. FLACC2													-	0.81*	0.67*	0.57*
14. FLACC3														-	0.82*	0.72*
15. FLACC4															-	0.85*
16. FLACC5																-

Note. FLACC= Face Legs Cry Consolability scale; HR= Heart Rate; * Correlation is significant at < 0.05 level (2-tailed).

Table 8. Correlations among respiratory sinus arrhythmia variables observed at 18 months.

	1.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Time since last feeding	-	0.11	-0.03	-0.07	0.02	0.02	0.07	0.15	0.10	-0.05	0.05	0.10	0.09	0.07	0.08	0.02
2. Time since last nap		-	0.10	0.11	0.08	0.02	-0.09	0.02	-0.04	-0.11	-0.13	-0.07	-0.09	-0.08	-0.10	-0.10
3. RSAB			-	0.43*	0.46*	0.43*	0.44*	0.43*	0.59*	-0.32*	-0.02	-0.03	-0.01	-0.06	-0.03	-0.11
4. RSA0				-	0.43*	0.38*	0.46*	0.43*	0.43*	-0.19	-0.12	0.00	-0.04	-0.08	-0.13	-0.15
5. RSA1					-	0.56*	0.43*	0.41*	0.33*	-0.27*	-0.24*	-0.35*	-0.30*	-0.20	-0.14	-0.10
6. RSA2						-	0.69*	0.67*	0.58*	-0.20	-0.24*	-0.27*	-0.36*	-0.31*	-0.23*	-0.22*
7. RSA3							-	0.80*	0.72*	0.00	-0.11	-0.02	-0.12	-0.14	-0.11	-0.12
8. RSA4								-	0.70*	-0.01	-0.03	0.07	0.00	-0.05	-0.05	-0.04
9. RSA5									-	-0.08	0.06	0.12	0.05	-0.06	-0.04	-0.10
10. FLACCB										-	0.39*	0.40*	0.40*	0.42*	0.43*	0.52*
11. FLACC0											-	0.66*	0.59*	0.41*	0.41*	0.31*
12. FLACC1												-	0.74*	0.57*	0.51*	0.43*
13. FLACC2													-	0.81*	0.67*	0.57*
14. FLACC3														-	0.82*	0.72*
15. FLACC4															-	0.85*
16. FLACC5																-

Note. FLACC= Face Legs Cry Consolability scale; RSA= Respiratory Sinus Arrhythmia; * Correlation is significant at < 0.05 level (2-tailed).

Table 9. Estimates for autoregressive cross-lagged path model of relations between behavioural pain scores and heart rate during 12-month vaccination.

	Standardized estimate	Unstandardized estimate	z	*p*
FLACC0				
FLACCB	0.40	0.35	4.13	< 0.001
HRB	-0.19	-0.02	-1.95	0.05
Time since last nap	-0.06	-0.00	-0.77	0.44
Time since last fed	0.05	0.00	0.74	0.46
HR0	3			
HRB	0.47	0.74	4.66	<0.001
FLACCB	0.15	1.75	1.65	0.10
Time since last nap	-0.00	-0.00	-0.03	0.98
Time since last fed	-0.02	-0.01	-0.28	0.78
FLACC1				
FLACC0	0.69	1.12	10.25	<0.001
HR0	0.04	0.01	0.67	0.51
HR1				
HR0	0.70	0.76	11.11	<0.001
FLACC0	0.25	3.80	5.21	<0.001
FLACC2				
FLACC1	0.72	0.83	10.70	<0.001
HR1	0.07	0.01	1.04	0.30
HR2				
HR1	0.85	0.77	18.18	<0.001

FLACC1	0.01	0.11	0.28	0.78
FLACC3				
FLACC2	0.63	0.63	8.66	<0.001
HR2	0.05	0.01	0.56	0.58
HR3				
HR2	0.91	0.82	19.70	<0.001
FLACC2	-0.05	-0.35	-1.28	0.20
FLACC4				
FLACC3	0.65	0.64	8.77	<0.001
HR3	0.23	0.03	2.69	0.01
HR4				
HR3	0.88	0.0.81	13.51	<0.001
FLACC3	-0.03	-0.21	-0.61	0.54
FLACC5				
FLACC4	0.71	0.72	6.87	<0.001
HR4	-0.04	-0.01	-0.38	0.70
HR5				
HR4	0.84	0.82	13.58	<0.001
FLACC4	-0.13	-0.83	-1.22	0.22

Note. FLACC= Face Legs Cry Consolability scale; HR= Heart Rate.

Table 10. Estimates for autoregressive cross-lagged path model of relations between behavioural pain scores and respiratory sinus arrhythmia during 12-month vaccination.

	Standardized estimate	Unstandardized estimate	z	*p*
FLACC0				
FLACCB	0.27	0.23	3.82	<0.001
RSAB	-0.06	-0.08	-0.72	0.47
Time since last nap	-0.05	-0.00	-0.62	0.54
Time since last fed	0.04	0.00	0.74	0.46
RSA0				
RSAB	0.18	0.35	1.38	0.17
FLACCB	-0.22	-0.26	-2.12	0.03
Time since last nap	-0.01	-0.00	-0.06	0.95
Time since last fed	0.04	0.00	0.44	0.66
FLACC1				
FLACC0	0.70	1.12	10.61	<0.001
RSA0	0.05	0.05	0.77	0.44
RSA1				
RSA0	0.30	0.21	3.31	0.001
FLACC0	-0.23	-0.23	-3.10	0.002
FLACC2				
FLACC1	0.76	0.87	13.61	<0.001
RSA1	0.02	0.03	0.26	0.79
RSA2				
RSA1	0.39	0.33	4.00	<0.001

FLACC1	-0.03	-0.01	-0.33	0.74
FLACC3				
FLACC2	0.67	0.67	11.14	<0.001
RSA2	0.16	0.35	2.37	0.02
RSA3				
RSA2	0.57	0.54	6.21	<0.001
FLACC2	-0.03	-0.01	-0.35	0.73
FLACC4				
FLACC3	0.74	0.72	13.04	<0.001
RSA3	-0.03	-0.06	-0.30	0.76
RSA4				
RSA3	0.47	0.43	5.01	<0.001
FLACC3	0.05	0.02	0.61	0.54
FLACC5				
FLACC4	0.74	0.75	12.37	<0.001
RSA4	0.12	0.29	1.51	0.13
RSA5				
RSA4	0.44	0.50	5.31	<0.001
FLACC4	0.05	0.02	0.36	0.72

Note. FLACC= Face Legs Cry Consolability scale; RSA= Respiratory Sinus Arrhythmia.

Table 11. Estimates for autoregressive cross-lagged path model of relations between behavioural pain scores and heart rate during 18-month vaccination.

	Standardized estimate	Unstandardized estimate	z	*p*
FLACC0				
FLACCB	0.45	0.35	4.11	<0.001
HRB	-0.11	-0.01	-0.90	0.37
Time since last nap	-0.10	-0.00	-0.96	0.34
Time since last fed	0.06	0.00	0.75	0.45
HR0				
HRB	0.65	0.85	7.13	<0.001
FLACCB	0.13	1.11	1.70	0.09
Time since last nap	-0.13	-0.03	-1.84	0.07
Time since last fed	-0.03	-0.01	-0.36	0.72
FLACC1				
FLACC0	0.62	0.86	7.08	<0.001
HR0	0.07	0.01	0.76	0.45
HR1				
HR0	0.72	0.75	10.18	<0.001
FLACC0	0.20	2.23	3.32	0.001
FLACC2				
FLACC1	0.66	0.66	8.27	<0.001
HR1	0.13	0.02	1.46	0.14
HR2				
HR1	0.82	0.75	8.43	<0.001

FLACC1	0.04	0.31	0.44	0.66
FLACC3				
FLACC2	0.82	0.82	12.18	<0.001
HR2	-0.00	-0.01	-0.07	0.95
HR3				
HR2	0.86	0.84	11.22	<0.001
FLACC2	-0.05	-0.34	-0.59	0.55
FLACC4				
FLACC3	0.80	0.79	11.34	<0.001
HR3	0.01	0.01	0.48	0.63
HR4				
HR3	0.91	0.81	12.77	<0.001
FLACC3	-0.04	-0.29	-0.70	0.48
FLACC5				
FLACC4	0.85	0.91	17.34	<0.001
HR4	-0.01	-0.00	-0.21	0.83
HR5				
HR4	0.84	0.76	12.02	<0.001
FLACC4	0.05	0.33	0.86	0.39

Note. FLACC= Face Legs Cry Consolability scale; HR= Heart Rate.

Table 12. Estimates for autoregressive cross-lagged path model of relations between behavioural pain scores and respiratory sinus arrhythmia during 18-month vaccination.

	Standardized estimate	Unstandardized estimate	z	*p*
FLACC0				
FLACCB	0.42	0.32	5.60	<0.001
RSAB	0.12	0.20	1.11	0.27
Time since last nap	-0.11	-0.00	-1.03	0.30
Time since last fed	0.08	0.00	1.03	0.30
RSA0				
RSAB	0.39	0.60	4.51	<0.001
FLACCB	-0.06	-0.04	-0.49	0.63
Time since last nap	-0.06	0.00	0.68	0.50
Time since last fed	-0.05	-0.00	-0.51	0.61
FLACC1				
FLACC0	0.67	0.93	10.42	<0.001
RSA0	0.09	0.14	1.23	0.22
RSA1				
RSA0	0.40	0.43	4.50	<0.001
FLACC0	-0.16	-0.19	-2.38	0.02
FLACC2				
FLACC1	0.74	0.73	12.36	<0.001
RSA1	-0.06	-0.02	-0.20	0.84
RSA2				
RSA1	0.44	0.54	4.79	<0.001

FLACC1	-0.03	-0.06	-0.60	0.55
FLACC3				
FLACC2	0.80	0.79	15.44	<0.001
RSA2	-0.02	-0.05	-0.44	0.66
RSA3				
RSA2	0.72	0.76	8.16	<0.001
FLACC2	0.14	0.08	1.57	0.12
FLACC4				
FLACC3	0.82	0.81	15.64	<0.001
RSA3	0.00	0.01	0.05	0.96
RSA4				
RSA3	0.81	0.81	11.45	<0.001
FLACC3	0.08	0.04	1.13	0.26
FLACC5				
FLACC4	0.84	0.90	20.87	<0.001
RSA4	-0.00	-0.00	-0.01	1.0
RSA5				
RSA4	0.70	0.63	9.30	<0.001
FLACC4	0.02	0.01	0.23	0.82

Note. FLACC= Face Legs Cry Consolability scale; RSA= Respiratory Sinus Arrhythmia.

Chapter 5: Moving from An Examination of Interrelationships Between Distress Indicators to Describing the Patterns of the Interrelationships

The primary aim of the last chapter was to implement methodological recommendations outlined in the first study (Chapter 2) to examine the direction and magnitude of the relation between toddlers' pain-related behavioural distress (as measured by FLACC) and cardiac responses (as measured by HR and RSA) during 12- and 18-month vaccinations. We used cross-lagged path analyses to investigate the predictive within-measure, predictive between-measure, and concurrent (residual) relations between behavioural and cardiac distress indicators across six consecutive 30-second epochs that spanned across 3-minutes post-needle. As well, we examined how contextual factors (i.e., baseline responses, time since last feeding/nap) impacted these relations.

Across 12- and 18-month vaccinations, predictive within-measure relations were consistent for FLACC, HR, and RSA (albeit stronger for FLACC and HR than for RSA), reflecting good stability of these pain indicators. Regarding predictive between-measure relations, higher pain-related behavioural distress immediately post-needle consistently predicted higher and lower subsequent HR and RSA 30-seconds post-needle, respectively. Contextual factors (i.e., baseline behaviour and HR/RSA) consistently predicted future pain-related behavioural distress and cardiac responses. After controlling for these predictive relationships, there were significant concurrent associations between behavioural pain responses and HR as well as behavioural pain responses and RSA at 12 and 18 months. However, RSA had smaller and more inconsistent relations with behaviour than HR. When comparing effect sizes (i.e., standardized *B*s) in the current

study to average effect sizes gleaned in Chapter 2 (Systematic Review), predictive within-measure and concurrent between-measure relations, as well as relations with contextual factors (i.e., baseline responses) were moderate to strong. However, predictive within-measure relations were considered small. The results parallel findings from Chapter 2 (Study 1), and suggest that HR and emotion expression behaviours measured concurrently are the most consistently related distress indicators within a high distress context. As well, the results highlight the need to account for contextual factors (i.e., baseline responses) and phases of distress (i.e., initial reactivity vs. regulation from peak distress) to fully understand the relation among distress indicators. This research is novel in that we have moved away from static, single behavioural and cardiac distress responses in toddlerhood that collapse across distress paradigms toward using more sophisticated analyses that capture different phases of distress.

However, recent work (i.e., Kahle et al., 2018; Qu & Leerkes, 2018) has found individual differences in the patterns of behavioural and physiological indicators of distress responding depending on the phase of distress. Thus, there has been a call from the above-mentioned researchers to investigate different profiles of reactivity and recovery processes from distress in toddlerhood. The goal of the final study (Study 3, Chapter 6) was to describe the variability in patterns of regulatory trajectories (i.e., concurrent toddler behavioural distress and HR) post-needle during the 12- and 18-month vaccinations according to distinct, prototypical patterns of change over time. The analyses were conducted using the same participants described in Chapter 4 (Study 2). Parallel-process growth-mixture modeling (Muthén & Shedden, 1999) was used to represent variability in toddler distress responding over the first 3 minutes post-needle.

This is the first known study to use person-centered analyses to document dynamic autonomic processes and how they converge or diverge from behavioural distress during a naturalistic high-distress paradigm in toddlerhood.

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Chapter 6: Investigating Convergence of Cardiac and Behavioral Indicators of Distress During Routine Vaccinations Over the Second Year of Life[4]

1. Introduction

Distress regulation can be defined as one's ability to modulate emotions and behaviors in order to recover from distressing events and return to homeostasis (Kopp, 1989; Thompson, 1990). The dynamic and complex nature of distress regulation has been highlighted in infancy and toddlerhood and involves sequencing expressed emotion, regulatory strategies, and biology (Ekas, Braungart-Rieker, & Messinger, 2018). Toddlerhood is a critical developmental phase to examine the multidimensional nature of distress regulation because it encompasses the transition from passive caregiver-directed regulation to more active and purposeful self-regulation (Kopp, 1982). As well, many abilities and important systems that are involved in the experience and expression of emotions are established in toddlerhood, leading to some of the greatest developmental changes in emotion regulation and emotion regulation skills (Ekas et al., 2018; Kahle, Miller, Helm, & Hastings, 2018).

Most empirical work on the underlying physiological components of distress regulation in toddlerhood highlights the maturation of the autonomic nervous system (ANS) as fundamental in the regulation of emotion (Santucci et al., 2008). However, several studies have shown that emotion expressions in young children are not

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concurrently associated with individual differences in physiological activity (Buss et al., 2005; Fortunato, Dribin, Granger, & Buss, 2008; Quas et al., 2000). Therefore, cardiac responses and behavioral regulatory skills should be considered distinct yet interrelated indicators of distress regulation (Barrett, 2013; Cole, Marin, & Dennis, 2004; Thompson, Lewis, & Calkins, 2008) that are malleable early in life. Yet, research suggests that there are individual differences in how toddlers respond behaviorally and physiologically during distressing events (Aureli, Grazia, Cardone, & Merla, 2015; Dale, O'Hara, Keen, & Porges, 2011; Lewis, Hitchcock, & Sullivan, 2004), and these patterns of behavioral and physiological responding may be differentially associated with subsequent adaptive or maladaptive developmental outcomes (Beauchaine, 2001; Del Giudice et al., 2011; Gunnar & Vasquez, 2006; Matthews & Phillips, 2010; Seifer et al., 1996; Stifter & Fox, 1990; Qu & Leerkes, 2018). Given the predictive nature of these patterns of distress regulation, it is important to understand how these different systems coordinate in times of distress in typically developing toddlers in order to identify risk pathways that extend beyond early childhood.

