UNDERSTANDING PATTERNS OF CAREGIVER-TODDLER BIOLOGICAL ATTUNEMENT IN A DISTRESS CONTEXT

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ABSTRACT

This dissertation consists of three studies that examine caregiver-toddler biological attunement within distress contexts in toddlerhood. Study 1 is a published systematic review (Di Lorenzo et al., 2021) that synthesizes the direction and magnitude of the relations between caregiver and young child (0 to 3 years of age) biological outcomes according to different methodology used to measure distress. Majority of articles examined cortisol outcomes of caregivers and their children in distress contexts. The magnitude of the association between caregiver and child cortisol indicators were moderate to large; however, results differed depending on the analysis used and measurement epochs examined. Relations between caregiver and child cardiac, saliva alpha amylase (sAA), and electroencephalography (EEG) outcomes were generally weak or inconsistent. Limitations of methodological approaches used to study caregiver-child attunement likely contribute to the heterogeneity of findings. To address limitations highlighted in Study 1, we used parallel process growth mixture modelling in Study 2 (Di Lorenzo-Klas et al., 2022a) to capture the dynamic nature of the attunement process and discern various patterns of caregiver-toddler coregulatory trajectories in a vaccination context during the second year of life. Three groups of dyads were discerned, with one group (80% of the sample) that demonstrated an adaptive regulatory attunement pattern (i.e., most regulated), and two groups (20% of the sample) that showed either a lack of attunement or misattunement between dyad members which demonstrated less adaptive child regulation from pain-related distress. To better understand what is driving the patterns of regulatory attunement, Study 3 (Di Lorenzo-Klas et al., 2022b) examined whether caregiver distress (operationalized as caregiver worry associated with their toddler's vaccination) and child distress (operationalized as behavioural pain-related distress) are associated with patterns of caregiver-toddler attunement

discerned in Study 2. Findings from Study 3 revealed that caregivers who experience higher distress associated with their toddlers' vaccination demonstrated higher probability of being associated with groupings reflecting lack of attunement or misattunement with their toddlers during vaccination, and these toddlers are at risk of experiencing higher levels of behavioural pain-related distress. Clinical implications and suggestions for future research are discussed.

DEDICATION

To my parents—you have been instrumental in shaping me into the person I am today, and you have always encouraged me to follow my dreams. Your dedication as parents continues to inspire my work in understanding the importance of caregiver-child relationships.

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PUBLICATION DISCLOSURE

The studies in this dissertation (Chapters 2, 4, and 6) are currently published or under review. The pre-published manuscripts are included in this document. The citations are as follows:

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DISSERTATION SYNOPSIS

The ability to adaptively regulate distress early in life is developed through repeated interactions with one's caregiver who serves as a source of external regulatory support. Specifically, distress is coregulated between children and their caregivers until children gain capacity to regulate their own emotions more independently. An understanding of how coregulatory dynamics between parent and child, also known as attunement, emerge early in life can provide insight into how the early building blocks of distress regulation are developed. Arguably, many studies to date have not used methodological approaches that have fully captured the dynamic process of biological attunement. Moreover, no studies have examined variable patterns of biological attunement within a high distress context (i.e., vaccinations). Differences in young children's regulatory abilities have been linked to future developmental outcomes, highlighting the importance of studying regulatory response patterns that emerge with one's caregiver early in life.

The current dissertation aimed to address gaps in the literature with three broad research aims: (1) Systematically review the literature on caregiver and child biological attunement, with special consideration of methodological approaches used to examine relations between caregiver and child biological outcomes; (2) To examine variability in patterns of caregiver-child regulatory attunement during vaccinations across the second year of life; (3) To determine if caregiver self-reported and child behavioural distress were associated with patterns of dyadic regulatory attunement. The research aims were addressed in three separate studies that are either published or currently under peer-review (Di Lorenzo et al., 2021; Di Lorenzo-Klas et al., 2022a; DiLorenzo-Klas et al., 2022b). In addition to this synopsis, a two-page overview of the main statistical findings for each of the three studies is briefly summarized in Appendix A.

The first study was a systematic review that organized and synthesized the literature on caregiver-child (0 to 3 years) biological attunement (i.e., relations between caregiver and child biological outcomes) in a distress context. The majority of studies examined cortisol outcomes, and there were generally moderate to large positive relations between caregiver and child cortisol, although findings varied depending on the analysis used and epochs examined. Most findings examining relations between caregiver and child cardiac, sAA, and EEG indicators were weak or inconsistent. The review addresses how the heterogeneity in results reflects limitations in methodological practices used to capture attunement, and recommendations for future research are discussed.

To address gaps elucidated in the systematic review, the second and third studies studied caregiver-toddler dyads that were part of a longitudinal cohort observed at 12-, 18, or 24-month routine vaccination appointments. Collapsing over the second year of life, a subsample of dyads (N = 189) was examined with both caregiver and toddler cardiac data available.

In study 2, profiles of caregiver-toddler regulatory attunement, indexed by high-frequency heart rate variability (HF-HRV), were discerned using parallel-process growth mixture modeling. Three groups of dyads emerged: Group 1 dyads (80% of sample) demonstrated attunement, with stable and parallel regulatory patterns of HF-HRV from baseline to post-needle; Group 2 (7.9% of sample) dyads showed lack of attunement, with both caregivers and toddlers demonstrating less regulated trajectories (i.e., consistently lower HF-HRV values); and Group 3 (11.1% of sample) dyads demonstrated misattunement with diverging trajectories, whereby caregivers showed a regulated trajectory, but their toddlers mounted an over-regulated or exaggerated withdrawal response (i.e., steep decrease followed by large increase in HF-HRV). Study 3 focused on examining whether caregiver self-reported and child behavioural distress

were associated with the caregiver-toddler attunement patterns discerned from Study 2. Results demonstrated that greater behavioural pain-related distress at various times after needle was associated with membership in groups that showed less adaptive attunement patterns. Additionally, caregivers who self-reported higher pre-needle worry, as well as lower post-needle worry, were more likely to belong to groups that demonstrated less adaptive attunement patterns.

Taken together, the findings from the three studies presented in this dissertation offer novel contributions to the developmental attunement literature. The systematic literature review provides an important call to researchers to carefully consider their methodology when studying biological attunement and provides concrete suggestions for improving the quality of study methods. For the first time in the literature, studies 2 and 3 identified unique patterns of dyadic attunement, including both adaptive and suboptimal patterns, as well as a greater understanding of these patterns through examining relations with caregiver and child pain-related distress. Overall, this dissertation provides compelling evidence supporting the essential role of the caregiver in shaping their child's distress regulation capacity and highlights the need to support caregivers in supporting their children to regulate from distress.

Chapter 1: Introduction

The Origins of Distress Regulation in the Context of the Early Caregiver-Child Relationship

The development of distress regulation skills is central to a child's emerging ability to adaptively respond to environmental demands and to engage in goal-directed behaviours (Calkins & Howse, 2004). Research indicates that the first few years of life are marked by a rapid acquisition of a set of skills (i.e., cognitive, biological, behavioural, emotional) that facilitate a child's ability to regulate their own distress (Calkins, 2007). The capacity to regulate distress provides a foundation for adaptive social, emotional, and cognitive functioning across the lifespan (Thompson, 1994). However, early in life, variable patterns in a child's ability to regulate distress begin to emerge (Pillai Riddell et al., 2013; Waxman et al., 2017). Children who show early challenges with emotion or distress regulation are at greater risk of developing later social and psychological adjustment difficulties, such as aggressive behaviour, anxiety symptomology, mood difficulties, and peer rejection (Eisenberg et al., 2010; Kim & Cicchetti, 2010; Robson et al., 2020). A critical element to mitigating the risks associated with poor regulatory abilities necessitates understanding the factors that influence how regulatory processes develop early in life.

There is a multitude of internal (e.g., temperament, cognition) and external (e.g., caregiving context) factors that affect the development of children’s regulatory skills (Calkins, 2011). Arguably, the early caregiver-child relationship is one of the most important roles in shaping and organizing a child's capacity to regulate distress (Feldman, 2017; Thompson, 2019), as young children are not born with skills to regulate distress on their own. The caregiver-infant relationship not only helps organize early biological and behavioural functioning related to

emotion regulation, but also supports the infant's ability to learn and build capacity for self-regulation (DiCorcia & Tronick, 2011).

A promising area of research that provides new insights into the role of caregiving on the development of distress regulation examines how caregivers and their children interact to work towards a mutually regulated state in distress contexts. These interactions typically involve contingent and reciprocal responses between dyad members (i.e., each dyad member produces and responds to the other's communicative signals; DiCorcia & Tronick, 2011). For example, the child may change their response based on the caregiver's behaviour if the child is using their caregiver as a source of information to respond to their environment. Additionally, the child's state may drive changes in the parent's response if they are interpreting the child's signals and responding accordingly. Regardless of the direction of influence, the caregiver is primarily responsible for facilitating regulation, as young children have less capacity to regulate on their own. Ideally, when a child is in distress, the caregiver regulates their own distress, is able to accurately discern the needs of the child, and then responds accordingly to the emotional needs of the child (Creavy et al., 2020; Provenzi et al., 2018). As a result, the caregiver and child can adjust and regulate their distress together. However, this adaptive pattern may not always occur, as the caregiver's inability to regulate their own distress can interfere with their ability to support their child's regulation from distress (DiCorcia & Tronick, 2011). Many researchers have studied caregiver-child interactions in stress contexts, and more specifically, the degree to which biological or behavioural regulatory states of caregivers and their infants are temporally coordinated or uncoordinated before and after a stressor. This relational process is referred to using various terms including caregiver child *attunement* (the term we will use in the current

dissertation), *synchrony*, *mutuality, matching*, *concordance*, and *affect contagion* (Bernard et al., 2017).

Theoretical Underpinnings of Dyadic Attunement in a Pain Context

Much of the research examining the development of children's regulatory abilities is rooted in early work on caregiver-child attachment (Bowlby, 1969, 1982). In the context of the attachment relationship, caregivers play a critical role in children's emotional development when they function as a "secure base", allowing their children to feel supported in exploring their environments. A history of supportive caregiver-child interactions is thought to lead to the formation of a secure attachment relationship which serves an important role in organizing children's behavioral and biological regulatory abilities. Ainsworth and colleagues (Ainsworth et al., 1974) built on Bowlby's work to understand what caregivers do to promote attachment. The researchers identified the concept of *maternal sensitivity*, which involves the mother perceiving a child's signals, interpreting them accurately, and responding promptly and appropriately to comfort the child when they are distressed. These early efforts to soothe a distressed child help build the child's expectations of the caregiver as available to support them when expressing various emotions. In a secure attachment, the caregiver is sensitive to the child's needs, helps the child tolerate and manage their distress, and guides the child back to a regulated state that allows them to explore again (Ainsworth et al., 1974; Ainsworth, 1979). However, if the child does not perceive their caregiver as being available to them due to inconsistency of responses or insensitivity, the child may begin to feel insecure in the caregiver-child relationship. The link between insecure attachment, poor maternal sensitivity, and suboptimal regulatory and mental health outcomes in children has been studied extensively (Deans, 2020; Koehn & Kerns, 2018; Madigan et al., 2013). While these concepts are critical for understanding relational patterns in

early caregiver-child relationships, there is more to be learned about underlying mechanisms that contribute to adaptive caregiver-child interactions.

Caregivers' own regulatory capacities may also play a critical role in shaping their children's ability to regulate distress. The Self-Regulation Intergenerational Transmission Model (Bridgett et al., 2015) postulates that self-regulation capacity is transmitted across generations from caregiver to child within rearing contexts via biological and social pathways. Specifically, the transmission starts with caregivers who have their own self-regulatory capacities that vary based on their own genetics and social contexts (e.g., the caregiver's early rearing context). To respond effectively to their child, caregivers need to flexibly engage self-regulatory processes to manage their own affect and behaviour, as well as structure and support interactions with their children in ways that promote their children's capacity for behavioural and biological regulation of distress. However, if caregivers demonstrate self-regulatory deficits, they may have more difficulty accessing parenting practices that would typically help their child navigate distressing situations. In contrast, caregivers who can regulate their distress may be better positioned to think flexibly and use different coregulatory strategies in distressing contexts. Through multiple interactions with caregivers in a rearing context, children's regulatory capacity develops and passes on to the next generation through similar pathways. To provide further support of this theory, research is needed that examines how caregivers regulate their own distress which then gives them the capacity to support their children's attempts to regulate distress.

Routine vaccination appointments provide a unique rearing context where caregivers need to help their child regulate distress from a needle. In Canada, healthy children are exposed to repeated needle procedures in early life (i.e., upwards of 10 vaccines before preschool; Government of Ontario, 2022). Although these procedures reflect routine healthcare, previous

research has found that majority of infants are unable to regulate to low levels of pain-related distress following vaccination across the first year of life (Pillai Riddell et al., 2013). Further, approximately one quarter of preschoolers (i.e., ages 4 to 6) have difficulty regulating their pain-related distress following vaccination (Waxman et al., 2017). These patterns of dysregulation underscore the need for caregivers to support their children in regulating their pain-related distress in the vaccination context.

The Development of the Infant Acute Pain Response - Revised model (DIAPR-R) is informed by attachment theory and purports that a child's experience of pain-related distress is shaped by caregiver-child interactions as well as broader biopsychosocial factors (Pillai Riddell et al., in press). The model emphasizes the importance of the caregiver in supporting children during vaccination, as young children signal their distress to their caregiver and the caregiver ideally responds sensitively to the child's needs. However, like other models discussed, the DIAPR-R model has yet to integrate evidence on how caregivers' ability to regulate their own distress supports their ability to attune to their children's needs and coregulate with their children to low levels of pain-related distress following vaccination. Gaps in theory highlight the need for research that examines the dyadic coregulatory system and examining caregiver-child coordination of biological responses may reflect an important mechanism that underlies the adaptive functioning of the coregulatory system.

Dyadic Biological Attunement

Extensive research has established associations between caregiver-child behavioural attunement and positive developmental outcomes for children (Feldman, 2007; Leclère et al., 2014). However, there are some limitations to examining behavioural attunement. Research has demonstrated that behavioural responses are sometimes too subtle to measure, or stress responses

do not consistently manifest through behaviour systems (Braren et al., 2019; Hibel & Mercado, 2019; Waters et al., 2014). More recently, a growing portion of the literature examining caregiver-child attunement has focused on understanding how the biological systems of caregivers and their children attune during distress, as underlying biological responses can provide insight into how caregivers and children have the internal regulatory capacity to interact and respond to each other. This idea is consistent with Hofer's "hidden regulator" hypothesis (Hofer, 2010). Hofer postulated that mechanisms that were not observable in the caregiver-child relationship (i.e., underlying biological processes) shaped caregiver-child interactions and the regulatory abilities of the child. Biological coregulation is an important process that is observable from very early in a child's life (Atzil & Gendron, 2017; Feldman, 2007). As soon as a child is born, babies vitally depend on their caregivers for biological regulation. For example, caregivers and their babies coregulate temperature and heart rate through bodily contact (Atzil & Gendron, 2017; Gupta et al., 2021). Thus, caregiver-child biological attunement is an important process that is hardwired from early in life to support children's regulatory capacity and provides a unique window into understanding how coregulatory dynamics develop in the context of early caregiver-child relationships. However, more research is needed in this area, particularly addressing the lack of methodological clarity in measuring caregiver-child biological attunement.

Methodological Approaches Used to Study Biological Attunement in Distress Contexts

The attunement literature has progressed our understanding of the sociobiological developmental origins of emotion regulation. However, there is a lot of variability in methodological approaches used to study biological attunement which makes it difficult for researchers to draw conclusions from this literature (Bernard et al., 2017). One common source of variation across studies includes the different biological indicators that are used to measure

caregiver and child stress responses. The most common biological systems that have been examined are the hypothalamic pituitary adrenal axis (HPA) and the autonomic nervous system (ANS). One of the most studied biological systems that elucidates the body's reaction to stress is the HPA axis. The system acts via a cascade of hormonal responses that culminate to release cortisol into the bloodstream (Smith & Vale, 2006). The measurement of cortisol as a stress response reflects a slower, long-term response to challenge, as cortisol levels generally peak 15 to 20 minutes after a stressor. The ANS, which is further subdivided into the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS), has also been examined to measure biological attunement in stress contexts. PNS activity is commonly captured with high-frequency heart rate variability (HF-HRV) or respiratory sinus arrythmia (RSA). The indices reflect fluctuations in instantaneous heart period over time, reflecting the strength of parasympathetic influence on the heart via the vagus nerve (Berntson et al., 1997; Quintana et al., 2012). At rest, the vagus nerve acts as a "brake" on the heart promoting homeostatic functions and social behaviour. However, in the face of threat, vagal tone is inhibited (i.e., a release of the "brake" on the heart) which triggers a cascade of responses to meet situational demands (e.g., increased heart rate, blood flow to limbs). Polyvagal theory (Porges, 2007; Porges & Furman, 2011) suggests that poor control of the vagal brake (i.e., low HF-HRV levels) hinders social interaction, as the individual cannot efficiently regulate to be able to optimally engage with their environment. On the other hand, high levels of HF-HRV are thought to reflect greater ability to adjust physiological arousal and regulate distress, allowing an individual to efficiently engage and disengage with their environment as needed. Thus, measuring PNS activity via HF-HRV provides a useful marker of physiological flexibility and regulation. There are a few other biomarkers that have been used to capture caregiver-child biological attunement in the literature,

including salivary alpha amylase (sAA; SNS system), heart period (HP; index of joint SNS and PNS activity), and electroencephalography (EEG). Although studies that include these measures are very limited, findings will be briefly summarized in Chapter 2 to capture the entirety of the literature on biological attunement in distress contexts.

Arguably, one of the most complex features of methodological approaches used by researchers to capture attunement is the use of statistical analyses that do not align with how the process is conceptualized (Bernard et al., 2017). Commonly, researchers may define attunement as dynamic, within-dyad coordination of biological responses that unfolds over time, but they use static correlations to examine relations between caregiver and child outcomes at specific time points. The reliance on a measure of attunement based on correlations between static measurements reflects a simplification of the complex process unfolding between caregivers and their children. However, there are more sophisticated analyses such as cross-lagged analyses, albeit less used in the literature, that better capture aspects of the attunement process, including the dynamic changes in biological responses and reciprocal influences between caregivers and their children. Given the state of the literature, a synthesis of findings is needed with special consideration of the methodological approaches used to study biological attunement. Summarizing results based on methodology can further our understanding of specific biological mechanisms of attunement and inform best methodological practices for studying attunement moving forward.

Key Gaps in the Literature

In addition to the need for a comprehensive synthesis of the biological attunement literature, there are other research gaps to fill, including examining attunement using cardiac measures. As mentioned previously, most of the literature has focused on examining cortisol

outcomes which is highlighted in our synthesis of the literature in Chapter 2. Cardiac measures have greater temporal resolution compared to measures like cortisol, and thus may be more suited to capturing dynamic changes that occur in the process of attunement. Specifically, high-frequency heart rate variability (HF-HRV) measures the parasympathetic influence on the heart and is thought to reflect an individual's capacity for distress regulation. As such, caregiver and child trajectories of HF-HRV can capture dynamic changes in regulatory outcomes over time.

Another gap in the literature relates to examining patterns of biological attunement. Based on previous research (Pillai Riddell et al., 2013; Waxman et al., 2017), we know that young children present with variable patterns of regulatory responses in a high distress context (i.e., the acute pain context), with some children showing difficulty regulating from distress after vaccination. In the attunement literature, researchers have yet to examine whether dyads demonstrate different patterns of biological regulatory attunement over time. Building off previous research examining trajectories of distress in a vaccination context (Pillai Riddell et al., 2013; Waxman et al., 2017), we hypothesize that dyads can experience either adaptive attunement (i.e., matched coregulation of distress responses), reflecting optimal responsiveness to child's needs and regulation of caregiver's own distress, or suboptimal (i.e., less adaptive) attunement (e.g. dyad members are either matched or mismatched in their coregulatory responses, but either one or both dyad members have difficulty regulating distress), reflecting poor dyadic regulation or poor responsiveness of the caregiver (Ostlund et al., 2017). Indeed, if caregivers report greater distress and are unable to regulate their own distress, their ability to attune to their child's needs may be impacted and lead to a greater experience of distress for the child. Previous research has yet to examine distinct patterns of the attunement and variables associated with these patterns (i.e., caregiver self-reported distress and child behavioural

distress). Addressing this and the other literature gaps identified above forms the foundation of the current dissertation.

Current Dissertation

The overall aim of the current dissertation is to garner a better understanding of the emergence of caregiver-child biological attunement in early life. Thus far, the literature examining biological attunement in stress contexts has reported inconsistent findings, partly due to the variety of methodology used to examine attunement. A comprehensive examination of findings related to biological attunement according to different methodologies has yet to be conducted. Additionally, there is no research that has examined variable patterns of biological attunement between caregivers and their children in distress contexts and potential caregiver and child factors that may be associated with variable coregulatory trajectories. Of note, the terms *biological attunement* and *physiological attunement* are used throughout the current dissertation. Biological attunement is a more encompassing term, as it refers to attunement of different biological systems including hormone systems, cardiac activity, and brain activity, whereas physiological attunement more specifically refers to coregulation of cardiac regulatory responses in the current dissertation.

The current body of work is a compilation of three studies, including a systematic review and narrative synthesis of biological attunement according to different methods (e.g., biological indicators, statistical analyses) used to study the process of dyadic attunement (Chapter 2, Di Lorenzo et al., 2022); the second study utilized dyad-centered analyses to describe the variability in caregiver-child regulatory attunement based on HF-HRV outcomes during vaccination (Chapter 4; Di Lorenzo-Klas et al., 2022a); and the third study examined whether caregiver distress (operationalized by self-reported worry before and after the vaccination) and child

distress (operationalized by behavioural pain-related distress) were associated with attunement patterns discerned from Study 2 (Chapter 6: Di Lorenzo-Klas et al., 2022b). There are bridging chapters (Chapter 3 and 5) that discusses how each study builds from the previous one. To aid readership, Appendix A provides a short summary of the findings across studies, including research questions, analyses, and results.

References

Ainsworth, M. D., Bell, S. M., & Stayton, D. J. (1974). Infant-mother attachment and social development: Socialization as a product of reciprocal responsiveness to signals. *The Integration of a Child into a Social World*, *1*, 9–135.

Ainsworth, M. S. (1979). Infant–mother attachment. *American Psychologist*, *34*(10), 932–937. https://doi.org/10.1037/0003-066X.34.10.932

Atzil, S., & Gendron, M. (2017). Bio-behavioral synchrony promotes the development of conceptualized emotions. *Current Opinion in Psychology*, *17*, 162–169. https://doi.org/10.1016/j.copsyc.2017.07.009

Bernard, N. K., Kashy, D. A., Levendosky, A. A., Bogat, G. A., & Lonstein, J. S. (2017). Do different data analytic approaches generate discrepant findings when measuring mother-infant HPA axis attunement? *Developmental Psychobiology*, *59*(2), 174–184. https://doi.org/10.1002/dev.21474

Berntson, G. G., Bigger, J. T., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., Nagaraja, H. N., Porges, S. W., Saul, J. P., Stone, P. H., & Molen, M. W. V. D. (1997). Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology*, *34*(6), 623–648. https://doi.org/10.1111/j.1469-8986.1997.tb02140.x

Bowlby, J. (1969). Attachment and loss: Volume I: attachment. In *Attachment and Loss: Volume I: Attachment* (pp. 1–401). London: The Hogarth Press and the Institute of Psycho-Analysis.

Bowlby, J. (1982). Attachment and loss: Retrospect and prospect. *American Journal of Orthopsychiatry*, *52*(4), 664–678. https://doi.org/10.1111/j.1939-0025.1982.tb01456.x

Calkins, S. D. (2007). The emergence of self-regulation: Biological and behavioral control mechanisms supporting toddler competencies. In *Socioemotional development in the toddler years: Transitions and transformations* (pp. 261–284). The Guilford Press.

Calkins, S. D. (2011). Caregiving as coregulation: Psychobiological processes and child functioning. In *Biosocial foundations of family processes* (pp. 49–59). Springer.

Calkins, S. D., & Howse, R. B. (2004). Individual differences in self-regulation: Implications for childhood adjustment. In *The regulation of emotion* (pp. 307–332). Lawrence Erlbaum Associates Publishers.

Creavy, K. L., Gatzke-Kopp, L. M., Zhang, X., Fishbein, D., & Kiser, L. J. (2020). When you go low, I go high: Negative coordination of physiological synchrony among parents and children. *Developmental Psychobiology*, *62*(3), 310–323. https://doi.org/10.1002/dev.21905

Deans, C. L. (2020). Maternal sensitivity, its relationship with child outcomes, and interventions that address it: A systematic literature review. *Early Child Development and Care*, *190*(2), 252–275.

DiCorcia, J. A., & Tronick, E. (2011). Quotidian resilience: Exploring mechanisms that drive resilience from a perspective of everyday stress and coping. *Neuroscience & Biobehavioral Reviews*, *35*(7), 1593–1602. https://doi.org/10.1016/j.neubiorev.2011.04.008

Di Lorenzo, M. G., Bucsea, O., Rumeo, C., Waxman, J. A., Flora, D. B., Schmidt, L. A., & Pillai Riddell, R. (2022). Caregiver and young child biological attunement in distress contexts: A systematic review and narrative synthesis. *Neuroscience & Biobehavioral Reviews*, *132*, 1010-1036. doi: 10.1016/j.neubiorev.2021.10.045

Di Lorenzo-Klas, M. G., Waxman, J. A., Pillai Riddell, R., Flora, D. B., Schmidt, L. A., Garfield, H., Flanders, D., Weinberg, E., & Savlov, D., Pillai Riddell, R. (2022a). *Distinct trajectories of caregiver-toddler physiological attunement during routine vaccinations*. Manuscript submitted for publication.

Di Lorenzo-Klas, M. G., Waxman, J. A., Pillai Riddell, R., Flora, D. B., Schmidt, L. A., Garfield, H., Flanders, D., Weinberg, E., & Savlov, D., Pillai Riddell, R. (2022b). *Caregiver and child distress as predictors of dyadic physiological attunement during vaccination*. Manuscript submitted for publication.

Eisenberg, N., Spinrad, T. L., & Eggum, N. D. (2010). Emotion-related self-regulation and its relation to children's maladjustment. *Annual Review of Clinical Psychology*, *6*(1), 495–525. https://doi.org/10.1146/annurev.clinpsy.121208.131208

Feldman, R. (2007). Parent–infant synchrony and the construction of shared timing; physiological precursors, developmental outcomes, and risk conditions. *Journal of Child Psychology and Psychiatry*, *48*(3–4), 329–354. https://doi.org/10.1111/j.1469-7610.2006.01701.x

Feldman, R. (2017). The neurobiology of human attachments. *Trends in Cognitive Sciences*, *21*(2), 80–99. https://doi.org/10.1016/j.tics.2016.11.007

Government Of Ontario. (2022, June). *Ontario's Publicly Funded Immunization Schedules*. Ontario Ministry of Health and Long-Term Care. https://health.gov.on.ca/en/pro/programs/immunization/schedule.aspx

Gupta, N., Deierl, A., Hills, E., & Banerjee, J. (2021). Systematic review confirmed the benefits of early skin-to-skin contact but highlighted lack of studies on very and extremely preterm infants. *Acta Paediatrica*, *110*(8), 2310–2315. https://doi.org/10.1111/apa.15913

Hofer, M. (2010). Hidden regulators within the mother–infant interaction. *Nurturing Children and Families: Building on the Legacy of T. Berry Brazelton*, 154–163.

Kim, J., & Cicchetti, D. (2010). Longitudinal pathways linking child maltreatment, emotion regulation, peer relations, and psychopathology. *Journal of Child Psychology and Psychiatry*, *51*(6), 706–716. https://doi.org/10.1111/j.1469-7610.2009.02202.x

Koehn, A. J., & Kerns, K. A. (2018). Parent–child attachment: Meta-analysis of associations with parenting behaviors in middle childhood and adolescence. *Attachment & Human Development*, *20*(4), 378–405.

Leclère, C., Viaux, S., Avril, M., Achard, C., Chetouani, M., Missonnier, S., & Cohen, D. (2014). Why synchrony matters during mother-child interactions: A systematic review. *PLOS ONE*, *9*(12), e113571. https://doi.org/10.1371/journal.pone.0113571

Madigan, S., Atkinson, L., Laurin, K., & Benoit, D. (2013). Attachment and internalizing behavior in early childhood: A meta-analysis. *Developmental Psychology*, *49*(4), 672–689. https://doi.org/10.1037/a0028793

Ostlund, B. D., Measelle, J. R., Laurent, H. K., Conradt, E., & Ablow, J. C. (2017). Shaping emotion regulation: Attunement, symptomatology, and stress recovery within mother–infant dyads. *Developmental psychobiology*, *59*(1), 15-25.

Pillai Riddell, R., Flora, D. B., Stevens, S. A., Stevens, B., Cohen, L. L., Greenberg, S., & Garfield, H. (2013). Variability in infant acute pain responding meaningfully obscured by averaging pain responses. *PAIN®*, *154*(5), 714–721. https://doi.org/10.1016/j.pain.2013.01.015

Pillai Riddell, R., Jasim, S., & Hamwi, L. (in press). Out of the mouth of babes: A lot about pain has nothing to do with pain. *PAIN.*

Porges, S. W. (2007). The polyvagal perspective. *Biological Psychology*, *74*(2), 116–143. https://doi.org/10.1016/j.biopsycho.2006.06.009

Porges, S. W., & Furman, S. A. (2011). The early development of the autonomic nervous system provides a neural platform for social behaviour: A polyvagal perspective. *Infant and Child Development*, *20*(1), 106–118.

Provenzi, L., Scotto di Minico, G., Giusti, L., Guida, E., & Müller, M. (2018). Disentangling the dyadic dance: Theoretical, methodological and outcomes systematic review of mother-infant dyadic processes. *Frontiers in Psychology*, *9*, 348. https://doi.org/10.3389/fpsyg.2018.00348

Quintana, D. S., Guastella, A. J., Outhred, T., Hickie, I. B., & Kemp, A. H. (2012). Heart rate variability is associated with emotion recognition: Direct evidence for a relationship between the autonomic nervous system and social cognition. *International Journal of Psychophysiology*, *86*(2), 168–172. https://doi.org/10.1016/j.ijpsycho.2012.08.012

Robson, D., Allen, M., & Howard, S. (2020). Self-regulation in childhood as a predictor of future outcomes: A meta-analytic review. *Psychological Bulletin*, *146*. https://doi.org/10.1037/bul0000227

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience*, *8*(4), 383–395.

Thompson, R. A. (2019). Emotion dysregulation: A theme in search of definition. *Development and Psychopathology*, *31*(3), 805–815. https://doi.org/10.1017/S0954579419000282

Waxman, J. A., DiLorenzo, M. G., Riddell, R. R. P., Flora, D. B., Greenberg, S., & Garfield, H. (2017). Preschool needle pain responding: Establishing 'normal.' *The Journal of Pain*, *18*(6), 739–745.

Chapter 2: Caregiver and young child biological attunement in distress contexts: A systematic review and narrative synthesis[1]

The first few years of life are marked by acquisition and differentiation of a set of skills implicated in the process of regulation (e.g., language development, executive attentional control, theory of mind; Thompson et al., 2008). As these skills develop, a transition occurs from relying heavily on one's caregiver, as an external regulator, to more self-directed regulation attempts (Ekas et al., 2018). Longitudinal research using a behavioural distress indicator has shown substantial variability in patterns regarding how children regulate from distress across the first years of life (i.e., from infancy to preschool; Pillai Riddell et al., 2013; Waxman et al., 2017). Thus, understanding the developmental origins of emotion regulation is crucial for determining implications of regulation deficits and the risk of long-term psychopathology. A child's immediate response and ability to recover from distress in early life is multi-determined, involving an interplay between individual (e.g., genetics, temperament) and contextual characteristics (e.g., relationship with caregiver) (Calkins & Hill, 2007). Although individual factors are important, significant developments in regulation skills early in life occur in the context of the caregiver-child relationship (Ainsworth, 1979; Bowlby, 1982). Tronick (1989) proposed a theoretical model explaining the reciprocal nature of caregiver-child interactions and the importance of these interactions for scaffolding affect regulation. According to Tronick's Mutual Regulation model (MRM; Beeghly & Tronick, 2011; Tronick, 1989), the capacity to recover from distress emerges early in life from within a dyadic mutually regulating

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communication system, which includes a child subsystem, a caregiver subsystem, and ongoing communication between them. Both children and caregivers use communicative signals (e.g., crying, touching, physical separation) to convey their biobehavioural regulatory states.

For decades, researchers have observed the nature of caregiver-child interactions within stressful contexts as the dyad works toward a mutually regulated state. The caregiver-child interactions in stress contexts typically involve dynamic, contingent, and reciprocal responses between dyad members to aid in recovery from distress (i.e., each dyad member produces and responds to the other's communicative signals; Bell, 2020; DiCorcia & Tronick, 2011). However, the caregiver is primarily responsible for driving regulation in young children given the child's limited capacity early in life. If a child is distressed, ideally the caregiver will be able to understand the child's inner state of distress, regulate their own behavioural and biological responses to stress, and respond sensitively and contingently to regulate the child. Caregiver self-regulatory abilities influence how they respond to their child's distress, which in turn influences their child's ability to regulate from distress (Bridgett et al., 2015) and subsequently contributes to the caregiver's regulation of distress. As a result, the caregiver and child are able to adjust and regulate their distress together (Creavy et al., 2020; Provenzi et al., 2018). During this process, the caregiver's use of regulatory strategies within the dyadic interaction helps build the child's repertoire of cognitive, behavioural, and biological strategies (Calkins & Hill, 2007; Tronick, 2017). However, this pattern may not always occur, as caregiver-child interactions can be disrupted by the caregiver's inability to regulate their own distress (DiCorcia & Tronick, 2011; Tronick, 2017).

Many researchers have studied these caregiver-child exchanges and the degree to which biological or behavioural regulatory states of caregivers and their children are temporally

coordinated. These interaction patterns have been referred to using various terms such as caregiver-child *attunement*, *synchrony*, *matching*, *coregulation*, *concordance*, and *affect contagion* (Bernard et al., 2017). Based on our theoretical understanding of early caregiver-child dynamics, for the purpose of this review, attunement was the term used and defined as the dynamic and reciprocal influence between caregivers and their children over time as they work towards a regulated state (Atkinson et al., 2016; Beeghly & Tronick, 2011). While there are a number of dimensions of attunement (e.g., behavioural, cognitive-emotional), the current work focused exclusively on biological attunement.

Dyadic biological attunement in stress contexts

Within the last two decades, there has been a greater focus on studying biological attunement as a critical underlying mechanism of caregiver-child distress regulation. Basic control of biological processes is necessary to support use of behavioural emotion regulation strategies (Calkins & Hill, 2007). Cycles of mutual influence and feedback are then established between biological and behavioural levels of regulation (Feldman, 2007; Feldman, 2016). The link between caregiver and child biological systems is a primitive process evidenced very early in the child's life, including prenatally (e.g. the effect of prenatal stress on a child's HPA axis and brain development; Lautarescu et al,. 2020; Oberlander et al., 2008) and during the neonatal period (e.g. skin-to-skin contact that modulates infant heart rate, sleep patterns, and temperature; Feldman et al., 2002; Moore et al., 2016). Caregiver physiology, through its effect on caregiver behaviours in response to their child's distress (e.g., sensitive or insensitive behaviours), bodily contact with the child (e.g., skin-to-skin contact), and the sensory experience of the child (e.g., the child hearing caregiver's calm heart rate during skin-to-skin contact), operates as a "hidden regulator" of young children's physiological response (Braren et al., 2019; Hofer, 2010). As

such, biological attunement provides a unique window into understanding coregulatory dynamics of the caregiver-child relationship.

Common tasks used to examine the coregulatory dynamics between caregivers and their children include parent-child interaction tasks that disrupt or threaten the attachment relationship through separation (e.g., the Strange Situation Procedure [Ainsworth et al., 1978]) or disrupted behavioural communication (e.g., the Still Face Paradigm [Tronick et al., 1978]). There are also tasks that elicit fear and frustration in the infant or toddler through exposure to novel stimuli or situations, such as the LabTAB paradigm (Goldsmith et al., 1993). These various tasks have shown evidence of eliciting a biological stress response in young children, particularly the paradigms that interfere with the child's main source of coping and security (i.e., their caregiver) (Gunnar et al., 2009; Jones-Mason et al., 2018; Provenzi et al., 2016).

The current review is focused on examining patterns of attunement during distress across different biological indicators and statistical procedures used by researchers. Specifically, there are multiple systems that have been studied to capture the biological attunement process, including the hypothalamic pituitary adrenal axis (HPA), the autonomic nervous system (ANS), which is further subdivided into the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS), and neural systems. The HPA axis plays a central role in one's bodily response to stress, as it manages reactivity to challenging situations via a cascade of hormones released in the body and ends with the production of cortisol (Smith & Vale, 2006). When individuals are exposed to stressful stimuli, activity of the HPA axis increases and an individual's stress response is reflected in their cortisol levels. The PNS also plays an important role in the body to regulate stress-related arousal via the influence of the vagus nerve on one's heart. A commonly indexed measure of parasympathetic activity is respiratory sinus arrythmia

(RSA), which reflects the rhythmic fluctuation in heart period at the respiratory frequency (Berntson, Cacioppo, & Quigley, 1993). Porges' polyvagal theory (Porges, 1995; Porges, 2011) suggests PNS activity is an important indicator of self-regulatory capacity, as it allows the dynamic regulation of one's arousal to either foster engagement or disengagement in response to situational demands. In addition to cortisol and RSA, other biomarkers used to index stress include salivary alpha amylase (sAA; SNS system), electroencephalography (EEG; neural activity) and heart period (HP; index of joint PNS and SNS arousal). The literature on these biomarkers in the attunement literature is nascent but are summarized briefly herein to provide a comprehensive overview of the biological attunement literature to date.

Arguably, the greatest complexity in examining attunement involves the analytic approach used to capture the dyadic process. Researchers have used various analytic approaches that significantly differ in terms of their underlying assumptions about the concept under study (Bernard et al., 2017). For example, a simple correlation suggests that attunement is an association between caregiver and child responses at one point in time, whereas a cross-lagged model considers both changes in individual response patterns over time and the reciprocal influence between caregivers and their children. Thus, to draw conclusions about caregiver-child biological attunement, patterns across different measurement and analytic approaches needs to be examined to clarify and further our understanding of this phenomenon.

Current Study

The primary goal of the current review is to provide an in-depth summary of the available studies examining caregiver-child biological attunement in early life, particularly within a distress context. To our knowledge, this is the first review to focus on synthesizing research over the first few years of life. A narrative synthesis methodology was undertaken because the

complexity inherent in this literature precluded a meta-analysis. This complexity related to the variety of a) biological indicators, b) types of statistical analyses, c) epoch definitions (i.e., time periods such as the immediate response [initial reactions to distressing stimulus] or recovery response [return to homeostasis]) between and within biological indicators, d) developmental ages or stages, and e) covariates and moderators used to examine caregiver-child biological attunement.

Thus, the review synthesized results according to biological indicator and analysis approach. Results are discussed separately for each biological indicator (cortisol, sAA, cardiac indicators [RSA, HP], and EEG) and further subdivided according to three broad statistical analysis groupings: *static concurrent and non-reciprocal* (e.g., correlations or regressions), *dynamic concurrent and non-reciprocal* (e.g., multilevel modeling examining rates of change), and *dynamic concurrent and reciprocal* (e.g., cross-lagged modeling). Further details of analysis groupings are included in the methods section (see section 2.7. Data Synthesis). Results were also synthesized based on epoch type (i.e., outcomes examined separately for baseline, immediate response, and recovery periods or collapsed across time). Broad syntheses of developmental patterns (Infancy [0-11months], Early Toddlerhood [12-23 months], and Middle/Late Toddlerhood [24-47 months]) and covariates and moderators are discussed. For each indicator by analysis grouping of articles, the synthesis included a general description of the indicator and its measurement, a description of the relationship direction (positive, negative), and a general summary comment on the overall quality, magnitude, and consistency of the findings.

Methods

Protocol and Registration

The current study followed an *a priori* protocol using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA; Moher et al., 2009). Our protocol was submitted for registration prior to data extraction with the International Prospective Register of Systematic Reviews (PROSPERO; registration no. CRD42019137782).

Eligibility Criteria

Prospective-observational studies were included if: 1) participants included caregivers and their infants or toddlers (0 to 3 years of age), 2) biological indicators for both caregivers and children were measured, 3) biological indicators were measured within the context of an acutely distressing stimulus or event, and 4) relationships between caregiver and child biological indicators were estimated. Studies were excluded if 1) animal subjects were used, 2) children were older than 3 years of age, 3) methods did not include a distressing event, 4) either caregiver or child biological indicators were not measured, 5) caregiver and child biological indicators were not measured during the same period (i.e., within the same paradigm), or 6) estimates of the associations between caregiver and child biological indicators were not reported. Further, non-empirical articles (reviews, book chapters, commentaries, conference proceedings) and non-English articles were also excluded.

Systematic Search

A systematic literature search was conducted by an academic librarian from the Hospital for Sick Children in Toronto, Ontario, Canada. The original search was completed in January 2019 using Medline, Embase, PsycINFO, CINAHL, and Scopus databases. Ongoing weekly Google Scholar updates were conducted to include any relevant articles published between January 2019 and August 2020. An updated systematic search was conducted in December 2020 to find relevant articles published after the date of the original search. Database-specific subject

headings and text word fields fell into three categories related to the constructs of (1) the caregiver-child relationship, (2) biological measures (cardiac indicators, cortisol, salivary alpha amylase, electroencephalography), and (3) children ages zero to three years (see Appendix B for search terms and pairings). References of all included studies were also searched. No date limits were applied to ensure the review encompassed relevant historical and current literature.

Study Selection

Two authors, with previous systematic review experience, designed the abstract selection criteria. Following removal of duplicates, all titles and abstracts from the systematic search were reviewed to determine eligibility for inclusion by three independent reviewers. Covidence online software, a systematic review management tool (www.covidence.org), was used to support and improve the efficiency of screening abstracts for inclusion among the independent reviewers. Approximately one-third of the abstracts (33%) were double-coded, and the overall average agreement rate was 94.4%. All disagreements reached a consensus through discussion.

Articles that met inclusion criteria based on the title and abstract were flagged for full-text review (see Figure 1 for PRISMA flowchart). Of note, studies with either distress or non-distress contexts were originally considered for inclusion (PROSPERO; registration no. CRD42019137782). However, given the substantial amount of literature found in the distress context, which is our primary interest, we narrowed our focus to the current analyses. Articles that were confirmed to meet the inclusion criteria during full-text review were included in the narrative review.

Data Collection Process

The following data were obtained from the final sample of 36 studies, including research group, demographic and study characteristics (country, study type [cross-sectional, longitudinal],

age of child), methodology (biological indices, distress paradigm, the number and timing of pre- and post-distress epochs, type of analyses), study results, and additional variables (i.e., covariates, moderators) that were included when examining the relation between caregiver and child biological indicators. It is important to consider covariates and moderators because there are many procedural and individual factors that can affect biological systems (Clements, 2013; Provenzi et al., 2016; Waxman et al., 2016). Effect sizes were prioritized for data extraction, particularly standardized effects (i.e., correlations, standardized path coefficients), as they can be effectively interpreted and compared across studies with different biological indicators and measurement approaches (Flora, 2020). When data were not provided (e.g., Pearson *r*s not reported for null associations, baseline data collected but not reported), authors were contacted via email on two occasions. Of twelve authors contacted, six responded to the email request for data. Three authors provided missing data, and three authors were unable to provide data requested, as the data were inaccessible. Three authors extracted data from included studies, with the lead author double extracting all studies to ensure consistency in the process. Discrepancies were minimal and resolved through discussion.

Risk of Bias Assessment

To examine the validity of results of the included studies, a 15-item checklist was developed based on the National Heart, Blood, and Lungs Institute Quality Assessment (National Heart, Lungs and Blood Institute, 2014), Downs and Black (1998), and Crombie (2007) checklists. For each item, scores reflected whether the quality index was present (1), absent (0), or not applicable. Quality assessments for each study were double-coded by the lead author and one of the co-authors. There were minimal disgagreements (inter-rater agreement was 93.8%) which were resolved through discussion.

An overall quality score was caculated for each article by summing the quality indices. The summed scores were converted to a percentage, excluding non-applicable ratings. Higher percentages reflect higher quality study methodology that was also well reported. Quality scores are reported for each study in Table 1 and discussed in the results section to inform the validity of results reviewed.

Data Synthesis

The extracted data were stratified by biological indicator (cortisol, sAA, cardiac indicators, and EEG) and type of analytic approach. Although researchers may be trying to measure the same concept of attunement, many of the analysis approaches used have different underlying assumptions about the phenomenon under study (Bernard et al., 2017). The type of analyses used to study attunement differed based on whether they considered the dynamic nature of the process (i.e., changes over time) or the reciprocal influence (i.e., who is responding to whom) between dyad members. Analyses were organized based on the following broad groupings: (1) *static concurrent and non-reciprocal analyses*—includes bivariate correlations, regressions, and multilevel analyses that assume attunement is an association between caregiver and child biological indicators at individual time points (i.e., "match of phase"; Feldman, 2007) and does not account for bidirectional reciprocal influences (i.e., current biological responses of each dyad member influences their partner's subsequent biological response), (2) *dynamic concurrent and non-reciprocal analyses*—includes correlations or regressions of change scores (i.e., change from pre-to-post stressor) and multilevel growth models, suggesting attunement occurs when caregivers and children "match in the direction of change" (Feldman, 2007) but does not consider the reciprocal influences, and (3) *dynamic concurrent and reciprocal analyses*—includes cross-lagged analyses that assume attunement involves concurrent changes in

caregiver and child biological indicators over time and reciprocal influence between dyad members. The Actor-Partner-Independence Model (APIM; Kenny 1996) describes the theoretical basis of using a cross-lagged model to examine the interdependence within dyadic interactions, as dyadic data are not independent. There are three important components of the model, including (1) stability of responses within the same individual (i.e., actor effects) for children and their caregivers, (2) the prediction of child responses from caregiver responses at an earlier time point and vice versa (i.e., partner effects), and (3) the concurrent relations between caregiver and child responses at any one time point (Bader et al., 2021). Lastly, broad syntheses of data according to developmental patterns, covariates (i.e., variables examined to have additive effects on outcomes), and moderators (i.e., variable that interacts with another predictor to have multiplicative effect on outcome) are also reported. The narrative synthesis includes a description of consistency, magnitude (standardized effect sizes were reported if quantitative results were provided), and general direction (positive, negative) of findings, as well as a general comment on quality of studies. If quantitative results were not reported, often due to lack of statistical significance, results were summarized accordingly.

Results

Study Description

Study Selection

After removing duplicate records, the electronic searches identified 9,932 unique abstracts, including five additional records found from Google Scholar updates. Using the *a priori* selection criteria, a total of 218 articles were selected for full-text review. Of these, 36 articles fulfilled the inclusion criteria for the narrative synthesis. See Figure 1 for the PRISMA flow chart outlining the stages of article selection.

Study Characteristics

The key characteristics of the studies included in this review are outlined in Table 1. The studies are organized by research group or sample. There are 26 unique samples and most of the research was conducted in the United States (69%). The remaining studies were completed in Canada, Brazil, Scotland, Israel, Sweden, and the Netherlands. Most studies were cross-sectional (83%). Of the five articles that included longitudinal data, two examined data separately at each age using the same distress context (Braren et al., 2019; Davis & Granger, 2009) and three collapsed across ages in the main analyses (Atkinson et al., 2013; Hibel et al., 2015; Laurent et al., 2020). All articles reported data on children between 0 and 3 years of age, with most being within the first year of life (Infancy [0-11 months] = 20 articles, Early Toddlerhood [12-23 months] = 12 articles, Mid-Late Toddlerhood [24-47 months] = 8 articles). Sixteen articles, most using cortisol as a biological indicator, included a clinical or high-risk sample. All studies included mothers as the primary caregiver except for one study that included a very small subsample of fathers (2 out of 83 parents; van Bakel & Riksen-Walraven, 2008). Thus, results are summarized according to mother and child outcomes.

Regarding study methodology, a variety of biological indicators (Cortisol = 24 articles, sAA = 5, RSA = 10, HP = 2, EEG = 2; 8 articles measured two biological indicators) were used to measure mother and child stress responses and recovery, though most articles used cortisol. Many different distress paradigms were used, with the most common being the Still-Face Paradigm (Tronick et al., 1978), the Strange Situation Procedure (Ainsworth et al., 1978), and the Lab-TAB paradigm (Goldsmith & Rothbart, 1993). All studies measured mother and child stress responses either before (i.e., baseline), after the stressor (i.e., immediate response or recovery), or both. The number of post-stress epochs ranged from one to four. Timing of post-

stressor epochs varied widely across studies, both for studies that used different biological indicators across epochs and those that used the same indicator. The *baseline* epoch was considered a measurement taken while the mother and child were at rest before the distress paradigm. The *immediate response* epoch was defined as a measurement during (cardiac indicators) or subsequently after (cortisol and sAA) the stressor. The *recovery response* epoch included a measurement taken after the stressor during which time the mother and child are working to return to baseline. A variety of variables were controlled across studies (see Table 5), including various maternal, child, and procedural factors, as well as moderators, which were often either individual (e.g., age), risk (e.g., maternal mental health), or protective factors (e.g., caregiver sensitivity).

Handling Multiple Effects

Several studies included multiple results as evidence of attunement for one or more of the following reasons: 1) participants underwent two different distress tasks; 2) there was more than one cortisol or sAA sample taken to capture the immediate response or recovery response; 3) two different groups were examined (e.g. very preterm and preterm infants); 4) the data were examined using more than one biological indicator (e.g. cortisol and sAA) or analysis (e.g. correlations and growth curve modeling); and 5) children participated at multiple ages. If participants underwent two different distress tasks within a particular age grouping (e.g., early toddlerhood [12-23 months of age]), the results from the more distressing task (as indicated by the paper or author consensus) were summarized. Biological samples taken post-stressor that closely resembled the methodologies for other included studies were summarized to minimize heterogeneity. For example, the cortisol sample taken closest to 20 minutes post-stressor was considered the immediate response and the sample taken closest to 40 minutes post-stressor was

considered the recovery response. However, for studies that used multivariate analyses and collapsed across epochs, all samples were included. Data from all participants examined, regardless of sample group, were reported. If data were examined using multiple analyses within the same analysis grouping (e.g., static concurrent, non-reciprocal analyses: correlation and regression), results that could be reported as a standardized effect size, most often correlations, were included. Articles that examined data with different biological indicators or analysis groupings were included in the summary, as the findings could be differentiated. Finally, because broad developmental patterns were summarized according to three age groups (infancy, early toddlerhood, and middle to late toddlerhood), articles that presented results within different age groupings were included for the narrative synthesis.

Quality Assessment

Quality percentage scores ranged from 53% to 87%, with a median score of 73%. The distributions of articles that received credit for each quality item are illustrated in Figure 2. The following are items that commonly reduced an article's quality score (i.e., the items for which at least 50% of articles did not receive credit): study population was not clearly defined (i.e., type of sample, the range of dates when participants were recruited), recruitment rate of 50% or more of eligible participants was not reported or was not reached, blinding of outcome assessors was not detailed, and exact *p*-values were not reported (e.g., $p < .05$ instead of $p = .03$).

Synthesis of Results: Relations between caregiver and child biological indicators

Below is a narrative summary of existing evidence on the relation between caregiver and child biological indicators in distress contexts. Tables 2 (a, b and c), 3, 4, and 5 summarize findings for each study in the narrative synthesis, according to biological indicator and further organized by analysis groupings. Results within each table also summarize results by epoch

(baseline, immediate response, recovery response). Broad patterns of findings related to age and covariates or moderators across all articles are also discussed.

Salivary Cortisol

Cortisol is the final glucocorticoid released and is the primary biomarker of HPA activity (Clements, 2013). For this review, all articles used saliva samples of cortisol. For sampling, there are theoretical rationales and empirical evidence to suggest that cortisol peak responses (i.e., reactivity) occurs around 20 minutes post-stressor, and a recovery response is to be expected around 30 to 40 minutes post-stressor, though timing tends to vary widely among studies (Provenzi et al., 2016), which is evident in the current review.

The current review included 25 studies, representing 18 distinct samples, that examined relations between mother and child cortisol in a distress context (see Table 1). Five articles (Atkinson et al., 2013; Braren et al., 2019; Davis & Granger, 2009; Hibel et al., 2015; Laurent et al., 2020) included longitudinal data. The samples in 16 studies (64%) were identified as clinical or high-risk. Timing of cortisol samples varied across studies from during the stressor to 50 minutes post-stressor. Samples ranging from 5 to 20 minutes post-stressor were considered the immediate distress response and samples taken between 35- and 50-minutes post-stressor were considered to capture the recovery response. Articles reporting cortisol outcomes were of higher quality on average (mean quality score = 76.16%, range = 67 – 87%). Results are summarized in Tables 2a–c according to analysis groupings.

Static Concurrent and Non-Reciprocal (Cortisol). As summarized in Table 2a, 23 articles examined the association between mother and child cortisol using static concurrent, non-reciprocal analyses, including correlations, regressions, and multilevel modeling (concurrent model). Only one study did not use a validated lab paradigm (Hendrix et al., 2018) and three

studies used a routine vaccine or heel lance as the stress-inducing stimulus (Castral et al., 2015; Davis & Granger, 2009; Spratt et al., 2016). Partial standardized effect sizes are reported for five studies that controlled for various procedural or individual characteristics that commonly affect cortisol outcomes (see Table 6 for list of covariates; Hibel et al., 2015; Kalomiris & Kiel, 2018; Kivlighan, 2006; Laurent et al., 2012; Laurent et al., 2020).

Seventeen studies examined relations between mother and child cortisol according to baseline or post-distress epochs. Most studies that examined the relation between mother and child cortisol at baseline (14 of 16 articles) reported a positive association, with effects ranging from small to large (r = .14 to .71; partial b^* = 0.47). Most of the reported effects (12/14) are moderate to large, positive relations between mother and child baseline cortisol (r > .23). Associations during the immediate response period were variable (r = -.09 to .49, partial b^* = 0.23, or non-significant and unreported). However, most studies (10/15) that examined the immediate response period found evidence of moderate, positive associations between mother and child cortisol (r = .22 to .49; partial b^* = 0.23). The relations between mother and child cortisol during the recovery period ranged from weak to moderate (r = -.07 to .54, partial b^* = 0.05, or non-significant and unreported). Evidence of covariation between mother and child cortisol during the recovery response remains unclear, as half of the studies (6/12) found moderate, positive effects and the other half found weak or non-significant effects. Most articles (8/10) that reported weak (< .20) or non-significant effects across the different epochs had sample sizes of fewer than 100 dyads.

Six studies in this section collapsed across epochs (baseline, immediate response, and recovery). Evidence of covariation between mother and child cortisol using this approach is unclear, as half of the articles (3/6) reported moderate, positive effects (r =.32, r = .58, and

partial *b = 0.49), and the other half reported weak (partial b* = -0.05 to 0.09) or non-significant relations. Again, the articles that reported weak or non-significant effects had sample sizes of fewer than 100 dyads.

Dynamic Concurrent and Non-Reciprocal (Cortisol). Nine studies from six distinct samples reported on the covariation between mother and child cortisol over time (see Table 2b). The different analyses used included correlations between mother and child slopes (correlated growth curve modeling), correlations between the change in child's cortisol from baseline with the change in mother's cortisol from baseline (difference score/dynamic correlation), and correlations and regressions using area under the cure increase (AUC_I), which captures cortisol change over time without accounting for baseline. Eight studies used a validated lab paradigm and one study used a heel stick procedure (Spratt et al., 2016).

Four studies examined changes in cortisol from baseline to the immediate response or recovery periods. Three out of four studies evidenced weak associations between mother and child cortisol (r = -.14 to .15). Five studies in this section collapsed across epochs, most of which (4/5) provided evidence of moderate to large, positive relations (r = .21 to .60) between changes in mother and child cortisol over time. Sample sizes of studies that collapsed across epochs and found stronger effects were much larger (N > 200 dyads) compared to the studies that evidenced weaker effects and analyzed epochs separately.

Dynamic Concurrent and Reciprocal (Cortisol). Three studies (Bernard et al., 2017; Hendrix et al., 2018; Nofech-Mozes et al., 2020) used a cross-lagged model to examine stability in cortisol levels over time (e.g. child's previous cortisol levels predict their subsequent cortisol levels) and reciprocity between mother and child cortisol (e.g. mother's cortisol levels predict

child's subsequent cortisol levels, and vice-versa; see Table 2c). Two of the three studies used a validated lab distress paradigm (Bernard et al., 2017; Nofech-Mozes et al., 2020).

Only one study examined results for epochs separately (Hendrix et al., 2018). All three studies provided evidence of moderate to large, positive relations (partial $b^* = 0.55$ to 0.83) between prior and subsequent cortisol levels for individual dyad members (i.e., stability of cortisol levels over time). Further, all three articles provided evidence of positive, albeit weak to moderate reciprocal relations (partial $b^* = 0.04$ to 0.23) between mother and child cortisol. All three articles in this section had large samples ($N > 150$ dyads).

Salivary Alpha Amylase

Salivary alpha amylase (sAA) is an enzyme in saliva that reflects activity of the SNS. sAA levels can be measured from 2 months of age onwards, though levels remain lower than among adults until 24 months of age (Davis et al., 2007). Further, reactivity to stress has not been demonstrated until 6 months of age. Compared to salivary cortisol, sAA demonstrates a quicker response to stress, peaking within 5 to 10 minutes post-stressor (Davis & Granger, 2009). Recovery is typically measured within 15 minutes, as return to baseline occurs by 20 minutes post-stressor. While both salivary cortisol and sAA have shown to be valid indicators of stress, they respond differently to stressors. For example, Mize and colleagues (2005) found that more children showed greater increases in sAA compared to cortisol on the same battery of tasks. Researchers have suggested that the HPA axis may be more responsive to stressors that evoke higher distress, whereas the SNS is more sensitive to a wider range of stressors (Hill-Soderlund et al., 2008).

A total of five studies from four distinct samples examined associations between mother and child sAA. One article (Davis & Granger, 2009) provided longitudinal data across infancy,

early, and mid-late toddlerhood. Timing of sAA samples ranged from 5 to 20 minutes post-stressor for the immediate response and 20 to 45 minutes for the recovery response. The quality scores for articles in this section were generally high (mean quality score = 76.4%, range = 67 – 87%). Three studies included a clinical or high-risk sample. Results are discussed below and are summarized in Table 3.

Static Concurrent and Non-Reciprocal (sAA). All sAA studies included in the current review examined the association between mother and child cortisol using static concurrent, non-reciprocal analyses, including correlations, regressions, and multilevel modeling. Four studies used a validated lab paradigm and one study used a vaccine as the stress-inducing stimulus (Davis & Granger, 2009). Partial standardized effect sizes are reported for three studies, as procedural, child, or maternal characteristics were adjusted for in the main analyses (see Table 6 for list of covariates; Davis & Granger, 2009; Kivlighan, 2006; Laurent et al., 2012).

Most studies (4/5) analyzed results separately for baseline, immediate response, and recovery response epochs. Three of four studies that analyzed baseline evidenced positive associations between mother and child sAA, ranging from small to large effects (r = .12 to .65). The strongest associations (r = .47 to .65) were reported by a study with small sample sizes across ages ($N \leq 22$; Davis & Granger, 2009). Three studies examined post-stressor epochs and consistently found non-significant associations between mother and child sAA during the immediate response and recovery phases. Effect sizes were not reported for these epochs. Only one study (Laurent et al., 2011) examined relations between mother and child sAA collapsed across epochs. A small, positive partial effect (b^* = 0.23) was found, whereby increases in mother's sAA predicted higher levels of infant sAA across the duration of the stress task.

Cardiac Indicators (Respiratory Sinus Arrythmia and Heart Period)

There is also a small but growing literature on caregiver and child attunement using cardiac indicators. Respiratory sinus arrythmia (RSA), which is one of the most common indices of heart rate variability, reflects the variability in heart rate that occurs at the frequency of spontaneous breathing (Hastings & Miller, 2014). In the absence of a perceived stressor, the parasympathetic nervous system acts as a "brake" on the heart via the vagus nerve (Porges, 2007). This adaptive resting state is indexed by high RSA (i.e., steady and low heart rate), suggesting homeostasis is being maintained. In stress contexts, the vagal brake is withdrawn which facilitates mobilization of the SNS and allows for emotional and behavioural responses. Vagal withdrawal, indexed by decreasing RSA (i.e., RSA suppression), allows individuals to orient, respond, and engage in active coping (Hastings & Miller, 2014; Porges, 2007).

Another cardiac biomarker that has been examined in the attunement literature is heart period. However, research using this biological indicator is very limited. Heart period refers to the timed interval between heartbeats (Bazhenova et al., 2001). It reflects a combination of parasympathetic and sympathetic activity and is inversely related to heart rate (i.e., low heart period corresponds to high heart rate). Heart period and RSA are typically highly interrelated in both theory and practice; however, they can behave differently since HP also integrates sympathetic activity (Stevenson-Hinde & Marshall, 1999).

A total of ten studies, representing seven distinct samples, examined the relation between mother and child cardiac indicators. Across all studies, 15 or 30 second epochs were analyzed and averaged across the duration of the stressor to capture the immediate response or the recovery response. All studies examined RSA in mothers and their children, and two of the ten studies also captured heart period (Busuito et al., 2019; Moore et al., 2009). Compared to the articles in other sections, the quality scores for articles reporting on cardiac indicators were lower

(mean quality score = 64.6%, range = 53 – 80%). Furthermore, these articles often did not report on the reliability of cardiac editing procedures. One study (Ostlund et al., 2017) included a clinical or high-risk sample. Results are summarized in Table 4.

Static Concurrent and Non-Reciprocal (Cardiac Indicators). All ten studies that examined the relations between mother and child cardiac indicators used static concurrent and non-reciprocal analyses, including correlation, regression, or multilevel modeling. Nine of the ten studies used a validated stress paradigm. Only half of the studies (5/10) reported correlations or standardized partial slopes. The one study that reported standardized partial coefficients adjusted for within-individual variability in RSA, overall mean RSA for mother's or infants, and mother's baseline RSA (Skoranski et al., 2017).

All studies examined cardiac indicators for separate baseline, immediate response, and recovery epochs. Generally, there was a consistent pattern of weak (r = .07 to .17; partial b^* = 0.11 to 0.19) or non-significant relations between mother and child RSA across epochs. Results for mother and child heart period at baseline were either small or moderate positive associations (r = .13 and r = .32). There were weak (r = .01 and .09) or non-significant relations between mother and child heart period during the immediate response and recovery period. Studies examining cardiac may not have had sample sizes large enough to detect small effects, as nine out of ten studies had a sample size of fewer than 100 dyads.

Electroencephalography (EEG)

The newest line of research examining caregiver-child attunement uses measures of brain activity, commonly with electroencephalography (EEG), and specifically, frontal asymmetric activity within the alpha band (8-13 Hz for adults and 6-9 Hz for infants and young children). Frontal EEG asymmetry is an indicator of the balance of brain activation in left and right frontal

areas of the brain and serves as a biomarker of emotional reactivity and regulation (Fox, 1991). Positive emotions and approach-related behaviours are thought to be organized and processed within the left frontal hemisphere, whereas negative emotions and avoidance behaviours are organized and processes within the right frontal hemisphere (Davidson, 2000; Fox, 1991). Thus, during a distress task that elicits negative affect, greater right frontal asymmetry would be expected.

Two studies examined associations between mother and child frontal asymmetry patterns using EEG (Atzaba-Poria et al., 2017; Krzeczkowski et al., 2020). EEG data were acquired from both mothers and children during a baseline period and a distress task. One of the studies used a validated paradigm, a musical piece shown to elicit fear or negative affect in adults and infants (Krzeczkowski et al., 2020). Atzaba-Poria et al., (2017) used a challenging puzzle task as their distress paradigm. The quality of studies in this section were 67% (Krzeczkowski et al., 2020) and 73% (Atzaba-Poria et al., 2017). Both studies collected data from normative populations. Results are summarized below and presented in Table 5.

Static Concurrent and Non-reciprocal (EEG). Both studies examined mother and child frontal asymmetry patterns using static, concurrent, and non-reciprocal analyses, specifically correlations. Baseline associations between mother and child frontal asymmetry were consistently weak (r = -.09 and r = .002). During the tasks (i.e., the immediate reactivity period), relations between mother and child frontal asymmetry differed across studies, as one study reported a weak association (r = -.01; Krzeczkowski et al., 2020) and the other reported a moderate, positive association (r = .25; Atzaba-Poria et al., 2017). Both studies had small sample sizes (Ns < 35).

Dynamic Concurrent and Reciprocal (EEG). One of the studies in this section also examined associations between mother and child frontal asymmetry patterns using a cross-lagged model (Krzeczkowski et al., 2020); that is, stability of frontal asymmetry within individuals and reciprocity between mother and child outcomes over time. Both mothers and their infants evidenced positive, moderate stability in frontal asymmetry activity during baseline and the stress task (b^* = 0.35 and b^* = 0.41). However, similar to the correlation results reported by this study, there were very weak reciprocal relations (b^* = 0.03 and b^* = -0.01) between mother and infant frontal asymmetry from baseline to the distress task.

Summary of Covariates/Moderators

Patterns of findings according to the different age groupings were examined. Developmental patterns were not discernable according to the age groupings. The majority of studies examined children during the infancy period (25/36), highlighting the need for more examinations of caregiver-child attunement during the second and third years of life.

Broad covariate or moderator patterns were examined across all studies included in the current review. A summary of covariates and moderators across studies is in Table 6. Most studies (30/36) examined potential covariates (e.g., common variables that are known to affect different biological measures but were not associated with outcomes) or included covariates in main analyses. Generally, there was lack of consistency in covariates examined across studies, as well as for studies using the same biomarker which precluded synthesis. Generally, covariates examined included infant variables (e.g., time of last feed), maternal (e.g., maternal body mass), and procedural factors (e.g., time of day).

Similar to covariates, a variety of moderators was examined including those hypothesized to negatively impact (i.e., risk factors) and those expected to augment (i.e., protective factors)

mother-child attunement. The most common moderator examined across biological indicators was maternal mental health. Two studies demonstrated that higher maternal depressive symptoms were associated with greater concordance between mother and child cortisol (Khoury et al., 2016; Laurent et al., 2011) and three studies reported that maternal depression did not significantly interact with maternal biological indicators to predict infant responses (Hendrix et al., 2018; Lunkenheimer et al., 2018; Ostlund et al., 2017). Other moderators examined in more than one study had inconsistent results; these included: intimate partner violence (Bernard et al., 2017; Hibel et al., 2009), negative parenting behaviours (Hibel et al., 2009; Lunkenheimer et al., 2018; Skoranski et al., 2017), and attachment status (Hill-Soderlund et al., 2008; Nofech-Mozes et al., 2020). Parent sensitivity was the only moderator that evidenced consistent results across two studies, as higher parent sensitivity was related to more concordance between parent and child cortisol (Atkinson et al., 2013; van Bakel & Riksen-Walraven, 2008).

Discussion

The current study is the first known to systematically review evidence of caregiver-child biological attunement within distress contexts and during the first few years of life. The main goal of this review was to qualitatively summarize and synthesize the literature based on methodological characteristics, including biological indicators used to measure distress, type of analyses used to examine attunement, and measurement epochs examined pre- and post-distress. Further, a broad summary of covariates and moderators was provided. Findings varied across methodological approaches used to study attunement. The variability in findings is likely due, in part, to the lack of consensus regarding a conceptualization of attunement which typically informs the methodology researchers use to study the dyadic process (Bernard et al., 2017; Nofech-Mozes et al., 2020). The heterogeneity of definitions and methods used poses an issue

for the attunement literature, as studies cannot be compared, or consistent result patterns cannot be easily discerned. There were some patterns gleaned from the literature that are discussed below, along with limitations of the extant literature and important avenues for future research.

Associations between caregiver and child biological indicators

Cortisol

Cortisol studies provide the bulk of evidence that can be summarized across different types of analyses. Findings suggest moderate to large, positive relations between mother and child cortisol responses during baseline, as well as moderate, positive associations between mother and child cortisol during the immediate response period. Evidence of covariation between mother and child cortisol during the recovery period and when outcomes were collapsed across epochs is unclear, as weak to moderate effects were equally prevalent. There was consistent evidence of moderate to large, positive relations between changes in mother and child cortisol over time and collapsed across epochs. These static and dynamic (non-reciprocal) results should be carefully contextualized, as examining static individual phases, one-way predictions (mother cortisol predicting infant cortisol), and collapsing across epochs over time oversimplifies the complex interaction between caregivers and their children.

There was also evidence of prospective associations between dyad members' cortisol during infancy and early toddlerhood (6 to 17 months of age), with weak to small, positive reciprocal relations between mother and child cortisol from baseline to post-distress epochs. There were no studies that examined dynamic and reciprocal relations during mid to late toddlerhood. Considering the significant growth and differentiation of infant emotion and skills required for regulation within the first year to two years of life (Feldman, 2007; Izard et al., 2011), concurrent coordination of distress with mothers may be less stable during this time. The

process of attunement is undergoing transformation from *external regulation* (i.e., infants rely on caregivers to facilitate their regulation from distress) to *mutual regulation* (i.e., greater contingency and reciprocity as both partners attempt to regulate together) as the infant gains more skills (e.g. cognitive, motor, etc.) to interact with their primary caregiver in an attuned manner (Feldman, 2007). Thus, it is important to examine the dynamic and reciprocal components of biological attunement, with more research including mid to late toddlerhood, to capture how the attunement process initially develops as being primarily constructed by the caregiver and transforms into a process of mutual regulation (Feldman, 2007).

Weak effects or non-significant results were often reported by studies with samples sizes that were smaller than 100 dyads. Thus, future studies should aim to have at least 100 dyads in order to obtain precise estimates of small effects. Challenges in measuring cortisol may have also weakened findings in the current review. Capturing cortisol early in life is particularly challenging because infants and toddlers sometimes have very little or no response to stressors (Clements, 2013). Further, research has demonstrated considerable variability in timing of peak distress for infants (Ramsay & Lewis, 1994). It is also important to consider that dyad reciprocal interactions are very dynamic which should be reflected in the biological measure (Nofech-Mozes et al., 2020). Three or four cortisol measurements may not accurately capture dynamic changes. An attempt to decrease measurement error would require increasing the frequency of cortisol samples taken, with smaller time increments between samples to increase the likelihood of observing peak distress and dynamic changes during recovery. It is also important to note that a wide variety of variables can affect cortisol levels (e.g. biological sex, medications, time of day, oral contraceptives, etc.; Clements, 2013; Provenzi et al., 2016). However, a portion of studies (5/25 cortisol articles) in this review did not report controlling for known sources of

cortisol variation. Thus, if researchers are using cortisol as a measure, careful consideration of which variables should be controlled is necessary to reduce measurement error. Researchers are encouraged to think about their measurement procedure (e.g., frequency of measure, controlling for factors, etc.) and whether the indicator accurately captures caregiver-child attunement.

Salivary Alpha Amylase (sAA)

Consistent with cortisol findings, results indicate caregiver and child sAA positively covary during baseline, with effect sizes ranging from small to large. There were consistent non-significant associations between dyad sAA during the immediate response and recovery periods. No studies examined sAA using dynamic concurrent and non-reciprocal or dynamic concurrent and reciprocal analyses. More research is needed to determine whether sAA is a sensitive and reliable biological indicator of attunement. Since sAA is a measure of SNS activity, it reflects immediate arousal or responsivity to an environmental stressor (Augustine & Leerkes, 2019). If measured on its own, it may not adequately represent the attunement process which largely involves the recovery process, which is a dynamic and transactional process that unfolds between caregivers and their children to reach a regulated state. As such, inconsistent findings may have occurred because sAA as a biomarker is not aligned theoretically with attunement as a coregulatory process. If researchers use it as an biological indicator of stress, it should be coupled with another indicator that measures regulatory processes (e.g. RSA; Augustine & Leerkes, 2019).

Further, there is a lack of stability in sAA levels for the first two years of life, with substantial variability in overall sAA levels (Bright et al., 2014; Davis et al., 2007). Given that it is not a very stable indicator early in life, it may be difficult for sAA to capture reliable patterns of attunement between caregivers and their children during this time period. All of the studies

that examined attunement using sAA in the current review included children 24 months age or younger. sAA also has a similar limitation as cortisol related to variability in peak distress (Hill-Soderlund et al., 2008) and is influenced by numerous contextual factors (e.g., teeth development, diet) that may contribute to sources of variation in the data (Davis & Granger, 2009).

Cardiac Indicators (RSA and HP)

Similar to analysis limitations of sAA, the relation between mother and child cardiac indicators was examined using only static concurrent and non-reciprocal analyses. Relations between mother and child RSA and heart period during baseline and post-stressor epochs were generally weak. Overall, these data suggest that dyadic regulatory processes are not captured by cardiac indicators. These findings may be partly due to how the studies were designed. All studies either averaged 15 or 30 second epochs over the duration of a task or used static analysis approaches that do not capture the temporal dynamics of the attunement process. Researchers should analyze smaller epoch intervals that have been used to detect dynamic changes in RSA (e.g., 30-second epoch; Hastings & Kahle, 2019). In addition, nine of ten studies that examined cardiac indicators had a sample size of fewer than 100 dyads. These samples may not have been large enough to estimate the population effect precisely.

Another important consideration is the rapid developmental changes that occur in the PNS, similar to the SNS and HPA axis, which may affect biological attunement. More research is needed to determine whether RSA is a stable indicator of distress within the first few years of life (also see Waxman et al., 2020). The cardiac studies included in the current review had the lowest quality scores on average, as most studies did not estimate or report reliability coefficients for cardiac editing procedures. Despite these limitations, RSA holds promise as an indicator to

uniquely capture the attunement process, as the PNS is suited to support interpersonal interactions due to its ability to rapidly modulate arousal through neural innervation of the heart (Porges, 2007). Thus, the measurement of RSA can capture rapid and dynamic changes. Researchers are encouraged to continue developing this area of research with consideration of methodological limitations discussed. Heart period is an understudied biological indicator and its ability to capture attunement process is currently unknown.

Electroencephalography (EEG)

The summary of relations between mother and child EEG (more specifically frontal asymmetry) in the current review is limited, as only two studies with small sample sizes were available for synthesis. This finding is not unexpected given that brain measures have only recently been used to capture caregiver-child dynamics. Both articles included in the current review examined the data using static concurrent and non-reciprocal approaches. The evidence available indicated weak baseline associations between mother and child frontal asymmetry. Further, weak and small, positive associations between mother and child frontal asymmetry were found during distressing tasks. One article also examined the dynamic concurrent and reciprocal relations between mother and child frontal asymmetry, but only weak reciprocal associations were found.