A recent systematic review by our group (Waxman et al., 2020) synthesized the literature on the relation between behavioral and cardiac indicators (i.e., heart rate [HR], heart period [HP], respiratory sinus arrhythmia [RSA], pre-ejection period [PEP]) of distress in toddlerhood. Several methodological challenges were outlined that may provide additional explanations for the discordance between behavioral and cardiac indicators of distress regulation, including: 1) the myriad of cardiac indicators of distress regulation; 2) a lack of delineation of phases of distress responding (i.e., initial reactivity

versus regulation from the peak response); and 3) a lack of research in high distress contexts.

Cardiac indicators. Both the parasympathetic (PNS) and sympathetic nervous system (SNS) have been implicated in the development of distress responses. Our review suggested that the only consistent relation between indicators was between HR (number of contractions of the heart per minute, reflecting of both sympathetic and parasympathetic activity) (Bornstein & Seuss, 2000) and concurrent behavioral indicators measured during times of distress (standardized mean differences in the range of .05 to .54). These results were posited to be due to metrics, such as RSA (variability in the time between heartbeats that occurs at the frequency of respiration, reflecting greater parasympathetic influence) (Berntson, Quigley, & Lozano, 2007), being more reflective of physiological regulation than simply emotional reactivity or arousal in toddlerhood (Kahle et al., 2018). As such, in the current study, we focused on the relation between behavior and HR responses during distress.

Phases of distress responding. Rothbart and Derryberry (1981) distinguished between different phases of distress. Whereas *reactivity* reflects an individual's behavioral and biological initial reaction to changes in the environment, *regulation* reflects the behaviors and biological processes used to modulate this response (e.g., behavioral approach or avoidance). Reactivity and regulation reflect divergent biological, psychological, and social mechanisms (Kopp, 1982), and as such responses underlying distress reactivity and regulation may not be identically associated across these phases of distress. Only recently have researchers begun to utilize more temporally sensitive techniques that capture dynamic change in distress regulation in infancy and preschool

(e.g., Brooker & Buss, 2010; Kahle et al., 2018; Miller et al., 2013; Obradovic & Finch, 2016; Qu & Leerkes, 2018; Thompson, Lewis, & Calkins, 2008). Findings from the abovementioned studies indicate that in response to distress, cardiac and behavioral indicators are interactive and are likely to fluctuate over the course of the distressing event. As such, the focus of the current study is to investigate different profiles of reactivity and recovery processes across time.

High distress contexts. Our systematic review revealed that there are few studies on the relation between behavioral and physiological indicators of high distress in toddlerhood, such as distress from pain (e.g., Waxman et al., 2020). Indeed, studies on the relation between behavioral and cardiac indicators of pain in infancy have found equivocal relations (Craig, Whitfield, Grunau, Linton, & Hadjistavropoulos, 1993; Gibbins et al., 2008; Grunau et al., 2010; Johnston et al., 1995; Johnston & Strada, 1986; Lewis & Ramsay, 1995; Owens & Todt, 1984; Stevens, Johnston, Petryshen, & Taddio, 2010). Therefore, it is unknown whether dynamic cardiac recovery is linked in the moment with toddlers' behavioral responses to high distress. Given the particular salience of high distress experiences during development, it is important to investigate toddler responses to high distress. A fruitful context to study high distress is during routine vaccination. Not only has clear variability in infant negative affect regulation been established with behavioral indicators (Pillai Riddell et al. 2013), but given the frequency of vaccinations over the first years of life, this pain context can provide valuable insight for understanding toddler norms for regulating distress.

The Present Study

This study examined a longitudinal sample of healthy toddlers observed over vaccinations at 12 and 18 months of age. Pain-related behavioral distress and HR were measured concurrently for 3-minutes post-vaccination. The main aim of this study was to model variability in regulatory trajectories (i.e., concurrent toddler pain-related behavioral distress and HR) immediately to 3 minutes post-needle during the 12- and 18-month vaccinations. Parallel-process growth-mixture modeling (GMM; Muthén & Shedden, 1999) was used to represent variability in toddler distress responding over the first 3 minutes post-needle. The 12- and 18-month ages were investigated separately because cognitive and physical development has been characterized by more differentiated behavioral and physiological responses to pain at 18 months than at 12 months (Anand & Craig, 1996; McGrath & Unruh, 1994). Because of this increased differentiation in pain responses across the second year of life and past work with infant and preschool GMM of distress (e.g., Pillai Riddell et al., 2013, Qu & Leerkes, 2018; Waxman et al., 2017), we hypothesized that there would be substantial heterogeneity with distinct trajectory patterns of co-occurring behavioral and cardiac indicators following 12- and 18-month vaccinations.

2. Method

2.1 Participants

Ethical approval was obtained through the research ethics review board at the participating university. After agreeing to speak to a researcher about the study,

caregivers were approached by a research assistant who explained the study and then asked them to sign informed consent forms.

The data are part of an ongoing longitudinal study in which caregiver-toddler dyads were recruited from two pediatric clinics in the greater Toronto area and observed in a cohort-sequential design during vaccinations over the second year of life (12, 18, and 24 months). Toddlers were recruited at 12 or 18 months of age. Of the 374 families approached for recruitment, 41 were ineligible based on exclusion criteria (i.e., child was hospitalized in a neonatal intensive care unit, was more than three weeks premature, suspected of a developmental delay, had a known heart condition, or the caregiver was not fluent in English). In total, 158 and 122 caregiver-toddler dyads were successfully recruited at the 12-month and 18-month vaccinations, respectively. A total of 72 participants were observed at both the 12- and 18-month vaccinations. Table 1 lists participants' demographic characteristics. Overall, participants were healthy, from middle-class families, and had caregivers who were well-educated.

Participants were from diverse cultural backgrounds. Most primary caregivers were born in Canada (59%) whereas caregivers were born outside of Canada came from Asia [23%], Europe [9%], South America [6%], Australia [2%], or the United States [1%]. In addition, their rating of acculturation suggested an integrated cultural background, with strong identification with both their heritage culture (a culture that influenced generations of their family) and mainstream North American culture (the culture they current live in).

2.2 Procedure

During the 12- and 18-month vaccination appointments, caregiver-toddler dyads were simultaneously videotaped and connected to equipment to measure their HR before and after their vaccinations. Caregivers filled out a short demographic questionnaire before each vaccination appointment. Toddlers were observed 1 minute before, immediately after the final needle, 1 minute after the final needle, and 2 minutes after the final needle. Given that this is a naturalistic study, the families were observed during their vaccination appointments with minimal interference from the research team, aside from videotaping and cardiac monitoring procedures. At both the 12- and 18-month vaccinations, caregivers were given a sheet outlining evidence-based pain management strategies, as per ethical practice in the pain context (Taddio et al., 2010). All infants were in caregiver's arms for the vaccination and 1% of the caregivers used EMLA cream prior to the vaccination at 12 and 18 months.

2.3 Measures

2.3.1 Caregiver demographic information

Caregivers were asked to complete a short demographic questionnaire that asked about caregiver age, relation to the child, self-reported heritage culture, and child age and sex. Caregivers were also asked to report on important factors that are known to impact physiological indicators, such as time since last feeding and since last nap.

2.3.2 Observed pain-related behavioral distress

The Face, Legs, Activity, Cry, Consolability coding system (FLACC; Merkel, Voepel-Lewis, & Malviya, 2002) was used to assess the degree of expressed pain

behaviors across the vaccinations at 12 and 18 months. The degree of pain-related behavioral distress was measured through five types of pain behaviors (face, legs, activity, cry, consolability) during seven different epochs (60 to 1 seconds prior to the first needle [Baseline]; 0 to 29 seconds immediately after the last needle [Needle]; 30 to 59 seconds after the last needle [Post 1]; 60 to 89 seconds after the last needle [Post 2]; 90-119 seconds after the last needle [Post 3] 120-149 seconds after the last needle [Post 4]; and finally, 150-179 seconds after the last needle [Post5]). Each behavior was scored on a 0 to 2 scale (e.g., on the Face scale: no expression or smile = 0; occasional grimace, frown, or withdrawn behaviour = 1; and constant frown, clenched jaw, or quivering chin = 2), resulting in possible total scores between 0 and 10 for each 30- (Needle to Post 5) or 60-second (Baseline) epoch. There were no significant differences between two distinct 30-second baseline epochs, and so they were collapsed to provide a more robust baseline indicator (Novak, Saul, & Eckberg, 1997). The FLACC scale captures infants' vocalizations (Cry scale), facial expression (Face scale) and body tension (Legs scale), which were criteria discussed in the seminal paper by Braungart-Rieker and Stifter (1996) in order to properly encapsulate behavioral distress. Moderate to high concurrent and construct validity as well as item-total and inter-rater reliability have been demonstrated for the FLACC in the acute pain context (Merkel, Voepel-Lewis, Shayevitz, & Malviya, 1997). To ensure high reliability, coders were trained by a primary FLACC coder. A total of 20% of the sample was reliability coded throughout the duration of the coding process, with unreliable codes being recoded. The coders were blind to the study hypotheses and inter-rater reliability between the coders was high (intraclass correlations between 0.90 and 0.93). In the present paper, FLACC operationalizes pain-related behavioral distress.

As well, we consider FLACC scores in the 7 to 10 range indicative of high pain-related distress, scores in the 4 to 6 range represent moderate pain-related distress, and scores in the 1 to 3 range indicate low pain-related distress (Merkel et al., 1997).

2.3.3 Heart rate

Cardiac data were collected continuously using MindWare ambulatory monitors (MW 1000A) at a sampling rate of 500 Hz. Three adhesive electrodes collected electrocardiography (ECG), with one electrode placed above the right shoulder blade, one electrode placed on the bottom most left rib, and a ground electrode placed on the bottom most right rib. Using MindWare Biolab 3.3, ECG signals were continuously acquired. ECG data were edited in MindWare HRV 3.1.5 with HR computed through identification of R-waves (Berntson, Quigley, & Lozano, 2007). To ensure high reliability, coders were trained by an experienced primary coder. A total of 20% of the sample was reliability coded throughout the duration of the coding process, with unreliable codes being recoded. The coders were blind to the study hypotheses and inter-rater reliability between the coders was high (intraclass correlations between 0.95 and 0.99).

Trained coders inspected the raw physiological data and identified any misidentified R-waves. Editing issues (e.g., cutting segments of data, identifying R-waves on data with artefact) were addressed and corrected in consultation with the experienced primary coder. In the case of artifact, the decision to include the data was made on an epoch-by-epoch basis in consultation with the primary coder. The primary reason for excluding an epoch of HR data was serial missing R waves (where a "midbeat" could not be estimated). In all cases, the key decision rule was whether edited epochs were consistent with the individual's other data. Supplementary Table 1

summarizes reasons that toddlers' HR data that were not a result of editing challenges could not be used. The amount of artefact editing did not exceed 5% and did not systematically relate to any of the study measures.

Heart rate was calculated during the seven different epochs named earlier (Baseline, Needle, and Post 1 through Post 5). In the present paper, results are contextualized based on the average awake HR for toddlers between the ages of one and two years of age, which is 98 to 140 beats per minute (Fleming et al., 2011).

3.2.4 Analysis Plan

All of the main analyses were performed with Mplus, version 7.0 (Muthen & Muthen, 2006), using a robust full-information maximum likelihood estimator (FIML) to incorporate incomplete cases and account for the degree of non-normality in the data. Prior to performing the main analyses, mixed ANOVAs were conducted in order to investigate whether the sex of participant impacted behavioural pain-related distress or cardiac responses across the post-vaccination epochs at 12 and 18 months. Sex did not impact behavioral pain-related distress responses at 12 months, $F(1, 119) = 1.03$, $p = .31$ or at 18 months, $F(1, 106) = 0.03$, $p = .86$. Additionally, sex did not impact cardiac responses at 12 months, $F(1, 100) = 1.97$, $p = .16$ or at 18 months, $F(1, 78) = .25$, $p = .62$. As such, sex was not investigated in the main or post-hoc analyses.

3.2.4.1 Parallel-process modeling growth mixture modeling

Growth mixture modeling (GMM) is a statistical technique to summarize individual variation on a set of longitudinal repeated measures (i.e., trajectories) using a small number of homogenous subgroups within a sample (Muthén & Shedden, 1999).

Our goal was to identify subgroups of prototypical trajectories to represent heterogeneity in how toddlers recover from peak distress by examining both pain-related behavioral distress and HR scores simultaneously (i.e., parallel processes) from Needle to Post 5 at 12 and 18 months of age. It is important to note that toddlers are not assigned to groups deterministically; instead, each participant receives a score representing the probability that she or he would be assigned to each of the discerned groups.

Separate parallel-process GMM analyses were used to model simultaneous trajectories of pain-related behavioral distress and HR at 12 and 18 months. Separate latent growth curve models (Bollen & Curran, 2006) were first estimated to determine the functional form (e.g., linear vs. non-linear) of trajectories of pain-related behavioral distress and HR individually at 12 and 18 months. Upon inspecting the means for FLACC and HR across post-vaccination epochs at 12 and 18 months, we decided to specify quadratic growth curve models to account for non-linear trends (see Bollen & Curran, 2006). Quadratic trajectories were estimated from immediately post-vaccination (Needle) to 3 minutes post-vaccination (Post 5).

Next, a single-group parallel-process GMM was specified and compared to a series of models formed by increasing the number of groups (with quadratic trajectories for FLACC and HR in each model). The models were compared using Akaike's Information Criterion (AIC) (Akaike, 1973) and the Bayesian Information Criterion (BIC) (Schwarz, 1978). Smaller values of AIC and BIC are associated with improved model fit. We increased the number of groups until the AIC and BIC no longer warranted additional groups.

3.2.4.2 Post-hoc analyses

After the optimal number of groups was discerned for the two ages (12 and 18 months), each participant was assigned to the group which had the highest probability of them belonging based on their pattern of data. Group membership was exported from Mplus to SPSS (Version 24), and post-hoc binary or multinomial logistic regression models were estimated to assess how well contextual factors (i.e., baseline pain-related behavioral distress, baseline HR, time since last feeding, and time since last nap) predicted group membership at 12 or 18 months. Separate models were estimated for each variable (e.g., baseline pain-related behavioral distress) to maximize sample size per analysis. Baseline responses (i.e., baseline pain-related behavioral distress and baseline HR) were investigated given that The Law of Initial Value asserts that the size of a psychophysiological response depends on the initial baseline level of the measure (Bernston, Uchino, & Cacioppo, 1994). Additionally, level of arousal has recently shown to be a determinant of pain-related brain activity (Jones et al., 2017). Time since last feeding and since last nap (in minutes) were also investigated as predictors because these factors are known to impact physiology (Oberlander, & Saul, 2002; Waxman et al., 2016).

3. Results

Table 2 presents the overall means and SDs of all variables included in the 12- and 18-month GMMs, and Tables 3 and 4 present the correlations among these variables. As FIML was used to incorporate cases with incomplete data, the sample sizes for the GMMs were 147 and 118 at 12 and 18 months, respectively.

3.1 Concurrent changes in pain-related behavioral distress and heart rate following 12-month vaccination

3.1.1 Unconditional growth curve model

Supplementary Table 2 presents the intercept, linear, and quadratic growth factor means and standard deviations for the latent growth curve model of behavioral distress and HR. As can be seen, average pain-related behavioral distress (FLACC scores) and heart rate (HR) decreased across the three-minutes post-needle at the 12-month vaccination.