Many different approaches can be used (functional magnetic resonance imaging [fMRI], magnetoencephalography [MEG], EEG, or functional near-infrared spectroscopy [fNIRS]) to index brain activity. These measures have been used to monitor activity patterns during a variety of tasks, though typically social interactions (Mayo & Gordon, 2020). There is great potential in assessing attunement via EEG given the high temporal resolution of the measure which allows for the capture of real-time dynamic changes in dyadic interactions. Previous research in a non-

distress context has shown that EEG changes are evident when there are subtle shifts in an adult-infant dyadic interaction (Leong et al., 2017; Wass et al., 2018). For example, Leong and colleagues (2017) demonstrated that brief aversions of adult eye gaze produced a significant change in EEG synchronicity between adult-infant pairs. More research is needed examining attunement using brain measures to determine their contributions as biomarkers in capturing early dyadic processes that support distress regulation.

Additional conceptual and methodological challenges

One of the most widespread inconsistencies in the attunement literature regards how the dyadic process is conceptualized, which ends up informing the methods researchers use, particularly analyses approaches. Some researchers have defined attunement as an 'empathic psychophysiological response' or 'matching of states' which is reflected by the static analyses (i.e., time-specific correlations and regressions) used to examine this concept (Thompson & Trevathan, 2009; van Bakel & Riksen-Walraven, 2008). More recently, researchers have started to probe the complexity of the attunement process by examining dynamic changes in interactions over time and the interactive reciprocity between dyad members (Bernard et al., 2017; Nofech-Mozes et al., 2020). These features that are intrinsic to attunement have been observed behaviourally, as caregivers and infants engage, respond, and adjust to one another, as they regulate from distress over time (Tronick & Beeghly, 2011).

Static concurrent and non-reciprocal analyses are the most common approaches used to study attunement to date. However, this analysis approach is unable to map the complexity of the attunement process (Calkins & Hill, 2007), as it represents an association at one time point and does not consider reciprocal influences unfolding between dyad members. Another limitation of using correlations or ordinary linear regression is the inability to account for repeated measures,

which is often needed when examining biomarkers of distress (Kivlighan, 2006). Further, some researchers using regression techniques only examined a one-way prediction (i.e., mothers predicting infants, or infants predicting mothers), overlooking the bidirectional nature of the attunement process.

Compared to static approaches, dynamic concurrent and non-reciprocal analyses (e.g., correlated growth models) capture the temporal aspect of attunement. Specifically, the dynamic and concurrent aspect of these analyses is consistent with a conceptualization of attunement as a concurrent process between caregivers and their children that unfolds across time. It is limiting as an analysis approach because it does not account for the 'lead-and-lag' or 'back-and-forth' structure of the attunement process. Further, researchers that have used growth models within the attunement literature often do not distinguish the patterns of growth that occur over time, despite the availability of this information (Nofech-Mozes et al., 2020). This makes it difficult to distinguish what positive or negative covariation means. For example, caregivers and children could have positive correlations between their cortisol trajectory slopes, but both members are either regulating from distress together (i.e., adaptive attunement), or demonstrating correlated increases in biological reactivity that may look attuned, though each member of the dyad is reacting to the same challenging circumstance separately and unable to mutually regulate (i.e., lack of attunement) (Nofech-Mozes et al., 2020). Dyadic response profiles should be mapped to visualize how each partner is reacting to the same challenging circumstance. Further, consideration of the psychosocial factors impacting the dyad is necessary to further elucidate whether dyads may be regulating together or reacting to their own stressors (Nofech-Mozes et al., 2020). For example, a caregiver experiencing intimate partner violence (e.g., Hibel et al., 2009) may process a challenging situation as threatening to themselves rather than responding to

their infant's distress. Furthermore, as highlighted by Bernard and colleagues (2017), researchers' statistical analysis should ultimately reflect the definition of dyadic attunement which involves two important components, including time and reciprocity.

It is possible to specify and estimate models (e.g., autoregressive cross-lagged models also known as APIM models; Kenny, 1996) that can assess both the concurrent temporal and reciprocal nature of the attunement process. APIM is a modelling strategy that captures the interdependence of a dyadic interactions, including the stability of caregiver and child responses across time (actor effects) and concurrent relations between caregiver and child responses at any time point. Most importantly, the APIM model can capture the 'lead-and-lag' structure of attunement (partner effects) that is thought to emerge within the first few years of life when caregivers serve primarily as the external regulator for the infant and thus drive the dyadic regulatory response. Similar to other analyses discussed, positive and negative coefficients provided through cross-lagged analysis do not provide information about the distinct pattens of the distress response and recovery over time. Thus, although cross-lagged models capture important aspects of attunement, additional analyses should be explored to map the trajectory patterns of attunement (Nofech-Mozes et al., 2020).

Moving forward, researchers need to better capture trajectory patterns of positive, negative, and weak (i.e., close to 0) covariation. The dominant tone in the literature is 'the more in sync, the better' (Mayo & Gordon, 2020). However, caregivers and children who appear to have correlated biological responses at one time or correlated changes over time may be experiencing attunement or lack of attunement. In addition, patterns of mismatch, manifesting as weak and/or negative associations in distress contexts, can be adaptive. Tronick and colleagues have suggested a lack of harmony in biobehavioural states between caregivers and their children

is not only typical but also crucial for a child's development (DiCorcia & Tronick, 2011; Tronick & Gold, 2020). In typical interactions, dyads oscillate between mismatched and matched biobehavioural states (Beeghly & Tronick, 2011; DiCorcia & Tronick, 2011). Mismatches can occur due to many different factors, including rapidly changing regulatory demands of a child, response time of caregivers to their child's distress signals, and the likelihood of missed distress cues (DiCorcia & Tronick, 2011). Despite these times of interactive disorganization, missteps within the interaction can be corrected and the dyad can repair into a more organized, regulated state, as caregiver regulatory input begins to match infant regulatory needs. This process of repairing mismatches and working towards a regulated state is known as *reparation* (Beeghly et al., 2016; DiCorcia & Tronick, 2011). Challenges with the reparation process (also known as *interactive ruptures*; Montirosso & McGlone, 2020) may reflect lack of attunement (i.e., when a caregiver is unable to regulate themselves or and has little regard for infant's emotional state or attempts to communicate). The process of reparation allows caregivers to serve as an external regulator but also scaffold skills in their children by providing them with moments to self-regulate. As such, the form and quality of the reparatory process is crucial for developing a young child's adaptive regulatory capacity.

Considering this notion, it is possible that many of the weak static associations evidenced in this review reflect the typical mismatches and subsequent reparations that occur between caregivers and their children. Analyses that map trajectory patterns, such as parallel-process growth modeling, are necessary to elucidate characteristic patterns of attunement and lack of attunement, as it is unlikely all dyads display similar attunement patterns. Further, as researchers are encouraged to move beyond simple conceptualizations of positive, negative, or weak covariation, interactions patterns should not just be considered as attuned or not attuned, as

attunement is not an all-or-none condition (Harrist & Waugh, 2002). With more research and evidence to support this notion, the definition of attunement should include consideration of how quickly dyads can repair interactive errors and successfully move back and forth between discordant and attuned states.

Timing of outcome measurement

Another important methodological consideration is the timing of measurement. Baseline, immediate reactivity, and regulation have been clearly distinguished in research examining biological indicators (Leerkes & Parade, 2015; Rothbart & Bates, 2006). Researchers have suggested that distress outcomes should be measured with temporally sensitive approaches due to dynamic changes of biology. Dynamic changes are expected to occur as part of the attunement process, as caregivers and children respond to a distressing stimulus and subsequently regulate from distress together. However, most studies in the current review either collapsed across epochs when analyzing data or only examined certain epochs (e.g., only baseline). These studies likely did not truly capture the concept of attunement that changes with time (i.e., reparation process), as the demands of the environment change. More research is needed to distinguish differences in attunement across these epochs, with more complex analyses (e.g., cross-lagged and trajectory models) than can capture dynamic changes and reciprocity across time.

Developmental Patterns and Moderators

Caregiver-child attunement was examined according to different developmental stages, including infancy (0-11 months), early toddlerhood (12-23 months), and mid to late toddlerhood (24-47) months). However, patterns of findings were not apparent across these different age groups. Developmental patterns may not have emerged, as the bulk of the studies (70%) examined attunement during infancy. More studies are needed that examine attunement in

caregivers and their children during toddlerhood, as attunement is purported to be an early life process that changes over time as a child develops (Feldman, 2007).

A variety of moderators was examined including those hypothesized to hinder (i.e., risk factors) or augment (i.e., protective factors) adaptive attunement. Results were variable for common moderators examined, including maternal mental health, intimate partner violence, attachment status, and negative parenting behaviour. These inconsistent findings further highlight the need to examine the data with more complex analyses to map patterns of attunement according to different levels of the moderator (e.g., high vs. low depressive symptoms). Parent sensitivity was the only moderator to evidence consistent results across studies, with higher parent sensitivity associated with greater concordance of distress outcomes between children and their parents. This finding is consistent with theory and research that demonstrate sensitive caregivers structure their interactions in a manner that is contingent and appropriate to the child's distress responses (Bowlby, 1982; Leerkes et al., 2016; Spanglar et al., 1994).

Limitations

We carried out a narrative synthesis due to the wide variety of methodology used to study attunement. Until there is greater consistency in moderator tracking, experimental controls, epoch timing, and statistical technique, our ability to gather a nuanced understanding of the conditions under which adaptive or maladaptive biological attunement exists is limited. The current review has discussed the improvements in methodology needed to better capture the biological process of attunement. The synthesis of literature was also limited by the number of unique samples available. Some studies were from an affiliated group of researchers that used the same sample which should be considered when interpreting results. Additionally, there are a

lack of studies within the mid to late toddlerhood age range which may result in important developmental patterns being missed. Almost all studies used a cross-sectional design which highlights the need for longitudinal investigations. Further, most studies examined North American samples which limits the generalizability of findings. A handful of studies did not report an estimate of the relation between caregiver and child biological indicators if the effect was non-significant, which interfered with consistent reporting of the size of effects. Finally, there is a need for more complex analyses to examine the dynamic and reciprocal patterns of attunement, as a majority of the studies in the current review used oversimplified approaches.

Conclusions and Future Directions

The present study aimed to contribute a better understanding of the emergence and patterns of caregiver-child biological attunement during the first few years of life. Overall, we assert that the types and variability of methodological approaches have hampered the ability to optimally capture the early caregiver-child attunement process and move the field forward. Despite these limitations, there were some patterns of results gleaned from the synthesis including: (1) consistent evidence of positive covariation at rest (i.e., baseline) between maternal and child cortisol (moderate to large effects) and sAA (small to large effects); (2) moderate, positive covariation between maternal and child cortisol during the immediate reactivity period (i.e., response to stressor); (3) emergence of moderate to large positive associations between changes in maternal and child cortisol occurring from baseline through to the recovery period; (4) weak to small, positive reciprocal relations were found between mother and child cortisol within infancy and early toddlerhood (less than 17 months old); (5) weak associations or limited evidence of associations between caregiver and child cardiac and EEG indicators were found; and (6) higher parent sensitivity (protective factor) was associated with positive covariation

between caregiver and child cortisol levels. Significant gaps in the literature were highlighted, including the need for better measurement procedures of biological indicators, the use of more nuanced analyses that better capture the concept of attunement as a dynamic and reciprocal process and patterns of change that occur over time, more research examining mid to late toddlerhood, consistent covariates used across studies, and more examinations of key risk and protective factors that influence attunement.

It is possible that inconsistent findings were due to the limitations of methodological practices used to study attunement. Based on our review of the literature, we recommend the following for future work examining caregiver-child biological attunement:

1. Choose biomarkers that theoretically align with the concept under study (i.e., attunement as a coregulatory process).
2. Consider limitations of biomarkers being used and address them when possible (e.g., control for variables that impact biomarkers, use more frequent measurement to capture variability in distress responses).
3. Use multiple biomarkers to better determine unique contributions of different systems (e.g., PNS, SNS and HPA axis) in measuring attunement.
4. Improve measurement approaches (e.g., distinguishing between pre- and post-stimuli responses, utilizing frequent and consecutive epochs to capture peak distress and dynamic changes) and reporting practices (e.g., calculate reliability of data editing, determine recruitment rates, detail study characteristics, and report blinding procedures).
5. Use analysis approaches that better capture the process of attunement, including cross-lagged models that can capture the dynamic and reciprocal nature of the dyadic

process, and parallel-process growth models that can capture distinct patterns of attunement.

There are additional areas to highlight for future research. There was only one study (van Bakel & Riksen-Walraven, 2008) in the current review that included two fathers in their sample. This underrepresentation originates from traditional gender-based roles which may not widely reflect current caregiving practices (Davison et al., 2017). This is an important area for future research, as research has demonstrated greater affect attunement between same-gender parent-infant dyads (Feldman, 2003). Relatedly, sex differences have been overlooked in the biological attunement literature. Parents may have different expectations for children's emotion experiences depending on the sex of the child, which may result in differences in how they respond to children's distress responses (Creavy et al., 2020).

References

Ainsworth, M. D. S., Blehar, M. C., Waters, E., & Wall, S. (1978). *Patterns of attachment: A psychological study of the strange situation.* Lawrence Erlbaum

Ainsworth, M. S. (1979). Infant–mother attachment. *American Psychologist*, *34*(10), 932–937. https://doi.org/10.1037/0003-066X.34.10.932

Atkinson, L., Jamieson, B., Khoury, J., Ludmer, J., & Gonzalez, A. (2016). Stress physiology in infancy and early childhood: Cortisol flexibility, attunement and coordination. *Journal of Neuroendocrinology*, *28*(8). https://doi.org/10.1111/jne.12408

Atkinson, Leslie, Gonzalez, A., Kashy, D. A., Santo Basile, V., Masellis, M., Pereira, J., Chisholm, V., & Levitan, R. (2013). Maternal sensitivity and infant and mother adrenocortical function across challenges. *Psychoneuroendocrinology*, *38*(12), 2943–2951. https://doi.org/10.1016/j.psyneuen.2013.08.001

Atzaba-Poria, N., Deater-Deckard, K., & Bell, M. A. (2017). Mother-child interaction: Links between mother and child frontal electroencephalograph asymmetry and negative behavior. *Child Development*, *88*(2), 544–554. https://doi.org/10.1111/cdev.12583

Augustine, M. E., & Leerkes, E. M. (2019). Associations between maternal physiology and maternal sensitivity vary depending on infant distress and emotion context. *Journal of Family Psychology*, *33*(4), 412–421. https://doi.org/10.1037/fam0000538

Bader, L. R., Tan, L., Gonzalez, R., Saini, E. K., Bae, Y., Provenzi, L., & Volling, B. L. (2021). Adrenocortical interdependence in father-infant and mother-infant dyads: Attunement or something more?. *Developmental Psychobiology*.

Bazhenova, O. V., Plonskaia, O., & Porges, S. W. (2001). Vagal reactivity and affective adjustment in infants during interaction challenges. *Child Development*, *72*(5), 1314–1326. https://doi.org/10.1111/1467-8624.00350

Beeghly, M., Perry, B. D., & Tronick, E. (2016). *Self-regulatory processes in early development* (S. Maltzman, Ed.; Vol. 1). Oxford University Press. https://doi.org/10.1093/oxfordhb/9780199739134.013.3

Beeghly, M., & Tronick, E. (2011). Early resilience in the context of parent-infant relationships: A social developmental perspective. *Current Problems in Pediatric and Adolescent Health Care*, *41*(7), 197–201. https://doi.org/10.1016/j.cppeds.2011.02.005

Bell, M. A. (2020). Mother-child behavioral and physiological synchrony. *Advances in child development and behavior*, 58, 163-188.

Bernard, N. K., Kashy, D. A., Levendosky, A. A., Bogat, G. A., & Lonstein, J. S. (2017). Do different data analytic approaches generate discrepant findings when measuring mother-infant HPA axis attunement? *Developmental Psychobiology*, *59*(2), 174–184. https://doi.org/10.1002/dev.21474

Berntson, G. G., Cacioppo, J. T., & Quigley, K. S. (1993). Respiratory sinus arrhythmia: autonomic origins, physiological mechanisms, and psychophysiological implications. *Psychophysiology*, *30*(2), 183-196.

Bowlby, J. (1982). Attachment and loss: Retrospect and prospect. *American Journal of Orthopsychiatry*, *52*(4), 664–678. https://doi.org/10.1111/j.1939-0025.1982.tb01456.x

Braren, S. H., Perry, R. E., Ursache, A., & Blair, C. (2019). Socioeconomic risk moderates the association between caregiver cortisol levels and infant cortisol reactivity to emotion

induction at 24 months. *Developmental Psychobiology*, *61*(4), 573–591. https://doi.org/10.1002/dev.21832

Bridgett, D. J., Burt, N. M., Edwards, E. S., & Deater-Deckard, K. (2015). Intergenerational transmission of self-regulation: A multidisciplinary review and integrative conceptual framework. *Psychological Bulletin*, *141*(3), 602–654. https://doi.org/10.1037/a0038662

Bright, M. A., Frick, J. E., Out, D., & Granger, D. A. (2014). Individual differences in the cortisol and salivary α-amylase awakening responses in early childhood: Relations to age, sex, and sleep. *Developmental Psychobiology*, *56*(6), 1300–1315. https://doi.org/10.1002/dev.21209

Busuito, A., Quigley, K. M., Moore, G. A., Voegtline, K. M., & DiPietro, J. A. (2019). In sync: Physiological correlates of behavioral synchrony in infants and mothers. *Developmental Psychology*, *55*(5), 1034–1045. https://doi.org/10.1037/dev0000689

Calkins, S. D., & Hill, A. (2007). Caregiver influences on emerging emotion regulation. *Handbook of emotion regulation*, *229248*.

Castral, T. C., Warnock, F., dos Santos, C. B., Daré, M. F., Moreira, A. C., Antonini, S. R. R., & Scochi, C. G. S. (2015). Maternal mood and concordant maternal and infant salivary cortisol during heel lance while in kangaroo care: Maternal and infant salivary cortisol during pain. *European Journal of Pain*, *19*(3), 429–438. https://doi.org/10.1002/ejp.566

Clements, A. D. (2013). Salivary cortisol measurement in developmental research: Where do we go from here? *Developmental Psychobiology*, *55*(3), 205–220. https://doi.org/10.1002/dev.21025

Creavy, K. L., Gatzke-Kopp, L. M., Zhang, X., Fishbein, D., & Kiser, L. J. (2020). When you go low, I go high: Negative coordination of physiological synchrony among parents and

children. *Developmental Psychobiology*, *62*(3), 310–323. https://doi.org/10.1002/dev.21905

Crockett, E. E., Holmes, B. M., Granger, D. A., & Lyons-Ruth, K. (2013). Maternal disrupted communication during face-to-face interaction at 4 months: Relation to maternal and infant cortisol among at-risk families. *Infancy*, *18*(6), 1111-1134.

Crombie IK. *The pocket guide to critical appraisal: a handbook for health care professionals*. Hoboken, NJ: Blackwell Publishing; 2007.

Davidson, R. J. (2000). Affective style, psychopathology, and resilience: brain mechanisms and plasticity. *American Psychologist*, *55*(11), 1196.

Davis, E. P., Kannan, I., Marucut, J., Granger, D. A., & Sandman, C. A. (2007). Salivary alpha-amylase response to an inoculation stressor. *Biennial Meeting of the Society for Research on Child Development (Boston, MA)*.

Davis, E. P., & Granger, D. A. (2009). Developmental differences in infant salivary alpha-amylase and cortisol responses to stress. *Psychoneuroendocrinology*, *34*(6), 795–804. https://doi.org/10.1016/j.psyneuen.2009.02.001

Davison, K. K., Charles, J. N., Khandpur, N., & Nelson, T. J. (2017). Fathers' perceived reasons for their underrepresentation in child health research and strategies to increase their involvement. *Maternal and Child Health Journal*, *21*(2), 267–274. https://doi.org/10.1007/s10995-016-2157-z

DiCorcia, J. A., & Tronick, E. (2011). Quotidian resilience: Exploring mechanisms that drive resilience from a perspective of everyday stress and coping. *Neuroscience & Biobehavioral Reviews*, *35*(7), 1593–1602. https://doi.org/10.1016/j.neubiorev.2011.04.008

Downs, S. H., & Black, N. (1998). The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *Journal of Epidemiology & Community Health*, *52*(6), 377-384.

Ekas, N. V., Braungart-Rieker, J. M., & Messinger, D. S. (2018). The development of infant emotion regulation: Time is of the essence. In *Emotion regulation* (pp. 31-51). Routledge.

Feldman, R. (2003). Infant–mother and infant–father synchrony: The coregulation of positive arousal. *Infant Mental Health Journal: Official Publication of The World Association for Infant Mental Health*, *24*(1), 1–23.

Feldman, R. (2007). Parent–infant synchrony and the construction of shared timing; physiological precursors, developmental outcomes, and risk conditions. *Journal of Child Psychology and Psychiatry*, *48*(3–4), 329–354. https://doi.org/10.1111/j.1469-7610.2006.01701.x

Feldman, R. (2016). The neurobiology of mammalian parenting and the biosocial context of human caregiving. *Hormones and behavior*, *77*, 3-17.

Feldman, R., Granat, A. D. I., Pariente, C., Kanety, H., Kuint, J., & Gilboa-Schechtman, E. (2009). Maternal depression and anxiety across the postpartum year and infant social engagement, fear regulation, and stress reactivity. *Journal of the American Academy of Child & Adolescent Psychiatry*, *48*(9), 919-927.

Feldman, R., Singer, M., & Zagoory, O. (2010). Touch attenuates infants' physiological reactivity to stress. *Developmental science*, *13*(2), 271-278.

Feldman, R., Weller, A., Sirota, L., & Eidelman, A. I. (2002). Skin-to-skin contact (kangaroo care) promotes self-regulation in premature infants: Sleep-wake cyclicity, arousal

modulation, and sustained exploration. *Developmental Psychology*, *38*(2), 194–207. https://doi.org/10.1037/0012-1649.38.2.194

Flora, D. B. (2020). Thinking about effect sizes: From the replication crisis to a cumulative psychological science. *Canadian Psychology*.

Fox, N. A. (1991). If it's not left, it's right: Electroencephalograph asymmetry and the development of emotion. *American psychologist*, *46*(8), 863.

Goldsmith, H. H., & Rothbart, M. K. (1993). The laboratory temperament assessment battery (LAB-TAB). *University of Wisconsin*.

Granger, D. A., Kivlighan, K. T., Blair, C., El-Sheikh, M., Mize, J., Lisonbee, J. A., ... & Schwartz, E. B. (2006). Integrating the measurement of salivary α-amylase into studies of child health, development, and social relationships. *Journal of Social and Personal relationships*, *23*(2), 267-290.

Gunnar, M. R., Talge, N. M., & Herrera, A. (2009). Stressor paradigms in developmental studies: What does and does not work to produce mean increases in salivary cortisol. *Psychoneuroendocrinology*, *34*(7), 953-967.

Harrist, A. W., & Waugh, R. M. (2002). Dyadic synchrony: Its structure and function in childrenÕs developmentq. *Developmental Review*, 38.

Hastings, P. D., & Kahle, S. (2019). Get bent into shape: The non-linear, multi-system, contextually-embedded psychophysiology of emotional development. In *Handbook of Emotional Development* (pp. 27–55). Springer.

Hastings, P. D., & Miller, J. G. (2014). Autonomic regulation, polyvagal theory, and children's prosocial development. *Prosocial Development: A Multidimensional Approach*, 112–127.

Hendrix, C. L., Stowe, Z. N., Newport, D. J., & Brennan, P. A. (2018). Physiological attunement in mother–infant dyads at clinical high risk: The influence of maternal depression and positive parenting. *Development and Psychopathology*, *30*(2), 623–634. https://doi.org/10.1017/S0954579417001158

Hibel, L. C., Granger, D. A., Blair, C., Cox, M. J., & The Family Life Project Key Investigators. (2009). Intimate partner violence moderates the association between mother–infant adrenocortical activity across an emotional challenge. *Journal of Family Psychology*, *23*(5), 615–625. https://doi.org/10.1037/a0016323

Hibel, L. C., Granger, D. A., Blair, C., Finegood, E. D., & The Family Life Project Key Investigators. (2015). Maternal-child adrenocortical attunement in early childhood: continuity and change. *Developmental Psychobiology*, *57*(1), 83–95. https://doi.org/10.1002/dev.21266

Hill-Soderlund, A. L., Mills-Koonce, W. R., Propper, C., Calkins, S. D., Granger, D. A., Moore, G. A., Gariepy, J.-L., & Cox, M. J. (2008). Parasympathetic and sympathetic responses to the strange situation in infants and mothers from avoidant and securely attached dyads. *Developmental Psychobiology*, *50*(4), 361–376. https://doi.org/10.1002/dev.20302

Hofer, M. (2010). Hidden regulators within the mother–infant interaction. *Nurturing children and families: Building on the legacy of T. Berry Brazelton*, 154-163.

Izard, C. E., Woodburn, E. M., Finlon, K. J., Krauthamer-Ewing, E. S., Grossman, S. R., & Seidenfeld, A. (2011). Emotion knowledge, emotion utilization, and emotion regulation. *Emotion Review*, *3*(1), 44–52.

Jones-Mason, K., Alkon, A., Coccia, M., & Bush, N. R. (2018). Autonomic nervous system functioning assessed during the still-face paradigm: A meta-analysis and systematic review of methods, approach and findings. *Developmental Review*, *50*, 113-139.

Kalomiris, A. E., & Kiel, E. J. (2018). Mother-toddler cortisol synchrony moderates risk of early internalizing symptoms. *Infancy*, *23*(2), 232–251. https://doi.org/10.1111/infa.12216

Kenny, D. A. (1996). Models of non-independence in dyadic research. *Journal of Social and Personal Relationships*, *13*(2), 279-294.

Khoury, J. E., Gonzalez, A., Levitan, R., Masellis, M., Basile, V., & Atkinson, L. (2016). Maternal self-reported depressive symptoms and maternal cortisol levels interact to predict infant cortisol levels. *Infant Mental Health Journal*, *37*(2), 125–139. https://doi.org/10.1002/imhj.21554

Kivlighan, K. T. (2006). Dyadic stress vulnerability in mother-infant dyads: Sympathetic nervous system and adrenocortical reactivity to infant challenge.

Krzeczkowski J.E., Van Lieshout R.J., & Schmidt L.A. (2020). Transacting brains: Testing an actor-partner model of frontal EEG activity in mother-infant dyads. *Development and Psychopathology*, Advance online publication. https://doi.org/10.1017/S0954579420001558

Laurent, H. K., Ablow, J. C., & Measelle, J. (2011). Risky shifts: How the timing and course of mothers' depressive symptoms across the perinatal period shape their own and infant's stress response profiles. *Development and Psychopathology*, *23*(2), 521–538. https://doi.org/10.1017/S0954579411000083

Laurent, H. K., Ablow, J. C., & Measelle, J. (2012). Taking stress response out of the box: Stability, discontinuity, and temperament effects on HPA and SNS across social stressors

in mother–infant dyads. *Developmental Psychology*, *48*(1), 35–45. https://doi.org/10.1037/a0025518

Laurent H.K., Sbrilli M., Dawson D., Finnegan M., & Ramdas-Neal D. (2020). Disentangling levels of mother-infant neuroendocrine attunement and longitudinal relations with maternal risk and protective factors. *Developmental Psychobiology*, *(Laurent) University of Oregon Psychology, Champaign, United States*. https://doi.org/10.1002/dev.21997

Lautarescu, A., Craig, M. C., & Glover, V. (2020). Prenatal stress: effects on fetal and child brain development. *International review of neurobiology*, *150*, 17-40.

Leerkes, E. M., & Parade, S. H. (2015). A psychobiological perspective on emotional development within the family context. *Handbook of Infant Biopsychosocial Development*, 206–231.

Leerkes, E. M., Su, J., Calkins, S. D., Supple, A. J., & O'Brien, M. (2016). Pathways by which mothers' physiological arousal and regulation while caregiving predict sensitivity to infant distress. *Journal of Family Psychology*, *30*(7), 769–779. https://doi.org/10.1037/fam0000185

Leong, V., Byrne, E., Clackson, K., Georgieva, S., Lam, S., & Wass, S. (2017). Speaker gaze increases information coupling between infant and adult brains. *Proceedings of the National Academy of Sciences*, *114*(50), 13290-13295.

Luecken, L. J., Crnic, K. A., Gonzales, N. A., Winstone, L. K., & Somers, J. A. (2019). Mother-infant dyadic dysregulation and postpartum depressive symptoms in low-income Mexican-origin women. *Biological Psychology*, *147*, 107614. https://doi.org/10.1016/j.biopsycho.2018.10.016

Lunkenheimer, E., Tiberio, S. S., Buss, K. A., Lucas-Thompson, R. G., Boker, S. M., & Timpe, Z. C. (2015). Coregulation of respiratory sinus arrhythmia between parents and preschoolers: Differences by children's externalizing problems. *Developmental Psychobiology*, *57*(8), 994-1003.

Lunkenheimer, E., Tiberio, S. S., Skoranski, A. M., Buss, K. A., & Cole, P. M. (2018). Parent-child coregulation of parasympathetic processes varies by social context and risk for psychopathology. *Psychophysiology*, *55*(2), e12985. https://doi.org/10.1111/psyp.12985

Mayo, O., & Gordon, I. (2020). In and out of synchrony—Behavioral and physiological dynamics of dyadic interpersonal coordination. *Psychophysiology*, *57*(6). https://doi.org/10.1111/psyp.13574

Mize, J., Lisonbee, J., & Granger, D. A. (2005). Stress in child care: Cortisol and alpha-amylase may reflect different components of the stress response. *Biennial Meeting of the Society for Research in Child Development, Atlanta, GA*.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & Group, T. P. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLOS Medicine*, *6*(7), e1000097. https://doi.org/10.1371/journal.pmed.1000097

Montirosso, R., & McGlone, F. (2020). The body comes first. Embodied reparation and the co-creation of infant bodily-self. *Neuroscience & Biobehavioral Reviews*, *113*, 77–87. https://doi.org/10.1016/j.neubiorev.2020.03.003

Moore, E. R., Bergman, N., Anderson, G. C., & Medley, N. (2016). Early skin-to-skin contact for mothers and their healthy newborn infants. *Cochrane database of systematic Reviews*, (11).

Moore, G. A. (2009). Infants' and mothers' vagal reactivity in response to anger. *Journal of Child Psychology and Psychiatry*, *50*(11), 1392-1400.

Moore, G. A., Hill-Soderlund, A. L., Propper, C. B., Calkins, S. D., Mills-Koonce, W. Roger., & Cox, M. J. (2009). Mother-infant vagal regulation in the face-to-face still-face paradigm is moderated by maternal sensitivity. *Child Development*, *80*(1), 209–223. https://doi.org/10.1111/j.1467-8624.2008.01255.x

Mörelius, E., Örtenstrand, A., Theodorsson, E., & Frostell, A. (2015). A randomised trial of continuous skin-to-skin contact after preterm birth and the effects on salivary cortisol, parental stress, depression, and breastfeeding. *Early human development*, *91*(1), 63-70.

National Heart, Lung, and Blood Institute. (2014). Quality assessment tool for observational cohort and cross-sectional studies. National Institute of Health. https://www.nhlbi.nih.gov/health-pro/guide lines/in-develop/cardiac-risk-reduction/tools/cohort.

Nofech-Mozes, J. A., Jamieson, B., Gonzalez, A., & Atkinson, L. (2020). Mother–infant cortisol attunement: Associations with mother–infant attachment disorganization. *Development and Psychopathology*, *32*(1), 43–55. https://doi.org/10.1017/S0954579418001396

Oberlander, T. F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., & Devlin, A. M. (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics*, *3*(2), 97–106. https://doi.org/10.4161/epi.3.2.6034

Ostlund, B. D., Measelle, J. R., Laurent, H. K., Conradt, E., & Ablow, J. C. (2017). Shaping emotion regulation: Attunement, symptomatology, and stress recovery within mother-

infant dyads. *Developmental Psychobiology*, *59*(1), 15–25. https://doi.org/10.1002/dev.21448

Pillai Riddell, R., Flora, D. B., Stevens, S. A., Stevens, B., Cohen, L. L., Greenberg, S., & Garfield, H. (2013). Variability in infant acute pain responding meaningfully obscured by averaging pain responses. *PAIN®*, *154*(5), 714-721.

Porges, S. W. (1995). Orienting in a defensive world: Mammalian modifications of our evolutionary heritage. A polyvagal theory. *Psychophysiology*, *32*(4), 301-318.

Porges, S. W. (2007). The polyvagal perspective. *Biological Psychology*, *74*(2), 116–143. https://doi.org/10.1016/j.biopsycho.2006.06.009

Porges, S. W. (2011). *The polyvagal theory: neurophysiological foundations of emotions, attachment, communication, and self-regulation.* WW Norton & Company.

Provenzi, L., Giusti, L., Fumagalli, M., Frigerio, S., Morandi, F., Borgatti, R., ... & Montirosso, R. (2019). The dual nature of hypothalamic-pituitary-adrenal axis regulation in dyads of very preterm infants and their mothers. *Psychoneuroendocrinology*, *100*, 172-179.

Provenzi, L., Giusti, L., & Montirosso, R. (2016). Do infants exhibit significant cortisol reactivity to the face-to-face still-face paradigm? A narrative review and meta-analysis. *Developmental Review*, *42*, 34–55. https://doi.org/10.1016/j.dr.2016.07.001

Provenzi, L., Scotto di Minico, G., Giusti, L., Guida, E., & Müller, M. (2018). Disentangling the dyadic dance: theoretical, methodological and outcomes systematic review of mother-infant dyadic processes. *Frontiers in Psychology*, *9*, 348. https://doi.org/10.3389/fpsyg.2018.00348

Ramsay, D. S., & Lewis, M. (1994). Developmental change in infant cortisol and behavioral response to inoculation. *Child Development*, *65*(5), 1491–1502. https://doi.org/10.1111/j.1467-8624.1994.tb00831.x

Rothbart, M. K., & Bates, J. E. (2006). Temperament. In N. Eisenberg, W. Damon, & R. M. Lerner (Eds.), *Handbook of child psychology: Social, emotional, and personality development* (pp. 99–166). John Wiley & Sons, Inc.

Skoranski, A. M., Lunkenheimer, E., & Lucas-Thompson, R. G. (2017). The effects of maternal respiratory sinus arrhythmia and behavioral engagement on mother-child physiological coregulation. *Developmental Psychobiology*, *59*(7), 888–898. https://doi.org/10.1002/dev.21543

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in clinical neuroscience*, *8*(4), 383.

Spanglar, G., Schieche, M., Ilg, U., Maier, U., & Ackermann, C. (1994). Maternal sensitivity as an external organizer for biobehavioral regulation in infancy. *Developmental Psychobiology*, *27*(7), 425–437. https://doi.org/10.1002/dev.420270702

Spratt, E. G., Marsh, C., Wahlquist, A. E., Papa, C. E., Nietert, P. J., Brady, K. T., Herbert, T. L., & Wagner, C. (2016). Biologic effects of stress and bonding in mother–infant pairs. *The International Journal of Psychiatry in Medicine*, *51*(3), 246–257. https://doi.org/10.1177/0091217416652382

Stevenson-Hinde, J., & Marshall, P. J. (1999). Behavioral inhibition, heart period, and respiratory sinus arrhythmia: An attachment perspective. *Child Development*, *70*(4), 805–816. https://doi.org/10.1111/1467-8624.00058

Thompson, L. A., & Trevathan, W. R. (2009). Cortisol reactivity, maternal sensitivity, and infant preference for mother's familiar face and rhyme in 6-Month-Old infants. *Journal of Reproductive and Infant Psychology*, *27*(2), 143–167. https://doi.org/10.1080/02646830801918463

Thompson, R. A., Lewis, M. D., & Calkins, S. D. (2008). Reassessing emotion regulation. *Child Development Perspectives*, *2*(3), 124–131. https://doi.org/10.1111/j.1750-8606.2008.00054.x

Tronick, E. Z. (1989). Emotions and emotional communication in infants. *American Psychologist*, *44*(2), 112–119. https://doi.org/10.1037/0003-066X.44.2.112

Tronick, E. (2017). The caregiver–infant dyad as a buffer or transducer of resource enhancing or depleting factors that shape psychobiological development. *Australian and New Zealand Journal of Family Therapy*, *38*(4), 561-572.

Tronick, E, Als, H., Adamson, L., Wise, S., & Brazelton, T. B. (1978). The infant's response to entrapment between contradictory messages in face-to-face interaction. *Journal of the American Academy of Child Psychiatry*, *17*(1), 1–13. https://doi.org/10.1016/S0002-7138(09)62273-1

Tronick, E, & Beeghly, M. (2011). Infants' meaning-making and the development of mental health problems. *The American Psychologist*, *66*(2), 107–119. https://doi.org/10.1037/a0021631

Tronick, E, & Gold, C. M. (2020). *The power of discord: Why the ups and downs of relationships are the secret to building intimacy, resilience, and trust*. Scribe Publications Pty Limited.

Tronick, E., Mueller, I., DiCorcia, J., Hunter, R., & Snidman, N. (2021). A caretaker acute stress paradigm: Effects on behavior and physiology of caretaker and infant. *Developmental psychobiology*, *63*(2), 237-246.

van Bakel, H. J. A., & Riksen-Walraven, J. M. (2008). Adrenocortical and behavioral attunement in parents with 1-year-old infants. *Developmental Psychobiology*, *50*(2), 196–201. https://doi.org/10.1002/dev.20281

Wass, S. V., Noreika, V., Georgieva, S., Clackson, K., Brightman, L., Nutbrown, R., ... & Leong, V. (2018). Parental neural responsivity to infants' visual attention: how mature brains influence immature brains during social interaction. *PLoS biology*, *16*(12), e2006328.

Waters, S. F., West, T. V., Karnilowicz, H. R., & Mendes, W. B. (2017). Affect contagion between mothers and infants: Examining valence and touch. *Journal of Experimental Psychology: General*, *146*(7), 1043.

Waxman, J. A., DiLorenzo, M. G., & Pillai Riddell, R. R. (2020). Convergence of behavioral and cardiac indicators of distress in toddlerhood: A systematic review and narrative synthesis. *International Journal of Behavioral Development*, 016502542092261. https://doi.org/10.1177/0165025420922618

Waxman, J. A., DiLorenzo, M. G., Riddell, R. R. P., Flora, D. B., Greenberg, S., & Garfield, H. (2017). Preschool needle pain responding: Establishing 'normal'. *The Journal of Pain*, *18*(6), 739-745.

Waxman, J. A., Pillai Riddell, R. R., Tablon, P., Schmidt, L. A., & Pinhasov, A. (2016). *Development of Cardiovascular Indices of Acute Pain Responding in Infants: A Systematic Review* [Review Article]. Pain Research and Management; Hindawi. https://doi.org/10.1155/2016/8458696

Figure 1

Study inclusion flow chart based on PRISMA guidelines

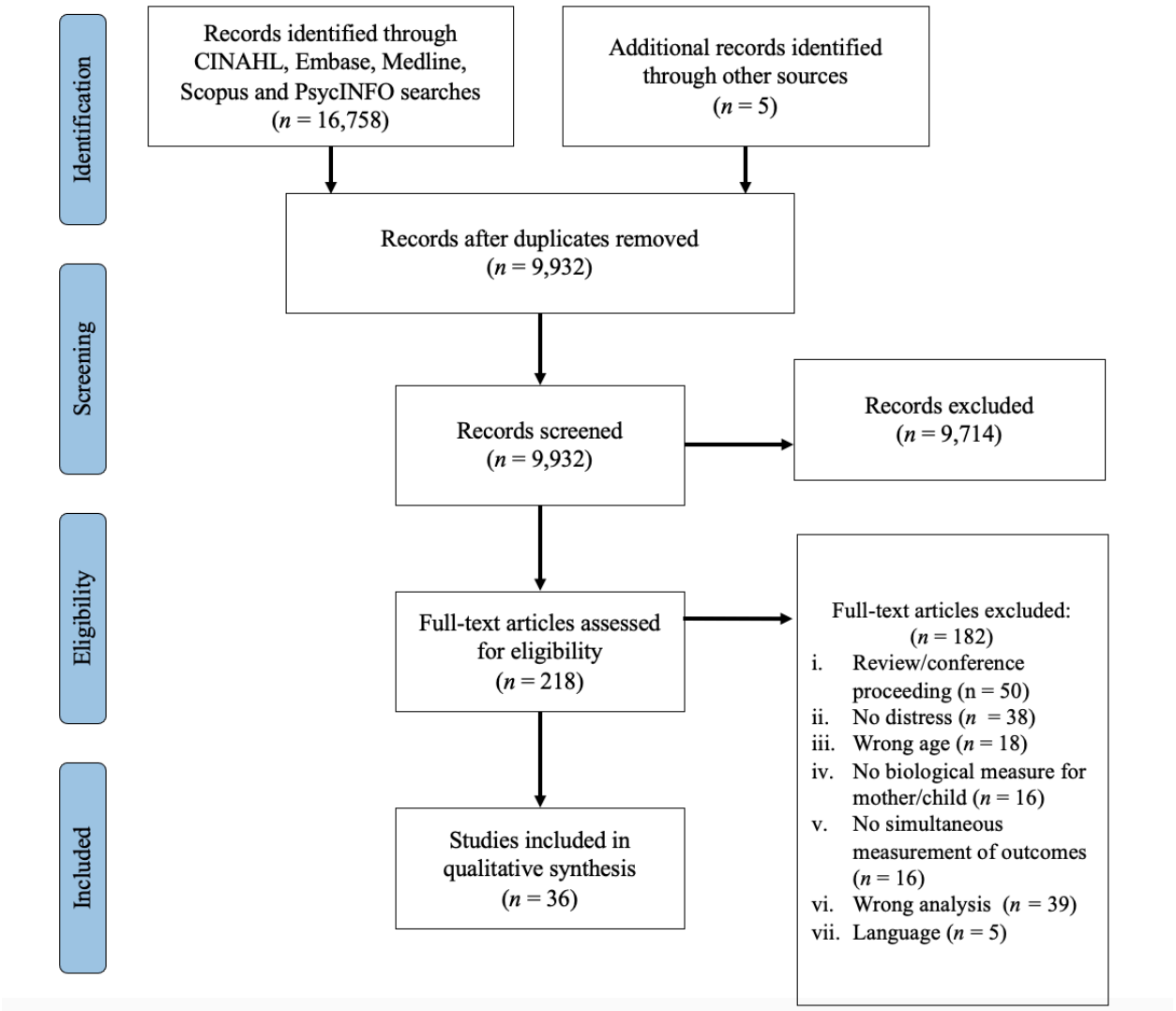

Figure 2

Distribution of studies meeting each quality assessment item

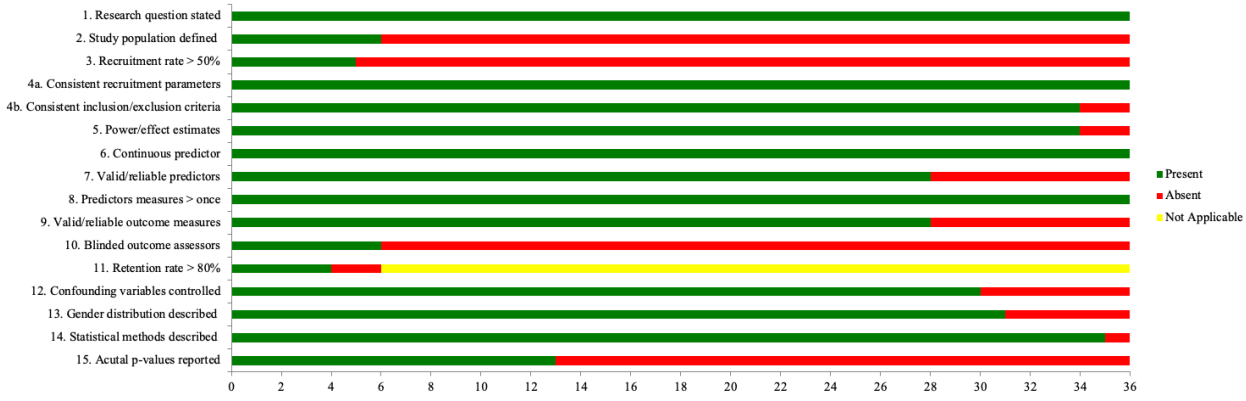

Table 1

Study Characteristics

Research Group/ Sample	Study	Biological Measure[a]	Country	Type of Study[b]	Sample Type	Child Age[c]	Distress Paradigm[d]	Epochs[e]	Overall Quality (%)
Atkinson 1	Atkinson 2013	Cortisol	Canada	B	Normative	Infant	*16 months:* Toy Frustration Procedure *17 months:* Strange Situation Procedure	Pre-task (B) +20 (IR) +40 (RR)	81
Atkinson 2	Khoury 2016	Cortisol	Canada	CS	Normative	Infant	Strange Situation Procedure	Pre-task (B) +20 (IR) +40 (RR)	75
Atkinson 3	Nofech-Mozes 2020	Cortisol	Canada	CS	Normative	Infant	Strange Situation Procedure	Pre-task (B) +20 (IR) +40 (RR)	80
Family Life Project 1	Braren 2019	Cortisol	USA	B	Clinical/Risk	Infant, Early Toddler, Mid-Late Toddler	Lab-TAB paradigm: *Infant*: masks, barrier, arm restraint *Toddler*: masks, toy removal	Pre-task (B)	75
Family Life Project 2	Granger 2006	Cortisol, sAA	USA	CS	Clinical/Risk	Infant	Lab-TAB paradigm: reach, masks, barrier and arm restraint	*Cortisol & sAA:* Pre-task (B) +20 (IR) +40 (RR)	67
Family Life Project 3	Hibel 2009	Cortisol	USA	CS	Clinical/Risk	Infant	Lab-TAB paradigm: masks, barrier, arm restraint	Pre-task (B) +20 (IR) +40 (RR)	80
Family Life Project 4	Hibel 2015	Cortisol	USA	B	Clinical/Risk	Infant, Early Toddler, Mid-Late Toddler	Lab-TAB paradigm: *Infant*: masks, barrier, arm restraint	Pre-task (B) +20 (IR) +40 (RR)	75

							Toddler: masks, toy removal		
Family Life Project 5	Kivlighan 2006	Cortisol, sAA	USA	CS	Clinical/Risk	Infant	Lab-TAB paradigm: mask, barrier and arm restraint	*Cortisol & sAA:* Pre-task (B) +20 (IR) +40 (RR)	87
Laurent 1	Laurent 2011	Cortisol	USA	CS	Clinical/Risk	Early Toddler	Strange Situation Procedure	Pre-task (B) +5 (IR) +20 (IR) +50 (RR)	80
Laurent 2	Laurent 2012	Cortisol, sAA	USA	CS	Clinical/Risk	Early Toddler	1. Clean-up task 2. Lab-TAB paradigm (fear, frustration, and joy tasks)	*Cortisol:* Pre-task (B) +5 (IR) +20 (IR) +40 (RR) *sAA:* Pre-task (B) +5 (IR) +20 (RR)	80
Lunkenheimer 1	Lunkenheimer 2015	RSA	USA	CS	Normative	Mid-Late Toddler	1. Free play 2. Clean up Task 3. Parent-Child Challenge Task	30s epochs averaged within Challenge Task (IR)	60
Lunkenheimer 2	Lunkenheimer 2018	RSA	USA	CS	Normative	Mid-Late Toddler	1. Free play 2. Clean up Task 3. Parent-Child Challenge Task	30s epochs averaged within Challenge Task (IR)	60
Lunkenheimer 3	Skoranski 2017	RSA	USA	CS	Normative	Mid-Late Toddler	1. Free play 2. Clean up Task 3. Parent-Child Challenge Task	Thirty-six 30s epochs analyzed across tasks (B & IR)	60
Moore 1	Moore 2009	RSA	USA	CS	Normative	Infant	Still-Face Paradigm	15/30s epochs averaged within episode: Pre-task (B) Normal Play	53

								Still-Face (IR) Reunion (RR)	
Moore 2	Moore, Hill-Soderlund 2009	RSA, HP	USA	CS	Normative	Infant	Still-Face Paradigm	15s epochs averaged within episode: Pre-task (B) Normal Play Still-Face (IR) Reunion (RR	60
Unique	Atzaba-Poria 2017	EEG	USA	CS	Normative	Mid-Late Toddler	Puzzle Interaction Task	Pre-task (B) Puzzle Task (IR)	73
Unique	Bernard 2017	Cortisol	USA	CS	Clinical/Risk	Early Toddler	Modified Lab-TAB arm restraint task	Pre-task (B) +20 (IR) +40 (RR)	73
Unique	Busuito 2019	RSA, HP	USA	CS	Normative	Infant	Still-Face Paradigm	30s epochs averaged within episode: Pre-task (B) Normal Play Still-Face (IR) Reunion (RR)	73
Unique	Castral 2015	Cortisol	Brazil	CS	Clinical/Risk	Infant	Heel lance	Pre-task (B) +20 (IR)	87
Unique	Crockett 2013	Cortisol	Scotland	CS	Clinical/Risk	Infant	Still-Face Paradigm	Pre-task (B) +18 (IR) +38 (RR)	80
Unique	Davis 2009	Cortisol, sAA	USA	B	Normative	Infant, Early Toddler, Mid-Late Toddler	Vaccine	Pre-task (B)	75
Unique	Feldman 2009	Cortisol	Israel	CS	Clinical/Risk & Normative	Infant	Lab-TAB paradigm: masks	Pre-task (B) +20 (IR) +35 (RR)	80

Unique	Feldman 2010	Cortisol, RSA	Israel	CS	Normative	Infant	Still-Face Paradigm	*Cortisol:* Pre-task (B) +20 (IR) +35 (RR) *RSA:* 15s epochs averaged within episode: Free play (B) Still-Face (IR) Reunion (RR)	67
Unique	Hendrix 2018	Cortisol	USA	CS	Clinical/Risk	Infant	1. Infant-Mother Separation 2. Arm Restraint 3. Noise Burst	Pre-task (B) *After Separation:* +20 *After Arm Restrain & Noise Burst:* +15-20 (IR) +30-40 (RR)	73
Unique	Hill-Soderlund 2008	RSA, sAA	USA	CS	Normative	Early Toddler	Strange Situation Procedure	*sAA:* Pre-task (B) +15 (IR) +45 (RR) *RSA:* 15/30s epochs averaged within episode: Mother-Infant Interaction (B) Separations (IR) Reunions (RR)	73
Unique	Kalomiris 2018	Cortisol	USA	CS	Normative	Mid-Late Toddler	Risk Room and Spider Procedure	Pre-task (B) +20 (IR)	67
Unique	Krzeczkowski 2020	EEG	Canada	CS	Normative	Infant	Emotion-Eliciting Music Fear Condition	Pre-task (B) Fear condition (IR)	67

Unique	Laurent 2020	Cortisol	USA	B	Clinical/Risk	Infant, Early Toddler	*Infant*: Still-Face Procedure *Early Toddler*: Strange Situation Procedure (12 months), Lab-TAB maternal separation and stranger approach	Pre-tasks (B) +0 (IR) +20 (IR) +30 (RR)	69
Unique	Luecken 2019	Cortisol	USA	CS	Clinical/Risk	Infant	1. Free play 2. Lab-TAB Arm Restraint 3. Teaching task (skill 1-2 months beyond capacity) 4. Peek-a-boo	Pre-Task (B) *After peek-a-boo:* +0 (IR) +20 (IR) +40 (RR)	73
Unique	Morelius 2015	Cortisol	Sweden	CS	Clinical/Risk & Normative	Infant	Still-Face Procedure	Pre-task (B), +30 (IR)	87
Unique	Ostlund 2017	RSA	USA	CS	Clinical/ Risk	Infant	Still-Face Procedure	5s epochs averaged within Reunion Episode (RR)	80
Unique	Provenzi 2019	Cortisol	Italy	CS	Clinical/Risk & Normative	Infant	Double Still-Face Paradigm	Pre-task (B) +10 +20 (IR) +30 (RR)	73
Unique	Spratt 2016	Cortisol	USA	CS	Normative	Infant	Heel Stick	Pre-task (B) +20 (IR)	67
Unique	Tronick 2021	Cortisol	USA	CS	Normative	Infant	1. Caregiver Acute Stress Paradigm 2. Still-Face Procedure	Pre-task (B) *After SFP:* +10 +20 (IR) +40 (RR)	73

Unique	van Bakel 2008	Cortisol	Netherlands	CS	Normative	Early Toddler	Stranger-Robot Procedure	Pre-task (B) +21 (IR)	80
Unique	Waters 2017	RSA	USA	CS	Normative	Early Toddler	1. Trier Social Stress Test 2. Dyad reunion 3. Child Interaction with Adult Stranger 4. Free play	30s epochs averaged within episode: Dyad Reunion (RR) Stranger Interaction with child (IR) Free play	60

Note. [a]Biological Measure: sAA = Salivary Alpha Amylase, RSA = Respiratory Sinus Arrythmia, EEG = Electroencephalogram.

[b]Type of Study: Cross-sectional (CS), Both (B) = Longitudinal and Cross-Sectional.

[c]Child Age: Infant = 0-11 months, Early Toddler = 12-23 months, Mid-Late Toddler = 23-47 months.

[d]Distress Paradigms: Caregiver Acute Stress Paradigm (Tronick et al., 2021), Double Still Face Paradigm (DiCorcia et al., 2015), Lab-TAB (LT; Goldsmith & Rothbart, 1988; Goldsmith & Rothbart, 1993), Modified Lab-TAB Arm Restraint task (Goldsmith & Rothbart, 1996), Parent-Child Challenge Task (PCCT; Lunkenheimer et al., 2017), Puzzle Interaction Task (Atzaba-Poria et al., 2017), Risk Room and Spider procedure (Buss and Goldsmith, 2000), Still Face Paradigm (SFP; Tronick et al., 1978), Stranger-Robot Procedure (SRP; adapted from Mullen, Snidman, and Kagnan, 1993), Strange Situation Procedure (SSP; Ainsworth et al., 1978), Toy Frustration Procedure (TFP; Braungart-Rieker & Stifter, 1996); Trier Social Stress Test (TSST; Kirschbaum & Hellhammer, 1994).

[e]Epochs**:** B = Baseline, IR = Immediate response, RR = Recovery response.

Table 2a

Studies examining static concurrent and non-reciprocal relationships between mother and child cortisol outcomes

Study	Age	N (Dyads)	Analysis	Baseline	Immediate Response	Recovery
Bernard 2017	Infant	Mothers: 174 Infants: 163	Correlations	*r* = .29	*r* = .22	*r* = .40
Braren 2019	Infant	1133	Correlations	*r* = .29		
	Early Toddler	1070	Correlations	*r* = .26		
	Mid-Late Toddler	1014	Correlations	*r* = .30		
Castral 2015	Infant	42	Correlations		*r* = .25	
Crockett 2013	Infant	62	Correlations	*r* = .16	*r* = -.09	*r* = -.07
Davis 2009	Infant	19	Correlations	∅		
	Early Toddler	22		∅		
	Mid-Late Toddler	22		∅		
Feldman 2009	Infant	100	Correlations	*r* = .71	*r* = .42	∅
Feldman 2010	Infant	53	Correlations	*r* = .27	∅	∅
Granger 2006	Infant	86	Correlations	*r* = .31	∅	∅
Hendrix 2018	Early Toddler	233	Correlations	*r* = .51	*r* = .35	*r* = .33

Hibel 2015	Infant, Early & Mid-Late Toddler	Infant: 1141 Early Toddler: 1058 Mid-Late Toddler: 1036	MLM Concurrent	Mother → Child: *b** = .47	Mother → Child: *b** = .23	Mother → Child: *b** = .05
Hibel 2009	Infant	702	Correlations	*r* = .27	*r* = .26	*r* = .25
Kivlighan 2006	Infant	284	Correlations (partial)	*r* = .23	∅	∅
Morelius 2015	Infant	19 (standard care group)	Correlations	*r* = .14	*r* = .49	
Nofech-Mozes 2020	Early Toddler	256	Correlations	*r* = .34	*r* = .39	*r* = .38
Provenzi 2019	Infant	49 (full-term group)	Correlations	*r* = .31	*r* = .42	*r* = .31
Spratt 2016	Infant	42	Correlations	*r* = .68	∅	
Tronick 2021	Infant	52	Correlations	∅	*r* = .27	*r* = .54
Collapsed across epochs:						
Kalomiris 2018	Mid-Late Toddler	70	Regressions (AUCG)	Mother → Child: *b** = .49		
Khoury 2016	Early Toddler	256	Correlations (AUCG)	*r* = .58		
Luecken 2019	Infant	204	Correlations (AUCG)	*r* = .32		
Laurent 2011	Early Toddler	86	MLM Concurrent	Mother → Child: ∅		
Laurent 2012	Early Toddler	86	MLM Concurrent	Mother → Child: *b** = .09		

Laurent 2020	Infant & Early Toddler	63	MLM Concurrent	Mother → Child *b** = -.05 Child → Mother *b** = .01

Note. Infant = 0-11 months of age, Early Toddler = 12-23 months of age, Mid-Late Toddler = 24-47 months of age. MLM = Multilevel Model. N reflects number of participants with full data used in analyses. → = direction of prediction, *r* = correlation coefficient, *b** = standardized slope, ∅ = coefficients not provided due to non-significant results. Where no data is available, grey shading is used.

Table 2b

Studies examining dynamic concurrent and non-reciprocal relationships between mother and child cortisol outcomes

Study	Age	N (Dyads)	Analysis	Baseline	Immediate Response	Recovery
Bernard 2017	Infant	Mothers: 174 Infants: 163	Difference Score Correlations		*r* = -.08	*r* = .06
Kalomiris 2018	Mid-Late Toddler	70	Regressions (AUC_I)		Mother → Child: *b** = .12	
Spratt 2016	Infant	42	Difference Score Correlations		r = -.14	
van Bakel 2008	Early Toddler	83	Difference Score Regressions		Child → Parent: *High parent sensitivity b** = .29 *Low parent sensitivity b** = -.24	
Collapsed across epochs:						
Atkinson 2013	Early Toddler	297	MLM Correlated Growth Model	*r (slopes)* = .60		
Hibel 2009	Infant	702	MLM Correlated Growth Model	*r (slopes)* = .01		
Khoury 2016	Early Toddler	256	Correlations (AUC_I)		*r* = .25	
Luecken 2019	Infant	204	Correlations (AUC_I)		*r* = .21	
Nofech-Mozes 2020	Early Toddler	256	MLM Correlated Growth Model	*r (slopes)* = .39		

Note. Infant = 0-11 months of age, Early Toddler = 12-23 months of age, Mid-Late Toddler = 24-47 months of age. MLM = Multilevel Model. N reflects number of participants with full data used in analyses. → = direction of prediction, *r* = correlation coefficient, *b** = standardized slope, ∅ = coefficients not provided due to non-significant results. Where no data is available, grey shading is used.

Table 2c

Studies examining dynamic concurrent and reciprocal relationships between mother and child cortisol outcomes

Study	Age	N (Dyads)	Analysis	Baseline	Immediate Response	Recovery
Hendrix 2018	Infant	233	Cross-lagged Model	*Stability over time:* Mother B→ Mother IR1: *b**= .83 Infant B → Infant IR1: *b**= .72 *Concurrent relationships*: Not reported *Reciprocal influence:* Mother B → Infant IR1: *b**= .02 Infant B→ Mother IR1: *b**= .04	*Stability over time:* Mother IR1→ Mother IR2: *b**= .83 Infant IR1 → Infant IR2: *b**= .55 *Concurrent relationships*: Not reported *Reciprocal influence:* Mother IR1 → Infant IR2: *b**= .16 Infant IR1→ Mother IR2: *b**= .12	*Stability over time:* Mother IR2→ Mother RR: *b**= .81 Infant IR2 → Infant RR: *b**= .61 *Concurrent relationships*: Not reported *Reciprocal influence:* Mother IR2 → Infant RR: *b**= .12 Infant IR2→ Mother RR: *b**= .04
					Collapsed across epochs:	
Bernard 2017	Infant	Mothers: 174 Infants: 163	Cross-lagged Model	*Stability over time:* Mother → Mother: *b** = .74 Infant → Infant: *b** = .59 *Concurrent relationships*: Not reported *Reciprocal influence:* Mother → Infant: *b** = .23 Infant → Mother: *b** = .04		

Nofech-Mozes 2020	Early Toddler	256	Cross-lagged Model	*Stability over time:* Mother → Mother: $b^* = .75$ Infant → Infant: $b^* = .60$ *Concurrent relationships*: Not reported *Reciprocal influence:* Mother → Infant: $b^* = .11$ Infant → Mother: $b^* = .09$

Note. Infant = 0-11 months of age, Early Toddler = 12-23 months of age. MLM = Multilevel Model. N reflects number of participants with full data used in analyses. → = direction of prediction, r = correlation coefficient, b^* = standardized slope, ∅ = coefficients not provided due to non-significant results. Where no data is available, grey shading is used.

Table 3

Studies examining static concurrent and non-reciprocal relations between mother and child salivary alpha amylase (sAA) outcomes

Study	Age	N (Dyads)	Analysis	Baseline	Immediate Response	Recovery Response
Davis 2009	Infant	19	Correlations (partial)	*r* = .65		
	Early Toddler	22	Correlations (partial)	*r* = .58		
	Mid-Late Toddler	22	Correlations (partial)	*r* = .47		
Granger 2006	Infant	86	Correlations	*r* = .29	∅	∅
Kivlighan 2006	Infant	284	Correlations (partial)	*r* = .12	∅	∅
Hill-Soderlund 2008	Early Toddler	Mothers: 98 Infants: 96	Regressions	Mother → Child: ∅	Mother → Child: ∅	Mother → Child: ∅
Collapsed across epochs:						
Laurent 2012	Early Toddler	86	MLM Concurrent	Mother → Child *b** = .23		

Note. Infant = 0-11 months of age, Early Toddler = 12-23 months of age, Mid-Late Toddler = 24-47 months of age. MLM = Multilevel Model. N reflects number of participants with full data used in analyses. → = direction of prediction. *r* = correlation coefficient. *b** = standardized slope. ∅ = coefficients not provided due to non-significant results. Where no data is available, grey shading is used.

Table 4

Studies examining static concurrent and non-reciprocal relations between mother and child cardiac (RSA and heart period) outcomes

Study	Age	N (Dyads)	Analysis	Baseline	Immediate Response (IR)	Recovery (RR)
Busuito 2019	Infant	140	Correlations	RSA: *r* = .12 HP: *r* = .13	RSA: *r* = .02 HP: *r* = .01	RSA: *r* = .10 HP: *r* = .09
Feldman 2010	Infant	53	Correlations	∅	∅	∅
Hill-Soderlund 2008	Early Toddler	Mothers: 95 Infants: 87	Regressions	Mother → Child: ∅	Mother → Child: ∅	Mother → Child: ∅
Lunkenheimer 2015	Mid-Late Toddler	47	Correlations		*r* = .17	
Lunkenheimer 2018	Mid-Late Toddler	47	Correlations		*Same results as Lunkenheimer 2015*	
Moore 2009	Infant	47	Correlations	∅	∅	∅
Moore, Hill-Soderlund 2009	Infant	Baseline: 66 IR: 86 RR: 83	Correlations	RSA: ∅ HP: *r* = .32	RSA: ∅ HP: ∅	RSA: ∅ HP: ∅
Ostlund 2017	Infant	95	Correlations			*r* = .07
Skoranski 2017	Mid-Late Toddler	47	MLM Concurrent		Mother → Child: **b* = .19 Child → Mother: **b* = .11	
Waters 2017	Early Toddler	98	Regressions		Child → Mother: ∅	Child → Mother: ∅

Note. Infant = 0-11 months of age, Early Toddler = 12-23 months of age, Mid-Late Toddler = 24-47 months of age. MLM = Multilevel Model. N reflects number of participants with full data used in analyses. *r* = correlation coefficient. **b* = standardized slope. ∅ = coefficients not provided due to non-significant results. Where no data is available, grey shading is used.

Table 5

Studies examining relations between mother and child EEG (frontal asymmetry) outcomes

Study	Age	N (Dyads)	Analysis	Baseline	Immediate Response (IR)	Recovery (RR)
Static Concurrent and Non-reciprocal:						
Atzaba-Poria 2017	Mid-Late Toddler	34	Correlations	*r* = -.09	*r* = .25	
Krzeczkowski 2020	Infant	29	Correlations	*r* = .002	*r* = -.006	
Dynamic Concurrent and Reciprocal:						
Krzeczkowski 2020	Infant	29	Cross-lagged Model	*Stability over time:* Mother → Mother: *b** = .35 Infant → Infant: *b** = .41 *Concurrent relationships*: Not reported *Reciprocal influence:* Mother → Infant: *b** = .03 Infant → Mother: *b** = -.01		

Note. Infant = 0-11 months of age, Early Toddler = 12-23 months of age, Mid-Late Toddler = 24-47 months of age. MLM = Multilevel Model. N reflects number of participants with full data used in analyses. *r* = correlation coefficient. *b** = standardized slope. ∅ = coefficients not provided due to non-significant results. Where no data is available, grey shading is used.

Table 6

Summary of study covariates and moderators

Research Group/ Sample	Study	Biological Measure	Covariates	Moderator Effect (Y/N)	Summary of Moderator Effect
Atkinson 1	Atkinson 2013	Cortisol	Child wake time, mother and child average cortisol levels, change in cortisol across challenges, samples across epochs, maternal sensitivity	Maternal sensitivity (Y)	↑ Maternal sensitivity → ↑ Mother-child concordance
Atkinson 2	Khoury 2016	Cortisol	Maternal sensitivity, child breakfast time, ethnicity	Maternal depressive symptoms (Y)	↑ Maternal depressive symptoms → ↑ Mother-child concordance
Atkinson 3	Nofech-Mozes 2020	Cortisol	Family income, maternal education, maternal relationship status, maternal age, maternal and Child feeding times, wake times, medication status, sleep disruptions the previous night, maternal smoking, menstrual stage, breastfeeding status, and insomnia status	Attachment disorganization (Y)	Attachment disorganization → ↓ Mother-child concordance *Moderation emerged when using growth curve modeling but not cross-lagged modeling
Family Life Project 1	Braren 2019	Cortisol	Child race, child gender, child age, mother age, pregnancy status, medication use, tobacco use, parity status, breastfeeding duration and status, child and mother body mass index,	Socioeconomic risk (N) Age (N)	

			child and mother body temperature, parenting, time of day at visit, time between each saliva collection, SES risk, maternal depression, intimate partner violence, child emotional reactivity		
Family Life Project 2	Granger 2006	Cortisol			
		sAA			
Family Life Project 3	Hibel 2009	Cortisol	Time of day at visit	Maternal experience of intimate partner violence (Y) Restrictive and punitive parenting behaviours (Y)	Maternal experience of intimate partner violence → ↑ Mother-Child Concordance Restrictive and punitive parenting behaviours → ↑ Mother-Child Concordance
Family Life Project 4	Hibel 2015	Cortisol	Time of day, race, income-to-needs ratio, child emotional reactivity, positive parenting	Child emotional reactivity (Y) Positive maternal behaviours (Y) Negative maternal behaviours (N)	↑ Child emotional reactivity → ↓ Mother-child concordance ↓ Positive maternal behaviour → ↓ Mother-child concordance
Family Life Project 5	Kivlighan 2006	Cortisol	Maternal: Time of day at visit, hours since eating, hormonal contraceptives, age, race, marital status *Child:* Time of day at visit, hours since eating, total hours of sleep, age, race, weight		
		sAA	Maternal: Hours since eating, time of day at visit, hours since sleeping, body temperature, cigarettes per day,		

			hormonal contraceptives, NSAIDs, age, years of education, marital status *Child:* Hours since eating, cough-cold over the counter medications, race		
Laurent 1	Laurent 2011	Cortisol	Medications, recent eating/drinking or brushing teeth, dental work, illness, sleep time/duration, body mass index, age, arrival time to lab, infant birth outcomes and attachment status	Maternal depressive symptoms (Y)	↑ Maternal depressive symptoms from prenatal to 18 months postnatal → ↑ Mother-child concordance
Laurent 2	Laurent 2012	Cortisol	Maternal body mass index, maternal age		
		sAA	Maternal dental work		
Lunkenheimer 1	Lunkenheimer 2015	RSA	SES, maternal education, child sex, child age, stability of within person RSA, individual differences in overall mean RSA	Child externalizing (Y) *Interaction with RSA outcomes collapsed across tasks	↑ Child externalizing → ↓ Mother-child concordance
Lunkenheimer 2	Lunkenheimer 2018	RSA	SES, maternal education, child sex, child age, stability of within person RSA, individual differences in overall mean RSA	Child externalizing (N) Child internalizing (N) Maternal depression (N) Maternal aggression (N) *Interactions with RSA outcomes during challenge task	
Lunkenheimer 3	Skoranski 2017	RSA	SES, maternal education, child age, stability of within person RSA,	Maternal baseline RSA (Y) Maternal teaching (i.e., time spent teaching during task) (Y)	↑ Maternal Baseline RSA → ↓ Mother-child concordance

			individual differences in overall mean RSA	Maternal disengagement (Y)	↑ Maternal teaching → ↑ Mother-child concordance ↑ Maternal disengagement → ↓ Mother-child concordance
Moore 1	Moore 2009	RSA	Ethnicity, income, maternal age, child age, child sex		
Moore 2	Moore, Hill-Soderlund 2009	RSA HP	Ethnicity, income, Child sex, Child negative affect		
Unique	Atzaba-Poria 2017	EEG	Child age, maternal age, child negativity, maternal negativity		
Unique	Bernard 2017	Cortisol	Time of afternoon, time from child's last feeding, time from when child last slept	Maternal experience of intimate partner violence (Y)	↓ Maternal experience of intimate partner violence → ↑ Mother-Child Concordance
Unique	Busuito 2019	RSA HP	Child age, time from Child's last feeding, Child sex, whether Child was fed during lab visit, feeding method, parity, race, maternal age, maternal education		
Unique	Castral 2015	Cortisol	Child sex, gestational age, post-natal age, painful experiences prior to 24h, sleep-wake state at baseline, previous experience with kangaroo care, number of heel punctures, duration of blood collection, and maternal use of corticosteroids		

Unique	Crockett 2013	Cortisol	Maternal age, maternal education, household income, food and dairy consumption, maternal anxiety and depression, and time of day cortisol was assessed	Maternal Disrupted Communication (Y)	Severely disrupted communication → ↓ Mother-Child Concordance
Unique	Davis 2009	Cortisol sAA	Time of day at visit		
Unique	Feldman 2009	Cortisol			
Unique	Feldman 2010	Cortisol RSA			
Unique	Hendrix 2018	Cortisol	Child sex, child age, birth weight, feeding time, number of siblings, number of hours away from mother per week, maternal age, tobacco use, current menstruation, aerobic activity before lab visit, hours slept night before, medication use, stress, breastfeeding, number of pregnancy complications	Maternal depressive symptoms (N) Time spent gazing at Child (N) Maternal positive affect (Y)	↑ Maternal positive affect → ↑ Mother-child Concordance
Unique	Hill-Soderlund 2008	RSA sAA	Income, Child race, Child sex, Child age, attachment group	Avoidant vs. secure attachment (N)	
Unique	Kalomiris 2017	Cortisol	Time of day at visit, # hours toddler was awake		
Unique	Krzeczkowski 2020	EEG		Maternal social approach (Y) Maternal social avoidance (Y)	↑ Maternal social avoidance → ↑ Mother-child Concordance

					↓ Maternal social approach → ↑ Mother-child Concordance
Unique	Laurent 2020	Cortisol	Use of medications or other substances, sleep/wake times, sickness, body mass index, and infant feeding during the session	Social Support Satisfaction (Y) Family Resources (Y) Parental Stress (N)	↑ Social support satisfaction → ↓ Mother-child Concordance ↑ Family resources → ↓ Mother-child Concordance
Unique	Luecken 2019	Cortisol	Maternal age, country of birth, number of children, marital status, time of day, child birthweight, gestational age, child sex and breastfeeding status		
Unique	Morelius 2015	Cortisol	Hospital site, maternal sensitivity		
Unique	Ostlund 2017	RSA	Maternal and Child baseline RSA, maternal postpartum anxiety and depressive symptoms	Maternal anxiety symptoms (N) Maternal depressive symptoms (N)	
Unique	Provenzi 2019	Cortisol			
Unique	Spratt 2016	Cortisol	Child age, maternal age, child sex, race, feeding type, mother present for blood draw		
Unique	Tronick 2021	Cortisol			

Unique	van Bakel 2008	Cortisol	Child sex, home vs. daycare, time since morning feeding, parental time since morning awakening, maternal reported pregnancy	Parent sensitivity (Y)	↑ Parent sensitivity → ↑ Parent-child concordance
Unique	Waters 2017	RSA	Maternal body mass index	Touch manipulation (N) Episode type (N)	

Note. Results displayed as direction of association (↑ = higher, ↓ = lower, → = moderation effect).

Chapter 3: Addressing gaps in the literature by examining caregiver-toddler regulatory attunement patterns

The primary goal of Study 1 (Chapter 2) was to synthesize the literature examining caregiver-child biological attunement during the first few years of life, particularly within distress contexts. Previous research highlighted variability in findings among existing studies of attunement that made it difficult for researchers to draw conclusions about the literature (Bernard et al., 2017). A major contributor to the inconsistencies in findings is the variety of methodologies used to examine biological attunement, including using different biological indicators, types of statical analyses, epoch definitions (i.e., immediate response to distress, recovery response), child ages, and covariates and moderators. To make sense of the available literature, a synthesis was warranted with special consideration of the methodological approaches used to study biological attunement.

A narrative synthesis approach was taken due to the complexity of the literature. The results of 36 studies were summarized according to biological indicators used to measure distress (cortisol, sAA, cardiac indicators [RSA, HP] and EEG) and broad statistical analysis groupings (static concurrent and non-reciprocal [e.g., correlations, regressions], dynamic concurrent and non-reciprocal [e.g., multilevel modeling], and dynamic concurrent and reciprocal [e.g., cross-lagged modeling]). Findings varied considerably across methodological approaches used to study attunement. However, some patterns emerged from the synthesis. Most of the articles (25 of 36 studies) examined caregiver-child cortisol outcomes. Relations between caregiver and child cortisol outcomes were moderate to large, though findings differed depending on the analysis used and measurement epochs examined (i.e., baseline, immediate response to stressor, and recovery response). Many of the findings examining relations between mother and child cardiac,

sAA, and EEG indicators were weak or inconsistent. We made several suggestions for improving the consistency and quality of methods used to study caregiver-chid biological attunement. The widespread inconsistencies in the literature likely reflect differences in how researchers conceptualize attunement which informs the methods they use, especially the analyses approaches. Further, it is possible that many of the inconsistent results reflect variability in caregiver-child coregulatory dynamics that is not fully captured, as it is unlikely that all dyads display similar attunement patterns. As explained in Chapter 2, researchers are encouraged to move past simple conceptualizations of positive or negative covariation as representing dyads as attuned or not attuned, as there are likely variable patterns of attunement across dyads that have not been adequately captured in the literature.

To address gaps in the literature that were described in Chapter 2, the goal of the next chapter (Chapter 4, Dissertation Study 2) was to examine patterns of caregiver-toddler physiological regulatory attunement, as indexed by high-frequency heart rate variability, during routine vaccinations across the second year of life. This study used a subsample of 189 dyads with both caregiver and toddler cardiac data available from vaccination appointments. Chapter 4 offers novel insights into the coregulatory dynamics between caregivers and their toddlers during vaccination. Groups of dyads were discerned with differing adaptive or suboptimal attunement patterns of physiological pain-related distress.