3.1.2 Parallel-process growth mixture model

Supplementary Table 3 provides the model fit indices for one- to four-group models; in each of these models, the quadratic factor variances and covariances were fixed to 0 to obtain proper model solutions. Although the three-group model fit the data slightly better than the two-group model, only 2% of the sample was represented by the third group. Therefore, we considered the two-group model optimal. Supplementary Table 4 presents means and standard deviations of the latent growth factors of pain-related behavioral distress and HR (i.e., intercept, slope, quadratic) for Groups 1 and 2, and Supplementary Table 5 presents the inter-factor correlations within the two groups. Figure 1 shows the mean trajectories of the two subgroups.

Group 1 (76% of the sample) showed high pain-related behavioral distress immediately post-needle that decreased across the three minutes post-needle (i.e., FLACC = 7.88 at Needle to 3.23 at Post 5). By the end of the third minute, the group displayed low pain-related behavioral distress. The HR scores reflected a similar pattern,

with HR decreasing from 157 beats per minute (bpm) immediately following the needle to 135 bpm at the end of the third minute following the vaccination, reflecting a return to typical awake HR in toddlerhood. Group 2 (24% of the sample) displayed moderate pain-related behavioral distress immediately following the needle that quickly regulated to low distress across the remaining vaccination epochs (FLACC = 5.30 at Needle to 2.27 at Post 5). The HR scores decreased from 149 bpm to within the average range across the remaining vaccination epochs (i.e., 140 bpm at Post 1 to 135 bpm at Post 5). Both pain-related behavioral distress and HR decreased from Post 1 to Post 3 and levelled off from Post 3 to Post 5. See Table 5 for the model-estimated means for pain-related behavioral distress and HR across the post-vaccination period as well as mean baseline scores calculated post-hoc (i.e., based on assigned group membership).

3.1.3 Post-hoc analyses

Four binary logistic regression models were estimated to ascertain the effect of time since last feeding (minutes), time since last nap (minutes), baseline HR, and baseline pain-related behavioral distress on group membership (see Table 6). Higher baseline pain-related behavioral distress was significantly associated with a decreased likelihood of being in Class 2, $OR = .52$, $p < 0.001$. No other variable significantly predicted group membership.

3.2 Concurrent changes in pain-related behavior distress and heart rate following 18-month vaccination

3.2.1 Unconditional growth curve model

Supplementary Table 2 presents the intercept, linear, and quadratic factor means and standard deviations for the latent growth curve model of behavioral distress and HR. At 18-months, FLACC scores and HR decreased across the three-minutes post-vaccination.

3.2.2 Parallel-process growth mixture model

Supplementary Table 6 provides the model fit indices for one- to four-group models. The three-group model had optimal fit to the data; Supplementary Table 4 presents means and standard deviations of the growth factors of behavioral distress and HR from this model and Supplementary Table 7 presents the inter-factor correlations for this three-group model. Figure 2 shows the mean trajectories of the three subgroups. Group 1 (31% of the sample) started with high pain-related behavioral distress that decreased to a moderate level by three minutes post-needle (i.e., FLACC = 7.44 at Needle to 5.73 at Post 5). Group 1 HR scores increased from immediately post-needle to 30-seconds post-needle and then decreased across the subsequent post-needle epochs (i.e., 161 bpm at Post 2 to 142 bpm at Post 5). Mean HR was greater than the average awake HR for toddlers across the vaccination period (i.e., 159 bpm at Needle to 142 bpm at Post 5). Group 2 (56% of the sample) began at a moderate level of pain-related behavioral distress immediately following the needle that decreased to a low level of behavioral distress within the second minute post-needle (i.e., FLACC = 6.96 at Needle to 2.29 at

Post 5). The HR scores showed a similar pattern, with HR decreasing from 152 bpm immediately following the needle to within the average awake HR for toddlers by the second minute post needle (142 bpm at Post 1 to 130 bpm at Post5). Group 3 (13% of the sample) displayed low pain-related behavioral distress across the vaccination period (FLACC = 2.12 at Needle to 1.45 Post 5). Patterns of HR were akin to patterns of pain-related behavioral distress, as Group 3 remained within the average HR for toddlers across the vaccination period (122 bpm at Needle to 123 bpm at Post 5). See Table 5 for the model-estimated means for pain-related behavioral distress and HR across the post-vaccination period as well as mean baseline scores calculated post-hoc.

3.2.3 Post-hoc analyses

Four multinomial logistic regression models were estimated to ascertain the effect of time since last feeding (minutes), time since last nap (minutes), baseline HR, and baseline pain-related behavioral distress on group membership (see Table 7). Because it was the largest group, Group 2 was the reference category. Higher baseline pain-related behavioral distress was significantly associated with a decreased likelihood of being in Class 3 compared to Class 2, $OR = 0.55$, $p < 0.001$. No other variable significantly predicted group membership.

4. Discussion

To our knowledge, this is the first study to utilize dynamic, person-centered analyses to represent the variability in toddler pain-related behavioral distress and HR responses within a naturalistic high distress context (i.e., vaccination pain). We examined whether there were distinct patterns of co-occurring pain-related behavioral distress and

HR across six post-vaccination epochs at 12 and 18 months of age. Given the lack of research in high distress contexts, we did not hypothesize about the specific number of patterns that would be discerned. However, we posited that there would be substantial heterogeneity in toddlers' trajectories of behavioral and HR responses at both 12 and 18 months of life. The results supported this hypothesis, with distinct subgroups of toddlers being discerned at both 12 (2 groups) and 18 months (3 groups).

Lewis (1992) proposed features of the nervous system that can help explain and differentiate between children's responses to distress, two of which are relevant to the discussion of results of our study: *threshold* and *dampening*. Threshold refers to the amount of stimulation necessary to produce a response, with some children requiring a very high amount of stimulation to produce a response (i.e., high threshold) while others require very little stimulation to elicit a response (i.e., low threshold). Dampening refers to the ability to inhibit arousal and return to homeostasis following distress, with some children calming quickly (i.e., high dampening ability) while others are unable to reduce their response (i.e., low dampening ability). Lewis proposes that there are four categories of children based on these factors, which may explain why emotional expressions and cardiac responses are not always concurrently associated. Specifically, children may be *difficult to soothe* (i.e., low threshold, low dampening ability), *high-reactive* (i.e., low threshold, high dampening ability), *low-reactive* (high threshold, low dampening ability), or *easy* (high threshold, high dampening ability). We will refer to these categories throughout our commentary.

4.1 Trajectories at 12 and 18 months

12 Months. Parallel-process GMMs discerned two characteristic trajectory patterns of pain-related behavioral distress and HR responses post-vaccination at 12 months of age. Neither initial pain-related behavioral distress nor changes in pain-related behavioral distress post-vaccination were significantly associated with initial HR or changes in HR post-vaccination at 12 months within either of the two subgroups. Group 1 represented the majority of participants (76%) and was characterized by similar behavioral and HR trajectories across the vaccination epochs. Group 1 began at baseline with low pain-related behavioral distress and within the average active HR range for toddlers (i.e., 98 bpm to 140 bpm; Fleming et al., 2011). Immediately following the needle, there was a high behavioral distress response that was paralleled by HR greater than the highest average active HR for toddlers. Both pain-related behavioral distress and HR returned to baseline levels by three-minute post-needle. This profile is somewhat analogous to Lewis' (1992) group of "high-reactive" children who are easily aroused (i.e., low threshold) yet display a high ability to inhibit this response once it has occurred (i.e., high dampening ability).

In contrast, Group 2 was a smaller class of participants (24%) who displayed corresponding, yet slightly differentiated pain-related behavioral distress and HR responses post-vaccination compared to Group 1. At baseline, Group 2 was within the average active HR range for toddlers and displayed very low pain-related behavioral distress. Immediately following the needle, their pain-related behavioral distress was moderate and HR increased to slightly greater than the average active HR level for toddlers. Within the first minute post-needle, there was a swift decrease in both pain-

related behavioral distress and HR down to a low level of behavioral distress and average active toddler HR. However, pain-related behavioral distress and HR levelled off approximately 1-minute post-needle and did not return to baseline levels within three-minutes post-needle. This pattern was similar to Lewis' (1992) proposition of the "low-reactive" child who is more difficult to arouse (i.e., high threshold) yet takes longer to calm down after they have become aroused (i.e., little dampening ability). Indeed, although the Group 2 means reflect low levels of distress and average toddler HR, the post-needle means never return to baseline levels. These results may be linked to regulatory strategies employed by the toddler or caregiver during the vaccination period. Kahle and colleagues (2018) found a similar pattern when investigating 3.5 year-olds during an anger induction, where certain regulatory strategies (e.g., attention diversion) employed immediately following anger induction predicted attenuated autonomic arousal concurrently, but increased sympathetic arousal within regulatory epochs.

18 months. At 18 months, the optimal model consisted of three distinct trajectory patterns of pain-related behavioral distress and HR responses post-vaccination. At this age, initial pain-related behavioral distress (FLACC) was positively associated with initial HR post-vaccination for all three groups. The largest group (Group 2; 56%) displayed a similar trajectory to Group 1 (i.e., high-reactive) observed at 12 months of age. Specifically, following a display of low pain-related behavioral distress and average HR prior to the needle, toddlers in Group 2 had moderate pain-related behavioral distress and slightly above average HR immediately post-needle that regulated to low distress and average HR by the second-minute post-needle. However, two smaller groups (i.e., Group 1 with 31% of the sample, Group 3 with 13%) had behavioral and HR trajectories that

were much different than those deciphered at 12 months of age. Specifically, Group 1 began at low pain-related behavioral distress and average HR, with a steep increase to severe distress and above average HR immediately following the needle. Group 1 remained at a moderate to severe pain-related behavioral distress level across the three minutes post-needle, and mean HR never returned to within the average active HR range. This group displayed a high and sustained pain-related behavioral distress reaction with only moderate physiological regulation (i.e., HR), consistent with the "difficult to soothe" child (i.e., low threshold, low dampening ability) described by Lewis (1992). Group 3 displayed little to no pain-related behavioral distress and average HR at baseline. Across the post-vaccination epochs, these toddlers displayed low pain-related behavioral distress that slowly decreased across the three-minutes post-vaccination. Mean HR remained stable, not increasing substantially from baseline to post-needle and remaining within the average active HR range across the post-vaccination epochs. This limited distress reaction followed by recovery with no evidence of physiological regulation is consistent with Lewis' "low-reactive" child (i.e., high threshold, low dampening ability).

Developmental Differences

When investigating individual differences in toddlers' responses to high distress, a majority of participants at both ages (12 months: 76%; 18 months: 56%) fit a trajectory of pain-related behavioral distress and HR characterized by high reactivity and high dampening ability (i.e., "high-reactive" child). These results extend previous work by our group investigating vaccination reactivity and regulation at 12 months and preschool age. Specifically, distinct trajectory patterns of pain-related behavioral responses to vaccination pain and distress during infancy were greater (i.e., increased number of

groups) and regulatory trajectories were steeper than at preschool (Pillai Riddell et al., 2013; Waxman et al., 2016). These results illustrate developmental changes across toddlerhood towards organization and increased specificity of behavioral and physiological distress responses (Johnston, Stevens, Craig, & Grunau, 1993). As well, given our pain-related distress context, this consistency in the majority of participants displaying high reactivity can be linked with a survival perspective. At 12 months and to a lesser extent 18 months, most toddlers signal with intense diffuse distress to communicate pain to their caregivers so that caregivers can help them regulate from distress.

Despite the "high-reactive" group being a majority at both ages, two additional distinct trajectory patterns (i.e., difficult to soothe, low-reactive) emerged at 18 months of age. Research investigating the development of behavioral and cardiac measures of negative emotions has found a lack of stability in responses across toddlerhood (Baker et al., 2012; Buss et al., 2005). As well, findings from Lewis' (1992) seminal work, in which the stability of behavioural stress responses were investigated in an acute pain context (i.e., heel stick at 2 days, inoculation at two months), substantiate these results. Specifically, infants who were "high" responders at two postnatal days were likely to remain "high" responders at two months of age (86% consistency). However, "moderate-to-low" responders displayed less stability across the two time-points (56%). Lewis ascertained that "moderate-to-low" responders are likely to be impacted by environmental differences (e.g., caregiver sensitivity).

Indeed, links between patterns of distress responding and attachment style have been found across moderate and high distress contexts. Qu and Leerkes (2018) described

profiles of infant observed distress and physiological regulation indexed by respiratory sinus arrhythmia (RSA) level during the still-face paradigm at 6 months, and found that the profile characterized by high RSA levels and low negative affect (i.e., low-reactive) exhibited lower attachment avoidance at 4.5 years of age. However, research in the vaccination context has found that avoidant infants tend to exhibit less distress than secure infants immediately prior to the needle at 12 months of age, and temperament has an interactive effect with attachment to predict either faster (low temperamental fear) or slower regulation (higher temperamental fear) in avoidant and disorganized infants (Horton, Pillai Riddell, Flora, Moran, & Pederson, 2015). These results are consistent with an attachment perspective that the most adaptive strategy a child can implement when experiencing high pain and fear is to signal a caregiver for the duration of time they are experiencing these emotional reactions. Therefore, it is possible that optimal distress regulation patterns may be different in moderate than high distress contexts. Future research is warranted to understand whether children who do not respond to or regulate from pain are at higher risk for suboptimal developmental outcomes. As well, an important future direction is to examine how caregivers can impact these regulatory patterns.

Contextual Factors Impacting Findings

Contextual factors, including baseline responses (i.e., baseline pain-related behavioral distress responses and baseline HR) and time since last feeding and since last nap, were also investigated to determine whether these factors predict group membership (i.e., regulation trajectory patterns) at 12 and 18 months of life. At both 12 and 18 months of age, baseline pain-related behavioral distress predicted group membership.

Specifically, at 12 months, higher baseline behavioral distress predicted membership in the "high-reactive" group (Group 1). At 18 months, higher behavioral distress predicted membership in the "high-reactive" group (Group 2) as opposed to the "low-reactive" group (Group 3). These results suggest a stable pattern whereby high-reactive toddlers expressed higher amounts of behavioral distress pre-needle than low-reactive toddlers. Toddlers in the "high-reactive" group ultimately displayed the greatest degree of regulation by the end of the vaccination period compared with other groups. These results parallel Kahle and colleagues' (2018) finding that toddlers who utilized more sophisticated forms of emotion regulation during an anger induction task had the greatest physiological reactivity but also steeper regulatory trajectories. At 18 months, baseline pain-related behavioral distress did not significantly differentiate the "high-reactive" (Group 2) and "difficult to soothe" (Group 1) toddlers. However, the highest levels of pain-related behavioral distress responses were in the "difficult to soothe" group, which also displayed heightened pain-related behavioral distress and HR throughout the vaccination appointment. These results are not surprising given that earlier infant pain behavior has been found to strongly predict subsequent infant pain behaviors within the same immunization appointment (Campbell, Pillai Riddell, Garfield, & Greenberg, 2013; Pillai Riddell et al., 2011).

Limitations

Generalizability of the current study is limited due to the sample only including healthy toddlers born full term. The high education level of our participants also affects generalizability. As such, the results may not be applicable to clinical populations and non-healthy neonates or premature infants who may have a history of exposure to

multiple acutely painful or stressful procedures. Additionally, important caregiver factors (i.e., caregiver behavioural regulatory or pain management strategies, attachment style, caregiver mental health) were not investigated as potential mediators or moderators of toddlers' distress responses and regulation as this was beyond the scope of the paper.

Conclusions: Implications for Future Research and Clinical Practice

This study identified distinct profiles (i.e., trajectory subgroups) of toddlers by examining joint trajectories of HR and pain-related behavioral distress during vaccinations at 12 and 18 months. At both ages and across profiles, the HR trajectory was similar to the pain-related behavioral distress trajectory, suggesting that HR can be used to represent the hedonic tone of an emotional state (Buss et al., 2005; Baker et al., 2012; Hay et al., 2017; Provost & Gouin-Décarie, 1979) and extends this finding to a high distress context. Importantly, however, patterns of responding became increasingly differentiated across toddlerhood. At 12 and 18 months of age, the majority of participants displayed high (12 months) to moderate (18 months) pain-related behavioral distress and increased HR immediately post-needle, but were able to calm down to baseline levels of pain-related behavioral distress and HR within three-minutes post-needle. However, at 18 months, almost 50% of toddlers displayed pain-related behavioral distress and HR that did not completely regulate across the three-minutes post needle or were blunted (i.e., no change from baseline in either pain-related behavior distress or HR). Given that our participants' ages coincide with the theorized development of attachment relationships with primary caregivers (Ainsworth, 1973), and differences in distress regulation have been linked to differences in attachment style (i.e., Horton et al., 2015; Qu & Leerkes, 2018), future studies may examine the child's social world

(including parenting behavior and attachment security) to understand which features may predict these distinct distress trajectories. Our findings are slightly divergent from research by Kahle and colleagues (2018) that found weak associations between expressed anger and sympathetic activity, while the greatest associations were between children's sympathetic responses and regulatory strategies. Thus, it is important for future research to include multiple physiological (e.g., heart rate, oxygen saturation, electroencephalography, skin conductance, cortisol) and behavioral (e.g., expressed emotion behaviors, self-regulation of emotion behaviors, caregiver behaviors) indicators to better understand individual differences in behavioral and physiological convergence in high distress contexts.

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Figure 1. 12-Month Model, Group 1 (76%) and Group 2 (24%): Concurrent behavioral distress (FLACC) and heart rate (HR) responding post-vaccination.

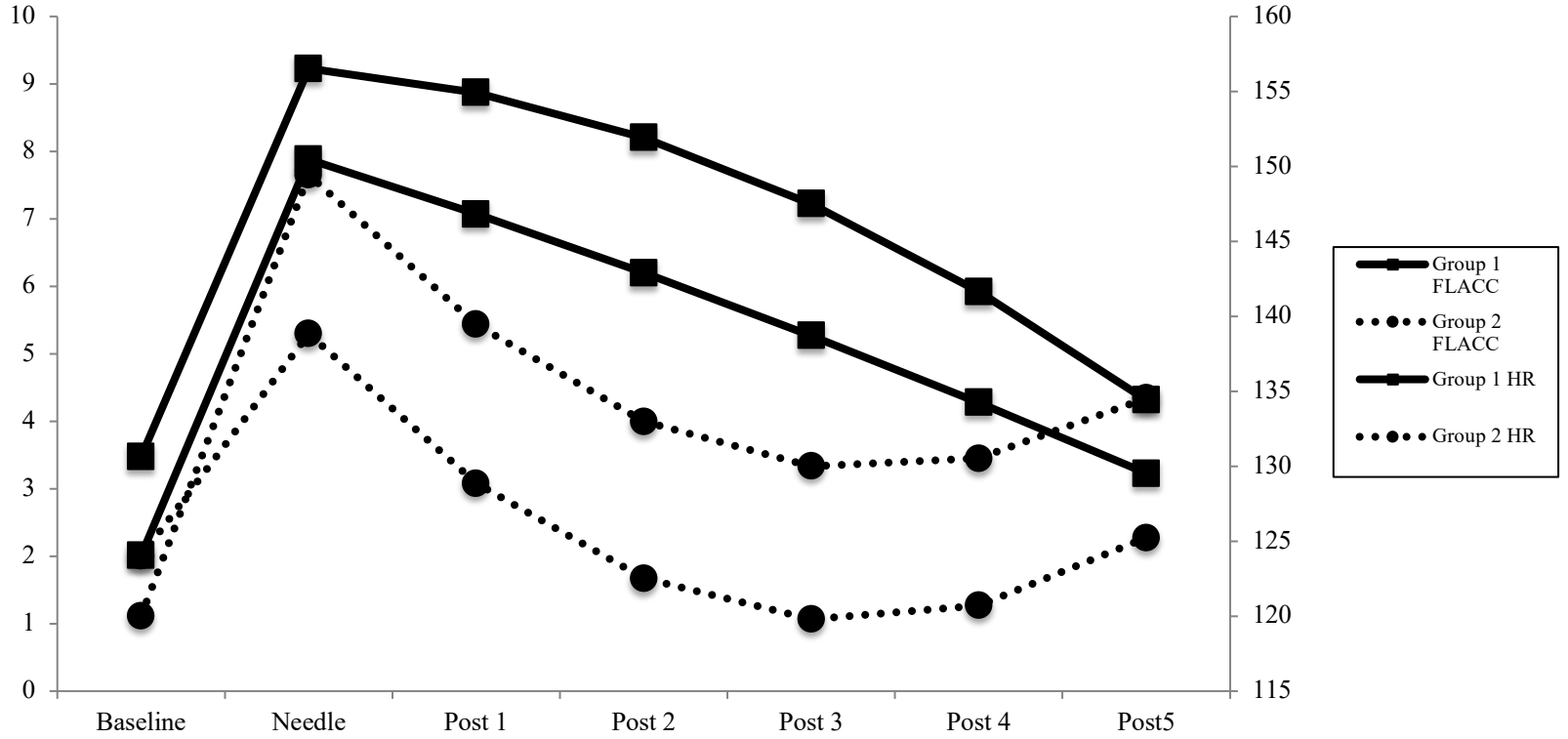

Figure 2. 18-Month Model, Group 1 (31%), Group 2 (56%) and Group 3 (13%): Concurrent behavioral distress (FLACC) and heart rate (HR) responding post-vaccination.

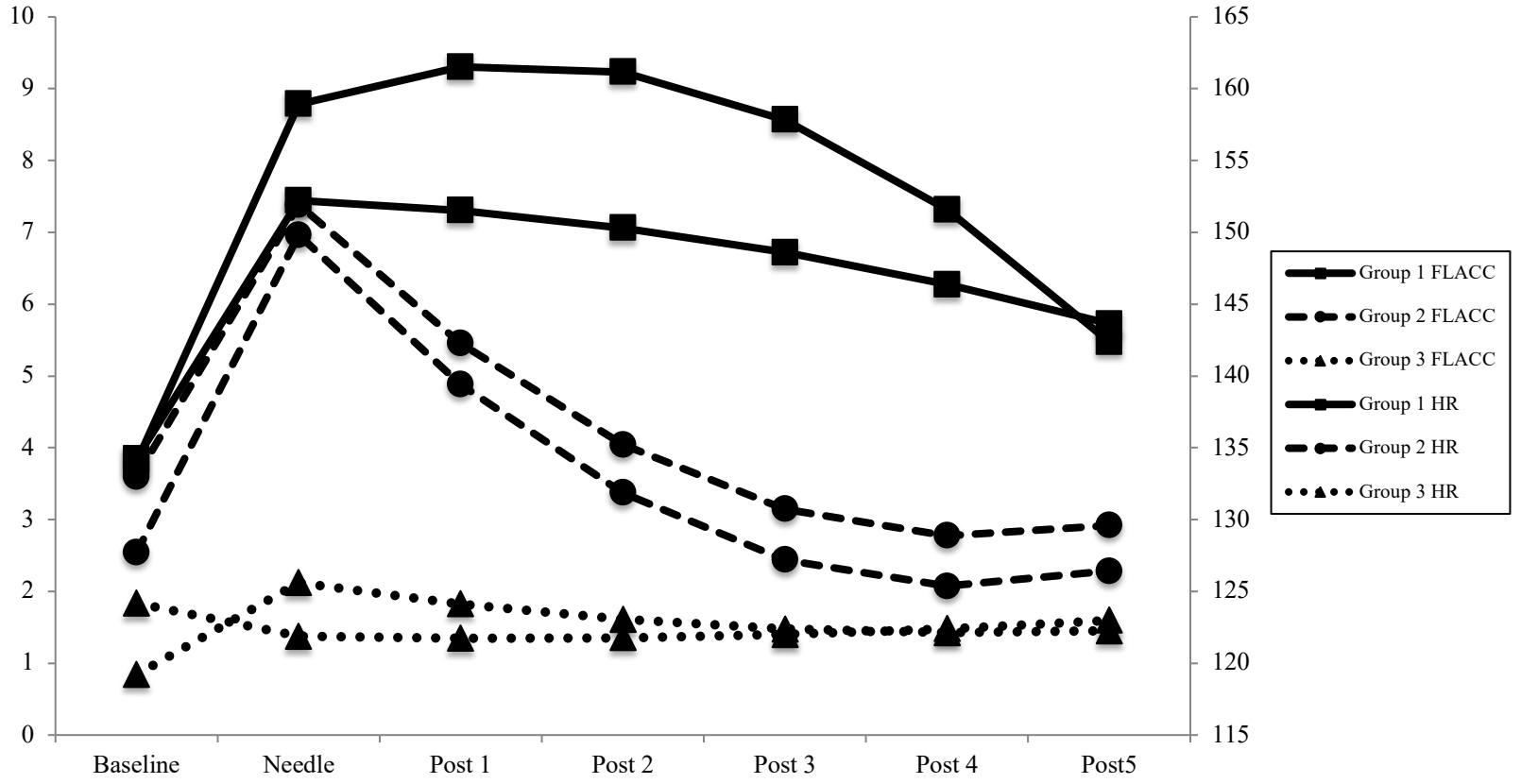

Table 1. Demographic information.

	Frequency (%)	
	12 months (n=158)	18 months (n= 121)
Sex of Infant		
Male	81 (54.4)	68 (58.1)
Female	68 (45.6)	49 (41.9)
Relationship to infant		
Mother	127	98
Father	12	17
Other	1	1
Education		
Graduate School/Professional Training	72	55
University Graduate (4 Years)	42	39
Partial University (at least 1 year)	4	3
Trade School/Community College	15	11
High School Graduate	2	1
Caregiver Age	35.91 (5.19)	36.63 (6.19)
Acculturation status		
Way of life reflects heritage culture	6.63 (2.79)	5.66 (2.43)
Way of life reflects mainstream North American/Canadian culture	7.62 (2.28)	7.75 (1.66)

Note. Certain data points were missing; thus, frequencies do not add to the total sample size.

Table 2. Means and SDs of variables included in 12- and 18-month models.

	12- Month Mean	12- Month SD	12-Month Scale Range	18-Month Mean	18-Month SD	18-Month Scale Range
Time since last feeding (minutes)	103.97	74.94	0-420	100.12	61.16	0-300
Time since last nap (minutes)	110.40	76.71	0-390	148.83	85.70	0-420
FLACCB	1.68	1.83	0-8	2.81	2.71	0-9.75
FLACC0	7.30	1.55	1-10	6.46	2.11	0-9
FLACC1	6.28	2.5	0-10	5.10	2.91	0-9.38
FLACC2	5.21	2.86	0-10	4.37	2.90	0-9
FLACC3	4.2	2.85	0-9.5	3.81	2.84	0-9.17
FLACC4	3.39	2.84	0-9.5	3.38	2.82	0-9.5
FLACC5	3.03	2.85	0-9	3.34	2.02	0-10
HRB	129.92	13.94	81.17-180.48	132.01	17.5	102.78-186.02
HR0	150.82	21.40	84.54-207.41	147.80	23.27	98.66-198.20
HR1	154.59	22.90	77.45-191.58	146.74	23.65	97.86-193.38
HR2	146.06	20.90	85.89-195.91	143.02	21.70	100.64-188.08
HR3	140.36	18.66	84.12-193.89	136.20	20.39	105.18-191.65
HR4	136.38	17.53	80.03-183.94	133.68	18.48	99.82-179.83
HR5	134.07	17.08	77.54-191.58	132.24	16.53	103.18-170.58

Note. FLACC= Face Legs Cry Consolability (behavioral distress); HR = heart rate (beats per minute).

Table 3. Correlations among variables included in 12-month models.

	1.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Time since last feeding	-	0.11	0.02	0.01	-0.07	-0.15	-0.10	-0.86	-0.13	0.09	0.08	0.11	0.07	0.05	0.05	0.00
2. Time since last nap		-	0.02	0.00	0.02	0.07	0.02	0.06	-0.04	0.23*	0.02	0.25*	0.16	0.05	0.18	0.13
3. HRB			-	0.57*	0.49*	0.53*	0.60*	0.65*	0.60*	0.56*	0.03	0.11	0.14	0.14	0.25*	0.15
4. HR0				-	0.72*	0.61*	0.51*	0.47*	0.42*	0.40*	0.18	0.14	0.20*	0.12	0.15	0.14
5. HR1					-	0.85*	0.74*	0.65*	0.51*	0.39*	0.36*	0.45*	0.42*	0.31*	0.35*	0.30*
6. HR2						-	0.88*	0.75*	0.61*	0.35*	0.30*	0.40*	0.46*	0.32*	0.35*	0.30*
7. HR3							-	0.86*	0.72*	0.27*	0.25*	0.31*	0.37*	0.39*	0.45*	0.32*
8. HR4								-	0.77*	0.20*	0.09	0.22*	0.26*	0.34*	0.53*	0.34*
9. HR5									-	0.23*	0.14	0.15	0.20*	0.22*	0.39*	0.38*
10. FLACCB										-	0.29*	0.34*	0.23*	0.24*	0.27*	0.27*
11. FLACC0											-	0.70*	0.55*	0.38*	0.33*	0.35*
12. FLACC1												-	0.76*	0.54*	0.44*	0.42*
13. FLACC2													-	0.64*	0.49*	0.48*
14. FLACC3														-	0.74*	0.65*
15. FLACC4															-	0.73*
16. FLACC5																-

Note. FLACC= Face Legs Cry Consolability scale; HR= Heart Rate; *$p < .05$.

Table 4. Correlations among variables included in 18-month models.

	1.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Time since last feeding	-	0.11	0.09	0.06	0.14	0.13	0.06	0.06	0.08	-0.05	0.05	0.10	0.09	0.07	0.08	0.02
2. Time since last nap		-	-0.22	-0.24*	-0.11	0.01	0.07	0.02	-0.02	-0.11	-0.13	-0.07	-0.09	-0.08	-0.10	-0.10
3. HRB			-	0.75*	0.57*	0.46*	0.43*	0.42*	0.57*	0.67*	0.20	0.19	0.20	0.23*	0.18	0.31*
4. HR0				-	0.82*	0.66*	0.58*	0.48*	0.55*	0.59*	0.55*	0.41*	0.36*	0.32*	0.34*	0.36*
5. HR1					-	0.85*	0.66*	0.54*	0.52*	0.56*	0.60*	0.62*	0.56*	0.44*	0.36*	0.36*
6. HR2						-	0.83*	0.74*	0.65*	0.42*	0.48*	0.58*	0.63*	0.50*	0.40*	0.39*
7. HR3							-	0.88*	0.79*	0.26*	0.30*	0.35*	0.46*	0.49*	0.41*	0.39*
8. HR4								-	0.87*	0.21	0.25*	0.23*	0.36*	0.43*	0.46*	0.39*
9. HR5									-	0.31*	0.17	0.16	0.30*	0.41*	0.47*	0.50*
10. FLACCB										-	0.39*	0.40*	0.40*	0.42*	0.43*	0.52*
11. FLACC0											-	0.66*	0.59*	0.41*	0.41*	0.31*
12. FLACC1												-	0.74*	0.57*	0.51*	0.43*
13. FLACC2													-	0.81*	0.67*	0.57*
14. FLACC3														-	0.82*	0.72*
15. FLACC4															-	0.85*
16. FLACC5																-

Note. FLACC= Face Legs Cry Consolability scale; HR= Heart Rate; * $p < .05$.