References

Bernard, N. K., Kashy, D. A., Levendosky, A. A., Bogat, G. A., & Lonstein, J. S. (2017). Do different data analytic approaches generate discrepant findings when measuring mother–infant HPA axis attunement? *Developmental psychobiology*, *59*(2), 174-184.

Chapter 4: Distinct trajectories of caregiver-toddler physiological attunement during routine vaccinations[2]

Research has increasingly elucidated the fundamental role of the caregiver in the acute pediatric pain context, particularly early in life (Pillai Riddell et al., in press; Waxman et al., 2017). The 'development of the infant acute pain response - revised model' (DIAPR-R; Pillai Riddell et al., in press) is grounded in attachment theory (Bowlby, 1982) and conceptualizes the impact of biopsychosocial factors on the development of early pain experiences. The model asserts the primacy of the caregiver-child relationship, particularly how dyad members respond to and adjust to one another, as the toddler regulates their pain-related distress (e.g., a child signals distress to their caregiver, and the caregiver responds sensitively to child's needs). The dynamic and reciprocal responding between caregivers and their toddler is known as attunement (Atkinson et al., 2016; Feldman, 2007). Much of the research examining the influence between caregivers and their toddlers in the pain context has focused on the links between caregiver behaviours (e.g., caregiver sensitivity) and infant and toddler pain-related distress behaviours (Atkinson et al., 2015; Campbell et al., 2013, 2018; Pillai Riddell et al., 2011). To our knowledge, no research has examined young child and caregiver attunement during an acute pain experience using cardiac physiology.

Caregiver physiology, through its influence on caregiver behaviours in response to their child's distress (e.g., sensitive or insensitive behaviours) and the sensory experience of the child (e.g., the child hearing caregiver's calm heart rate during skin-to-skin contact), operates as a

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[2] This is the author's version of the submitted manuscript:
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coregulator of young children's physiological response to stress (Braren et al., 2019; Hofer, 2010). Thus, gaining an understanding of caregiver-child co-occurring physiological responses in the pain context is important, as sustained dysregulation in the caregiver or child may indicate a dyad that is struggling to adaptively respond to and regulate distress, which may in turn then influence future pain experiences for the child (Campbell et al., 2013; McMurtry et al., 2015; Pillai Riddell et al., 2013).

Extensive research examining physiological responses to distress has demonstrated that the response of the parasympathetic system, commonly indexed with high-frequency heart rate variability (HF-HRV), reflects a regulatory response to distress (Appelhans & Luecken, 2006; Berntson et al., 1997; Calkins, 2011), including pain-related distress (Oberlander et al., 1997; Waxman et al., 2020). Within the broader developmental literature, many studies have examined caregiver-child HF-HRV attunement within an experimental distress context (Di Lorenzo et al., 2021). However, there are no studies that have examined the attunement patterns of caregiver and toddler HF-HRV during an acute pain procedure in early life.

Present Study

The current study examines the coregulatory patterns of caregiver and toddler HF-HRV from one minute pre-vaccination to three minutes post-vaccination. Attunement in this context reflects whether the patterns of cardiac responding are similar in caregivers and children, as they regulate from pain-related distress. Parallel-process growth-mixture modeling (GMM) was used to describe variability in caregiver-toddler attunement patterns over the course of the vaccination. Because previous work has demonstrated variable pain responses in young children (Pillai Riddell et al., 2013; Waxman et al., 2017), we hypothesized that there would be distinct groupings of co-occurring caregiver and child HF-HRV trajectory patterns. Furthermore, we

aimed to further contextualize the attunement patterns by examining the heart rate outcomes of dyads in each group derived from the GMM. Heart rate reflects both sympathetic and parasympathetic activity, and thus provides indication of sympathetically mediated arousal in response to a stressor (Berntson et al., 2007). Finally, post-hoc analyses were used to examine predictors of the attunement trajectory patterns.

Method

Participants

Caregiver-toddler dyads were recruited from two pediatric clinics in the greater Toronto area from December 2015 to December 2019. Dyads were observed at routine vaccination appointments at the age of 12, 18, or 24 months. A total of 437 dyads were approached for recruitment at either 12 or 18 months of age. Refusal rate was 36% at 12 months and 35% at 18-months. Forty dyads were ineligible based on exclusion criteria were NICU stay during infancy, prematurity (< 37 weeks gestation), suspected or confirmed developmental delay, chronic illness, or lack of caregiver fluency in English. In total, data was collected from 234 unique dyads at either 12-months (n = 182), 18-months (n=155), and/or 24 months (n = 64). Not all data points have both caregiver and toddler cardiac data due to equipment failure (i.e., devices malfunctioned, electrodes detached from participants) or data processing challenges (i.e., too many artifacts). The current study used a subsample of 189 dyads with both caregiver and toddler cardiac data available at the 12- (n = 81), 18- (n = 66), or 24-month (n = 42) vaccination appointments (if a dyad had data from multiple appointments, data from only the most recent appointment was included), with a mean age of 17.04 months (SD = 4.80). Table 1 contains demographic and other characteristics of the dyads included. Toddlers were 55% male, generally healthy, came from middle-class families, and had well-educated caregivers. The subsample of

dyads included in this study did not differ from those excluded due to missing data in terms of caregiver age, education, relation to the child, ethnicity, caregiver self-reported mainstream and heritage culture, toddler sex, time since last nap, time since last feed, and number of needles received. Previous analyses examining toddler pain-related distress responses from the current cohort are presented elsewhere (Waxman et al., 2020, 2021).

Primary caregivers had a mean age of 36 years (SD = 5.54). They reported diverse cultural backgrounds. The majority reported being born in Canada (57.6%), with a large proportion of caregivers born outside of Canada, including Asia (18.3%), South America (5.8%), Europe (5.2%), Africa (2.1%), Russia (2.1%), United States/Mexico (2.1%), or Australia (1.6%). On average, caregiver ratings of acculturation indicated a strong identification with both their heritage cultural (i.e., a culture inherited across generations of their family) and mainstream North American culture (i.e., the culture within which they reside), suggesting the sample reflected the integrated categorization of acculturation (Berry, 2003).

Procedure and Apparatus

Ethics approval was obtained for this study through the research ethics review board at the participating university. Caregivers were made aware of the study upon entering the pediatric clinic for their child's vaccination appointment. If they agreed to speak to a researcher, caregivers were approached by a research assistant who explained the study. Informed consent was obtained from all caregivers included in the study. Caregivers were asked to fill out a short demographic questionnaire immediately before the vaccination appointment. During the appointment, caregiver-toddler dyads were videotaped and both members of the dyad were connected to mobile monitoring devices to measure their high-frequency heart rate variability (HF-HRV) and heart rate (HR) before and after the needle. Cardiac data were collected using

Mindware wireless monitors (MW 1000A) with a sampling rate of 500 Hz. Three electrocardiograph (ECG) electrodes were placed on each caregiver and toddler, with one electrode placed over the right clavicle, one electrode placed under the rib cage on the left side, and a ground electrode placed under the rib cage on the right side. Mindware software (BioLab 3.3) was used to acquire ECG data continuously and Noldus technologies were used to synchronize video and cardiac data capture. Dyads were examined for 1 minute prior to the needle and up to three minutes after the needle. All caregivers held their toddler during the vaccination. Once monitoring equipment was in place, dyads were observed with minimal interference from the research team. Caregivers who participated in the study were given an information sheet on evidenced-based pain management strategies (Taddio et al., 2010).

Measures

Demographic information

The short demographic questionnaire included questions about caregiver age, education, relation to the child, and toddler age and sex. Caregivers were also asked to provide two separate ratings adapted from the Vancouver Index of Acculturation (Ryder et al., 2000) assessing the extent to which they believe their way of life reflects their self-reported heritage culture, and the extent to which their way of life reflects mainstream 'North American or Canadian culture'. Ratings were provided on a scale of 0 to 10, with a higher score indicating greater identification with mainstream culture or with their self-reported heritage culture. Child factors that are known to affect biological indicators were also reported (Oberlander & Saul, 2007; Waxman et al., 2016), including time since last feeding and since last nap, and number of needles the toddler received during the vaccination appointment.

Cardiac indicators: high-frequency heart rate variability and heart rate

High-frequency heart rate variability, an index of the parasympathetic system, refers to the variation in time between consecutive heart beats (Berntson et al., 2007). At rest, parasympathetic activity engages a "brake" on the heart via the vagus nerve, which promotes internal homeostasis (Hastings & Miller, 2014). This adaptive resting state is indexed by higher HF-HRV. However, in the presence of a perceived threat, the vagal brake is withdrawn which allows for mobilization and quick adaptation to different social environments and contexts. Temporary withdrawal of HF-HRV, indexed by decreasing HF-HRV, allows individuals to respond to a stressor and engage in active coping (Hastings & Miller, 2014; Porges, 2007). Although decreases in HF-HRV in response to stressors is considered a normative response, research suggests that excessive or prolonged vagal withdrawal reflects maladaptive regulation and predicts negative psychosocial outcomes (Beauchaine, 2015). Our understanding of cardiovascular markers as measures of pain-related distress in toddlers is a growing area of investigation and requires ongoing work, as these measures can be particularly helpful since they are easy to acquire and provide an indication of distress in pre-verbal populations. Some research has already shown that cardiac indicators reflect unique aspects of the nociceptive response when measured alongside behavioural pain-related distress in toddlers (Waxman et al., 2020).

Cardiac data were processed using Mindware HRV 3.1.5. HR was derived from the identification of R-waves and HF-HRV was calculated from spectral analysis of the RR intervals (i.e., the intervals between successive heart beats or R waves) (Cacioppo et al., 2000) using the Mindware HRV algorithm to identify each R wave. We used frequency bands within the range of spontaneous respiration (0.24 to1.04 Hz for toddlers and 0.12 to 0.40 for adults) (Bar-Haim et al., 2000; Cacioppo et al., 2000).

All coders were trained to edit artifact by an experienced primary coder. A total of 20% of the sample was double coded for reliability. Overall, interrater reliability was high (intraclass correlations between .96 and .99). Data were edited for artifacts due to software misidentification or equipment failure (i.e., device malfunction, electrodes fell off participant). Decisions to exclude data due to artifact were made in consultation with the primary coder and decided on an epoch-by-epoch basis. Editing of artifacts was below 5% for all participants.

Cardiac values (HF-HRV and HR) were calculated for eight 30-second epochs: 60 to 31 seconds before the first needle, 30 seconds to 1 second before the first needle, 1 to 30 seconds after the last needle, 31to 60 seconds after the last needle, 61 to 90 seconds after the last needle, 91 to 120 seconds after the last needle, 121 to 150 seconds after the last needle, and 151 and 180 seconds after the last needle. Our methods are consistent with official guidelines on HRV standards of measurement that suggest approximately one minute of data is needed at minimum to identify the high-frequency components of HRV (Camm et al., 1996).

Analysis Plan

Growth-mixture modeling (GMM) was used to describe variation in patterns of cardiac outcomes over time (i.e., trajectories) using a small number of latent classes or groups (Muthén & Shedden, 1999). Specifically, our goal was to identify distinct regulatory trajectory groups to represent heterogeneity in how caregivers and their toddlers respond and recover simultaneously (i.e., parallel processes) in a stressful context. With GMM, each dyad receives a score representing the probability of membership in each discerned group. We then classified each dyad into the group for which it had the highest probability of membership. These models were estimated using the robust maximum likelihood estimator (MLR) in Mplus version 8.0 (Muthén

& Muthén, 1998) to account for non-normality in the data, which also handles incomplete data using full-information maximum likelihood.

Separate univariate latent growth curve models were first estimated for caregiver and toddler HF-HRV individually to examine the form of the HF-HRV trajectories (i.e., linear or non-linear) (Jung & Wickrama, 2008). For the sake of estimating a smoother trajectory pattern, we averaged the caregiver and toddler 30 second HF-HRV epochs for every minute pre- and post-needle (i.e., our timepoints became Baseline [60 to 1 second before the needle], Post 1 [1-60 seconds post needle], Post 2 [61-120 seconds post needle], and Post 3 [121-180 seconds post needle]). Model fit was examined using the root mean square error of approximation (RMSEA), the comparative fit index (CFI), and the Tucker-Lewis index (TLI). As approximate guidelines, RMSEA values of .06 or lower indicate good fit as well as CFI and TLI values of .95 of higher (Hu & Bentler, 1999).

Next, we estimated a single group parallel-process GMM. This model was then compared to a set of GMM models specified by increasing the number of groups. Model fit was compared using Akaike's information criterion (AIC; Akaike, 1981) and the Bayesian information criterion (BIC; Schwarz, 1978). Smaller values of AIC and BIC are associated with better model fit, while accounting for model complexity. The number of groups specified increased until AIC and BIC no longer decreased. The optimal number of groups was determined with consideration of the information criteria and interpretability of the model (Jung & Wickrama, 2008).

Once the optimal model was determined, dyad group membership (i.e., the group for which they had the highest probability of belonging) was exported to SPSS (Version 26) (IBM Corp, 2019). Mean heart rate across epochs was calculated and trajectories were plotted for Groups 1 to 3 to further contextualize findings with another biological indicator. Post-hoc

multinomial logistic regression was used to examine whether dyadic or contextual factors (i.e., toddler sex, toddler age, relation to child, baseline caregiver and toddler HF-HRV, time of last nap, time of last feed, number of needles toddler received) predicted group membership. Baseline HF-HRV, time since last feeding, and time since last nap have been previously shown to impact child physiology (Oberlander & Saul, 2007; Waxman et al., 2016). Predictors were examined in separate models to maximize the sample size for each analysis. P-values were adjusted for multiple comparisons using the false discovery rate (FDR) method (Benjamini & Hochberg, 1995).

Results

Table 2 presents the overall sample means and SDs of caregiver and toddler HF-HRV. Table 3 presents the correlations among caregiver and toddler HF-HRV variables. Small, positive associations (r = .16 - .27) were consistently found between caregiver baseline, Post 1, Post 2, and Post 3 HF-HRV with toddlers Post 2 and Post 3 HF-HRV. There were less than eight missing caregiver and toddler HRV scores for each epoch.

Concurrent changes in caregiver and toddler HF-HRV during vaccination

Unconditional growth curve models

Table S1 provides the intercept and linear slope factor means, standard error estimates and model fit for the unconditional latent growth curve models of caregiver and toddler HF-HRV. Among toddlers, mean HF-HRV at Post 1 was 3.81 and the Post 3 mean was 3.96, suggesting a small average change from the reactivity to regulatory phases. The standard deviation of the toddler HF-HRV slope was 0.40, indicating that most data points clustered around the average slope. There was a small average decrease in caregiver mean HF-HRV scores from 4.84 at Post 1 to 4.73 at Post 3. The standard deviation of the caregiver slope factor was

0.26, suggesting low heterogeneity in caregiver HF-HRV outcomes. Fit indices for the caregiver and toddler growth curve models indicated good fit to the data (see Table S1), supporting the estimation of linear growth models using a growth mixture modeling approach.

Parallel-Process Growth-Mixture Model

Table S2 displays the fit indices of one- to four-group GMM models. The toddler slope factor variances were fixed to 0 to obtain proper model solutions. Based on the fit indices and interpretability of the model solution, we considered the three-group model optimal. Table S3 presents the intercept and linear slope factor means and standard error estimates for Groups 1 to 3. Table S4 includes the inter-factor correlations between groups. Figure 1 depicts the trajectories for the three-group model. See Table 2 for model-estimated means for caregiver and toddler HF-HRV pre- and post-vaccination across groups.

Group 1 (80.9% of the sample) caregivers and toddlers demonstrated generally stable levels of HF-HRV from Baseline to Post 3 epochs and mirrored one another in their responses. Specifically, beginning from baseline, the caregiver maintained a level of HRV close to normative levels for healthy adults at baseline (HF-HRV: 4.76; Nunan et al., 2010). Similar to their caregivers, toddlers in Group 1 demonstrated stable levels of HF-HRV during the vaccination. The HF-HRV baseline levels of toddlers in Group 1 are within the normative range reported by other researchers (HF-HRV between 3.97-4.74 for healthy children in the 2nd year of life (Bar-Haim et al., 2000). Both caregivers and toddlers in Group 1 demonstrated a small increase in HF-HRV immediately after the needle and returned to baseline levels by the third minute post-needle. Group 2 (7.9% of sample) dyads showed lower HF-HRV at baseline relative to Group 1. The caregivers and their toddlers demonstrated a parallel response pattern whereby their HF-HRV levels continued to decrease post-needle and did not return to baseline levels by

the third minute. Group 3 (11.1% of sample) displayed similar but slightly lower baseline HF-HRV levels compared to Group 1 caregivers and toddlers. The dyads in Group 3 showed diverging response patterns such that caregivers maintained stable levels of HF-HRV post-needle, comparable to the caregivers in Group 1, and toddlers' HF-HRV decreased immediately after the needle and then increased steeply during the second and third post-needle minutes, surpassing their baseline levels.

Heart Rate Patterns of Groups

Caregiver and toddler mean heart rate across 1-minute epochs were calculated post-hoc according to group membership from the GMM of HF-HRV and are plotted in Figure 2. Group 1 toddlers experienced an increased heart rate immediately post-needle and a return to baseline levels by the third minute post-needle. Caregivers in Group 1 maintained a stable heart rate across epochs. The low baseline and decreasing HF-HRV pattern of Group 2 dyads is also reflected in their heart rate responses. Both caregivers and toddlers display a high baseline heart rate, with caregivers maintaining that level of high heart rate across epochs. Toddlers showed an increase in heart rate from baseline right after the needle and maintained a high heart rate (i.e., no return to baseline) throughout all post-needle epochs. Group 3 caregivers maintained a stable heart rate level, similar to caregivers in Group 1, with slight fluctuations. In contrast, Group 3 toddlers demonstrated a steep increase in heart rate immediately after the needle and decreased to baseline levels by the third minute post-needle.

Post-hoc analyses

Eight multinomial logistic regression models were estimated to determine how well individual and contextual characteristics predict group membership; these characteristics included toddler sex, toddler age, caregiver relation to child, time since last nap, time since last

feed, number of needle toddlers received, and caregiver and toddler baseline HF-HRV. Results are presented in Table 4. Group 1 was the reference category because it is the largest and is considered the normative-stable group. Lower caregiver and toddler baseline HF-HRV were significantly associated with an increased likelihood of being in Group 3 compared to Group 1, OR = 0.29, $p < .001$, and OR = 0.29, $p < .001$, respectively. None of the other characteristics significantly predicted group membership.

Discussion

To our knowledge, this is the first study to examine the co-occurring physiological arousal and regulatory response patterns of caregivers and their toddlers, measured with HF-HRV, in a pain context. Correlations indicated that caregiver HF-HRV across baseline and post-needle epochs are positively related, albeit small relations, with toddlers' post-needle HF-HRV (Post 2 and Post 3). Based on correlations alone, it cannot be interpreted whether caregiver and their toddlers are regulated or dysregulated together. Thus, further analyses were conducted to discern distinct trajectory groupings that indicated meaningful differences in dyadic responses within an acute pain context. In addition, individual and contextual factors were examined as predictors of the groups. Our discussion focuses on the caregiver and toddler coregulatory patterns for each group. Considering the importance of early attachment relationships in serving as the primary context in which young children regulate distress (Ainsworth, 1979; Bowlby, 1982), we speculate about the attunement patterns for each group according to differences in attachment (i.e., secure vs insecure relationships) patterns that have been shown to be associated with physiological responses to non-pain related distress in children (Groh & Narayan, 2019; Qu & Leerkes, 2018).

Caregiver-toddler coregulatory trajectories

Parallel-process growth-mixture modeling was used to characterize heterogeneity in caregiver-toddler physiological coregulatory responses during routine vaccination. Specifically, three groups sufficiently characterized the variation in dyad HF-HRV outcomes. Group 1 represented most dyads (80.9%) and was characterized by parallel caregiver and toddler regulatory trajectories that were stable from pre- to post-needle. The initial arousal of toddlers in Group 1, as indexed by heart rate, demonstrated a normative peak distress response (i.e., activation of the sympathetic branch of the autonomic system) followed by a steady decline in heart rate to baseline levels by the third minute post-needle. Caregivers in Group 1 maintained a stable and normative heart rate pattern, indicating low levels of arousal in response to their child's vaccination. When confronted with a distressing situation, it is common for individuals to initially experience decreases in HF-HRV from baseline levels, reflecting withdrawal of parasympathetic control of the heart (i.e., leading to increases in heart rate) which mobilizes the individual to engage in active coping to return to homeostasis (Porges, 2007). However, the toddlers in Group 1 did not appear to activate their internal regulatory system (i.e., the parasympathetic system) to return to homeostasis. Instead, we postulate that the toddler's caregiver provided "external" coping resources, including remaining calm and responding to the child's needs with close-contact soothing, which allowed the toddler to adequately recover from pain-related distress (Waxman et al., 2017). The attuned physiological response patterns of dyads in Group 1 are consistent with what would be expected from a secure attachment relationship. Young children with a secure attachment rely on their caregiver as an external source of regulation (Ainsworth, 1979; Groh & Narayan, 2019). Engaging with one's caregiver via physical proximity – behaviour often exhibited by secure infants – has been shown to reduce

pain-related distress in the vaccination context (Hillgrove-Stuart et al., 2015; Horton et al., 2016).

Group 2 characterized 7.9% of dyads in the current study. Both members of the dyad demonstrated lower HF-HRV across baseline and post-needle epochs compared to the normative and stable responses of Group 1. Caregivers and toddlers in Group 2 mirrored each other in their regulatory responses, with continual decreases in HF-HRV until the third minute post-needle. It's important to highlight that the dyad did not demonstrate a HF-HRV withdrawal response (i.e., a clear lowering of HF-HRV values) followed by augmentation (i.e., increase in HF-HRV values), which indicates an internal regulatory response. According to their heart rate responses, the toddlers and caregivers also demonstrated higher peak arousal compared to Group 1 dyads and sustained distress that did not return to baseline levels by three minutes post-needle. These findings suggest that these toddlers were sub-optimally regulating, leaving them less able to return to baseline. The poorer regulatory coping resources likely results in prolonged activation of the parasympathetic system (i.e., stable, low HF-HRV scores across time). We speculate that the caregiver in Group 2 did not have the capacity to respond to their child's needs and soothe their child's pain-related distress given their own internal dysregulation (i.e., consistent low HF-HRV and high heart rate). Although the regulatory trajectories of the dyad look synchronous, each dyad member likely responds to their distress independently, indicating a lack of attunement (Nofech-Mozes et al., 2020). Further, this pattern of under-regulation in Group 2 toddlers has been linked to resistant attachment style in young children (Braungart-Rieker et al., 2001; Qu & Leerkes, 2018), whereby children both seek and resist contact with their primary caregiver which leads to difficulty calming down and responding to their caregiver's attempts to soothe distress.

Lastly, Group 3 dyads represented 11.1% of the sample. Unlike the other two groups, the caregivers and toddlers within this group demonstrated very different arousal and regulatory responses. Specifically, caregivers in Group 3 exhibited normative HF-HRV and heart rate trajectory responses comparable to the caregivers in Group 1. However, the toddlers demonstrated a large withdrawal response (i.e., steep decrease in HF-HRV) followed by large increase in HF-HRV that surpassed their baseline levels. When the initial arousal is very high, which was evident based on toddler peak heart rate, the parasympathetic system can overcompensate in attempt to regulate the extreme arousal (Berntson et al., 1997). This exaggerated regulatory response seen in Group 3 toddlers suggests they overly rely on their own internal physiological resources to cope with distress despite their caregiver's physical presence. The struggle to adjust one's responses appropriately among dyad members in a distress context (e.g., caregiver does not offer support to their child in distress) indicates potential mis-attunement (Nofech-Mozes et al., 2020). Further, this pattern of over-regulation in the toddler has been associated with an avoidant attachment style between caregivers and young children, whereby the caregiver does not provide adequate regulatory support to the child and the child does not rely on their caregiver for external regulatory support (Ainsworth, 1979; Qu & Leerkes, 2018).

Predictors of coregulatory groups

Several individual and contextual factors were examined as potential predictors of group membership (i.e., the caregiver-toddler coregulatory trajectories). Only caregiver and child baseline values of HF-HRV significantly predicted group membership. Specifically, lower caregiver and toddler baseline HF-HRV predicted membership in Group 2 (the group with consistently low HF-HRV levels) compared to Group 1 dyads, suggesting both caregivers and

toddlers had lower regulatory functioning from baseline. These results parallel prior work that suggests young children with lower baseline HF-HRV have less potential to engage their internal physiological regulatory system (i.e., demonstrate RSA withdrawal) and thus have reduced regulatory resources to cope with distress (Calkins, 1997). Further, previous research has shown that children who are distressed before a needle procedure experience more pain-related behavioural distress after receiving the needle (Campbell et al., 2018). This finding highlights the need for caregivers to provide support to their toddlers in regulating distress beginning before a vaccination procedure.

Limitations

As discussed in previous published papers using the same sample (Waxman et al., 2020, 2021), our study sample includes healthy toddlers and caregivers with high education levels which may affect the generalizability of our findings. Further, the sample sizes of dyads in Groups 2 and 3 (n = 15 and 21, respectively) were smaller than the number of dyads in Group 1 (n = 153), which limited our investigation of the predictors of group membership. Future work will benefit from validating the groupings discerned from the current study with other independent samples and collecting more detailed information about factors that can modify HRV (e.g., lifestyle factors, medications, medical conditions; Quintana & Heathers, 2014) and appropriately control for these variables.

Conclusions: research and clinical implications

This study presents a novel examination of distinct caregiver-child attunement profiles based on HF-HRV outcomes during toddler vaccinations. The majority of dyads in the sample displayed a stable and unchanging HF-HRV trajectory, indicating that toddlers relied on their caregivers as an external source of regulation to soothe their pain-related distress. In groups

where dyads experienced a lack of attunement or were misattuned, toddlers were either underregulated (i.e., less able to use internal or external resources to regulate pain-related distress) or overregulated (i.e., the toddler appears to be overly reliant on internal regulatory systems instead of their caregiver). Similar patterns of physiological responses have been found in prior research with young children in experimental distress contexts (Qu & Leerkes, 2018). Moreover, our findings are consistent with caregiver-child attachment relationship styles which have provided an important understanding of a caregiver's ability to respond effectively to their child and a child's ability to regulate their distress in the vaccination context (Hillgrove-Stuart et al., 2015; Horton et al., 2016; O'Neill et al., 2021).

The ability to regulate from distress within pain contexts and beyond develops over the course of early childhood and plays an important role in a variety of developmental and psychosocial outcomes (Calkins, 1997; Calkins & Dedmon, 2000; Stifter & Corey, 2001). This study highlights patterns of caregiver-toddler distress responding that reflect a lowered capacity to cope with pain-related distress, which ultimately may undermine a child's ability to cope with future pain-related distress. Researchers should continue to study less adaptive attunement patterns by further exploring caregiver and child factors that are associated with these groups, as well as examine whether different attunement patterns predict behavioural pain-related distress in the vaccination context. It is also critical for health care providers help caregivers support their children during vaccination procedures (e.g., inform caregivers about evidenced-based strategies to support their child in distress) and encourage caregivers to seek their own individual or parenting support if needed.

References

Ainsworth, M. S. (1979). Infant–mother attachment. *American Psychologist*, *34*(10), 932–937. https://doi.org/10.1037/0003-066X.34.10.932

Akaike, H. (1981). Likelihood of a model and information criteria. *Journal of Econometrics*, *16*(1), 3–14.

Appelhans, B. M., & Luecken, L. J. (2006). Heart rate variability as an index of regulated emotional responding. *Review of General Psychology*, *10*(3), 229–240.

Atkinson, L., Jamieson, B., Khoury, J., Ludmer, J., & Gonzalez, A. (2016). Stress physiology in infancy and early childhood: Cortisol flexibility, attunement and coordination. *Journal of Neuroendocrinology*, *28*(8), Article 8. https://doi.org/10.1111/jne.12408

Atkinson, N. H., Gennis, H., Racine, N. M., & Pillai Riddell, R. (2015). Caregiver emotional availability, caregiver soothing behaviors, and infant pain during immunization. *Journal of Pediatric Psychology*, *40*(10), 1105–1114.

Bar-Haim, Y., Marshall, P. J., & Fox, N. A. (2000). Developmental changes in heart period and high-frequency heart period variability from 4 months to 4 years of age. *Developmental Psychobiology*, *37*(1), 44–56. https://doi.org/10.1002/1098-2302(200007)37:1<44::AID-DEV6>3.0.CO;2-7

Beauchaine, T. P. (2015). Respiratory sinus arrhythmia: A transdiagnostic biomarker of emotion dysregulation and psychopathology. *Current Opinion in Psychology*, *3*, 43–47.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*(1), 289–300.

Berntson, G. G., Bigger, J. T., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., Nagaraja, H. N., Porges, S. W., Saul, J. P., Stone, P. H., & Molen, M. W. V. D. (1997). Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology*, *34*(6), 623–648. https://doi.org/10.1111/j.1469-8986.1997.tb02140.x

Berntson, G. G., Quigley, K. S., & Lozano, D. (2007). Cardiovascular psychophysiology. In *Handbook of psychophysiology, 3rd ed* (pp. 182–210). Cambridge University Press. https://doi.org/10.1017/CBO9780511546396.008

Berry, J. W. (2003). Conceptual approaches to acculturation. In *Acculturation: Advances in theory, measurement, and applied research* (pp. 17–37). American Psychological Association. https://doi.org/10.1037/10472-004

Bowlby, J. (1982). Attachment and loss: Retrospect and prospect. *American Journal of Orthopsychiatry*, *52*(4), 664–678. https://doi.org/10.1111/j.1939-0025.1982.tb01456.x

Braren, S. H., Perry, R. E., Ursache, A., & Blair, C. (2019). Socioeconomic risk moderates the association between caregiver cortisol levels and infant cortisol reactivity to emotion induction at 24 months. *Developmental Psychobiology*, *61*(4), 573–591. https://doi.org/10.1002/dev.21832

Braungart-Rieker, J. M., Garwood, M. M., Powers, B. P., & Wang, X. (2001). Parental sensitivity, infant affect, and affect regulation: Predictors of later attachment. *Child Development*, *72*(1), 252–270.

Cacioppo, J. T., Tassinary, L. G., & Berntson, G. G. (Eds.). (2000). *Handbook of psychophysiology* (2nd ed). Cambridge University Press.

Calkins, S. D. (1997). Cardiac vagal tone indices of temperamental reactivity and behavioral regulation in young children. *Developmental Psychobiology*, *31*(2), 125–135. https://doi.org/10.1002/(sici)1098-2302(199709)31:2<125::aid-dev5>3.0.co;2-m

Calkins, S. D. (2011). Caregiving as coregulation: Psychobiological processes and child functioning. In *Biosocial foundations of family processes* (pp. 49–59). Springer.

Calkins, S. D., & Dedmon, S. E. (2000). Physiological and behavioral regulation in two-year-old children with aggressive/destructive behavior problems. *Journal of Abnormal Child Psychology*, *28*(2), 103–118.

Camm, A. J., Malik, M., Bigger, J. T., Breithardt, G., Cerutti, S., Cohen, R. J., ... & Singer, D. H. (1996). Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation*, *93*(5), 1043–1065. https://doi.org/10.1161/01.CIR.93.5.1043

Campbell, L., Pillai Riddell, R., Cribbie, R., Garfield, H., & Greenberg, S. (2018). Preschool children's coping responses and outcomes in the vaccination context: Child and caregiver transactional and longitudinal relationships. *PAIN*, *159*(2), 314–330. https://doi.org/10.1097/j.pain.0000000000001092

Campbell, L., Pillai Riddell, R., Garfield, H., & Greenberg, S. (2013). A cross-sectional examination of the relationships between caregiver proximal soothing and infant pain over the first year of life. *Pain*, *154*(6), 813–823. https://doi.org/10.1016/j.pain.2013.02.006

Di Lorenzo, M. G., Bucsea, O., Rumeo, C., Waxman, J. A., Flora, D. B., Schmidt, L. A., & Riddell, R. P. (2021). Caregiver and young child biological attunement in distress contexts: A systematic review and narrative synthesis. *Neuroscience & Biobehavioral Reviews*.

Feldman, R. (2007). Parent–infant synchrony and the construction of shared timing; physiological precursors, developmental outcomes, and risk conditions. *Journal of Child Psychology and Psychiatry*, *48*(3–4), 329–354. https://doi.org/10.1111/j.1469-7610.2006.01701.x

Groh, A. M., & Narayan, A. J. (2019). Infant attachment insecurity and baseline physiological activity and physiological reactivity to interpersonal stress: A meta-analytic review. *Child Development*, *90*(3), 679–693.

Hastings, P. D., & Miller, J. G. (2014). Autonomic regulation, polyvagal theory, and children's prosocial development. *Prosocial Development: A Multidimensional Approach*, 112–127.

Hillgrove-Stuart, J., Riddell, R. P., Flora, D. B., Greenberg, S., & Garfield, H. (2015). Caregiver soothing behaviors after immunization and infant attachment: A longitudinal analysis. *Journal of Developmental & Behavioral Pediatrics*, *36*(9), 681–689.

Hofer, M. (2010). Hidden regulators within the mother–infant interaction. *Nurturing Children and Families: Building on the Legacy of T. Berry Brazelton*, 154–163.

Horton, R., Pillai Riddell, R., Moran, G., & Lisi, D. (2016). Do infant behaviors following immunization predict attachment? An exploratory study. *Attachment & Human Development*, *18*(1), 90–99. https://doi.org/10.1080/14616734.2015.1115113

Hu, L., & Bentler, P. M. (1999). Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. *Structural Equation Modeling: A Multidisciplinary Journal*, *6*(1), 1–55. https://doi.org/10.1080/10705519909540118

IBM Corp. (2019). *IBM SPSS Statistics for Mac, Version 26.0*. IBM Corp.

Jung, T., & Wickrama, K. a. S. (2008). An introduction to latent class growth analysis and growth mixture modeling. *Social and Personality Psychology Compass*, *2*(1), 302–317. https://doi.org/10.1111/j.1751-9004.2007.00054.x

McMurtry, C. M., Pillai Riddell, R., Taddio, A., Racine, N., Asmundson, G. J. G., Noel, M., Chambers, C. T., Shah, V., & HELPinKids&Adults Team. (2015). Far from "just a poke": Common painful needle procedures and the development of needle fear. *The Clinical Journal of Pain*, *31*(10 Suppl), S3-11. https://doi.org/10.1097/AJP.0000000000000272

Muthén, B., & Shedden, K. (1999). Finite mixture modeling with mixture outcomes using the EM algorithm. *Biometrics*, *55*(2), 463–469. https://doi.org/10.1111/j.0006-341X.1999.00463.x

Muthén, L. K., & Muthén, B. (1998). *Mplus user's guide: Statistical analysis with latent variables, user's guide* (Eighth Edition). Muthén & Muthén.

Nofech-Mozes, J. A., Jamieson, B., Gonzalez, A., & Atkinson, L. (2020). Mother–infant cortisol attunement: Associations with mother–infant attachment disorganization. *Development and Psychopathology*, *32*(1), 43–55. https://doi.org/10.1017/S0954579418001396

Nunan, D., Sandercock, G. R. H., & Brodie, D. A. (2010). A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *Pacing and Clinical Electrophysiology*, *33*(11), 1407–1417. https://doi.org/10.1111/j.1540-8159.2010.02841.x

Oberlander, T. F., Grunau, R. V. E., Whitfield, M. F., & Saul, J. P. (1997). Cardiac autonomic and behavioral responses to acute pain in 4 month old infants. *Pediatric Research*, *41*(4), 16–16. https://doi.org/10.1203/00006450-199704001-00101

Oberlander, Tim, & Saul, JP. (2007). Methodological considerations in heart rate variability analysis. In F. Rautaharju & P. Rautaharju (Eds.), *Investigative Electrocardiography in Epidemiological Studies and Clinical Trials* (pp. 68–73). Springer. https://doi.org/10.1007/978-1-84628-481-6_5

O'Neill, M. C., Pillai Riddell, R., Bureau, J.-F., Deneault, A.-A., Garfield, H., & Greenberg, S. (2021). Longitudinal and concurrent relationships between caregiver–child behaviours in the vaccination context and preschool attachment. *PAIN*, *162*(3), 823–834. https://doi.org/10.1097/j.pain.0000000000002091

Pillai Riddell, R., Campbell, L., Flora, D. B., Racine, N., Din Osmun, L., Garfield, H., & Greenberg, S. (2011). The relationship between caregiver sensitivity and infant pain behaviors across the first year of life. *PAIN*, *152*(12), 2819–2826. https://doi.org/10.1016/j.pain.2011.09.011

Pillai Riddell, R., Flora, D. B., Stevens, S. A., Stevens, B., Cohen, L. L., Greenberg, S., & Garfield, H. (2013). Variability in infant acute pain responding meaningfully obscured by averaging pain responses. *PAIN®*, *154*(5), 714–721. https://doi.org/10.1016/j.pain.2013.01.015

Pillai Riddell, R., Jasim, S., & Hamwi, L. (in press). Out of the mouth of babes: A lot about pain has nothing to do with pain. *PAIN.*

Porges, S. W. (2007). The polyvagal perspective. *Biological Psychology*, *74*(2), 116–143. https://doi.org/10.1016/j.biopsycho.2006.06.009

Qu, J., & Leerkes, E. M. (2018). Patterns of RSA and observed distress during the still-face paradigm predict later attachment, compliance and behavior problems: A person-centered approach. *Developmental Psychobiology*, *60*(6), 707–721.