Table 5. 12- and 18-month model estimated means.

	Baseline	Needle	Post 1	Post 2	Post 3	Post 4	Post 5
12-Month Model							
Group 1: FLACC Mean (SD)	2.01	7.88 (0.20)	7.07 (0.34)	6.20 (0.12)	5.27 (0.31)	4.28 (0.37)	3.23 (0.28)
Group 1: HR Mean (SD)	130.64	156.53 (5.38)	154.93 (4.64)	151.92 (1.55)	147.50 (0.86)	141.66 (3.51)	134.42 (0.58)
Group 2: FLACC Mean (SD)	0.68	5.30 (0.37)	3.08 (0.63)	1.67 (0.23)	1.07 (0.54)	1.27 (0.31)	2.27 (0.62)
Group 2: HR Mean (SD)	127.83	149.43 (0.39)	139.46 (0.45)	132.98 (0.59)	130.00 (1.24)	130.35 (0.59)	134.54 (0.18)
18-Month Model							
Group 1: FLACC Mean (SD)	3.85	7.44 (0.02)	7.30 (0.22)	7.06 (0.03)	6.72 (0.14)	6.27 (0.08)	5.73 (0.13)
Group 1: HR Mean (SD)	134.01	158.94 (2.01)	161.52 (1.04)	161.16 (1.79)	157.85 (0.82)	151.59 (1.64)	142.38 (0.10)

Group 2: FLACC Mean (SD)	2.54	6.96 (0.01)	4.88 (0.14)	3.37 (0.10)	2.44 (0.03)	2.08 (0.03)	2 .29 (0.04)
Group 2: HR Mean (SD)	132.99	151.93 (0.98)	142.27 (3.99)	135.21 (2.77)	130.74 (1.47)	128.87 (0.99)	129.60 (0.58)
Group 3: FLACC Mean (SD)	0.84	2.12 (0.02)	1.82 (0.16)	1.61 (0.32)	1.48 (0.26)	1.42 (0.06)	1.45 (0.07)
Group 3: HR Mean (SD)	124.18	121.87 (0.32)	121.73 (0.75)	121.77 (1.68)	121.00 (0.14)	122.41 (0.44)	123.00 (1.27)

Note. Baseline scores were derived post-hoc. For 12-month model , Group 1 represents 76% of participants. Group 2 represents 24% of the participants. For 18-month model, Group 1 represents 31% of participants. Group 2 represents 56% of the participants. Group 3 represents 13% of the participants.

Table 6. Summary of post-hoc logistic regression analyses predicting class membership at 12 months.

Predictor	*B*	*SE B*	OR
Time since last feeding	-0.003	.003	1.00
Time since last nap	-0.01	.003	1.00
Baseline heart rate	-0.02	0.2	0.99
Baseline behavioral distress (FLACC)	-0.66*	0.21	0.52

Note. OR= odds ratio. *$p < .01$. Logistic regressions were completed individually for each predictor in order to maximize sample size; time since last feeding *N*= 139; time since last nap *N*= 133; baseline heartrate *N*= 102; baseline behavioral distress *N*= 120.

Table 7. Summary of the post-hoc logistic regression analyses predicting class membership at 18 months.

Predictor	*B*	*SE B*	*OR*
Time since last feeding			
Class 2	0.002	0.003	1.00
Class 3	-0.003	0.54	1.00
Time since last nap			
Class 2	0.001	0.003	1.00
Class 3	0.002	0.003	1.00
Baseline heart rate			
Class 2	0.003	0.01	1.00
Class 3	-0.04	0.03	0.96
Baseline behavioral distress (FLACC)			
Class 2	0.15	0.08	1.16
Class 3	-0.60*	0.25	0.55

Note. *OR* = odds ratio. *$p < .05$. Logistic regressions were completed individually for each predictor in order to maximize sample size; time since last feeding *N*= 104; time since last nap *N*= 109; baseline heartrate *N*= 82; baseline behavioral distress *N*= 104.

Supplementary Table 1. Reasons for cardiac data not being usable.

	Frequency	
	12 months	18 months
Complete	129	101
Device Malfunction	19	15
Timing	5	4
No needle	2	2
Blocked	1	0
Electrode Removed	1	4
Declined Stickers	0	1
Lost to follow-up	0	23
Refused	0	14

Supplementary Table 2. Growth factor means (and SDs) for the latent growth curve models of behavioral distress and heart rate at 12 and 18 months.

	12- Month Intercept, mean (SD)	12-Month Linear, mean (SD)	12-Month Quadratic, mean (SD)	18-Month Intercept, mean (SD)	18-Month Slope, mean (SD)	18-Month Quadratic, mean (SD)
Behavioral distress (FLACC)	7.26 (1.53)	-1.21 (0.51)	0.07 (0.00)	6.45 (2.07)	-1.32 (1.41)	0.14 (0.25)
Heart rate	156.41 (22.20)	-4.61 (4.19)	0.05 (0.00)	148.45 (23.04)	-4.39 (10.79)	0.23 (1.78)

Note. Quadratic factor variances in 12-month model were fixed to 0 to obtain properly converged model solutions.

Supplementary Table 3. Model fit indices of parallel-process growth mixture models at 12 months.

Model	AIC	BIC	SSABIC
One group	9544	9624	9539
Two groups	9449	9550	9443
Three groups	9376	9498	9369
Four groups	9359	9503	9351

Note. AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; SSABIC = Sample-Size Adjusted Bayesian Information Criterion. Smaller values indicate better fit.

Supplementary Table 4. Growth factor means (and SDs) for parallel-process growth mixture models at 12 and 18 months.

	Intercept	Linear	Quadratic
12 Months- Group 1			
Behavior distress (FLACC)	7.88 (0.84)	-0.78 (0.50)	-0.03 (0.00)
Heart rate	156.53 (19.89)	-0.90 (3.68)	-0.71 (0.00)
12 Months- Group 2			
Behavior distress (FLACC)	5.30 (0.84)	-2.62 (0.50)	0.40 (0.00)
Heart rate	149.43 (19.89)	-11.72 (3.68)	1.75 (0.00)
18 Months- Group 1			
Behavior distress (FLACC)	7.44 (1.04)	-0.09 (0.79)	-0.05 (0.18)
Heart rate	158.94 (18.82)	4.06 (7.79)	-1.47 (1.24)
18 Months- Group 2			
Behavior distress (FLACC)	6.96 (1.04)	-2.37 (0.79)	0.29 (0.18)
Heart rate	151.93 (18.82)	-10.96 (7.79)	1.30 (1.24)
18 Months- Group 3			
Behavior distress (FLACC)	2.12 (1.04)	-0.34 (0.79)	0.04 (0.18)
Heart rate	121.87 (18.82)	-0.23 (7.79)	0.09 (1.24)

Note. In 12-month model, quadratic factor variances were fixed to 0 to obtain properly converged model solutions. In 18-Month model, inter-factor covariances were constrained to be equal across groups for model identification.

Supplementary Table 5. Inter-factor correlations for the two-group parallel-process growth mixture model at 12 months.

Behavior Distress (FLACC)	Heart rate	
	Intercept	Linear
Intercept	0.50	-0.25
Slope	0.17	0.28

Note. Inter-factor correlations were fixed to be equal across groups and all correlations with the quadratic factor variance were fixed to 0 to obtain a properly converged model solution.

Supplementary Table 6. Model fit indices of profiles at 18 months.

Model	AIC	BIC	ABIC
One group	7065	7170	7050
Two groups	7030	7155	7013
Three groups	7003	7147	6982
Four groups	6984	7148	6961

Note. AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; SSABIC = Sample-Size Adjusted Bayesian Information Criterion. Smaller values indicate better fit.

Supplementary Table 7. Inter-factor correlations for the three-group parallel-process growth mixture model at 18-month.

Behavior Distress (FLACC)	Heart rate		
	Intercept	Linear	Quadratic
Intercept	**0.40**	-0.25	0.02
Slope	-0.07	0.12	-0.13
Quadratic	0.10	**-0.34**	**0.49**

Note. Group 1, 2 and 3 have identical parameters. Significant correlations were bolded.

Chapter 7: Conclusion

This dissertation research provides a series of studies that contribute significantly to the field of early childhood distress regulation. The first study was a systematic review that used a narrative synthesis to examine the overall direction and magnitude of the relation between behavioural and cardiac indicators measured during distress in toddlerhood. The review revealed inconsistent relations between most behavioural and cardiac indicators of distress, which were posited to be related to the lower quality and methodological rigor of studies included in the synthesis. Study 2 built on Study 1 and adopted a dynamic, transactional perspective of distress regulation, using original longitudinal data to examine the concurrent and reciprocal relations between toddlers' pain-related behavioural distress and cardiac indicators (i.e., heart rate [HR], respiratory sinus arrhythmia [RSA]) during vaccinations at 12 and 18 months. The results parallel findings from Study 1 and found that HR and pain-related behavioural distress measured concurrently are the most consistently related distress indicators within a high-distress context (as compared with RSA). When compared to the effect sizes extracted in Chapter 2 (Systematic Review), these relations between HR and pain-related behavioural distress were moderate to strong. As well, the results highlighted the need to account for contextual factors (i.e., baseline responses) and phases of distress (i.e., initial reactivity vs. regulation from peak distress) to fully understand the relations among distress indicators. Subsequently, Study 3 built one Studies 1 and 2 and used person-centered analyses to describe the variability in regulatory trajectories (i.e., concurrent toddler pain-related behavioural distress and HR) post-needle during the 12- and 18-month vaccinations. Findings from Study 3 highlight the need to take a developmental approach

when studying distress regulation, as patterns of behavioural and cardiac distress responding became increasingly differentiated across toddlerhood.

This research has pushed the field of early childhood distress regulation forward, as a number of concrete suggestions for improving the quality and consistency of research investigating behavioural and cardiac measures of distress in toddlerhood have been provided (Study 1), and empirical work (Study 2 and 3) has used more sophisticated analyses that account for contextual factors and phase of distress to properly encapsulate toddler distress. Each study chapter (i.e., Chapter 2, 4, and 6) discussed the results of the study analyses individually. For ease of reader review, Appendix A presents a summary of the analyses and results for all three studies in point form. In the sections that follow, the findings from each of the three studies are briefly summarized. An integrative synthesis of all three studies, followed by a discussion of the clinical implications, limitations, and directions for future research is then presented.

Synthesizing the Literature on Behavioural and Cardiac Indicators of Distress in Toddlerhood: Study 1

The goal of Study 1 was to organize and synthesize the literature examining the direction and magnitude of the relation between behavioural and cardiac indicators of distress in toddlerhood. Studies were first organized according to conceptual categories (i.e., Cardiac Response Type [HR: baseline, task, change score; HP: baseline, standard deviation; RSA: baseline, task, change score; PEP: task] by Behavioural Response Type [emotion expression behaviour, emotion regulation behaviour]), and then further contextualized by methodological differences (*timing of measurement* [concurrent or predictive], *epoch length* [length of both behavioural and cardiac measurement epochs in

seconds], and *covariates/moderators* [were additional measures controlled for or investigated in analysis]). Given that the narrative synthesis was generally inconclusive due to heterogeneity in methodology and low study quality, the following synopsis is a general summary of findings and methodological challenges.

Relation between behavioural and cardiac distress responses

Overall, the only relation consistently found in the literature was between task HR and expressed emotion behaviours. These indicators were generally positively associated with standardized mean differences in the D = .05 to .54 range. These results suggest that cardiac-behaviour associations are strongest when they are concurrent instead of predictive. Inconsistent relations (i.e., positive and negative results found across studies included) or near-zero relations were found between expressed emotion behaviours and baseline HR, HP, RSA, RSA change score, and task PEP, and between emotion regulation behaviours and baseline HP and RSA, and RSA change score. A major gap in the literature was that no research has investigated the relation between HR and emotion regulation behaviours, or relations between behavioural and cardiac indicators during high distress contexts (e.g., pain). Taken together, the synthesis suggests that physiological and behavioural responses may be independent components of a toddler's distress response (Hastings et al., 2009), or that certain behavioural indicators (i.e., facial movements) may not have the sensitivity or specificity to represent specific emotions such as distress (Barrett et al., 2019). Additionally, it may be that heterogeneity in study findings result from methodological differences in the studies included.

Methodological challenges in the literature to date

Overall, the quality of the studies included was generally rated as *lower* and studies had less rigorous methodology for physiological data acquisition and analysis. Key methodological issues that Study 1 elucidated were: (1) a lack of reliable and valid behavioural coding systems; (2) inter-rater reliability coefficients not being reported for cardiac data analysis; (3) researchers not measuring the activated emotion (i.e., expressed emotion behaviours) and regulatory strategies (i.e., emotion regulatory behaviours) independently; (4) researchers collapsing measurement epochs that span across different phases of distress (i.e., initial reactivity versus recovery from distress); (5) differing operationalizations of cardiac indicators at baseline; and (6) few studies including relevant covariates (e.g., age, sex, baseline characteristics, time of day, activity level, and respiration).

In an effort to incorporate methodological recommendations set forth in the systematic review and the lack of research in high-distress contexts, Study 2 was an in-depth and dynamic investigation of the relation between behavioural and cardiac indicators of pain-related distress in toddlerhood.

Examining Reciprocal and Concurrent Relations Between Behavioural and Cardiac Indicators of Acute Pain in Toddlerhood: Study 2

The goal of Study 2 was to examine the predictive and concurrent within- and between-measure and contextual (i.e., baseline responses, time since last feeding and nap) relations between toddlers' pain-related behavioural distress (i.e., Face Legs Cry Consolability; Merkel et al., 2002) and cardiac responses (i.e., HR, RSA) during 12- and 18-month vaccinations. The method used for these analyses was cross-lagged path

analysis (Kessler & Greenberg, 1981). This was the first study to investigate the concurrent and predictive relations between behavioural and cardiac indicators of distress both within and across multiple phases of a high distress context (i.e., vaccination).

Behavioural and cardiac pain-related distress indicators were measured during a 60-second baseline epoch (i.e., pre-needle) and six different 30-second epochs across the first 3-minutes post-needle. The data for Study 2 came from a subsample of an ongoing longitudinal cohort of caregiver-toddler dyads who were seen at the 12- (N= 158) and/or 18-month (N= 122) vaccination. To summarize the dynamic relations between behavioural and cardiac indicators of distress during 12- and 18-month vaccinations: 1) Pain-related behavioural or physiological (i.e., HR, RSA) distress significantly predicts future pain-related behavioural or physiological distress, with moderate to strong effect sizes. The more behavioural or physiological pain-related distress a toddler expressed, the more he or she continues to express across the post-vaccination period. 2) Pain-related behavioural distress immediately post-needle significantly predicted physiological pain-related distress (i.e., HR, RSA) 30-seconds post-needle, albeit with small effect sizes. 3) After controlling for predictive autoregressive and cross-lagged effects, concurrent relations between behavioural and physiological pain-related distress were inconsistent; relations were stronger for HR than RSA and were categorized as moderate to strong effect sizes. 4) Finally, baseline behavioural and physiological pain-related distress consistently significantly predicted behavioural and physiological pain-related distress immediately post-needle, with moderate effect sizes.

Building on these cross-lagged path analyses, Study 3 examined how variability in behavioural and physiological reactivity and recovery processes from high distress in toddlerhood could be described by a small number of prototypical profiles.

Describing Toddler Patterns of Convergence between Cardiac and Behavioural Indicators of Pain-Related Distress: Study 3

The final study was descriptive, and examined whether there were distinct patterns of co-occurring pain-related behavioural distress and HR across six 30-second post-vaccination epochs at 12 and 18 months of age. Behavioural pain-related distress and HR were measured concurrently for 3-minutes post-vaccination. Two parallel-process growth-mixture models (Muthén & Shedden, 1999) were used to represent variability in toddler distress responding. Post-hoc binary or multinomial logistic regression models were then estimated to assess whether contextual factors (i.e., baseline behavioural distress, baseline HR, time since last feeding, and time since last nap) predicted group membership at 12 or 18 months. The analyses used the same participants as Study 2.

For the first time in the literature, patterns of concurrent behavioural and HR responding within a high distress context (i.e., vaccination pain) in toddlerhood were elucidated. Specifically, growth mixture modeling delineated two prototypical patterns of distress responding at 12 months and three patterns at 18 months. Across groups and ages, behavioural distress and HR responses post-vaccination followed similar trajectories, with most participants displaying high initial reactivity followed by regulation to baseline behavioural distress and HR levels following the vaccination. However, at 18 months, almost half of the participants displayed either a blunted

response (i.e., low reactivity, low regulation) or did not regulate to a low level of distress (i.e., high reactivity, low regulation) by 3 minutes post-needle. Post-hoc analyses revealed that higher baseline behavioural distress predicted membership in the majority groups (i.e., those participants who regulated to baseline levels of behavioural and cardiac distress) at 12 and 18 months.

In the following section, an integrative synthesis of all three studies is provided, which discusses what has been gleaned from the systematic review and variable- and person-centered analyses.

Integrative Synthesis

Informed by the broader distress regulation literature, the overarching goal of this dissertation was to provide an integrative understanding of the development of toddler distress regulation through careful examination of longitudinal patterns of cardiac and behavioural responding during distress. The three studies not only contribute to the broader distress regulation literature by providing methodological guidelines and standards for coding and analyzing behavioural and cardiac distress indicators, but also provide novel data, which adheres to these standards. Integrating all three studies, the experience of distress in toddlerhood is a complex orchestration of behavioural and physiological systems, which interact and become more differentiated over the second year of life.

Specifically, these studies provided a broad overview of cardiac indicators (i.e., HR, HP, RSA, PEP) used in conjunction with behavioural distress measures to encapsulate toddler distress, and noted that HR and RSA are the most commonly used

cardiac indicators of distress in toddlerhood. Based on the systematic literature review and the two empirical studies, it can be concluded that behavioural distress and HR measured concurrently have a stronger relation during distress in toddlerhood than behavioural distress and RSA. These results provide further support that HR can be used to represent the hedonic tone of an emotional state (Buss et al., 2005; Baker et al., 2012; Hay et al., 2017; Provost & Gouin-Décarie, 1979).

However, the relations among distress indicators are dynamic, as our results highlight that initial pain-related behavioural distress predicted cardiac responses (i.e., HR, RSA) 30-seconds post-needle. These results underscore the regulating or dysregulating impact of initial pain-related behavioural distress on toddler physiology in the initial reactivity period of the vaccination, which has carryover effects for behavioural and cardiac responses in the regulatory phase following high distress. Additionally, it is important to note that over the second year of life, this work showed that indicators of distress responding (i.e., concurrent behavioural pain-related distress and HR) became increasingly differentiated, with almost half of the toddlers examined not regulating to baseline levels or displaying a blunted distress response across three-minutes post-needle. Thus, researchers may obtain divergent results when examining distress regulation in toddlerhood according to the behavioural or cardiac indicator utilized, the age at examination, the length of the measurement epoch, and the nature of the study design (i.e., distress indicators observed in a cross-sectional or dynamic manner).

Another factor that was highlighted in this research was the importance of incorporating contextual factors when examining the relation between behavioural and cardiac distress indicators. Across studies, baseline behavioural and cardiac distress

responses predicted future distress responses and regulatory patterns, such that higher baseline behavioural and cardiac responses were positively associated with higher initial behavioural and cardiac responses post-needle. However, higher baseline behavioural and cardiac responses predicted more adaptive patterns of distress responding (i.e., toddlers that return to homeostasis).

Clinical Implications

Considering all three studies, several clinical implications are offered. For the first time, the results of this research provide researchers and medical professionals with normative data regarding how toddlers respond both behaviourally and physiologically (i.e., HR, RSA) to acute procedural pain, specifically the most commonly used painful procedure in general practice (i.e., vaccination pain). These norms provide valuable insight into how pain should be assessed and managed in later infancy and toddlerhood, as well as potential profiles of distress responding that may warrant follow-up.

Related to pain assessment, the shared variance between HR and pain-related distress behaviours suggests that these indicators are complementary measures of pain in toddlers, and confirms practice in neonatology where multimodal approaches to pain in nonverbal children is the evidence-based bedside approach (e.g., Premature Infant Pain Profile-Revised; Gibbins et al., 2014). However, there were inconsistent concurrent relations between behavioural and cardiac indicators across the post-needle period and differing patterns of distress responding (e.g., hypo- or hyper-responsive, regulating) at 12 and 18 months. Therefore, our research suggests that although multidimensional pain indicators accurately capture pain-related distress within the immediate reactivity period,

the reliability and validity of multidimensional measures may be questionable within the regulatory phase of distress.

Relatedly, the predictive nature of baseline behavioural and cardiac distress, as well as previous distress responses in predicting future distress responses, was highlighted. Thus, infant pain assessment tools should account for baseline levels of behavioural distress and cardiac responses in addition to observing infants and toddlers across the reactivity (e.g., 30-seconds post-need) and regulatory phases of pain-related distress to obtain a more complete assessment of pain levels. Additionally, given that toddlers' baseline distress levels predicted immediate distress responses following the vaccination and how toddlers regulated from distress, healthcare professionals should ensure that caregivers soothe their children before vaccination to reduce distress as much as possible prior to the needle. Indeed, Pillai Riddell and colleagues (2018) found that when caregivers were taught non-medical pain management strategies to keep themselves and their child regulated prior to 18-month vaccinations, their children displayed lower pain-related behavioural distress scores post-needle compared to a control group.

Regarding common patterns of distress responding, almost 50% of 18-month-old toddlers displayed behavioural distress and HR that did not completely regulate across the three-minutes post-needle or were blunted (i.e., no change from baseline in either behaviour distress or HR). Although developmental outcomes were not examined in relation to these trajectories, it is possible that screening for certain distress regulatory patterns would allow for earlier detection of deleterious regulatory strategies, facilitating referrals for ameliorative mental health treatments.

Limitations

There are several limitations to note. In Study 1, most studies included were from North American researchers, which may limit the generalizability of our findings. As well, the lower quality of the majority of studies must be taken into consideration when drawing conclusions from our synthesis. Additionally, many of the studies did not report data when the relation between indicators was non-significant. As such, the weaker relations between behavioural and most cardiac indicators may overestimate the true relation between distress indicators. For Studies 2 and 3, generalizability is affected by the education level of the sample and the self-selection bias associated with being a caregiver who agrees to be followed through the second year of vaccinations. As well, our study included healthy toddlers born full-term who underwent a standardized acutely painful procedure, which limits generalizability to non-healthy neonates or premature infants who must undergo multiple acute painful or stressful procedures. The observational design of Study 2 and 3 should also be acknowledged. It is also possible that the relationships between the variables could be explained by unmeasured variables such as parental behaviours, style or mental health, or attachment style. Indeed, autonomic activity is determined and impacted by many potentially unmeasured factors (Bush, Alkon, Obradovic, Stamperdalh, & Boyce, 2011). Finally, related to coding of behavioural pain-related distress, it is important to note that emotions are not subject to regulation (Thompson, 2011), but are themselves regulating processes. Although the current dissertation was not designed to disentangle this particular issue, or focus on emotion regulation behaviours specifically, it should be examined in future work. All clinical implications offered above should be considered in the context of these points.

Directions for Future Research

Several directions for future research in developmental psychophysiology and pediatric pain stem from this dissertation. First, this dissertation has highlighted the inconsistencies in methodological and reporting practices as well as gaps in the current literature. Future researchers are encouraged to utilize methodological standards outlined in the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996) and also within Chapter 2 of this dissertation (i.e., using behavioural coding schemes that have established psychometrics, reporting inter-rater reliability coefficients for cardiac data analysis, using temporal measurement epochs and statistical approaches that capture dynamic aspects of distress). Additionally, future research examining toddler responses to distress should include multiple physiological (e.g., heart rate, pre-ejection period, oxygen saturation, electroencephalography, skin conductance, cortisol) and behavioural (e.g., expressed emotion behaviours, self-regulation of emotion behaviours, caregiver behaviours) indicators to better understand individual differences in behavioural and physiological convergence in different distress contexts (e.g., fear, frustration, pain). Relatedly, given that the strength of the relations between behavioural pain-related distress and cardiac indicators differs across phases of distress regulation (i.e., reactivity vs. regulation), our research suggests possible revisions to the DIAPR-R model. Specifically, the DIAPR-R model should account for differences in the strength of the relation between different pain indicators (e.g., behavioural, autonomic, cortical) as the toddler transitions from initial pain reactivity to pain regulation. Future research should model individual patterns of brain, behaviour, and physiology during pain to validate these revisions to the DIAPR-R

model. Second, given that our participants' ages coincide with the theorized development of attachment relationships with primary caregivers (Ainsworth 1973), and differences in distress regulation have been linked to differences in attachment style (i.e., Horton et al., 2015; Qu & Leerkes, 2018), future studies should examine the impact of the child's social context (including parenting behaviour and attachment security) on patterns of behavioural and cardiac distress responding. Third, it would be informative to investigate whether patterns of distress responding are associated with subsequent socio-emotional, cognitive, language, and motor development. Finally, given that the empirical studies in the dissertation consisted of typically developing toddlers, these analyses should be replicated with other samples, paying attention to contextual factors that are known to impact distress regulation abilities (e.g., poverty, preterm birth, child maltreatment, family stress).

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Appendix A

Summary of Analyses and Results

STUDY 1: CONVERGENCE OF BEHAVIORAL AND CARDIAC INDICATORS OF DISTRESS IN TODDLERHOOD: A SYSTEMATIC REVIEW AND NARRATIVE SYNTHESIS

Research Question: What is the concurrent (i.e., within-session) relation between behavioral and cardiac measures of distress in toddlerhood?

Analysis: A search yielded 2424 studies, which were examined against inclusion criteria. 22 studies were included in the review.

Results: The narrative synthesis included an examination of the general direction (positive, negative) and magnitude (Cohen's D) of the findings.

- The overall relation between behavioural and cardiac indicators needs to be contextualized in the lower study quality and methodological rigor.
- The relation between behavioural (i.e., expressed emotion behaviours, emotion regulation behaviours) and 7 types of cardiac measures (i.e., baseline heart rate [HR], HR responding, baseline heart period, baseline respiratory sinus arrhythmia [RSA], RSA responding, RSA change score, pre-ejection period responding) was examined. Overall, findings suggested:
    - Relations with expressed emotion behaviours
        - Positive relations found with HR responding (D = .05 to .54).
        - Near-zero relations or mixed results with baseline HR, HP and RSA, RSA and PEP responding, and RSA change score.
    - Relations with emotion regulation behaviours
        - Near-zero or mixed relations with baseline HP and RSA, RSA responding, and RSA change score.

STUDY 2: AN EXAMINATION OF THE RECIPROCAL AND CONCURRENT RELATIONS BETWEEN BEHAVIORAL AND CARDIAC INDICATORS OF ACUTE PAIN IN TODDLERHOOD

Research Question: What are the predictive and concurrent within- and between-measure and contextual relations between toddlers' expressed pain behaviors and cardiac responses (i.e., HR, RSA) during 12- and 18-month vaccinations?

Analysis: Four autoregressive cross-lagged path models, two (HR, RSA) at each of the two time-points (12 and 18-months of age).

Results:

- ***Predictive within-measure relations***: Across 12 and 18 months, behavioural pain-related distress, HR and RSA positively predicated forward across all phases of vaccination.
- ***Predictive between-measure relations:*** Across 12 and 18 months, behavioural pain-related distress predicted HR (positive relation) and RSA (negative relation) responses at 30-seconds post-needle.
- ***Concurrent (residual) between-measure relations:*** Concurrent (i.e. at the same time epoch) associations were found between behavioural pain-related distress and cardiac responses (i.e., HR, RSA) at 12 and 18 months, over and above their predictive autoregressive and cross-lagged effects.
    - HR had stronger and more consistent relations with behaviour than RSA.
- ***Contextual factors:*** Baseline distress responses consistently predicted future pain scores and cardiac responses within the post-needle period at both 12 and 18 months.

STUDY 3: INVESTIGATING CONVERGENCE OF CARDIAC AND BEHAVIORAL INDICATORS OF DISTRESS DURING ROUTINE VACCINATIONS OVER THE SECOND YEAR OF LIFE

Research Question: Is there variability in regulatory trajectories (i.e., concurrent toddler behavior distress and HR) from immediately following needle to 3 minutes post-needle during the 12- and 18-month vaccinations?

Analysis: Two parallel process growth mixture models (12 and 18 months).

Results:

- At 12 months of age, two groups were discerned reflecting distinct patterns of behavioral distress and HR responses post-vaccination:
    - Group 1 (76% of the sample) were considered to be "high-reactive" children (high initial reactivity, high regulatory ability).
    - Group 2 (24% of the sample) were considered to be "low-reactive" children (low initial reactivity, low regulatory ability).
- At 18 months of age, three groups were discerned reflecting distinct patterns of behavioral distress and HR responses post-vaccination:
    - Group 1 (31% of the sample) were considered to be "difficult" children (high initial reactivity, low regulatory ability)
    - Group 2 (56% of the sample) were considered to be "high-reactive" children.
    - Group 3 (13% of the sample) were considered to be "low-reactive" children.
- At both 12 and 18 months of age, baseline behavioral distress predicted increased likelihood of group membership in the "high-reactive" group (i.e., the group with the greatest degree of regulation by the end of the vaccination period).