Quintana, D. S., & Heathers, J. A. J. (2014). Considerations in the assessment of heart rate variability in biobehavioral research. *Frontiers in Psychology*, *5*. https://www.frontiersin.org/article/10.3389/fpsyg.2014.00805

Ryder, A. G., Alden, L. E., & Paulhus, D. L. (2000). Is acculturation unidimensional or bidimensional? A head-to-head comparison in the prediction of personality, self-identity, and adjustment. *Journal of Personality and Social Psychology*, *79*(1), 49.

Schwarz, G. (1978). Estimating the dimension of a model. *Annals of Statistics*, *6*(2), 461–464.

Stifter, C. A., & Corey, J. M. (2001). Vagal regulation and observed social behavior in infancy. *Social Development*, *10*(2), 189–201. https://doi.org/10.1111/1467-9507.00158

Taddio, A., Appleton, M., Bortolussi, R., Chambers, C., Dubey, V., Halperin, S., Hanrahan, A., Ipp, M., Lockett, D., MacDonald, N., Midmer, D., Mousmanis, P., Palda, V., Pielak, K., Riddell, R. P., Rieder, M., Scott, J., & Shah, V. (2010). Reducing the pain of childhood vaccination: An evidence-based clinical practice guideline. *CMAJ*, *182*(18), E843–E855. https://doi.org/10.1503/cmaj.101720

Waxman, J. A., DiLorenzo, M. G., Pillai Riddell, R. R., Flora, D. B., Schmidt, L. A., Garfield, H., Flanders, D., Weinberg, E., & Savlov, D. (2021). Investigating convergence of cardiac and behavioral indicators of distress during routine vaccinations over the second year of life. *Developmental Psychobiology*, *63*(3), 437–451.

Waxman, J. A., DiLorenzo, M. G., Riddell, R. R. P., Flora, D. B., Greenberg, S., & Garfield, H. (2017). Preschool needle pain responding: Establishing 'normal.' *The Journal of Pain*, *18*(6), 739–745.

Waxman, J. A., DiLorenzo, M. G., Riddell, R. R. P., Flora, D. B., Schmidt, L. A., Garfield, H., Flanders, D., Weinberg, E., & Savlov, 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.

Waxman, J. A., Pillai Riddell, R. R., Tablon, P., Schmidt, L. A., & Pinhasov, A. (2016). Development of Cardiovascular Indices of Acute Pain Responding in Infants: A Systematic Review. *Pain Research and Management*. https://doi.org/10.1155/2016/8458696

Waxman, J., Martin, J., & Riddell, R. P. (2017). Understanding infant pain responding within a relational context. In *Neonatal Pain* (pp. 89–104). Springer.

Figure 1

Three-group parallel-process growth-mixture model of caregiver and toddler concurrent high-frequency heart rate variability (HF-HRV)

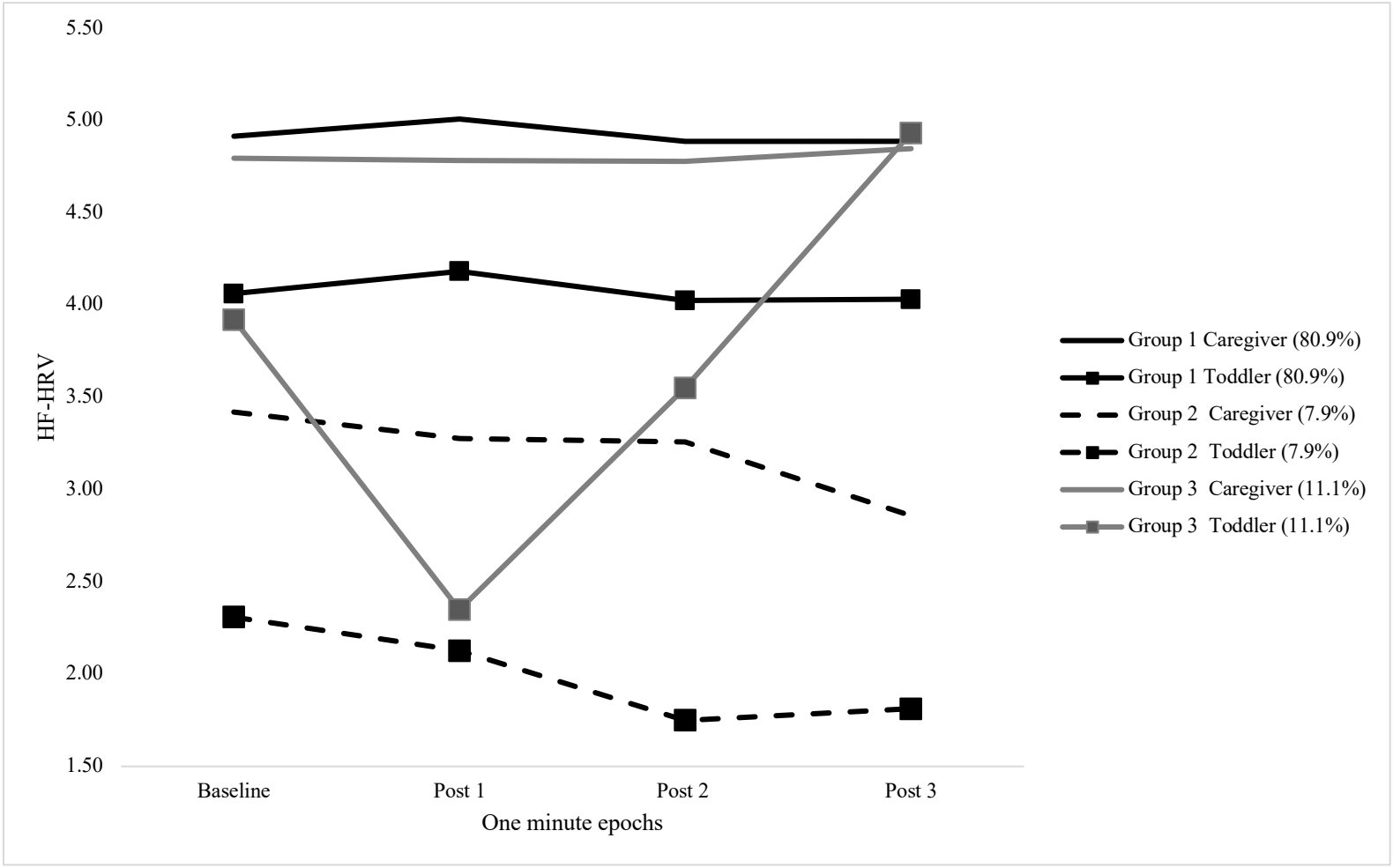

Figure 2

Distinct trajectories of caregiver and child concurrent heart rate based on groupings derived from the parallel-process growth-mixture model of HF-HRV outcomes

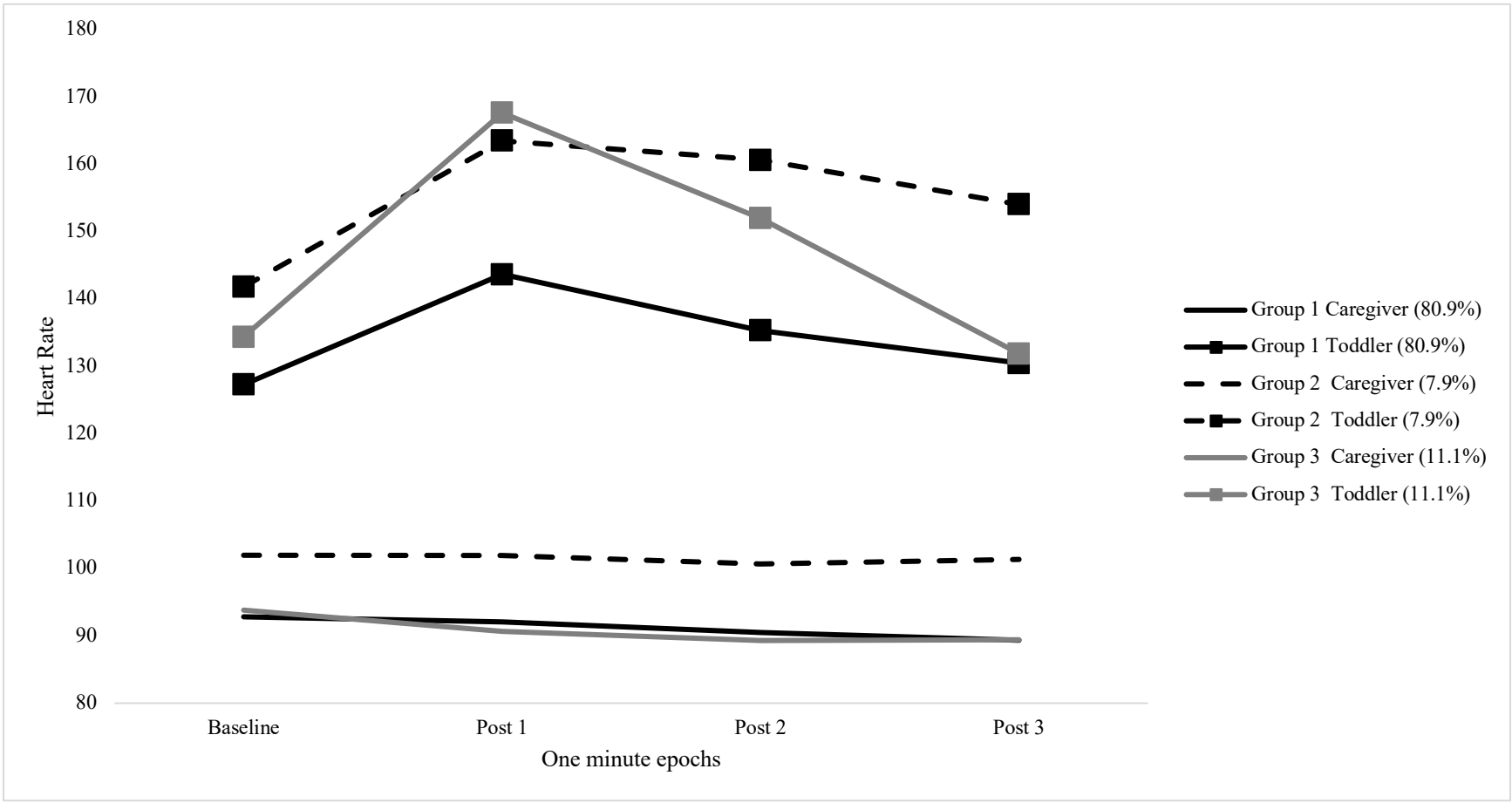

Table 1

Demographic and personal characteristics

Caregiver age in years, M (SD)	36 (5.54)
Toddler age in months, M (SD)	17.04 (4.80)
Toddler age by appointment, n (%)	
12 months	81 (42.9)
18 months	66 (34.9)
24 months	42 (22.2)
Sex of toddler, n (%)	
Male	104 (55.0)
Female	85 (45.0)
Relationship to toddler, n (%)	
Mother	163 (86.2)
Father	24 (12.7)
Other	2 (1.1)
Caregiver Education, n (%)	
Graduate school/professional training	92 (48.7)
University graduate	60 (31.7)
Partial university (at least 1 year)	5 (2.6)
Trade school/community college	26 (13.8)
High School Graduate	2 (1.1)
Missing	4 (2.1)
Acculturation status, M (SD)	
Way of life reflects heritage culture	7.78 (2.13)
Way of life reflects mainstream North American/Canadian Culture	6.33 (2.83)
At vaccination appointment:	
Minutes since last feed for toddler,	102.22 (71.46)

M (SD)	
Minutes since last nap for toddler, M (SD)	137.38 (101.53)
Number of needles toddler received, Mode (range)	1 (1-3)

Table 2

Means and SDs of caregiver and toddler high-frequency heart rate variability from the overall sample and the three groups discerned from the parallel-process growth-mixture model

	Baseline *M (SD)*	Post 1 *M (SD)*	Post 2 *M (SD)*	Post 3 *M (SD)*
Overall:				
Caregiver HF-HRV	4.78 (1.20)	4.84 (1.22)	4.74 (1.23)	4.73 (1.15)
Toddler HF-HRV	3.90 (1.26)	3.81 (1.47)	3.78 (1.22)	3.96 (1.19)
Group 1:				
Caregiver HF-HRV	4.93 (1.11)	5.01 (1.13)	4.89 (1.16)	4.89 (1.02)
Toddler HF-HRV	4.05 (1.13)	4.18 (1.31)	4.03 (1.06)	4.03 (1.01)
Group 2:				
Caregiver HF-HRV	3.29 (1.19)	3.28 (0.88)	3.26 (0.88)	2.86 (0.75)
Toddler HF-HRV	2.43 (1.00)	2.13 (1.10)	1.75 (.88)	1.81 (.78)
Group 3:				
Caregiver HF-HRV	4.80 (1.06)	4.78 (1.23)	4.78 (1.24)	4.85 (1.18)
Toddler HF-HRV	3.92 (1.55)	2.35 (0.91)	3.55 (1.09)	4.93 (0.82)

Note. Group 1 represents 80.9% of dyads, Group 2 represents 7.9% of dyads, and Group 3 represents 11.1% of dyads.

HF-HRV: High-frequency heart rate variability.

Table 3

Correlations between caregiver and toddler high-frequency heart rate variability (one-minute epochs included in GMMs)

	1.	2.	3.	4.	5.	6.	7.	8.
1. Caregiver HF-HRV Baseline	_	.54*	.52*	.57*	.07	.15	.16*	.17*
2. Caregiver HF-HRV Post 1		_	.73*	.73*	.12	.03	.17*	.16*
3. Caregiver HF-HRV Post 2			_	.79*	.15	.06	.22*	.20*
4. Caregiver HF-HRV Post 3				_	.20*	.08	.27*	.27*
5. Toddler HF-HRV Baseline					_	.53*	.63*	.63*
6. Toddler HF-HRV Post 1						_	.60*	.52*
7. Toddler HF-HRV Post 2							_	.74*
8. Toddler HF-HRV Post 3								_

Note. *p < .05; Abbreviations: HF-HRV = High-frequency heart rate variability.

Table 4

Post hoc logistic regressions analyses predicting caregiver-toddler high-frequency HRV (HF-HRV) trajectory groups

Predictor	B	SE B	OR	95% CI	*p*
Toddler sex					
Group 2	0.68	0.55	1.98	0.67-3.83	0.82
Group 3	0.18	0.47	1.20	0.48-3.00	0.86
Toddler age					
Group 2	0.21	0.34	1.23	0.63-2.40	0.86
Group 3	-0.32	0.32	0.73	0.39-1.35	0.83
Caregiver Relation to Toddler					
Group 2	-0.25	0.81	0.78	0.16-3.79	0.86
Group 3	-0.16	0.67	0.85	0.23-3.16	0.86
Time since last nap					
Group 2	-0.001	0.003	1.00	0.99-1.01	0.86
Group 3	0.002	0.002	1.00	0.99-1.01	0.85
Time since last feed					
Group 2	0.004	0.003	1.00	0.99-1.01	0.83
Group 3	0.002	0.003	1.00	0.99-1.01	0.86
Number of needles					
Group 2	-0.66	0.44	0.51	0.22-1.21	0.69
Group 3	0.04	0.32	1.04	0.55-1.97	0.90
Caregiver Baseline HF-HRV					
Group 2	-1.25	0.29	0.29	0.16-0.51	0.00
Group 3	-0.11	0.22	0.90	0.58-1.38	0.86
Toddler Baseline HF-HRV					
Group 2	-1.26	0.29	0.29	0.16-0.51	0.00
Group 3	-0.10	0.20	0.91	0.61-1.35	0.86

Note. B: unstandardized estimate, SE B: standard error of unstandardized estimate, OR: odds ratio, 95% CI: 95 percent confidence interval of odds ratio.

Supplementary Table 1

Growth factor means and standard error estimates for the latent growth curve models of caregiver and toddler high-frequency heart rate variability

	Caregiver		Toddler	
	Estimate	SE	Estimate	SE
Means				
Intercept	4.56	0.36	2.83	0.36
Slope	-0.16	0.14	0.22	0.12
Variance				
Intercept	1.12	0.17	1.68	0.41
Slope	0.07	0.06	0.16	0.09
CFI	1.00	--	0.99	--
TLI	1.00	--	0.98	--
RMSEA	0.03	--	0.06	--

Note. CFI = Comparative fit index; TLI = Tucker-Lewis index;

RMSEA = root mean square error of approximation.

Supplementary Table 2

Model fit indices of parallel-process growth mixture models

Model	AIC	BIC	SSABIC
One group	3039.14	3123.97	3040.62
Two groups	3035.37	3121.23	3037.22
Three groups	3032.69	3116.98	3034.62
Four groups	3034.88	3131.06	3036.85

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

Supplementary Table 3

Growth factor means and standard error estimates for the three-group parallel-process growth mixture model

	Intercept, *M* (SE)	Linear, *M* (SE)
Group 1		
Caregiver HF-HRV	4.98 (0.48)	-0.14 (0.15)
Toddler HF-HRV	4.83 (0.51)	--
Group 2		
Caregiver HF-HRV	3.61 (0.45)	-0.56 (0.77)
Toddler HF-HRV	2.57 (0.50)	--
Group 3		
Caregiver HF-HRV	5.04 (0.61)	0.02 (0.43)
Toddler HF-HRV	3.10 (0.61)	--

Note. Toddler slope factor variances were fixed to 0 to obtain properly converged model solutions.

Supplementary Table 4

Inter-factor correlations for the three-group parallel-process growth mixture model

		Toddler HF-HRV	
		Intercept	Linear Slope
Caregiver HF-HRV	Intercept	-0.03	--
	Linear Slope	0.13	--

Note. Correlations with the child slope factor variance were fixed to 0 to obtain a properly converged model solution.

Groups 1, 2 and 3 have identical parameters.

Chapter 5: Moving towards a better understanding of caregiver-toddler regulatory attunement trajectories

The primary aim of the preceding chapter was to address an important gap in the literature outlined in the systematic review (Chapter 2), which was to examine distinct patterns of caregiver-toddler regulatory attunement, as measured by HF-HRV, during routine vaccinations in the second year of life. We used parallel-process growth mixture modeling (GMM) to describe variability in caregiver-toddler attunement patterns over the course of the vaccination (pre- to post-needle) using a small number of latent classes or groups. Dyad regulatory trajectory patterns based on HF-HRV were further elucidated by examining the heart rate outcomes of dyads within these patters, which provides an indicator of arousal. Lastly, we examined whether contextual factors (i.e., toddler sex, toddler age, relation to child, baseline caregiver and toddler HF-HRV, time of last nap, time of last feed, number of needles toddler received) predicted dyadic group membership.

Three groups of dyads were discerned that differed in their patterns of physiological regulatory responses to pain-related distress. One group of dyads (Group 1: 80% of the sample) displayed an attuned regulatory pattern, and two groups of dyads (20% of the sample) showed less adaptive attunement patterns, particularly a lack of attunement (Group 2) or mis-attunement (Group 3) between dyad members. Toddlers in Groups 2 and 3 also demonstrated greater arousal, as indexed by heart rate, at various timepoints after the needle compared to toddlers in Group 1. The only contextual factor that predicted group membership was baseline values of HF-HRV. Specifically, lower caregiver and toddler baseline HF-HRV predicted membership in Group 2 compared to Group 1 dyads, suggesting that both caregivers and toddlers in Group 2 had lower regulatory functioning from before the needle. We postulated that the caregivers in

groups that demonstrated less adaptive attunement patterns did not have the capacity to respond to their child's needs due to their own dysregulation (Group 2: lack of attunement with toddler) or they struggled to offer appropriate support based on their child's internal state (Group 3: mis-attuned to toddler). This research is novel in that we have moved away from the bulk of the literature that has examined attunement using static analyses (e.g., correlations) as summarized in Chapter 2. To our knowledge, this was the first time that various patterns of physiological attunement have been described in a high distress context. To further our understanding of adaptative versus suboptimal patterns of attunement, an examination of factors that are associated with the various patterns is warranted.

Since regulatory patterns of a child are theorized to be shaped by the regulatory capacities of the caregiver (Bridgett et al., 2015), further understanding circumstances under which children signal distress but caregivers are unable to respond due to their own regulatory challenges can further elucidate dyads at-risk for experiencing poor coregulation in distress contexts. The main objective of the final study (Chapter 6) was to examine whether caregiver distress (operationalized by reported worry before and after the vaccination) and toddler distress (operationalized by behavioural pain-related distress) are associated with the regulatory attunement patterns that were described in Chapter 4. Multinomial logistic regression was used to examine the study's primary aim with group membership as the dependent variable. We also plotted FLACC (face, legs, activity, cry and consolability scale; Merkel et al., 1996) scores by group membership to examine the child pain-related distress responses of these groups. This is the first known study to examine relations between pain-related caregiver and child distress and caregiver-toddler regulatory attunement patterns during vaccination.

References

Bridgett, D. J., Burt, N. M., Edwards, E. S., & Deater-Deckard, K. (2015). Intergenerational transmission of self-regulation: A multidisciplinary review and integrative conceptual framework. *Psychological Bulletin*, *141*(3), 602–654. https://doi.org/10.1037/a0038662

Merkel, S., Voepel-Lewis, T., Shayevitz, J., & Malviya, S. (1996). The FLACC: A behavioral scale for scoring postoperative pain in young children. *Pediatric Nursing*, *23*, 293–297.

Chapter 6: The relations between caregiver and toddler pain-related distress and dyadic physiological attunement during vaccination[3]

The caregiver-toddler relationship plays a crucial role in shaping and organizing a toddler's capacity to regulate distress across many contexts, including the acute pediatric pain context (Pillai Riddell et al., in press). With the ability to express pain verbally still developing and with limited strategies to regulate themselves, young children rely on their attachment relationship with their primary caregiver to help them regulate pain-related distress (Pillai Riddell & Racine, 2009; Waxman et al., 2017).

Within the broader developmental literature, researchers have observed caregiver-toddler dyads working toward a mutually regulated state in distress contexts through moment-to-moment coordination of their biological, affective, and behavioural responses (Calkins, 2011; Feldman, 2012). These caregiver-toddler interactions typically involve contingent and reciprocal responses between dyad members (i.e., each dyad member produces and respond to the other's responses) (DiCorcia & Tronick, 2011). Over time, repeated interactions of mutual regulation with one's caregiver support the toddler's internalization of regulatory skills (Beeghly et al., 2016). Despite the dyadic nature of caregiver-toddler interactions, the caregiver is primarily responsible for facilitating regulation, given a toddler's limited capacity early in life. According to the Self-Regulation Intergenerational Transition Model (Bridgett et al., 2015), a caregiver's self-regulatory capacity influences their ability to effectively structure and support their child's needs, which in turn influences their child's ability to regulate distress. Ideally, the caregiver will be able to regulate their own distress, perceive and understand the distress of their child, and

[3] This is the author's version of the submitted manuscript:
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respond sensitively and contingently to their child. However, the caregiver not being able to effectively regulate their own distress may prompt a greater focus on their own distress and hinder their ability to respond to their child's needs (Augustine & Leerkes, 2019; Leerkes et al., 2016). The pattern of dynamic and reciprocal responding between caregivers and their children is known as attunement (Atkinson et al., 2016).

Patterns of physiological attunement in a distress context

Attunement is studied at various biobehavioural levels. Recently, there has been a greater focus on how caregivers and their children regulate their physiological responses in distress contexts as an underlying mechanism of caregiver-child attunement (Di Lorenzo et al., 2021). Regulation of physiological responses helps support one's ability to engage in cognitive and behavioural emotion regulation and respond to one's environment (Calkins & Hill, 2007). Several studies have shown that there are varying patterns of both physiological and behavioural regulation that infants and toddlers demonstrate during distressing events (Pillai Riddell et al., 2013; Roubinov et al., 2020; Waxman et al., 2021) and that different patterns are associated with various adaptive (e.g., secure attachment) or maladaptive (e.g., avoidant or resistant attachment, internalizing and externalizing behavioral problems) outcomes for children (Boyce et al., 2001; Braungart-Rieker et al., 2001; Hill-Soderlund et al., 2008; Qu & Leerkes, 2018). Given the differing regulatory patterns in young children that can emerge post-distress and the predictive nature of these patterns, it is important to further examine the different patterns of regulatory responses for both toddlers and their caregivers in a distress context. However, a systematic review by our group revealed that much of the research on caregiver-child physiological attunement has focused on interpreting overall relationships between caregiver and child physiological outcomes (Di Lorenzo et al., 2021). Given the variable distress response and

regulatory patterns that can emerge, it is important to examine trajectories of caregiver-child coregulatory patterns (Di Lorenzo et al., 2021; Pillai Riddell et al., 2013; Waxman et al., 2017).

Recently, Di Lorenzo-Klas and colleagues (2022) examined caregiver-child coregulatory response patterns within an acute pain context (see Figure 1 from Chapter 4). These authors found that having three latent groups of caregiver-toddler dyads could adequately represent variability in their regulatory responses to pain-related distress over the vaccination appointment (1-minute before needle to 3 minutes after needle), as measured by high-frequency heart rate variability. Specifically, the largest group (80%) demonstrated stable and parallel dyadic regulatory patterns from baseline to post-needle, reflecting an adaptive caregiver-toddler pattern or attunement. It was deemed adaptive because the caregiver's trajectory matched the child's regulatory trajectory pattern and both trajectories were close to normative baseline HF-HRV levels for healthy adults and toddlers (Bar-Haim et al., 2000; Nunan et al., 2010). Although the second group (7.9 precent of the sample) also had parallel caregiver-toddler regulatory trajectories, their HF-HRV values were notably lower, indicating that both caregivers and their children were dysregulated from baseline to post-needle. This pattern reflects a lack of regulatory attunement between dyad members, as the caregiver is distressed themselves and unlikely able to help their child regulate distress. The third group (11.1%) demonstrated divergent regulatory trajectories such that caregivers had a stable regulatory trajectory comparable to the normative group, but their toddlers showed an exaggerated withdrawal response (i.e., a steep decrease followed by an increase in HF-HRV values that surpassed their baseline levels). The regulatory responses of these dyads likely reflect mis-attunement, whereby the caregiver's attempts at providing external regulatory support are not matched to their toddler's internal state. Thus, the toddler is overly relying on their own internal resources to cope. This research describes the

variability in coregulatory patterns of caregivers and their toddlers in a distress context. However, there is a lack of understanding of the factors that are associated with the different adaptive and suboptimal attunement patterns.

Caregiver and child variables associated with physiological attunement patterns

Research has shown that measures of observed behavioural distress and physiological regulatory responses can reflect interrelated but also unique aspects of the distress response (Waxman et al., 2021). In a pain context, toddler behavioural pain-related distress reflects various behaviours (e.g., crying, facial expressions) that signal the toddler's level of distress (Merkel et al., 2002). The caregiver is meant to interpret these cues and respond sensitively to the needs of their child. However, in some cases, there is a mismatch between what a child signals and their internal state. For example, young children may express limited behavioural distress and higher physiological dysregulation. In prior research, this pattern has been associated with maladaptive outcomes, including avoidant attachment (Hill-Soderlund et al., 2008) and greater internalizing behaviors in children (Boyce et al., 2001). Alternatively, although toddlers may display behaviours to signal distress to their caregiver, caregivers may be unable to respond due to their own dysregulation (Leerkes et al., 2016, 2017). Thus, identifying whether observed behavioural distress is associated with variable patterns of caregiver-child attunement can further elucidate why dyads are demonstrating adaptive versus suboptimal patterns of attunement.

The broader physiological attunement literature has examined how various caregiver factors affect attunement, including caregiver sensitivity and mental health (e.g. depressive symptoms), although much of the evidence is mixed (Di Lorenzo et al., 2021). There is limited but consistent evidence to show that higher parent sensitivity is associated with more concordance between caregiver and child regulatory responses (Atkinson et al., 2013; van Bakel

& Riksen-Walraven, 2008). The influence of caregiver worry associated with the distressing event (i.e., state worry) on attunement patterns has yet to be examined. Because coregulatory patterns may be shaped by the regulatory capacities of the caregiver, an understanding of caregiver's state psychological distress may elucidate whether parent self-regulation and their ability to support their child's regulatory response is at-risk of being compromised. Indeed, previous research has shown that parent-reported state anxiety is associated with less emotional availability (Nicol-Harper et al., 2007; Woodruff-Borden et al., 2002) as well as greater child pain-related distress (Bearden et al., 2012; Bernard & Cohen, 2006).

Present Study

To address these issues, we examined whether caregiver distress (operationalized by reported worry before and after the vaccination) and child distress (operationalized by behavioural pain-related distress) are associated with caregiver-toddler cardiac attunement patterns (Di Lorenzo-Klas et al., 2022) in an acute pain context. We examined the separate contributions of caregiver worry and child pain-related distress to caregiver-child attunement patterns. Based on previous research, we hypothesized the following: (1) higher child behavioural pain-related distress would be associated with a greater likelihood of belonging to one of the two dyad groupings (Group 2 or 3) defined in Di Lorenzo-Klas and colleagues (2022) that demonstrated less adaptive coregulatory patterns (i.e., a lack of attunement or misattunement between dyad members) rather than the normative group (Group 1) who showed an adaptive attunement pattern, over and above parental worry; and (2) greater caregiver pre-needle and post-needle worry would also be associated with a greater likelihood of being in either Group 2 or 3 than Group 1, over and above child behavioural pain-related distress.

Methods

Participants

The data are part of a cohort-sequential longitudinal study in which caregiver-toddler dyads were recruited from two pediatric clinics in the greater Toronto area. Dyads were observed during routine vaccinations at 12, 18, and 24 months of age. Participant details and data collection procedures are described in detail elsewhere (Di Lorenzo-Klas et al., 2022); a synopsis is presented below. Exclusion criteria were NICU stay during infancy, prematurity (< 37 weeks gestation), suspected or confirmed developmental delay, chronic illness, or caregiver lack of fluency in English. The current analyses were based on a subset of 189 unique dyads with both caregiver and child cardiac data available at either 12 (n = 81), 18 (n = 66), or 24-month (n = 42) vaccination appointments, with a mean age of 17.04 months (SD = 4.80). See Table 1 for participant demographic characteristics. Overall, the toddlers were healthy, from middle-class families, and had caregivers who were well-educated. The subset of dyads included in this study did not significantly differ from those excluded due to missing data in terms of demographic factors (i.e., caregiver age, education, relation to the child, ethnicity, self-reported mainstream and heritage culture, toddler sex, time since last nap, time since last feed, and number of needles received).

The mean age of primary caregivers was 36 years old (SD = 5.54 years). Most caregivers were born in Canada (57.6%), with the remaining born in Asia (18.3%), South America (5.8%), Europe (5.2%), Africa (2.1%), Russia (2.1%), United States/Mexico (2.1%), or Australia (1.6%). On average, caregivers reported strong identification with both their heritage culture (i.e., a culture passed down in their family for generations) and mainstream North American culture (i.e., the culture within which they currently live).

Procedures

Approval to collect data was obtained through the research ethics review board of the participating university. Caregivers were approached prior to their child's vaccination appointment. If caregivers agreed to participate, informed consent was obtained, and caregivers were asked to fill out a short demographic form before the appointment. During the vaccination appointment, caregivers held their toddlers as part of the vaccination process and video and cardiac data were simultaneously collected before and after the needle. Both caregivers and toddlers were connected to mobile monitoring devices to measure their high-frequency heart rate variability and heart rate. Based on methods from previous longitudinal studies that examined caregiver-infant dyads during vaccination (Camm et al., 1996), toddlers and their caregivers were examined for 1 minute prior to the needle and up to three minutes after the needle. Technologies from Mindware and Noldus were used to synchronize video and cardiac data during the vaccination appointment and were used for data coding or editing procedures. Procedures allowed for naturalistic observation of participants with minimal interference from the research team except for videotaping and cardiac monitoring. All participating caregivers were provided with a sheet outlining evidenced-based pain management strategies at each vaccination appointment (Taddio et al., 2010).

Measures

Demographic information

Caregivers reported demographic information for themselves (i.e., age, education, relation to child) and their child (i.e., age, sex, ethnicity). Caregivers were also asked to provide two separate ratings adapted from the Vancouver Index of Acculturation (Ryder et al., 2000). They reported the extent to which they believe their way of life reflects their self-reported

heritage culture, as well as the extent to which their way of life reflects mainstream 'North American or Canadian culture'. The ratings were on a scale of 0 to 10, with a higher score reflecting greater identification with self-reported or mainstream culture. Variables that are known to affect cardiac indictors (e.g. time since last feed, time since last nap, and number of needles; Oberlander & Saul, 2007; Waxman et al., 2016) were are also collected.

Child pain behaviours

The Face, Legs, Activity, Cry, and Consolability (FLACC) scale was used to measure child pain behaviour during the vaccination appointment (Merkel et al., 1996). The degree of pain-related distress is measured with five types of pain behaviours (face, legs, activity, cry, and consolability) during 15-second epochs from one minute pre-needle to three minutes post-needle. Each item is scored with a scale from 0 to 2, resulting in a total score between 0 and 10 for each epoch. For ease of comparison and interpretation across measures used in the current study, 15-second epochs were averaged over one-minute phases. Four phases were used for the current analyses, including baseline (FLACC Baseline; 60 to 1 second before the first needle), one minute immediately after the last needle (FLACC Post 1; 1 to 60 seconds after the last needle), two minutes after the last needle (FLACC Post 2; 61 to 120 seconds after the last needle), and three minutes after the last needle (FLACC Post 3; 121 to 180 seconds after the last needle). The FLACC scale has evidenced moderate to high validity and strong interrater reliability in the acute pain context (Merkel et al., 2002). High pain-related distress is considered in the 7 to 10 range, moderate pain in the 4 to 6 range, low/mild pain in the 1 to 3 range, and scores of 0 represent no pain-related distress. All coders in the current study were trained by an experienced primary FLACC coder and were blind to the study hypotheses. A total of 20% of the sample was

reliability-coded throughout the coding process. Interrater reliability was high (intraclass correlations between 0.9 and 0.93).

Caregiver ratings of self-worry

Caregiver perceptions of their own worry level were assessed using a numeric rating scale from 0 to 10. Parents were asked to rate worry levels immediately before and following the immunization procedure (i.e., “On a scale from 0 to 10, how worried about the needle are you right now on a scale from 0 ‘no worry at all’ to 10 ‘the most worry possible’?”).

High-frequency heart rate variability

High-frequency heart rate variability (HF-HRV) is the variation in time between heart beats and reflects a measure of parasympathetic activity (Berntson et al., 2007). Under resting conditions, the parasympathetic nervous system maintains homeostasis in the body by controlling the heart via the vagus nerve (Hastings & Miller, 2014). Increased parasympathetic influence (i.e., a state of "rest-and-digest") is reflected in higher values of HF-HRV. Individuals with higher HF-HRV at rest have shown to have a greater capacity to regulate distress (Appelhans & Luecken, 2006). However, in the face of a distressing event, temporary withdrawal of parasympathetic influence elicits autonomic arousal and allows for active coping (i.e., flight or fight response) (Hastings & Miller, 2014; Porges & Furman, 2011). The withdrawal response is captured through decreasing HF-HRV. Of note, an excessive or prolonged withdrawal response reflects inflexibility of the autonomic system (i.e., poor ability to regulate distress and adaptability to changing situations) which is associated with poorer long-term mental health outcomes (Beauchaine, 2015). Since HF-HRV is an index of regulatory capacity, it can provide unique insights on children's responses to distress in a needle pain context.

Cardiac measures were collected using Mindware wireless monitors (MW 1000A) with a sampling rate of 500 Hz. Three electrocardiograph (ECG) electrodes were placed on caregivers and their toddlers including one electrode placed over the right clavicle, one electrode placed under the rib cage on the left side, and a ground electrode placed under the rib cage on the right side. Mindware software (BioLab 3.3) was used to acquire ECG data during the vaccination appointment. The data were processed offline using Mindware HRV 3.1.5. The software uses an algorithm to identify each R wave. HF-HRV was calculated from spectral analysis of the RR intervals (i.e., the intervals between successive heart beats or R waves) (Cacioppo et al., 2000). Frequency bands within the range of spontaneous respiration (0.24-1.04 Hz for toddlers and 0.15-0.40 for adults) (Bar-Haim et al., 2000; Cacioppo et al., 2000) were used. Data were edited for artifacts due to misidentification of R waves by the software or equipment failure in the clinic (e.g., device malfunction, electrodes fell off participant). Decisions to exclude data due to artifact were made in consultation with the primary coder on an epoch-by-epoch basis. Editing of artifacts was less than 5% across the sample.

All coders were trained to edit cardiac data by an experienced primary coder. A total of 20% of data files were double-coded for reliability throughout the coding process. Unreliable codes (i.e., intraclass correlation below 0.9) were consensus coded and reviewed by the primary coder. Overall, the interrater reliability between the coders was high (intraclass correlations between 0.96 and 0.99).

HF-HRV was calculated for 30-second epochs from 1 minute pre-needle to three minutes post-needle. For analyses purposes (see Di Lorenzo-Klas et al., 2022), 30-second epochs were averaged for every minute pre- and post-needle (i.e., Baseline [60 seconds to 1 second pre-needle], Post 1 [1 to 60 seconds post-needle], Post 2 [61 to 120 seconds post-needle], and Post 3

[121 to 180 seconds post-needle]). Our methods are consistent with Task Force guidelines on HRV standards of measurement that suggest approximately 1 minute of data is needed at minimum to identify the high-frequency components of HRV (Camm et al., 1996).

Analysis plan

Distinct groups of caregiver-toddler dyads were formed according to growth mixture models (GMM) of the simultaneous HRV trajectories of caregivers and toddlers (see Di Lorenzo-Klas et al., 2022 for original analyses). Group membership served as the dependent variable in the current analyses. In our previous study (Di Lorenzo-Klas et al., 2022), demographic characteristics (e.g., child sex, child age, caregiver relation to child) were not significantly associated with group membership and are not included in the current analyses. The study variables, caregiver self-worry pre- and post-needle and FLACC variables, were checked for outliers, and correlations among variables were examined to ensure there was no evidence of multicollinearity. We also plotted FLACC scores by group membership to examine the child pain-related responses of these groups (see Figure 1).

Multinomial logistic regression was used in SPSS (Version 26; IBM Corp, 2019) to examine whether child behavioural distress and parent worry pre- and post-needle were associated with various patterns of caregiver-child attunement during vaccination. Child behavioural distress variables (FLACC scores) were entered first in the model, as they were hypothesized to have a greater influence on physiological attunement compared to parent variables. Caregiver pre- and post-needle worry ratings were subsequently entered to examine the relations between caregiver factors and attunement patterns above and beyond the child pain variables.

Results

Preliminary analyses

Descriptive statistics for study variables are provided in Table 2. Correlations among study variables are in Table 3; all variables were weakly to moderately correlated (r = .01 to .60), and there were no evident outliers.

Displaying Child behavioural pain scores according to dyad coregulatory group membership

Figure 1 shows child behavioural distress, measured with FLACC, graphed according to the caregiver-child HF-HRV regulatory trajectories. On average, Group 1 (dyads with an attuned regulatory pattern) showed low pain-related behavioural distress at baseline (M = 2.01), followed by an increase to moderate pain levels immediately after the needle (M = 5.76), and a return to near-baseline levels by the third minute following the needle (M = 2.33). Regarding the two suboptimal dyadic regulatory patterns, Group 2 (dyads showing lack of regulatory attunement) demonstrated mild pain-related distress at baseline (M = 2.55) and a subsequent increase in pain-related distress at a moderate-to-high level immediately after the needle (M = 6.80). Mean FLACC scores for Group 2 decreased to a moderate level by the third minute post-needle (M = 4.73). Group 3 (dyads with a misattuned regulatory pattern) showed mild-to-moderate pain-related distress at baseline (M = 3.15) and a steep increase to a high level of pain-related distress immediately after the needle (M = 7.63), which decreased to a low-to-moderate level by the third minute post-needle (M = 3.43).

Associations with dyad coregulatory group membership

Results from the multinomial regression are in Table 4. Group 1 was chosen as the reference category because it is the largest and demonstrated a stable HF-HRV coregulatory

response. Post 1 (χ^2 (2) = 6.90, p = .03) and Post 3 (χ^2 (2) = 5.89, p = .043) FLACC scores, as well as parent ratings of pre- (χ^2 (2) = 5.80, p = .045) and post-needle (χ^2 (2) = 11.66, p = .003) self-worry significantly contributed to the model's ability to predict group membership.

Baseline, Post 1, and Post 2 FLACC scores were not significantly associated with greater odds of membership in Group 2 rather than Group 1. A higher FLACC score during the third minute post-needle was significantly associated with an increased likelihood of being in Group 2 versus Group 1, OR = 1.41, p = .020. Higher ratings of parent self-worry pre-needle was associated with greater odds of being in Group 2 versus Group 1, OR = 1.24, p = .044, while lower parent self-worry post-needle was significantly associated with a greater likelihood of being in Group 2 versus Group 1, OR = 0.719, p = .036.

Baseline, Post 2, and Post 3 FLACC scores were not significantly associated with greater odds of membership in Group 3 instead of Group 1. However, higher FLACC scores immediately after the needle (Post 1) were associated with a greater likelihood of being in Group 3 versus Group 1, OR = 1.73, p = .024. Higher ratings of parent self-worry pre-needle was associated with greater odds of being in Group 3 versus Group 1, OR = 1.22, p = .041. Lastly, lower parent ratings of self-worry post-needle are significantly associated with a higher likelihood of being in Group 3 versus Group 1, OR = 0.68, p = 0.012.

Discussion

The goal of the current study was to investigate whether caregiver distress (self-reported worry before and after the needle) and toddler distress during the vaccination appointment (behavioural pain-related distress) were associated with caregiver-toddler physiological attunement patterns during vaccination. This study builds on previous work (Di Lorenzo-Klas et al., 2022) that discerned three latent groups of dyads that demonstrated different attunement

patterns in an acute pain context. DiLorenzo-Klas et al. (2022) showed that the largest group (i.e., Group 1) demonstrated attunement with a stable and parallel pattern of HF-HRV from baseline to post-needle. The second group of dyads showed a parallel but low pattern of HF-HRV from baseline to post-needle, indicating both dyad members are dysregulated, and the caregiver is unlikely able to respond in a reciprocal and supportive manner (i.e., lack of attunement). Finally, the third group showed diverging regulatory patterns, whereby the caregiver showed a stable regulatory response, but the child mounted an excessive withdrawal response right after the needle. This pattern reflects misattunement between dyad members, as the caregiver's attempts to provide regulatory support did not reflect their child's internal regulatory state.

The current study is unique in investigating whether child and caregiver distress during the vaccination appointment are associated with differences in physiological attunement patterns. In addition to graphically describing toddler pain scores that were associated with each of the three dyadic regulatory patterns, the current study confirmed hypotheses that both caregiver and child pain-related distress differentiated the dyads that demonstrated an attuned regulatory response from those who did not.

Consistent with our first hypothesis, dyads with less adaptive attunement patterns had higher behavioural pain-related distress compared to the attuned group of dyads, though these findings emerged at specific time points post-needle. Specifically, higher pain-related behavioural distress during the third minute post-needle was associated with a greater likelihood of being in Group 2 (dyads showing lack of regulatory attunement) versus Group 1 (dyads with an attuned regulatory pattern), indicating that the children in Group 2 continued to experience considerable pain-related distress by the third minute post-needle. This result is consistent with

their HF-HRV trajectory which suggests an inflexible parasympathetic response (i.e., stable, low HF-HRV with no withdrawal response) and reduced capacity for regulation.

Contrary to expectations, there were no significant differences in behavioural pain-related distress between dyads showing an attuned regulatory pattern (Group 1) versus dyads showing lack of regulatory attunement (Group 2) at any other time points. One possible explanation is that there is typically little variability in distress responses immediately after the needle (Pillai Riddell et al., 2013), whereas pain regulatory responses more distal to the needle will differ due to individual (e.g., child's pain threshold, regulatory capacity) and external factors (e.g., parent support) (Pillai Riddell et al., in press). It was more unexpected that there were no significant differences between Groups 1 and 2 in terms of behavioural pain-related distress at baseline, as Group 2's HF-HRV values suggest lower parasympathetic influence (i.e., internal dysregulation) leading up to the needle. Research has shown that behavioural pain-related distress is more closely linked to a heart rate that reflects more of a sympathetic or arousal response, whereas HF-HRV captures more regulatory activity because it is an index of the parasympathetic system (Waxman et al., 2020). Thus, before a distressing event, toddlers in Group 2 maintain a baseline level of arousal. However, consistent with previous research, the low levels of baseline HF-HRV suggest that the toddlers will be slower in recovering from the stressor, which is also predictive of later challenges with regulation and behavioural problems (Calkins, 1997). Further, these results suggest that toddlers in Group 2 were signalling distress; however, their caregivers where unable to regulate themselves (i.e., they had stable, low HF-HRV values) which affected their ability to attune to the child's needs in a sensitive manner to support their regulatory response (Carreras et al., 2019).

There was a different pattern of results for dyads with a misattuned regulatory pattern (Group 3) when examining associations between pain-related behavioural distress and caregiver-child coregulatory patterns. Higher behavioural pain-related distress immediately after the needle was significantly associated with a greater likelihood of being in Group 3 versus Group 1 (dyads with an attuned regulatory pattern). This result reflects the pattern of HF-HRV responding of toddlers in Group 3, as they mounted an excessive withdrawal response followed by a steep increase in HF-HRV that surpassed their baseline levels. Based on the patterns of HF-HRV and behavioural pain-related distress responses, toddlers in Group 3 demonstrated an overarousal response. Despite displaying normative baseline and regulatory responses (i.e., steady declines to low-to-moderate pain-related distress by the third minute post-needle) that were similar to Group 1, the excessive withdrawal response may reflect parasympathetic dysfunction. Previous research has linked overarousal or excessive vagal withdrawal with emotional lability and later mental health outcomes such as panic, anxiety, and mood challenges (Beauchaine, 2001; Beauchaine, 2015). Toddlers in Group 3 were signalling distress to their caregivers. However, although caregivers appeared regulated enough to respond to their child, they may not have responded in an attuned and supportive way to scaffold an adaptive regulatory response (e.g., the use of distress-promoting behaviours such as verbal reassurance can increase child pain-related distress; Pillai Riddell et al., 2018), reflecting misattunement between dyad members.

Finally, consistent with our second hypothesis, we found that higher caregiver worry before the needle was associated with a greater likelihood of displaying patterns of misattunement or lack of attunement (Groups 2 and 3) compared to a more adaptive attunement pattern (Group 1). These findings are consistent with models such as the Self-Regulation Intergenerational Transition Model (Bridgett et al., 2015) which suggest that caregivers must be

able to regulate their own emotional reactions in order to model adaptive regulatory strategies and to respond to their child's distress in way to support their regulation. Therefore, caregivers' ability to regulate their own emotions precedes their ability to organize and engage sensitive caregiving behaviours to aid with their child's distress regulation.

Contrary to expectations, lower caregiver worry after the needle was associated with a greater likelihood of being in one of the same groups that reported higher worry before the needle (i.e., Group 2 or 3: misattunement or lack of attunement). This finding was unexpected given that these dyads were less attuned and the toddlers in these groups displayed less optimal reactivity or regulatory responses. One possible explanation is that some caregivers in these groups might have resorted to using a response-focused regulatory strategy (i.e., avoidance and suppression of emotion), which is often used to regulate a distress response when earlier efforts have failed, instead of using an early antecedent regulatory strategy (e.g., reappraisal) before the event (Gross & Thompson, 2007). It is possible that, before the procedure, caregivers in Group 2 and 3 experienced anticipatory feelings of distress, which then affected their ability to attune to their child's needs and support their regulation from pain-related distress. Consequently, their reduced report of worry post-needle may reflect attentional avoidance to reduce their prolonged distress associated with their child's needle procedure.

Limitations

The findings of the current study should be interpreted in the context of some limitations that offer directions for future research. The current study examined a low-risk sample (i.e., highly educated families with healthy toddlers) and analyses were limited by the small sample sizes of dyads in Groups 2 (n = 15) and Group 3 (n = 21). This study did not examine parent behaviours that are known to support toddler distress regulation (e.g., physical comfort and

distraction). Future work should examine these behaviours to elucidate whether discrete parent behaviours support or hinder attunement between dyads. Further, it would be informative to examine whether certain distress-promoting behaviours and lower parent emotional availability are associated with lack of attunement or misattunement patterns between caregivers and their children, as previous research has shown that parent insensitive behaviours predict greater pain-related distress (Badovinac et al., 2018).

Conclusions and clinical implications

The current results showed that caregiver and child factors are associated with differences in the coregulatory experience of caregivers and their toddlers during vaccination. Our findings suggest that caregivers who report psychological distress before vaccination are less connected to their toddlers in terms of their physiological regulatory responses and their toddlers experience greater pain-related distress. These caregivers likely lack the capacity to regulate themselves or offer resources to support their children's regulation from distress. Consequently, they resort to maladaptive regulatory strategies, such as suppressing or avoiding their distress, as these same caregivers reported lower worry after the needle compared to the attuned group of dyads.

Early vaccination procedures are a common source of pain, fear, and distress for young children which can lead to elevated anxiety, fear, and pain associated with needles in the future, as well as avoidance of other health care. An important precursor to understanding how to best support children in acute pain contexts is understanding the factors that shape these early experiences. It is widely acknowledged that caregivers play an important role in providing external regulatory support for their children during painful experiences. Identifying how caregivers support their children within the routine vaccination context is important given the importance of the first few years of life for laying the foundation of child regulation skills. The

findings from this study help to identify caregivers who may or may not be attuned to their child's needs, and how caregivers' own distress can hinder attunement leading to greater pain-related distress experienced by their toddlers. From a clinical perspective, encouraging caregivers to be mindful of their own arousal and worry pre-needle and providing support to help their distress regulation prior to the vaccination may enhance their capacity to shift focus to their children's needs and help their children's distress regulation. Future directions should include efforts to educate primary health care professionals to screen for parents who may require more support in helping their child regulate during vaccination.

References

Appelhans, B. M., & Luecken, L. J. (2006). Heart rate variability as an index of regulated emotional responding. *Review of General Psychology*, *10*(3), 229–240.

Atkinson, L., Gonzalez, A., Kashy, D. A., Santo Basile, V., Masellis, M., Pereira, J., Chisholm, V., & Levitan, R. (2013). Maternal sensitivity and infant and mother adrenocortical function across challenges. *Psychoneuroendocrinology*, *38*(12), 2943–2951. https://doi.org/10.1016/j.psyneuen.2013.08.001

Atkinson, L., Jamieson, B., Khoury, J., Ludmer, J., & Gonzalez, A. (2016). Stress physiology in infancy and early childhood: Cortisol flexibility, attunement and coordination. *Journal of Neuroendocrinology*, *28*(8). https://doi.org/10.1111/jne.12408

Augustine, M. E., & Leerkes, E. M. (2019). Associations between maternal physiology and maternal sensitivity vary depending on infant distress and emotion context. *Journal of Family Psychology*, *33*(4), 412–421. https://doi.org/10.1037/fam0000538

Badovinac, S., Gennis, H., Riddell, R. P., Garfield, H., & Greenberg, S. (2018). Understanding the relative contributions of sensitive and insensitive parent behaviors on infant vaccination pain. *Children*, *5*(6), 80. https://doi.org/10.3390/children5060080

Bar-Haim, Y., Marshall, P. J., & Fox, N. A. (2000). Developmental changes in heart period and high-frequency heart period variability from 4 months to 4 years of age. *Developmental Psychobiology*, *37*(1), 44–56.

Bearden, D. J., Feinstein, A., & Cohen, L. L. (2012). The influence of parent preprocedural anxiety on child procedural pain: Mediation by child procedural anxiety. *Journal of Pediatric Psychology*, *37*(6), 680–686. https://doi.org/10.1093/jpepsy/jss041

Beauchaine, T. (2001). Vagal tone, development, and Gray's motivational theory: Toward an integrated model of autonomic nervous system functioning in psychopathology. *Development and Psychopathology*, *13*(2), 183–214. https://doi.org/10.1017/S0954579401002012

Beauchaine, T. P. (2015). Respiratory sinus arrhythmia: A transdiagnostic biomarker of emotion dysregulation and psychopathology. *Current Opinion in Psychology*, *3*, 43–47.

Beeghly, M., Perry, B. D., & Tronick, E. (2016). *Self-Regulatory Processes in Early Development* (S. Maltzman, Ed.; Vol. 1). Oxford University Press. https://doi.org/10.1093/oxfordhb/9780199739134.013.3

Bernard, R. S., & Cohen, L. L. (2006). Parent anxiety and infant pain during pediatric immunizations. *Journal of Clinical Psychology in Medical Settings*, *13*(3), 282–287.

Berntson, G. G., Quigley, K. S., & Lozano, D. (2007). Cardiovascular psychophysiology. In *Handbook of psychophysiology, 3rd ed* (pp. 182–210). Cambridge University Press. https://doi.org/10.1017/CBO9780511546396.008

Boyce, W. T., Quas, J., Alkon, A., Smider, N. A., Essex, M. J., & Kupfer, D. J. (2001). Autonomic reactivity and psychopathology in middle childhood. *The British Journal of Psychiatry*, *179*(2), 144–150.

Braungart-Rieker, J. M., Garwood, M. M., Powers, B. P., & Wang, X. (2001). Parental sensitivity, infant affect, and affect regulation: Predictors of later attachment. *Child Development*, *72*(1), 252–270.

Bridgett, D. J., Burt, N. M., Edwards, E. S., & Deater-Deckard, K. (2015). Intergenerational transmission of self-regulation: A multidisciplinary review and integrative conceptual framework. *Psychological Bulletin*, *141*(3), 602–654. https://doi.org/10.1037/a0038662

Cacioppo, J. T., Tassinary, L. G., & Berntson, G. G. (Eds.). (2000). *Handbook of psychophysiology* (2nd ed). Cambridge University Press.

Calkins, S. D. (1997). Cardiac vagal tone indices of temperamental reactivity and behavioral regulation in young children. *Developmental Psychobiology*, *31*(2), 125–135. https://doi.org/10.1002/(sici)1098-2302(199709)31:2<125::aid-dev5>3.0.co;2-m

Calkins, S. D. (2011). Caregiving as coregulation: Psychobiological processes and child functioning. In *Biosocial foundations of family processes* (pp. 49–59). Springer.

Calkins, S. D., & Hill, A. (2007). Caregiver influences on emerging emotion regulation: biological and environmental transactions in early development. In *Handbook of emotion regulation* (pp. 229–248). The Guilford Press.

Camm, A. J., Malik, M., Bigger, J. T., Breithardt, G., Cerutti, S., Cohen, R. J., ... & Singer, D. H. (1996). Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *Circulation*, *93*(5), 1043–1065. https://doi.org/10.1161/01.CIR.93.5.1043

Carreras, J., Carter, A. S., Heberle, A., Forbes, D., & Gray, S. A. O. (2019). Emotion regulation and parent distress: Getting at the heart of sensitive parenting among parents of preschool children experiencing high sociodemographic risk. *Journal of Child and Family Studies*, *28*(11), 2953–2962. https://doi.org/10.1007/s10826-019-01471-z

DiCorcia, J. A., & Tronick, E. (2011). Quotidian resilience: Exploring mechanisms that drive resilience from a perspective of everyday stress and coping. *Neuroscience & Biobehavioral Reviews*, *35*(7), 1593–1602. https://doi.org/10.1016/j.neubiorev.2011.04.008

Di Lorenzo, M. G., Bucsea, O., Rumeo, C., Waxman, J. A., Flora, D. B., Schmidt, L. A., & Riddell, R. P. (2021). Caregiver and young child biological attunement in distress contexts: A systematic review and narrative synthesis. *Neuroscience & Biobehavioral Reviews*.

DiLorenzo-Klas, M. G., Waxman, J. A., Flora, D. B., Schmidt, L. A., Garfield, H., Flanders, D., Weinberg, E., Savlov, D., Pillai Riddell, R. R. (2022). *Distinct trajectories of caregiver-toddler physiological attunement during routine vaccinations*. Manuscript submitted for publication.

Feldman, R. (2012). Parent-infant synchrony: A biobehavioral model of mutual influences in the formation of affiliative bonds. *Monographs of the Society for Research in Child Development*, *77*(2), 42–51. https://doi.org/10.1111/j.1540-5834.2011.00660.x

Gross, J. J., & Thompson, R. A. (2007). Emotion regulation: Conceptual foundations. In *Handbook of emotion regulation* (pp. 3–24). The Guilford Press.

Hastings, P. D., & Miller, J. G. (2014). Autonomic regulation, polyvagal theory, and children's prosocial development. *Prosocial Development: A Multidimensional Approach*, 112–127.

Hill-Soderlund, A. L., Mills-Koonce, W. R., Propper, C., Calkins, S. D., Granger, D. A., Moore, G. A., Gariepy, J.-L., & Cox, M. J. (2008). Parasympathetic and sympathetic responses to the strange situation in infants and mothers from avoidant and securely attached dyads. *Developmental Psychobiology*, *50*(4), 361–376. https://doi.org/10.1002/dev.20302

IBM Corp. (2019). *IBM SPSS Statistics for Mac, Version 26.0*. IBM Corp.

Leerkes, E. M., Su, J., Calkins, S. D., O'Brien, M., & Supple, A. J. (2017). Maternal physiological dysregulation while parenting poses risk for infant attachment disorganization and behavior problems. *Development and Psychopathology*, *29*(1), 245–257. https://doi.org/10.1017/S0954579416000122

Leerkes, E. M., Su, J., Calkins, S. D., Supple, A. J., & O'Brien, M. (2016). Pathways by which mothers' physiological arousal and regulation while caregiving predict sensitivity to infant

distress. *Journal of Family Psychology*, *30*(7), 769–779. https://doi.org/10.1037/fam0000185

Merkel, S., Voepel-Lewis, T., & Malviya, S. (2002). Pain assessment in infants and young children: The FLACC scale. *The American Journal of Nursing*, *102*(10), 55–58.

Merkel, S., Voepel-Lewis, T., Shayevitz, J., & Malviya, S. (1996). The FLACC: A behavioral scale for scoring postoperative pain in young children. *Pediatric Nursing*, *23*, 293–297.

Nicol-Harper, R., Harvey, A. G., & Stein, A. (2007). Interactions between mothers and infants: Impact of maternal anxiety. *Infant Behavior & Development*, *30*(1), 161–167. https://doi.org/10.1016/j.infbeh.2006.08.005

Nunan, D., Sandercock, G. R. H., & Brodie, D. A. (2010). A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *Pacing and Clinical Electrophysiology*, *33*(11), 1407–1417. https://doi.org/10.1111/j.1540-8159.2010.02841.x

Oberlander, Tim, & Saul, JP. (2007). Methodological considerations in heart rate variability analysis. In F. Rautaharju & P. Rautaharju (Eds.), *Investigative Electrocardiography in Epidemiological Studies and Clinical Trials* (pp. 68–73). Springer. https://doi.org/10.1007/978-1-84628-481-6_5

Pillai Riddell, R., Gennis, H., Tablon, P., Greenberg, S., & Garfield, H. (2018). Developing a measure of distress-promoting parent behaviors during infant vaccination: Assessing reliability and validity. *Canadian Journal of Pain*, *2*(1), 135–144. https://doi.org/10.1080/24740527.2018.1471325

Porges, S. W., & Furman, S. A. (2011). The early development of the autonomic nervous system provides a neural platform for social behaviour: A polyvagal perspective. *Infant and Child Development*, *20*(1), 106–118.

Qu, J., & Leerkes, E. M. (2018). Patterns of RSA and observed distress during the still-face paradigm predict later attachment, compliance and behavior problems: A person-centered approach. *Developmental Psychobiology*, *60*(6), 707–721.

Pillai Riddell, R., Flora, D. B., Stevens, S. A., Stevens, B., Cohen, L. L., Greenberg, S., & Garfield, H. (2013). Variability in infant acute pain responding meaningfully obscured by averaging pain responses. *PAIN®*, *154*(5), 714–721.

Pillai Riddell, R., Jasim, S., & Hamwi, L. (in press). Out of the mouth of babes: A lot about pain has nothing to do with pain. *PAIN.*

Pillai Riddell, R., & Racine, N. (2009). Assessing pain in infancy: The caregiver context. *Pain Research and Management*, *14*(1), 27–32.

Roubinov, D., Tein, J.-Y., Kogut, K., Gunier, R., Eskenazi, B., & Alkon, A. (2020). Latent profiles of children's autonomic nervous system reactivity early in life predict later externalizing problems. *Developmental Psychobiology*. https://doi.org/10.1002/dev.22068

Ryder, A. G., Alden, L. E., & Paulhus, D. L. (2000). Is acculturation unidimensional or bidimensional? A head-to-head comparison in the prediction of personality, self-identity, and adjustment. *Journal of Personality and Social Psychology*, *79*(1), 49.

Taddio, A., Appleton, M., Bortolussi, R., Chambers, C., Dubey, V., Halperin, S., Hanrahan, A., Ipp, M., Lockett, D., MacDonald, N., Midmer, D., Mousmanis, P., Palda, V., Pielak, K., Pillai Riddell, R., Rieder, M., Scott, J., & Shah, V. (2010). Reducing the pain of childhood vaccination: An evidence-based clinical practice guideline. *CMAJ*, *182*(18), E843–E855. https://doi.org/10.1503/cmaj.101720

van Bakel, H. J. A., & Riksen-Walraven, J. M. (2008). Adrenocortical and behavioral attunement in parents with 1-year-old infants. *Developmental Psychobiology*, *50*(2), 196–201. https://doi.org/10.1002/dev.20281

Waxman, J. A., DiLorenzo, M. G., Pillai Riddell, R. R., Flora, D. B., Greenberg, S., & Garfield, H. (2017). Preschool needle pain responding: Establishing 'normal.' *The Journal of Pain*, *18*(6), 739–745.

Waxman, J. A., DiLorenzo, M. G., Pillai Riddell, R. R., Flora, D. B., Schmidt, L. A., Garfield, H., Flanders, D., Weinberg, E., & Savlov, D. (2021). Investigating convergence of cardiac and behavioral indicators of distress during routine vaccinations over the second year of life. *Developmental Psychobiology*, *63*(3), 437–451.

Waxman, J. A., DiLorenzo, M. G., Pillai Riddell, R. R., Flora, D. B., Schmidt, L. A., Garfield, H., Flanders, D., Weinberg, E., & Savlov, 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.

Waxman, J., Martin, J., & Pillai Riddell, R. (2017). Understanding infant pain responding within a relational context. In *Neonatal Pain* (pp. 89–104). Springer.

Waxman, J. A., Pillai Riddell, R. R., Tablon, P., Schmidt, L. A., & Pinhasov, A. (2016) . Development of cardiovascular indices of acute pain responding in infants: A systematic review. *Pain Research and Management*; Hindawi. https://doi.org/10.1155/2016/8458696

Woodruff-Borden, J., Morrow, C., Bourland, S., & Cambron, S. (2002). The behavior of anxious parents: Examining mechanisms of transmission of anxiety from parent to child. *Journal of Clinical Child & Adolescent Psychology*, *31*(3), 364–374. https://doi.org/10.1207/S15374424JCCP3103_08

Figure 1

Child pain-related distress scores according to dyad coregulatory group membership. Pain-related distress scores, measured with the Face, Legs, Activity, Cry and Consolability (FLACC) scale, were calculated post-hoc using the exported class membership variable.

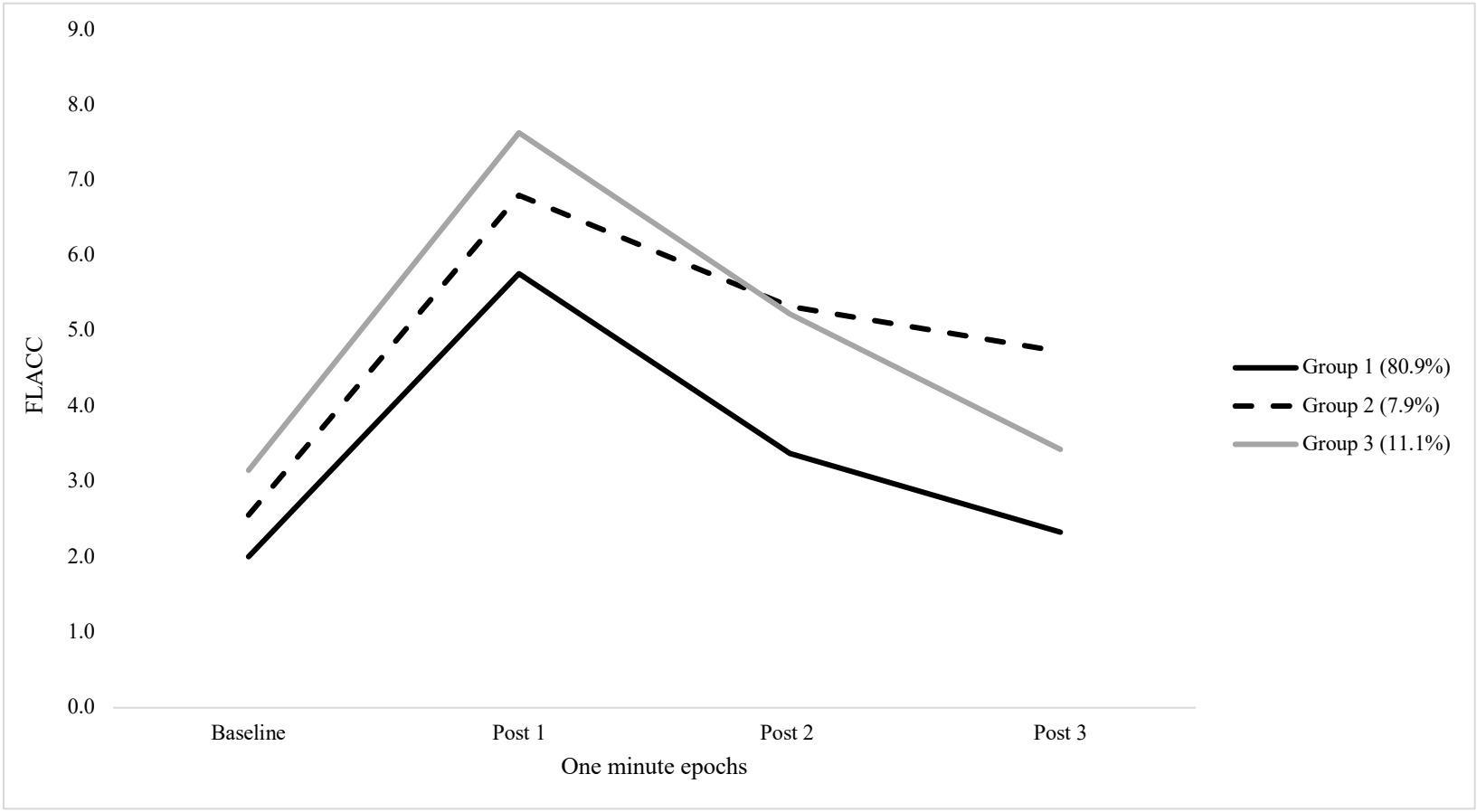


Table 1. Demographic and personal characteristics.

Caregiver age in years, M (SD)	36 (5.54)
Toddler age in months, M (SD)	17.04 (4.80)
Toddler age by appointment, n (%)	
12 months	81 (42.9)
18 months	66 (34.9)
24 months	42 (22.2)
Sex of toddler, n (%)	
Male	104 (55.0)
Female	85 (45.0)
Relationship to toddler, n (%)	
Mother	163 (86.2)
Father	24 (12.7)
Other	2 (1.1)
Caregiver Education, n (%)	
Graduate school/professional training	92 (48.7)
University graduate	60 (31.7)
Partial university (at least 1 year)	5 (2.6)
Trade school/community college	26 (13.8)
High School Graduate	2 (1.1)
Missing	4 (2.1)
Acculturation status, M (SD)	
Way of life reflects heritage culture	7.78 (2.13)
Way of life reflects mainstream North American/Canadian Culture	6.33 (2.83)
At vaccination appointment:	
Minutes since last feed for toddler, M (SD)	102.22 (71.46)

Minutes since last nap for toddler, M (SD)	137.38 (101.53)
Number of needles toddler received, Mode (range)	1 (1-3)

Table 2. Study variable descriptive statistics.

	Mean	SD	Range
Group 1 – Normative-stable (80.1%)			
FLACC Baseline	2.01	2.41	0-9.75
FLACC Post 1	5.76	2.39	0-10.00
FLACC Post 2	3.37	2.67	0-9.00
FLACC Post 3	2.33	2.54	0-8.46
Parent pre-needle worry	2.38	2.40	0-10.00
Parent post-needle worry	2.10	2.50	0-9.00
Group 2 – Dysregulated (7.9%)			
FLACC Baseline	2.56	2.46	0-8.25
FLACC Post 1	6.80	2.18	0.5-8.75
FLACC Post 2	5.33	2.93	0-8.75
FLACC Post 3	4.73	3.13	0-9.75
Parent pre-needle worry	2.73	3.26	0-8.00
Parent post-needle worry	1.80	2.46	0-7.00
Group 3 – Reactive & Overcompensation (11.0%)			
FLACC Baseline	3.15	2.72	0-8.00
FLACC Post 1	7.63	1.07	4.50-9.00
FLACC Post 2	5.23	1.98	0.50-8.25
FLACC Post 3	3.43	2.43	0-8.50
Parent pre-needle worry	3.19	3.63	0-10.00
Parent post-needle worry	1.71	2.26	0-7.00

Abbreviations: FLACC = Face, Legs, Activity, Cry, and Consolability Scale.

Table 3. Correlations among predictors.

	1.	2.	3.	4.	5.	6.
1. FLACC Baseline	_	.37*	.39*	.39*	.17*	.25*
2. FLACC Post 1		_	.55*	.42*	.03	.22*
3. FLACC Post 2			_	.60*	.01	.29*
4. FLACC Post 3				_	.01	.34*
5. Parent pre-needle self-worry					_	.48*
6. Parent post-needle self-worry						_

Note. *$p < .05$; FLACC = Face, Legs, Activity, Cry, and Consolability scale.

Table 4. Relations between caregiver and child pain-related distress and group membership

Predictor	*B*	*SE B*	OR	*p*
Group 2 vs. Group 1:				
FLACC Baseline	-0.07	0.13	0.93	.574
FLACC Post 1	0.08	0.19	1.08	.689
FLACC Post 2	0.12	0.19	1.13	.532
FLACC Post 3	0.34*	0.15	1.41	.020
Parent pre-needle self-worry	0.22*	0.12	1.24	.044
Parent post-needle self-worry	-0.33*	0.16	0.72	.036
Group 3 vs. Group 1:				
FLACC Baseline	0.04	0.10	1.04	.672
FLACC Post 1	0.55*	0.24	1.73	.024
FLACC Post 2	0.11	0.16	1.11	.503
FLACC Post 3	0.09	0.13	1.10	.522
Parent pre-needle self-worry	0.20*	0.10	1.22	.041
Parent post-needle self-worry	-0.39*	0.15	0.68	.012

Note. *$p < .05$; Model χ^2 (12) = 37.09, $p < 0.001$; Group 1 = Normative-stable, Group 2 = Dysregulated, Group 3 = Reactive with overcompensation; FLACC = Face, Legs, Activity, Cry and Consolability Scale.

Chapter 7: Conclusion

The biological substrates of emotion regulation are partly organized, early in life, through exchanges with one's caregiver (Bornstein, 2013; Feldman, 2007). When a caregiver is attuned with their young child, their ability to regulate their own distress and respond sensitively and contingently to the child can positively shape the child's regulatory capacity to handle stress (Kopp, 1989). Up to 75% of mental health diagnoses are said to be associated with maladaptive regulation of affective states (Jazaieri et al., 2013). Thus, it behooves researchers to understand how the substrates of regulatory capacity are organized or disrupted through repeated interactions with caregivers during early development. Specifically, understanding how caregiver-child regulatory attunement unfolds in early childhood is crucial to supporting caregiver and young child mental health interventions that focus on strengthening children's regulation of distress, while the skill is more developmentally malleable.

The current dissertation provided novel insights that advance our understanding of the development of caregiver-toddler biological attunement using a naturalistic, high-distress paradigm (i.e., the vaccination context). Three studies were conducted that made innovative contributions to the broader developmental attunement literature, but also deepened our understanding of the importance of the caregiver in supporting children in a pain context (consistent with DIAPR-R model; Pillai Riddell et al., in press). The objective of Study 1 was to systematically review the literature on caregiver-child biological attunement within distress contexts during the first few years of life. The synthesis revealed inconsistent relations between most of the caregiver and child biological outcomes examined in distress contexts across the literature. We posited that the lack of consistency in results was due to limitations of methodological approaches used to capture the complexity of the dyadic attunement process.

Suggestions for improving the quality and consistency of methodology used to study biological attunement were discussed. Limitations of the literature were also highlighted that set the stage for Study 2, which involved moving beyond examining attunement outcomes averaged across samples of dyads. Instead, Study 2 used person-centered analyses to examine variable patterns of caregiver-toddler regulatory attunement trajectories (i.e., concurrent caregiver and toddler HF-HRV) during vaccinations across the second year of life. The results revealed distinct adaptive and suboptimal caregiver-toddler attunement patterns and highlighted the need to further understand these distinct coregulatory patterns. Subsequently, Study 3 built on the findings of Study 2 by examining caregiver and child pain-related distress as predictors of caregiver-toddler attunement patterns. In the sections that follow, summaries of the findings from each of the three studies are presented, as well as an integrative synthesis and discussions of the clinical implications, limitations, and future directions for research. As a reminder, a summary of the objectives, analyses, and main findings for all three studies are also provided in Appendix A.

Study 1: Synthesizing the literature on caregiver and young child biological attunement in distress contexts

The overarching goal of Study 1 was to synthesize the literature examining caregiver and young child biological attunement in distress contexts. Specifically, the study aimed to conceptually organize previous literature according to the various methodological approaches that researchers have used to study attunement. Findings were synthesized according to biological indicators of distress (cortisol, saliva alpha amylase, cardiac indicators [respiratory sinus arrythmia and heart period], and electroencephalography) and broad statistical analysis groupings. The statistical grouping considered whether analyses captured the dynamic coordination and/or reciprocal nature of the attunement process: 1) static concurrent and non-

reciprocal (e.g., correlations, regressions), 2) dynamic concurrent and non-reciprocal (e.g., multilevel models that examine change over time), and 3) dynamic concurrent and reciprocal (e.g., cross-lagged modeling). The results were further contextualized based on the timing of measurement (i.e., epoch types: baseline, immediate response, and recovery response, or collapsed across time), developmental stage (Infancy [0-11 months], Early Toddlerhood [12-23 months], and Middle/Late Toddlerhood [24-47 months]), and covariates or moderators. Consistent patterns were not easily discerned due to the heterogeneity of methods used. The following discussion highlights some patterns that were obtained from the synthesis, as well as current methodological challenges and important gaps in the literature.