Appendix B

Systematic Review Search Strategies

MEDLINE Search Strategy:

1 cardiovascular physiological phenomena/ (11639)

2 heart rate/ (156653)

3 respiratory sinus arrhythmia/ (150)

4 vagus nerve/ (21510)

5 heart rate*.tw,kf. (146071)

6 (cardiac or cardiovascular).tw,kf. (858732)

7 (vagal or vagus).tw,kf. (31019)

8 respiratory sinus arrhythmia*.tw,kf. (1517)

9 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 (1031852)

10 child behavior/ (15976)

11 infant behavior/ (3000)

12 Facial Expression/ (11354)

13 Affect/ (28777)

14 Temperament/ (5149)

15 behavior*.tw,kf. (830001)

16 behaviour*.tw,kf. (243413)

17 (facial adj3 (express* or response*)).tw,kf. (8676)

18 affect.tw,kf. (561100)

19 temperament*.tw,kf. (7786)

20 observation*.tw,kf. (739849)

21 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 (2283517)

22 9 and 21 (98891)

23 exp Pain/ (358006)

24 Pain Measurement/ (75924)

25 Pain Threshold/ (11535)

26 stress, psychological/ (106396)

27 Frustration/ (2164)

28 exp Immunization/ (160931)

29 exp Vaccines/ (209532)

30 exp Injections/ (272512)

31 Phlebotomy/ (2801)

32 exp Fear/ (30979)

33 exp Anger/ (7444)

34 (pain* or stress* or distress*).tw,kf. (1346136)

35 strange situation*.tw,kf. (447)

36 separation*.tw,kf. (199855)

37 still face*.tw,kf. (1179)

38 interaction challenge*.tw,kf. (27)

39 frustrat*.tw,kf. (15347)

40 immuniz*.tw,kf. (123824)

41 immunis*.tw,kf. (11196)

42 vaccine*.tw,kf. (205337)

43 Vaccinat*.tw,kf. (134684)

44 injection*.tw,kf. (524218)

45 phlebotom*.tw,kf. (7386)

46 venesection*.tw,kf. (705)

47 venipuncture*.tw,kf. (3113)

48 heel prick*.tw,kf. (307)

49 heelprick*.tw,kf. (11)

50 heel stick*.tw,kf. (190)

51 heelstick*.tw,kf. (77)

52 heel lance*.tw,kf. (164)

53 (fear* or panic*).tw,kf. (84391)

54 (anger* or angry or rage*).tw,kf. (22071)

55 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54 (2824977)

56 22 and 55 (19614)

57 (infant* or infancy or baby* or babies or toddler* or pre-school* or preschool*).mp. (1611138)

58 56 and 57 (1291)

EMBASE Search Strategy:

1 exp heart rate/ (232966)

2 cardiovascular function/ (16783)

3 cardiovascular response/ (11427)

4 vagus nerve/ (25441)

5 heart rate*.tw,kw. (196104)

6 (cardiac or cardiovascular).tw,kw. (1226858)

7 (vagal or vagus).tw,kw. (43346)

8 respiratory sinus arrhythmia*.tw,kw. (1786)

9 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 (1441300)

10 child behavior/ (41055)

11 facial expression/ (18410)

12 affect/ (21420)

13 temperament/ (8397)

14 behavior*.tw,kw. (906632)

15 behaviour*.tw,kw. (327272)

16 (facial adj3 (express* or response*)).tw,kw. (10480)

17 affect.tw,kw. (692445)

18 temperament*.tw,kw. (9720)

19 observation*.tw,kw. (977978)

20 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 (2786761)

21 9 and 20 (141510)

22 exp pain/ (1137957)

23 pain measurement/ (4022)

24 exp pain threshold/ (16860)

25 exp stress/ (248189)

26 frustration/ (6704)

27 exp immunization/ (278332)

28 exp vaccine/ (322884)

29 exp injection/ (219646)

30 vein puncture/ (5818)

31 fear/ (55017)

32 exp anger/ (16878)

33 (pain* or stress* or distress*).tw,kw. (1854434)

34 strange situation*.tw,kw. (501)

35 separation*.tw,kw. (249658)

36 still face*.tw,kw. (1423)

37 interaction challenge*.tw,kw. (24)

38 frustrat*.tw,kw. (17490)

39 immuniz*.tw,kw. (155385)

40 immunis*.tw,kw. (14436)

41 vaccine*.tw,kw. (250073)

42 Vaccinat*.tw,kw. (166122)

43 injection*.tw,kw. (746639)

44 phlebotom*.tw,kw. (9905)

45 venesection*.tw,kw. (1206)

46 venipuncture*.tw,kw. (4421)

47 heel prick*.tw,kw. (397)

48 heelprick*.tw,kw. (18)

49 heel stick*.tw,kw. (239)

50 heelstick*.tw,kw. (98)

51 heel lance*.tw,kw. (185)

52 (fear* or panic*).tw,kw. (113045)

53 (anger* or angry or rage*).tw,kw. (29800)

54 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 (4008931)

55 21 and 54 (28763)

56 (infant* or infancy or baby* or babies or toddler* or pre-school* or preschool*).mp. (1302979)

57 55 and 56 (1470)

PSYCHINFO Search Strategy:

1 vagus nerve/ (1078)

2 cardiovascular reactivity/ (3439)

3 heart rate/ (11183)

4 heart rate*.tw. (20008)

5 (cardiac or cardiovascular).tw. (38946)

6 (vagal or vagus).tw. (3874)

7 respiratory sinus arrhythmia*.tw. (902)

8 1 or 2 or 3 or 4 or 5 or 6 or 7 (56371)

9 behavior/ (23541)

10 exp facial expressions/ (9860)

11 behavior*.tw. (809240)

12 behaviour*.tw. (113451)

13 (facial adj3 (express* or response*)).tw. (10609)

14 affect.tw. (166120)

15 temperament*.tw. (15630)

16 observation*.tw. (143891)

17 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 (1111822)

18 8 and 17 (15693)

19 exp pain/ (51148)

20 pain measurement/ (1274)

21 pain perception/ or pain thresholds/ (11569)

22 exp stress/ (95211)

23 frustration/ (2384)

24 exp fear/ (18501)

25 exp anger/ (12516)

26 immunization/ (3892)

27 exp injections/ (4715)

28 distress/ (18722)

29 (pain* or stress* or distress*).tw. (360894)

30 strange situation*.tw. (1133)

31 separation*.tw. (27918)

32 still face*.tw. (600)

33 interaction challenge*.tw. (26)

34 frustrat*.tw. (16702)

35 immuniz*.tw. (3086)

36 immunis*.tw. (234)

37 vaccine*.tw. (3853)

38 vaccinat*.tw. (3781)

39 injection*.tw. (43993)

40 phlebotom*.tw. (104)

41 venesection*.tw. (21)

42 venipuncture*.tw. (261)

43 heel prick*.tw. (30)

44 heelprick*.tw. (1)

45 heel stick*.tw. (51)

46 heelstick*.tw. (30)

47 heel lance*.tw. (66)

48 (fear* or panic*).tw. (86252)

49 (anger* or angry or rage*).tw. (33923)

50 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 (556609)

51 18 and 50 (6045)

52 limit 51 to (120 neonatal <birth to age 1 mo> or 140 infancy <2 to 23 mo>) (271)

53 (infant* or infancy or baby* or babies or toddler* or pre-school* or preschool*).mp. (149538)

54 51 and 53 (440)

55 52 or 54 (461)

CINAHL Search Strategy:

#	Query	Results
S51	S7 AND S38 AND S49 AND S50	526
S50	(infant* or infancy or baby* or babies or toddler* or pre-school* or preschool*)	387,305
S49	S39 OR S40 OR S41 OR S42 OR S43 OR S44 OR S45 OR S46 OR S47 OR S48	466,795
S48	observation*	96,535
S47	temperament*	2,943
S46	affect	105,908
S45	(facial N3 (express* or response*))	3,717
S44	behaviour*	51,146
S43	behavior*	256,831
S42	(MH "Temperament")	1,943
S41	(MH "Affect")	10,126
S40	(MH "Facial Expression")	3,048
S39	(MH "Child Behavior+")	10,545
S38	S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34 OR S35 OR S36 OR S37	546,951

S37	(anger* or angry or rage*)	9,103
S36	(fear* or panic*)	30,276
S35	heel lance*	88
S34	heelstick*	46
S33	heel stick*	86
S32	heelprick*	4
S31	heel prick*	76
S30	venipuncture*	1,559
S29	venesection*	56
S28	phlebotom*	1,580
S27	injection*	51,210
S26	Vaccinat*	18,055
S25	vaccine*	41,089
S24	immunis*	1,925
S23	immuniz*	26,270
S22	frustrat*	4,853
S21	interaction challenge*	10
S20	still face*	325
S19	separation*	7,811
S18	strange situation*	119
S17	(pain* or stress* or distress*)	391,000
S16	(MH "Venipuncture+")	2,035
S15	(MH "Injections+")	25,091
S14	(MH "Vaccines+")	34,347
S13	(MH "Immunization")	17,757
S12	(MH "Frustration") OR (MH "Fear+") OR (MH "Anger")	13,964
S11	(MH "Stress, Psychological+")	58,131
S10	(MH "Pain Threshold")	3,830

S9	(MH "Pain Measurement")	37,740
S8	(MH "Pain+")	151,105
S7	S1 OR S2 OR S3 OR S4 OR S5 OR S6	227,073
S6	respiratory sinus arrhythmia*	205
S5	(vagal or vagus)	2,615
S4	(cardiac or cardiovascular)	201,721
S3	heart rate*	34,263
S2	(MH "Arrhythmia, Sinus+")	498
S1	(MH "Cardiovascular System Physiology") OR (MH "Heart Rate")	29,533

Appendix C

Information Package for Participating Parents

We need you!

Our new study involves videotaping 12-, 18-, and 24-month-old children during their immunization appointments and completing parent questionnaires. Interested parents will also be given the opportunity to receive a free general developmental assessment at York University after the 24-month immunization. During both the immunizations and the assessment at York University, we would be attaching baby-friendly stickers to the children's and parent's shoulder blades and lower ribs so that equipment can capture their heart's reaction to pain and stress.

Why participate?

You will be helping us understand how kids learn to cope with stress. After every immunization, you will receive a DVD memento and a $10 coffee gift certificate. After participating in the York University assessment, you will receive a **developmental report** using gold standard child development tools. These reports contain valid measures of your child's cognitive, language, and motor development. Reports will help you optimize your child's learning potential.

Want to register?

Please email our Cohort Assessment Coordinator at cohort@yorku.ca or call at 416 736-2100, ext. 20177! Please call us before your child's 12-month immunization or let the receptionist know.

Ouch Lab

Opportunities to Understand Childhood Hurt Laboratory

YORK UNIVERSITÉ UNIVERSITY

redefine THE POSSIBLE.

Opportunities to Understand Childhood Hurt Laboratory

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Toronto, ON
M3J 1P3
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Email: ouchlab@yorku.ca
Website: www.yorku.ca/ouchlab

Have a heart for infants in pain!

Be a Part of Our New Cohort!

Who are we?

The Opportunities to Understand Childhood Hurt Laboratory, is located at York University. It opened in Summer 2004. Since then, under the leadership of Dr. Pillai Riddell, the lab has produced innovative research with infants and how they experience pain, particularly in the context of caregiver interactions.

The old cohort

The O.U.C.H. Lab team has ambitiously followed 760 babies during immunizations over their first year of life in Toronto pediatric clinics.

When the same children were 4 to 6 years of age, approximately 200 families came to the OUCH lab at York University and received a full preschool psychoeducational assessment. This allowed the OUCH lab to better understand how early infant behaviours during immunization relate to their functioning later in childhood.

The new cohort

Now that we understand how babies experience pain in the first year of life, we would like to better understand how infants function during immunizations and in other settings in the second year of life!

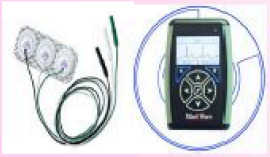
This will tell us how infant behaviours during distressing times relate to their functioning at the end of the second year of life, and what parent behaviours are important in optimizing this functioning.

The most exciting aspect of our study is that we have state-of-the-art technology that can tell how your child's heart is reacting to pain, since they cannot tell us themselves!

This 12-month old is having her heart beats counted!

What can this new technology do?

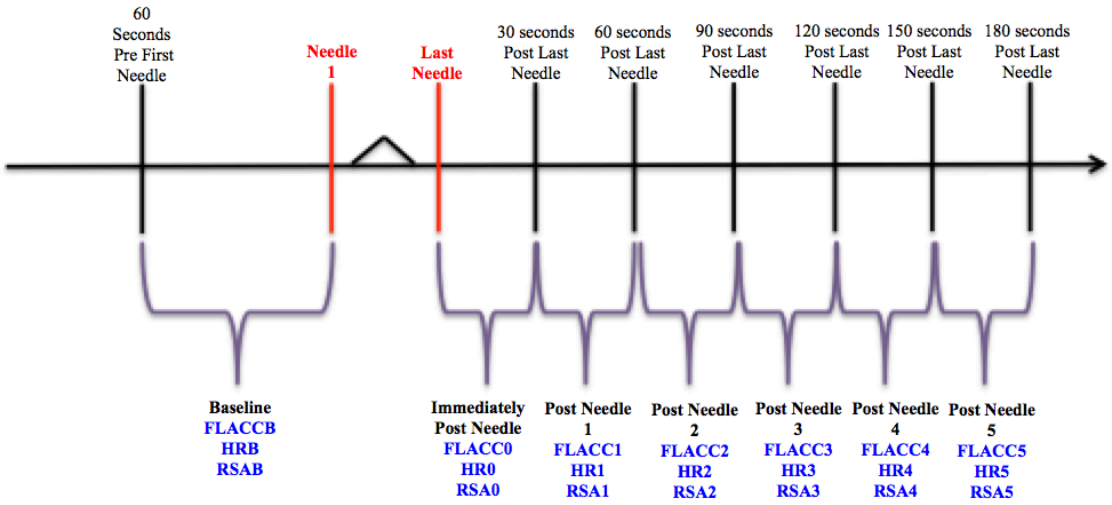
A wireless device can measure how fast your child's heart is beating through three baby-friendly stickers on your child's shoulder blade and lower ribs!



The wireless device tells us when your child is having strong emotional reactions by looking at how much your child's heart is beating! We use the technology to count parent heart beats too!

Appendix D

12- and 18-Month Vaccination Timeline



Note: FLACC= Face Legs Cry Consolability scale; HR= Heart Rate (beats per minute); RSA= Respiratory Sinus Arrhythmia.

Appendix E

Participant Information Sheet- 12 Months

Date of Appointment_____ Participant ID_____

PARTICIPANT INFORMATION SHEET – 12 MONTHS

PART 1: PARENT QUESTIONS – *These questions refer to the parent who will be most responsible for **soothing** child during needle.* (*If possible, all questions to be asked by Clinic RA)*

1. **Your birth date** (dd/mm/yyyy)_____
2. **Your relationship to Infant:** Mother Father Other _____
3. **Who is currently the primary caregiver of your infant?**
   Mother Father Equally between Other _____
   Mother & Father
4. **Time since last fed**: _____Hours _____Minutes
5. **Time since last napped**: _____Hours _____Minutes
6. **Number of Family Members living in your household**: Adults _____ Children_____
7. **For each child in your family please list their age and sex**.
   Age of infant brought in today: _____(months) **Male** **Female**
   Birth date of infant (dd/mm/yyyy): _____

   Ages/genders of your other children:
   Age: _____ Male Female
   Age: _____ Male Female
   Age: _____ Male Female
   Age: _____ Male Female
8. **Which caregivers are present at this immunization?** (circle one number):

1. Mom only
2. Dad only
3. Mom and Dad
4. Nanny
5. Grandparents
6. Parent(s) and Nanny
7. Parent(s) and Grandparent(s)
8. Other _____
9. Parent(s) and Other _____

b) How many other children are present, if any? (do not include child getting the needle) ____

9. Since your child's birth, have you taken any infant parenting classes/workshops? **Yes No**
   If yes, how many? _____

10. Since your child's birth, have you read any infant parenting books/watched videos? **Yes No**
    If yes, how many?_____

11. Since your child's birth, approximately how often do you visit parenting websites?

Never	Once a day	Once a week	Once every few weeks	Once a month	Once a year

12. Since your child's birth, have you received any guidance from an organization or professional to help with parenting your children (e.g. health unit nurse, midwife, Early Years Centre, Healthy Babies Healthy Children, Hincks-Dellcrest, Jessie's Place)? **Yes No**
    If yes, from how many organizations/professionals? _____

13. Has your child been given EMLA or TYLENOL prior to the appointment?:
    EMLA TYLENOL NONE

14. A) Since your child's birth, has your infant been separated from his/her primary caregiver for longer than 24 hours (e.g. infant hospitalization, parent hospitalization, Children's Aid involvement, parent travel, family emergency)?
    Circle: **YES NO**

    ***If you circled YES*:**
    Approximately, how many separations longer than 24 hours have occurred? _____
    How long was the longest period of separation _____(days)

15. **Child's Medical History**

Please check next to any illness or condition that your child has had since birth. When you check an item, also note the approximate date of the illness or your child's age at illness.

Illness or condition	Date(s) or age(s)	Illness or condition	Date(s) or age(s)
□Measles	_____	□Visual problems	_____
□German Measles	_____	□Fainting spells	_____
□Mumps	_____	□Loss of consciousness *(please specify cause)*	_____
□Chicken Pox	_____	□Lead Poisoning	_____
□Whooping Cough	_____	□Ear Problems	_____
□Diphtheria	_____	□TB	_____
□Scarlet Fever	_____	□Bone or joint disease	_____
□Meningitis	_____	□Anemia	_____
□Pneumonia	_____	□Jaundice/Hepatitis	_____
□Encephalitis	_____	□Cancer	_____

□High fever (>41°C or 105.8°F) _____
□Seizure _____
□Allergy _____
□Hay Fever _____
□Injuries to head _____
□Broken bones _____
□Hospitalization _____
(please specify reason)
□Operations _____
(please specify)
□Otitis media _____
□Heart disease _____
□Asthma _____
□Bleeding problems_____
□Eczema or hives _____
□Paralysis _____
□Stomach pumped_____
□Thrush _____
□Circumcision _____
□Other _____

16. Since birth, has your child been diagnosed with any other chronic illnesses not listed above?
Yes No
If <u>yes</u>, which chronic illness and at what age were they diagnosed? _____
_____

17. Since birth, has your child taken any medication long-term (i.e. longer than 2 weeks)?
Yes No
If <u>yes</u>, please list: _____
_____

<u>PART 2: PREGNANCY HISTORY</u> – If child is adopted please check box: □
(If so, please fill as much of the following information as you are aware of, which regards to the child's birth mother, her pregnancy, and delivery.)

1. During pregnancy, were you on medication? **Yes□ No□**
   If yes, what kind? _____
2. During pregnancy, did you smoke? **Yes□ No□**
   If yes, how many cigarettes each day? _____
3. During pregnancy, did you drink alcoholic beverages? **Yes□ No□**
   If yes, what did you drink? _____
   Approximately how much alcohol was consumed each day? _____
4. During pregnancy, did you use drugs? **Yes□ No□**

a. If yes, what kind?_____

5. Were there any complications during pregnancy (excessive vomiting, excessive staining/blood loss, threatened miscarriage, infections, toxemia, fainting, dizziness, etc.)
   _____
   _____
6. Duration of pregnancy (weeks):_____
7. Duration of labour (total hours):_____
8. Were there indications of fetal distress during labour or during birth? **Yes□ No□**
9. Were forceps used during delivery? **Yes□ No□**
10. Was delivery normal? **Yes□ No□**

11. Was a Caesarean section performed? **Yes□ No□**
12. Was delivery breach? **Yes□ No□**
13. Was delivery induced? **Yes□ No□**
    If yes on any of the above, for what reason? _____
14. Was your child premature? **Yes□ No□**

a. If so, by how many weeks?_____

15. What was your child's birth weight? (in pounds)_____
16. Were there any birth defects or complications? **Yes□ No□**

a. If yes, please describe: _____
_____

PART 3: PRIMARY CAREGIVER AND SPOUSE BACKGROUNDS – *(If possible, all questions to be asked by Clinic RA)*

1. **Your Highest Education (circle one number):**
   1. Graduate School/Professional Training
   2. University graduate (4 years college)
   3. Partial university (at least 1 year)
   4. Trade School/Community College
   5. High School Graduate
   6. Some high school
   7. Junior high school graduate
   8. Less than 7th grade

a) **Your Current Occupation** _____

2. If applicable, your Spouse's Highest Education (circle one number):
   1. Graduate School/Professional Training
   2. University graduate (4 years college)
   3. Partial university (at least 1 year)
   4. Trade School/Community College
   5. High School Graduate
   6. Some high school
   7. Junior high school graduate
   8. Less than 7th grade

a) **If applicable, Your Spouse's Current Occupation** _____

3. **What language is spoken most in your home (only put two languages if they are equally spoken)** _____

4. What country was the primary caregiver born in:_____

a) If applicable, what country was your spouse born in:_____

5. What country was the primary caregiver's parents born in:_____

a) If applicable, what country were your spouse's parents born in: _____

6. How long has the primary caregiver lived in Canada: _____

a) If applicable, how long has your spouse lived in Canada: _____

7. **On a scale of 0 to 10, where 0 is "Not at All" and 10 is "Completely", how much do you feel your way of life reflects mainstream North American/Canadian culture? _____**

a) If applicable, how much do you feel your spouse's way of life reflects mainstream North American/Canadian culture: _____

8. **What is your heritage culture (**It may be the culture of your birth, the culture in which you have been raised, or another culture that forms part of your background. Pick the culture that has influenced you *most*. If you do not feel that you have been influenced by any other culture, please try to identify a culture that may have had an impact on previous generations of your family.**):**

_____

a) If applicable, what is your spouse's heritage culture: _____

9. **On a scale of 0 to 10, where 0 is "Not at All" and 10 is "Completely", how much do you feel your way of life reflects your heritage culture? _____**

a) If applicable, how much do you feel your spouse's way of life reflects his/her heritage culture:

_____

PART 4: PARENT RATINGS (PRE-IMMUNIZATION)

Pre-Immunization **Self-Worry** Rating

*On a scale from 0 to 10, how worried about the needle pain are **YOU**, right now, before the needle, where 0 is "no worry at all" and 10 is "the most worry possible"?*

_____

PART 5: PARENT RATINGS (POST-IMMUNIZATION)

Post-Immunization **Self-Worry** Rating

*On a scale from 0 to 10, how worried about the needle pain are **YOU**, right now, after the needle, where 0 is "no worry at all" and 10 is "the most worry possible"?*

To be done approximately 5 minutes after last needle!

_____

Post-Immunization **Child Pain** Rating

On a scale from 0 to 10, how much pain do you think your child experienced from the needles they just received, where 0 is "no pain at all" and 10 is "the worst pain possible"?

_____

PART 6: VACCINES GIVEN BY IMMUNIZATION NEEDLE

RA to fill out (ask nurse or doctor for vaccine name and trade name)

Disease it Protects Against

- ☐ Prevnar _____
- ☐ Twinrix _____
- ☐ Bexsero _____
- ☐ Menjugate _____
- ☐ Pediacel _____
- ☐ Menveo _____
- ☐ Nimenrix _____
- ☐ Other (List Name) _____
- ☐ Pneumococcal-13 _____
- ☐ NeisVac-C (Menj C) _____
- ☐ MMR _____
- ☐ DPTP/H _____
- ☐ HAB (Hep A / B) _____
- ☐ Varicella _____

How many needles total: _____

1. Vaccines in needle #1:_____

2. Vaccines in needle #2:_____

Appendix F

Participant Information Sheet- 18 Months

Date of Appointment_____ Participant ID_____

PARTICIPANT INFORMATION SHEET – 18 MONTHS

PART 1: PARENT QUESTIONS – *These questions refer to the parent who will be most responsible for **soothing** child during needle.* (*If possible, all questions to be asked by Clinic RA)*

16. **Were you the primary soother at the 12-month appointment?** Yes No

17. **Your birth date** (dd/mm/yyyy)_____

18. **Your relationship to Infant:** Mother Father Other _____

19. **Who is currently the primary caregiver of your infant?**
Mother Father Equally between Other _____
Mother & Father

20. **Time since last fed**: _____Hours _____Minutes

21. **Time since last napped**: _____Hours _____Minutes

22. **Number of Family Members living in your household**: Adults _____ Children_____

23. **For each child in your family please list their age and sex**.
Age of infant brought in today: _____(months) **Male** **Female**
Birth date of infant (dd/mm/yyyy): _____

Ages/genders of your other children:
Age: _____ Male Female
Age: _____ Male Female
Age: _____ Male Female
Age: _____ Male Female

24. **Which caregivers are present at this immunization?** (circle one number):
10. Mom only
11. Dad only
12. Mom and Dad
13. Nanny
14. Grandparents
15. Parent(s) and Nanny
16. Parent(s) and Grandparent(s)
17. Other _____
18. Parent(s) and Other _____

b) How many other children are present, if any? (do not include child getting the needle) ____

25. Since we last asked, have you taken any infant parenting classes/workshops? **Yes No**
If <u>yes</u>, how many? _____

26. Since we last asked, have you read any infant parenting books/watched videos? **Yes No**
If <u>yes</u>, how many? _____

27. Since we last asked, approximately how often do you visit parenting websites?

Never	**Once a day**	**Once a week**	**Once every few weeks**	**Once a month**	**Once a year**

28. Since we last asked, have you received any guidance from an organization or professional to help with parenting your children (e.g. health unit nurse, midwife, Early Years Centre, Healthy Babies Healthy Children, Hincks-Dellcrest, Jessie's Place)? **Yes No**
If <u>yes</u>, from how many organizations/professionals? _____

29. Has your child been given EMLA or TYLENOL prior to the appointment?:
EMLA TYLENOL NONE

30. Since we last asked, has your infant been separated from his/her primary caregiver for longer than 24 hours (e.g. infant hospitalization, parent hospitalization, Children's Aid involvement, parent travel, family emergency)?
Circle: **YES NO**

***If you circled YES*:**
Approximately, how many separations longer than 24 hours have occurred? _____
How long was the longest period of separation _____(days)

31. **Child's Medical History**

Please check next to any illness or condition that your child has had **<u>since we last asked</u>**. When you check an item, also note the approximate date of the illness or your child's age at illness.

Illness or condition	Date(s) or age(s)	Illness or condition	Date(s) or age(s)
□Measles	_____	□Visual problems	_____
□German Measles	_____	□Fainting spells	_____

□Mumps _____

□Chicken Pox_____
□Whooping Cough_____
□Diphtheria _____
□Scarlet Fever _____
□Meningitis _____
□Pneumonia _____
□Encephalitis _____
□High fever (>41°C or 105.8°F) _____
□Seizure _____
□Allergy _____
□Hay Fever _____
□Injuries to head _____
□Broken bones _____
□Hospitalization _____
(please specify reason)
□Operations _____
(please specify)
□Otitis media _____

□Loss of consciousness_____
(please specify cause)
□Lead Poisoning _____
□Ear Problems _____
□TB _____
□Bone or joint disease_____
□Anemia _____
□Jaundice/Hepatitis_____
□Cancer _____

□Heart disease _____
□Asthma _____
□Bleeding problems_____
□Eczema or hives _____
□Paralysis _____
□Stomach pumped_____
□Thrush _____

□Circumcision _____

□Other _____

14. Since we last asked, has your child been diagnosed with any other chronic illnesses not listed above? **Yes No**
If <u>yes</u>, which chronic illness and at what age were they diagnosed?
_____
_____
_____

15. Since we last asked, has your child taken any medication long-term (i.e. longer than 2 weeks)?
Yes No
If <u>yes</u>, please list:
_____
_____
_____

<u>PART 2: PREGNANCY HISTORY</u> – If child is adopted please check box: □
(If so, please fill as much of the following information as you are aware of, which regards to the child's birth mother, her pregnancy, and delivery.)

Has the primary caregiver answered these questions at 12 months? Yes No
If **<u>yes</u>**, please proceed to Part 3 questions.

If **no,** please proceed to ask questions below.

17. During pregnancy, were you on medication? **Yes□ No□**
    If yes, what kind? _____
18. During pregnancy, did you smoke? **Yes□ No□**
    If yes, how many cigarettes each day? _____
19. During pregnancy, did you drink alcoholic beverages? **Yes□ No□**
    If yes, what did you drink?
    _____
    Approximately how much alcohol was consumed each day?
    _____
20. During pregnancy, did you use drugs? **Yes□ No□**
a. If yes, what kind?_____
21. Were there any complications during pregnancy (excessive vomiting, excessive staining/blood loss, threatened miscarriage, infections, toxemia, fainting, dizziness, etc.)
    _____
    _____
    _____
22. Duration of pregnancy (weeks):_____
23. Duration of labour (total hours):_____
24. Were there indications of fetal distress during labour or during birth? **Yes□ No□**
25. Were forceps used during delivery? **Yes□ No□**
26. Was delivery normal? **Yes□ No□**
27. Was a Caesarean section performed? **Yes□ No□**
28. Was delivery breach? **Yes□ No□**
29. Was delivery induced? **Yes□ No□**
    If yes on any of the above, for what reason?
    _____
30. Was your child premature? **Yes□ No□**
a. If so, by how many weeks?_____
31. What was your child's birth weight? (in pounds)_____
32. Were there any birth defects or complications? **Yes□ No□**
a. If yes, please describe: _____
    _____

PART 3: PRIMARY CAREGIVER BACKGROUND – *These questions are to be asked only if this is the first time the parent has participated in the study. (If possible, all questions to be asked by Clinic RA)*

Has the primary caregiver answered these questions at 12 months? Yes No
If **yes**, please proceed to Part 4 questions. If **no,** please proceed to ask questions below.

10. **Your Highest Education (circle one number):**
    1. Graduate School/Professional Training
    2. University graduate (4 years college)
    3. Partial university (at least 1 year)
    4. Trade School/Community College
    5. High School Graduate
    6. Some high school
    7. Junior high school graduate
    8. Less than 7th grade

    a) Your Current Occupation _____

11. **If applicable, your Spouse's Highest Education (circle one number):**
    1. Graduate School/Professional Training
    2. University graduate (4 years college)
    3. Partial university (at least 1 year)
    4. Trade School/Community College
    5. High School Graduate
    6. Some high school
    7. Junior high school graduate
    8. Less than 7th grade

    a) If applicable, Your Spouse's Current Occupation _____

12. **What language is spoken most in your home (only put two languages if they are equally spoken)** _____

13. **What country was the primary caregiver born in:_____**

a) If applicable, what country was your spouse born in:_____

14. **What country was the primary caregiver's parents born in:_____**

a) If applicable, what country were your spouse's parents born in: _____

15. **How long has the primary caregiver lived in Canada: _____**

a) If applicable, how long has your spouse lived in Canada: _____

16. **On a scale of 0 to 10, where 0 is "Not at All" and 10 is "Completely", how much do you feel your way of life reflects mainstream North American/Canadian culture?** _____

a) If applicable, how much do you feel your spouse's way of life reflects mainstream North American/Canadian culture: _____

17. **What is your heritage culture (**It may be the culture of your birth, the culture in which you have been raised, or another culture that forms part of your background. Pick the culture that has influenced you *most*. If you do not feel that you have been influenced by any other culture, please try to identify a culture that may have had an impact on previous

generations of your family.**): _____**

a) If applicable, what is your spouse's heritage culture: _____

18. On a scale of 0 to 10, where 0 is "Not at All" and 10 is "Completely", how much do you feel your way of life reflects your heritage culture? _____

a) If applicable, how much do you feel your spouse's way of life reflects his/her heritage culture: _____

PART 4: PARENT RATINGS (PRE-IMMUNIZATION)

Pre-Immunization **Self-Worry** Rating

*On a scale from 0 to 10, how worried about the needle pain are **YOU**, right now, before the needle, where 0 is "no worry at all" and 10 is "the most worry possible"?*

_____

PART 5: PARENT RATINGS (POST-IMMUNIZATION)

To be done approximately 5 minutes after last needle!

Post-Immunization **Self-Worry** Rating

*On a scale from 0 to 10, how worried about the needle pain are **YOU**, right now, after the needle, where 0 is "no worry at all" and 10 is "the most worry possible"?*

_____

Post-Immunization **Child Pain** Rating

On a scale from 0 to 10, how much pain do you think your child experienced from the needles they just received, where 0 is "no pain at all" and 10 is "the worst pain possible"?

_____

How many needles total: _____

1. Vaccines in needle #1:_____

2. Vaccines in needle #2:_____

3. Vaccines in needle #3:_____

PART 6: VACCINES GIVEN BY IMMUNIZATION NEEDLE

RA to fill out (ask nurse or doctor for vaccine name and trade name)

		Disease it Protects Against
☐	Prevnar	_____
☐	Twinrix	_____
☐	Bexsero	_____
☐	Menjugate	_____
☐	Pediacel	_____

_____

- ☐ Menveo _____
- ☐ Nimenrix _____
- ☐ Other (List Name) _____
- ☐ Pneumococcal-13 _____
- ☐ NeisVac-C _____
- ☐ MMR _____