Relations between caregiver and child biological distress responses

Overall, the most consistent relations found in the literature were between caregiver and child cortisol outcomes, though this finding is not surprising given that 25 of the 36 studies used cortisol as an indicator of distress. The following results were gleaned from cortisol studies: 1) moderate to large positive (static concurrent and non-reciprocal) relations were found between caregiver and child cortisol responses during baseline and the immediate response period; 2) moderate to large positive (dynamic concurrent and non-reciprocal) relations were found between changes in caregiver and child cortisol outcomes over time; and 3) only a few studies examined dynamic concurrent and reciprocal relations between caregiver and child cortisol outcomes during infancy and early toddlerhood (less than 17 months old) and found weak to small, positive reciprocal relations between dyad outcomes. These findings suggest that cortisol can capture dyadic coordination of distress states; however, there are some challenges in measuring cortisol that researchers should consider moving forward when examining dyadic attunement. Specifically, given the very dynamic nature of the attunement process (i.e., moment-

to-moment changes), and the variability in timing when cortisol peaks in early life following a stressor (Ramsay & Lewis, 1994), the typical three or four measurements of cortisol that researchers collect may not adequately capture the dynamic changes that occur in the attunement process. Further, most of the cortisol studies used static analyses that did not capture the dynamic or reciprocal nature of the attunement process.

Compared to cortisol, the relations between caregiver and child sAA, cardiac outcomes, and EEG were mostly weak. These findings are likely attributable to many factors including: 1) there were very few studies of these outcomes (e.g., only two studies on EEG outcomes); 2) all studies used static concurrent and non-reciprocal analyses; 3) sAA, a measure of sympathetic nervous system activity, reflects a measure of immediate arousal and may not adequately capture the attunement process which involves coregulation from distress; and 4) although cardiac measures provide a window into the dynamic changes that occur in response to stress, none of the studies examined dynamic changes in cardiac measures over time to capture the attunement process. Thus, more research is needed to determine whether these measures are reliable biological indicators of attunement. When examining different age groupings, there were no evident developmental patterns across age, likely because most studies (25/36) examined infancy, highlighting the need for more studies of attunement in other periods of early childhood (i.e., 12 to 47 months old). Finally, a variety of moderators was examined in the literature, including caregiver mental health, caregiver sensitivity, intimate partner violence, negative parenting behaviours, and attachment status. Caregiver sensitivity was the only moderator that emerged with consistent results, with higher caregiver sensitivity associated with positive relations between caregiver and child cortisol.

Methodological challenges and gaps in the literature

Taken together, the inconsistencies in findings from the synthesis result from certain types of methodology as well as variability in approaches that may not fully capture caregiver and young child biological attunement. There were key methodological considerations that were elucidated from Study 1, including: (1) biological indicators of distress should be carefully chosen to reflect the concept under study (i.e., attunement as a dynamic, coregulatory process); (2) in attempt to reduce measurement errors, limitations of biomarkers should be considered and addressed when possible (e.g., use more frequent measurements to capture dynamic changes in distress responses, control for variables that can influence biological outcomes, examine multiple biomarkers which highlight how attunement unfolds according to different biological systems [e.g. PNS, SNS, HPA axis]); (3) researchers should improve measurement approaches (e.g., capture changes from pre- to post-stressor, use frequent and consecutive epochs to capture changes in distress responses instead of collapsing across epochs) and reporting practices (e.g. report reliability of cardiac data editing); and (4) more research is needed that uses analyses that better capture the attunement process, including the dynamic and reciprocal nature of attunement (e.g. cross-lagged models), as well as potential distinct patterns of attunement (e.g. growth mixture modeling), as it is unlikely that all dyads display the same coregulatory pattern given the variability in children's distress regulatory responses that have been evidenced in high distress contexts (Pillai Riddell et al., 2013; Waxman et al., 2017)

To address gaps in the literature and methodological considerations outlined in the systematic review, Study 2 provided a unique investigation of dynamic patterns of cardiac regulatory attunement in an acute pain context.

Study 2: Examining patterns of caregiver-toddler regulatory attunement in an acute pain context

The goal of Study 2 was to examine patterns of caregiver-toddler attunement patterns, as indexed by high-frequency heart rate variability (HF-HRV), during routine vaccinations across the second year of life (12-, 18-, or 24-month vaccinations). Caregiver and child HF-HRV were measured concurrently from one minute pre-vaccination to three minutes post-vaccination. Parallel-process growth mixture modelling (GMM; Muthén & Shedden, 1999) was used to represent variability in caregiver-toddler coregulatory responses. Findings were further contextualized by examining heart rate outcomes of dyads from each group derived from the GMM. Lastly, post-hoc analyses were done to assess whether dyadic or contextual control variables (i.e., toddler sex, toddler age, relation to child, baseline caregiver and toddler HF-HRV, time of last nap, time of last feed, number of needles toddler received) predicted group membership.

The data for Study 2 came from a subsample of caregiver-toddler dyads (N = 189) who were a part of a longitudinal cohort, and dyads had cardiac data that were collected at either 12-, 18-, or 24-month vaccination appointments. For the first time in the literature, dyad cardiac regulatory attunement patterns were elucidated within a high distress context. Specifically, GMM uncovered three dyad groupings that differed in their cardiac regulatory responses during vaccination. The largest group (80 percent of dyads) demonstrated physiological attunement, whereby caregivers and their children had similar, stable HF-HRV trajectories, indicating adaptive regulatory responses in a prototypical high distress context. The second group (7.9 percent of dyads) showed a lack of attunement among dyad members, as both caregivers and their children evidenced parallel but less regulated (i.e., low HF-HRV) trajectories, suggesting caregivers were trying to regulate their own distress and unable to support their toddler in regulating from distress. The third group (11.1 percent of dyads) displayed a misattuned pattern

of coregulatory trajectories as the caregivers' regulatory supports did not reflect the children's distress response; caregivers showed a stable regulatory response, but their children mounted an excessive withdrawal response right after the needle (i.e., steep decrease in HF-HRV followed by an increase that surpassed baseline levels). Post hoc analyses revealed that lower caregiver and toddler baseline HF-HRV predicted an increased likelihood of Group 2 membership (dyads showing lack of regulatory attunement) over Group 1 membership (attuned dyads), indicating that both caregivers and toddlers in this group had lower regulatory functioning from before the needle.

Building on the findings from Study 2, the final study of this dissertation (Study 3) examined associations between caregiver and child pain-related distress and cardiac regulatory attunement patterns to further understand the dyad groupings.

Study 3: Examining relations between caregiver and child pain-related distress and dyadic regulatory attunement patterns

The primary aim of Study 3 was to examine potential correlates of the caregiver-toddler regulatory attunement patterns discerned in Study 2. Specifically, multinomial logistic regression was used to examine whether caregiver distress (operationalized by self-reported worry pre- and post-vaccination) and child distress (operationalized by behavioural pain-related distress pre- and post-vaccination) were related to group membership. The analyses used the same participants as Study 2.

The following findings emerged: 1) higher toddler pain-related distress at three minutes post-needle was associated with a greater likelihood of being in Group 2 (dyads showing lack of regulatory attunement) versus Group 1 (dyads with an attuned regulatory pattern), indicating a prolonged stress reaction and slower recovery from distress in toddlers in Group 2; 2) higher

toddler pain-related distress immediately after the needle was associated with a greater likelihood of being in Group 3 (dyads with a misattuned regulatory pattern) versus Group 1, which is consistent with the excessive withdrawal response shown by toddlers in Group 3 and corroborates an overarousal pattern in these toddlers in response to vaccination; and 3) higher caregiver worry before the needle and lower caregiver worry after the needle was associated with a greater likelihood of displaying patterns of misattunement or lack of attunement (Groups 2 and 3) instead of a more adaptive attunement pattern (Group 1). The final pattern of results indicates that caregivers who self-reported anticipatory feelings of distress associated with their child's vaccination struggled to attune to their child and support their child's regulation from distress, and subsequently, may have engaged in attentional avoidance strategies to try to reduce a prolonged pain-related distress response.

The next section presents a synthesis of all three studies from the current dissertation, with a focus on the contributions to the literature.

Integrative Synthesis

Building on the existing literature of dyadic attunement, the three studies in the current dissertation make important contributions to literature, as the studies highlight limitations of methodological approaches used to study biological attunement, offer methodological guidelines for future research, and provide novel findings which illustrate emerging patterns of adaptive and maladaptive biological attunement in an acute pain context. Based on all three studies, it is evident that caregiver-toddler attunement is a complex and dynamic interactional process that unfolds on a moment-to-moment basis, and diverse patterns can emerge early in life through interactions with one's caregiver in distress contexts.

Specifically, consistent with notions previously discussed in the literature (Bernard et al., 2017), capturing the process of attunement cannot be simplified into a single correlation. Collective findings from this dissertation show that caregiver-toddler attunement is better represented by using methodological approaches, including biological indicators and statistical analyses, that capture the dynamic and the reciprocal nature of the process. Moreover, consistent with previous research that captured distinct profiles of pain-related distress regulation in young children (Pillai Riddell et al., 2013; Waxman et al., 2017), there are distinct patterns of caregiver-toddler regulatory attunement that reflect the complexity of dyadic regulatory efforts which may be beneficial or suboptimal in supporting children from regulating from pain-related distress and influences the development of their distress regulation skills. A simple mean response ignores the variability of responding post-needle, both over the course of the appointment and between individuals.

The distinct patterns of attunement and associated pain-related distress variables (caregiver self-reported and child behavioural distress) can be understood in the context of attachment patterns. Specifically, most dyads demonstrated a coregulatory pattern (i.e., Group 1) that was consistent with what would be expected in a secure attachment relationship (Cassidy, 1994). Both caregivers and their toddlers demonstrated minimal internal dysregulation, but the child within the distressing vaccination context felt secure enough to signal pain-related distress behaviour to gain the external regulatory support of their caregiver as needed. The other two coregulatory patterns that emerged are more consistent with insecure attachment patterns. The pattern whereby toddlers and their caregivers demonstrated 'under/dysregulation' (i.e., Group 2; stable, low HRV levels with no vagal withdrawal) may reflect an anxious or ambivalent attachment pattern. Caregivers of children with anxious or ambivalent attachment are often

unable to accurately identify and appropriately respond to their child's distress (Cassidy, 1994; Cassidy & Berlin, 1994), which may partly reflect in their inability to regulate their own distress. The final attunement pattern (Group 3) was characterized by caregivers who showed a normative HRV trajectory; however, their children displayed a pattern of 'over-regulation' (i.e., an excessive HRV withdrawal response). This pattern potentially reflects an avoidant attachment pattern, as children are using an excessive suppression strategy when they cannot rely on external regulatory support from their caregiver (Marvin et al., 2002; Qu & Leerkes, 2018). Although caregivers in Group 3 did not appear internally dysregulated, they self-reported higher anticipatory distress compared to the normative group, which likely interfered with their ability to support their child in distress. It was unexpected that caregivers in both groups demonstrating less adaptive attunement patterns self-reported lower distress after the needle compared to the caregivers in the group demonstrating adaptive attunement. However, if the caregivers are having difficulty regulating themselves or helping their children regulate from distress, it follows that such a prolonged pattern of distress would be difficult to maintain overtime, leading to suppression or avoidance strategies (Gross & Thompson, 2007; Qu & Leerkes, 2018).

Clinical Implications

Several clinical implications are offered based on the findings from the current dissertation. First, identification of suboptimal coregulatory cardiac response profiles may be a useful marker for early risk. Specifically, identifying patterns of responding that confer risk for developing poor regulatory skills in young children has important implications for early screening to identify dyads in need of support. There is a multitude of intervention efforts designed to enhance caregiver awareness and responsiveness to child's needs, including Circle of Security (Cassidy et al., 2010) and Attachment and Biobehavioural Catch up (Bick & Dozier,

2013). Identifying coregulatory profiles at-risk can not only be useful to inform which dyads may need intervention, but also provides a useful marker to gauge intervention success if coregulatory profiles are monitored over time.

Second, findings demonstrated that parents' capacity to regulate their own distress plays a key role in allowing them to help their children regulate from distress. Healthcare professionals should examine whether the caregiver may need support regulating their own emotions. In relevant circumstances, parents should be empowered by health care professionals to seek their own mental health support as well as take part in interventions that provide skill-building around better allocating their own internal resources during parenting; a context that places a lot of demands on caregivers. Analogous to the 'oxygen masks on a plane' metaphor (i.e., put on your own mask before assisting a child) that is often used when working with caregivers, clinicians should encourage caregivers to attend to their own emotional needs to harness the capacity that is required to respond to their children's needs.

Third, dyads were studied in the vaccination context, which is a naturalistic setting and a unique window into observing parent-child coregulatory dynamics. Health care professionals can use this setting to screen for dyads that show poor coregulatory skills, as well as enhance the pain management skills of all caregivers by educating them on how to sensitively respond to and support their child's distress. Previous research has shown that presenting caregivers with a brief five-minute video that outlines four simple strategies to support their child's pain-related distress led to decreased pain-related distress in children and increased use of pain management strategies by caregivers during vaccination appointments (Pillai Riddell et al., 2017).

Taken together, examining caregiver-child attunement in an acute pain context early in life can be a useful way for clinical practitioners and researchers to attend to the quality of the

caregiver-child relationship and how it may influence the development of a child's regulatory skills.

Limitations

The findings from this dissertation should be interpreted in the context of the following limitations. In Study 1, we conducted a narrative synthesis, as the variability in methodology in the literature made it difficult to quantitively synthesize estimates of attunement and derive a nuanced understanding of how biological attunement manifests in distress contexts. Consistencies in terminology and methodological approaches would help build a more cohesive literature. Further, most studies in the synthesis included samples from North America and some of the studies used the same data sources, which may affect the generalizability of the findings. For studies 2 and 3, data was collected from generally healthy toddlers with highly educated caregivers, and most of the primary caregivers were mothers, which limits our ability to generalize findings across samples at higher-risk or other caregiver-child relationships (e.g., father-child dyads). There were no measures of caregiver behaviour (e.g., caregiver sensitivity) used in the reviewed literature, which left us to speculate about whether parents responded contingently to their child to support regulation from pain-related distress. Although the current dissertation was not designed to explore longitudinal outcomes, it is not known whether the observed physiological coregulatory patterns have meaningful implications for later regulatory or broader developmental outcomes for the child. Studies 2 and 3 controlled for factors that are known to affect heart rate variability (e.g., time since nap, time since last feed); however, we could not account for the potential effects of additional unmeasured variables known to impact autonomic activity (e.g., lifestyle factors, medications, medical conditions; Quintana & Heathers, 2014). Finally, given the nature of the study context (i.e., brief, fast-paced vaccination

appointment), we did not collect respiration data, which is thought to directly influence HF-HRV, in order to increase efficiency of set-up procedures and reduce participant burden. Research is mixed on the benefit of including respiration data, as adjusting for respiration has shown to minimally impact HRV outcomes (accounts for 2-10% of variance; Denver et al., 2007), and studies in pediatric populations have reported that artifacts created from movement of the respiration band outweighs the benefits of accounting for respiration (Graziano & Derefinko, 2013).

Directions for future research

Building off the findings of the current dissertation, there are several proposed areas for future research. This dissertation highlighted the challenge of synthesizing data on caregiver-child biological attunement due to variability in conceptualizations and methodology used to capture the dyadic process. Future researchers are encouraged to adapt the recommendations outlined in Chapter 2 (e.g., use analysis approaches that better capture the process of attunement). Moreover, it is important for future research to capture both biological responses to distress (e.g., heart rate variability, cortisol, electroencephalography) and behavioural responses (e.g., child regulatory behaviours, caregiver sensitivity) to better determine how attunement manifests at different biobehavioural levels. It would also be valuable to further examine different proximal risk and protective factors that may contribute to the different patterns of attunement found in the current dissertation. For example, in the context of risk (e.g., caregiver psychopathology, chronic stress, negative parenting behaviours), attunement has been shown to be disrupted or altered and may not be adaptive in these contexts (Birk et al., 2022). In addition to examining proximal factors, it is also important for future work to integrate broader contextual and cultural factors into the study of caregiver and child attunement. Buhler-Wassmann and

Hibel (2021) presented a 'call to action' for researchers to study attunement with consideration of culturally diverse contexts, as there may be important culture-specific patterns that emerge. Further, most of the literature has focused on examining only mothers and their children when studying attunement. Recent work suggests that biological attunement differs for mothers and fathers (Fuchs et al., 2021; Lunkenheimer et al., 2021); however, more research is needed in this area, particularly with samples of younger children (i.e., infancy and early toddlerhood). Finally, another important future direction is to examine whether different patterns of attunement are associated with later developmental and mental health outcomes.

References

Bernard, N. K., Kashy, D. A., Levendosky, A. A., Bogat, G. A., & Lonstein, J. S. (2017). Do different data analytic approaches generate discrepant findings when measuring mother-infant HPA axis attunement? *Developmental Psychobiology*, *59*(2), 174–184. https://doi.org/10.1002/dev.21474

Bick, J., & Dozier, M. (2013). The effectiveness of an attachment-based intervention in promoting foster mothers' sensitivity toward foster infants. *Infant Mental Health Journal*, *34*(2), 95–103.

Birk, S. L., Stewart, L., & Olino, T. M. (2022). Parent–Child Synchrony After Early Childhood: A Systematic Review. *Clinical Child and Family Psychology Review*, 1–23.

Bornstein, M. H. (2013). Mother-infant attunement: A multilevel approach via body, brain, and behavior. In *The infant mind: Origins of the social brain* (pp. 266–298). The Guilford Press.

Buhler-Wassmann, A. C., & Hibel, L. C. (2021). Studying caregiver-infant co-regulation in dynamic, diverse cultural contexts: A call to action. *Infant Behavior and Development*, *64*, 101586. https://doi.org/10.1016/j.infbeh.2021.101586

Cassidy, J. (1994). Emotion regulation: Influences of attachment relationships. *Monographs of the Society for Research in Child Development*, *59*(2–3), 228–283. https://doi.org/10.2307/1166148

Cassidy, J., & Berlin, L. J. (1994). The Insecure/Ambivalent Pattern of Attachment: Theory and Research. *Child Development*, *65*(4), 971–991. https://doi.org/10.2307/1131298

Cassidy, J., Ziv, Y., Stupica, B., Sherman, L. J., Butler, H., Karfgin, A., Cooper, G., Hoffman, K. T., & Powell, B. (2010). Enhancing attachment security in the infants of women in a jail-diversion program. *Attachment & Human Development*, *12*(4), 333–353.

Denver, J. W., Reed, S. F., & Porges, S. W. (2007). Methodological issues in the quantification of respiratory sinus arrhythmia. *Biological Psychology*, *74*(2), 286–294. https://doi.org/10.1016/j.biopsycho.2005.09.005

Feldman, R. (2007). Parent–infant synchrony and the construction of shared timing; physiological precursors, developmental outcomes, and risk conditions. *Journal of Child Psychology and Psychiatry*, *48*(3–4), 329–354. https://doi.org/10.1111/j.1469-7610.2006.01701.x

Fuchs, A., Lunkenheimer, E., & Lobo, F. (2021). Individual differences in parent and child average RSA and parent psychological distress influence parent-child RSA synchrony. *Biological Psychology*, *161*, 108077.

Graziano, P., & Derefinko, K. (2013). Cardiac vagal control and children's adaptive functioning: A meta-analysis. *Biological Psychology*, *94*(1), 22–37. https://doi.org/10.1016/j.biopsycho.2013.04.011

Gross, J. J., & Thompson, R. A. (2007). Emotion Regulation: Conceptual Foundations. In *Handbook of emotion regulation* (pp. 3–24). The Guilford Press.

Jazaieri, H., Urry, H. L., & Gross, J. J. (2013). Affective disturbance and psychopathology: An emotion regulation perspective. *Journal of Experimental Psychopathology*, *4*(5), 584–599. https://doi.org/10.5127/jep.030312

Kopp, C. B. (1989). Regulation of distress and negative emotions: A developmental view. *Developmental Psychology*, *25*(3), 343–354. https://doi.org/10.1037/0012-1649.25.3.343

Lunkenheimer, E., Brown, K. M., & Fuchs, A. (2021). Differences in mother–child and father–child RSA synchrony: Moderation by child self-regulation and dyadic affect. *Developmental Psychobiology*, *63*(5), 1210–1224. https://doi.org/10.1002/dev.22080

Marvin, R., Cooper, G., Hoffman, K., & Powell, B. (2002). The Circle of Security project: Attachment-based intervention with caregiver-pre-school child dyads. *Attachment & Human Development*, *4*(1), 107–124.

Muthén, B., & Shedden, K. (1999). Finite mixture modeling with mixture outcomes using the EM algorithm. *Biometrics*, *55*(2), 463–469. https://doi.org/10.1111/j.0006-341x.1999.00463.x

Pillai Riddell, R., Flora, D. B., Stevens, S. A., Stevens, B., Cohen, L. L., Greenberg, S., & Garfield, H. (2013). Variability in infant acute pain responding meaningfully obscured by averaging pain responses. *PAIN®*, *154*(5), 714–721. https://doi.org/10.1016/j.pain.2013.01.015

Pillai Riddell, R., Jasim, S., & Hamwi, L. (in press). Out of the mouth of babes: A lot about pain has nothing to do with pain. *PAIN.*

Pillai Riddell, R., O'Neill, M. C., Campbell, L., Taddio, A., Greenberg, S., & Garfield, H. (2017). The ABCDs of pain management: A double-blind randomized controlled trial examining the impact of a brief educational video on infants' and toddlers' pain scores and parent soothing behavior. *Journal of Pediatric Psychology*.

Qu, J., & Leerkes, E. M. (2018). Patterns of RSA and observed distress during the still-face paradigm predict later attachment, compliance and behavior problems: A person-centered approach. *Developmental Psychobiology*, *60*(6), 707–721.

Quintana, D. S., & Heathers, J. A. J. (2014). Considerations in the assessment of heart rate variability in biobehavioral research. *Frontiers in Psychology*, *5*. https://www.frontiersin.org/article/10.3389/fpsyg.2014.00805

Ramsay, D. S., & Lewis, M. (1994). Developmental Change in Infant Cortisol and Behavioral Response to Inoculation. *Child Development*, *65*(5), 1491–1502. https://doi.org/10.1111/j.1467-8624.1994.tb00831.x

Waxman, J. A., DiLorenzo, M. G., Riddell, R. R. P., Flora, D. B., Greenberg, S., & Garfield, H. (2017). Preschool needle pain responding: Establishing 'normal.' *The Journal of Pain*, *18*(6), 739–745.

Appendix A

Summary of Analyses and Results

Study 1: Caregiver and young child biological attunement in distress contexts: A systematic review and narrative synthesis

Research Question: What are the relations between caregiver and child biological outcomes in a distress context between 0 to 3 years of age?

Analysis: A search yielded 9932 unique studies that were examined against inclusion criteria. A total of 36 studies were included in the review.

Results: The narrative synthesis included a description of consistency, magnitude (standardized effect sizes), and general direction (positive, negative) of the findings.

- The variability of methodology used poses an issue for the attunement literature, as consistent patterns could not be easily discerned across studies.
- Despite the complexity of the literature, there were some patterns of results found:
    - Majority of articles examined caregiver and child cortisol outcomes (25 of 36 studies).
    - Relations between caregiver and child cortisol outcomes were generally moderate to large; however, results differed across the different analyses used and the epochs examined.
        - Moderate to large, positive relations between caregiver and child cortisol during baseline.
        - Moderate, positive relations between caregiver and child cortisol during the immediate reactivity period (i.e., response to stressor).
        - Moderate to large positive relations between changes in caregiver and child cortisol occurring from baseline through to the recovery period.
        - Higher caregiver sensitivity was associated with positive, moderate relations between caregiver and child cortisol levels.
    - Majority of the findings examining relations between caregiver and child cardiac, saliva alpha amylase (sAA), and electroencephalography (EEG) outcomes were weak or inconsistent.

Study 2: Distinct trajectories of caregiver-toddler physiological attunement during routine vaccinations

Research Question: Are there distinct trajectories of caregiver-child coregulatory responses (i.e., high-frequency heart rate variability; HF-HRV) during the second year of life in a vaccination context?

Analysis: Parallel-process growth-mixture model

Results: Three groups of dyads were discerned that differed in their physiological coregulatory patterns during vaccination:

- Group 1 (80% of the sample) demonstrated physiological *attunement*, with a stable and parallel regulatory pattern of HF-HRV from baseline to post-needle.
- Group 2 (7.9% of the sample) showed parallel regulatory trajectories but with notably lower (i.e., less regulated) HF-HRV values, reflecting independent regulatory responses (i.e., a *lack of attunement* among dyad members).
- Group 3 (11.1% of the sample) had diverging regulatory trajectories such that caregivers had a stable HF-HRV regulatory trajectory comparable to the normative group, but their toddlers showed an exaggerated withdrawal response (i.e., a steep decrease followed by an increase in HF-HRV values that surpassed their baseline levels); the regulatory responses of these dyads likely reflect *misattunement*.

Study 3: The relations between caregiver and toddler pain-related distress and dyadic physiological attunement during vaccination

Research Question: Are caregiver distress (operationalized by caregiver-reported worry before and after the vaccination) and child distress (operationalized by child behavioural pain-related distress) associated with caregiver-toddler physiological attunement patterns (i.e., groups identified in Study 2)?

Analysis: Multinomial logistic regression

Results:

- Toddler behavioural pain-related distress (FLACC) was associated with caregiver-child regulatory trajectories:
  - Higher behavioural pain-related distress during the third minute post-needle was associated with a greater likelihood of being in Group 2 (dyads showing lack of regulatory attunement) versus Group 1 (dyads with attuned regulatory pattern), indicating that the children in Group 2 continued to experience considerable pain-related distress by the third minute post-needle.
  - Higher behavioural pain-related distress immediately after the needle was significantly associated with a greater likelihood of being in Group 3 (dyads showing lack of regulatory attunement) versus Group 1, reflecting an overarousal response by toddlers in Group 3.
- Caregiver self-reported worry pre- and post-needle was associated with caregiver-child regulatory trajectories:
  - Caregivers with higher pre-needle worry and lower post-needle worry had a greater likelihood of belonging to groups that showed a maladaptive attunement pattern (i.e., Groups 2 and 3) when compared to the group that showed a more adaptive attunement pattern.

Appendix B

PsycINFO Search Terms and Pairings

#	Searches	Results
1	Parent Child Relations/ or Father Child Relations/ or Mother Child Relations/ or Parenting/	57622
2	("child mother relation*" or "child parent relation*" or "child parent spatial pattern*" or "father child relation*" or "father infant relation*" or "father-child relation*" or "maternal fetal relation*" or "maternal patterns of care" or "maternal-fetal relation*" or "mother child interaction*" or "mother child relation*" or "mother fetus relation*" or "mother infant interaction*" or "mother infant relation*" or "mother-child interaction*" or "mother-child relation*" or "mother-fetus relation*" or "mother-infant interaction*" or "mother-infant relation*" or "parent child correlation" or "parent child relation*" or "parent infant bonding" or "parent infant relation*" or "parental role*" or "parent-child relation*" or "parenting").tw.	54099
3	Mothers/ or Fathers/	41822
4	(father or fathers or mother or mothers).tw.	135908
5	or/1-4	182521
6	Hypothalamic Pituitary Adrenal Axis/ or ("adrenal hypophyseal axis" or "adrenohypophyseal axis" or "diencephalon adrenal axis" or "hypophyseal adrenal axis" or "hypophyseal adrenal system" or "hypophyseal adrenocortical system" or "hypophyseoadrenocortical system" or "hypophysis adrenal axis" or "hypophysis adrenal cortex system" or "hypophysis adrenal function" or "hypophysis adrenal gland" or "hypophysis adrenal system" or "hypophysoadrenal axis" or "pituitary adrenal axis" or "pituitary adrenal function" or "pituitary adrenal system" or "pituitary adrenocortical function" or "pituitary adrenocortical regulation" or "pituitary adrenocortical system" or "pituitary gland adrenal gland axis" or "pituitary-adrenal system").tw.	6986
7	Parasympathetic Nervous System/ or ("acetyl cholinergic system" or "cholinergic mechanism" or "cholinergic nerve system" or "cholinergic nervous system" or "cholinergic pathway" or "cholinergic receptor system" or "parasympathetic nerve plexus" or "parasympathetic nerve system" or "parasympathetic nervous system" or "parasympathetic nervous systems" or "parasympathetic system" or "parasymphatic nervous system").tw.	1033
8	Heart Rate/ or ("cardiac chronotropism" or "cardiac chronotropy" or "cardiac frequency" or "cardiac rate" or "cardiac rates" or "heart frequency" or "heart rate" or "heart rates" or "pulse rate" or "pulse rates" or "ventricle rate" or "ventricle rates").tw.	23875
9	Sympathetic Nervous System/ or ("adrenergic mechanism" or "adrenergic nerve system" or "adrenergic nervous system" or "adrenergic regulatory mechanism" or "adrenergic system" or "beta	3452

	adrenergic system" or "ortho sympathetic nervous system" or "orthosympathetic nervous system" or "sympathetic nerve system" or "sympathetic nervous system" or "sympathetic system" or "sympathic nervous system" or "sympathicoadrenal system" or "sympathoadrenal system" or "vasomotor system" or "vasomotor systems").tw.	
10	Hydrocortisone/ or ("11 epicortisol" or 11-epicortisol or acticort or "aeroseb hc" or "aeroseb-hc" or ala-cort or ala-scalp or alfacort or algicortis or "alpha derm" or alphaderm or anucort-hc or anumed-hc or anutone-hc or aquanil hc or balneol-hc or barseb hc or beta-hc or biacort or cetacort or cobadex or colocort or "compound f" or "cordicare lotion" or coripen or "cort dome" or cort-dome or cortef or "cortef cream" or cortenema or cortibel or corticorenol or cortifair or cortifan or cortiphate or cortisol or cortisole or cortispray or cortoderm or cortril or cotacort or covocort or cremicort-h or cutaderm or "dermacrin hc lotion" or dermaid or "derm-aid cream" or dermocare or dermocortal or dermolate or dioderm or eczacort or "ef cortelan" or efcortelan or egocort or eksalb or eldecort or emo-cort or epicort or epicortisol or ficortril or filocot or flexicort or glycort or gly-cort or "hc no. 1" or "hc no. 4" or h-cort or hebcort or "hemorrhoidal hc" or hemril-30 or "hemril-hc uniserts" or hi-cor or hidrotisona or hycor or hycort or hydracort or hydrasson or "hydro ricortex" or hydrocort or hydrocorticosteroid or hydrocortisate or hydrocortison or hydrocortisone or hydrocortisonum or hydrocortisyl or hydrocortone or hydrogalen or hydrokort or hydrokortison or hydro-rx or hydrotopic or hysone or hytisone or hytone or "incortin h" or "instacort 10" or kyypakkaus or "lacticare hc" or lacticare-hc or "lemnis fatty cream hc" or lenirit or "medihaler cort" or "medihaler duo" or medrocil or mildison or "mildison fet krem" or "mildison lipocream" or mildison-fatty or "mitocortyl demangeaisons" or munitren or "nogenic hc" or novohydrocort or nutracort or optef or "otosone f" or penecort or plenadren or prepcort or "prevex hc" or "pro cort" or procort or proctocort or "procto-kit 1%" or "procto-kit 2.5%" or "proctosert hc" or proctosol-hc or proctosone or procutan or rectasol-hc or rectocort or rederm or sanatison or scalp-aid or schericur or "schericur 0.25%" or "scherosone f" or "sistral hydrocort" or skincalm or stie-cort or "substance m" or synacort or texacort or triburon-hc or unicort or vasocort).tw.	15181
11	("SA nodal arrhythmia" or "SA node arrhythmia" or "sinoatrial arrhythmia" or "sinoatrial arrhythmias" or "sinoatrial node arrhythmia" or "sino-atrial node arrhythmia" or "sinus arrhythmia" or "sinus arrhythmias" or "sinus arrhythmica" or "sinus node arrhythmia" or "sinus node syndrome").tw.	1079
12	Autonomic Nervous System/ or ("automatic nervous system" or "autonomic nerve system" or "autonomic nervous system" or "autonomic nervous systems" or "autonomic system" or "autonomous nervous system" or "involuntary nervous system" or "neuroautonomic system" or "neurovegetative system" or "organic nervous system" or "splanchnic nervous system" or "systema	6129

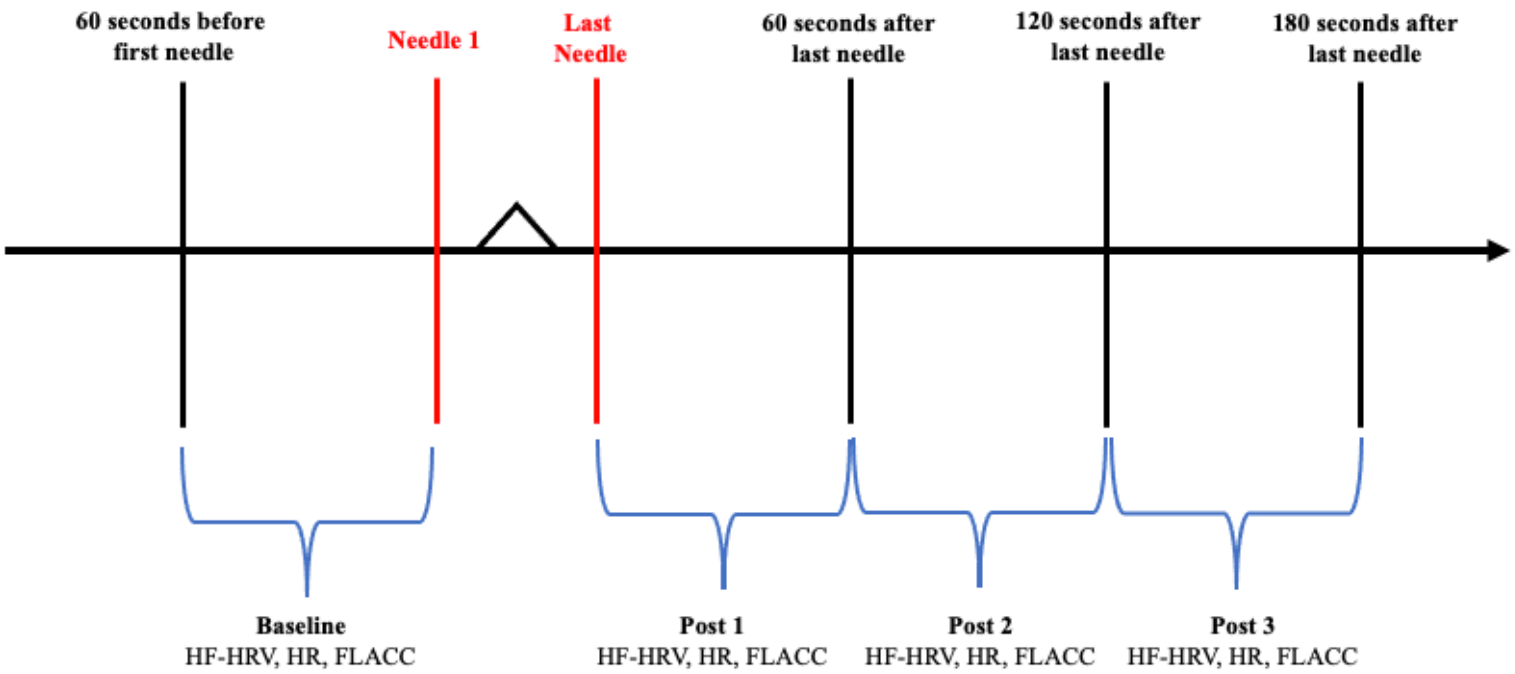
	nervosum autonomicum" or "vegetative nervous system" or "vegetative nervous systems" or "vegetative system" or "visceral nervous system" or "visceral nervous systems").tw.	
13	Galvanic Skin Response/ or ("cutaneous galvanic response" or "electric skin response" or "electrodermal response" or "electrodermal responses" or "galvanic skin reflex" or "galvanic skin response" or "galvanic skin responses" or "GSR" or "psychogalvanic reflex" or "psychogalvanic skin reflex" or "skin conductance response" or "skin electric conductance" or "skin galvanic response" or "skin potential response").tw.	5174
14	("adrenal cortex" or "adrenal cortical activity" or "adrenal cortical activities" or "adrenal cortical system" or "adrenal cortical systems" or "adrenal gland cortex" or "adreno cortical activity" or "adreno cortical activities" or "adrenocortical activity" or "adrenocortical activities" or "adrenocortical system" or "adrenocortical systems" or "adrenocortical tissue" or "cortex glandulae suprarenalis").tw.	964
15	Saliva/ or (spittle or saliva or salivas).tw.	4312
16	("parotid amylase" or "parotid amylases" or "saliva alpha amylase" or "saliva alpha amylases" or "saliva amylase" or "saliva amylases" or "salivary alpha amylase" or "salivary alpha amylases" or "salivary alpha amylase" or "salivary alpha amylases" or "salivary alpha-amylase" or "salivary alpha-amylases" or "salivary amylase" or "salivary amylases" or "salivary gland amylase" or "salivary gland amylases").tw.	374
17	("diencephalohypophyseal system" or "diencephalohypophyseal systems" or "diencephalohypophysis system" or "diencephalohypophysis systems" or "hypophyseal hypothalamic system" or "hypophyseal hypothalamic systems" or "hypophyseal portal system" or "hypophyseal portal systems" or "hypophysis hypothalamus system" or "hypophysis hypothalamus systems" or "hypothalamic hypophyseal axis" or "hypothalamic hypophyseal system" or "hypothalamic hypophyseal systems" or "hypothalamic neurohypophyseal system" or "hypothalamic neurohypophyseal systems" or "hypothalamic neuropituitary system" or "hypothalamic neuropituitary systems" or "hypothalamic pituitary axis" or "hypothalamic pituitary system" or "hypothalamic pituitary systems" or "hypothalamic pituitary unit" or "hypothalamic-pituitary unit" or "hypothalamo hypophysary axis" or "hypothalamo hypophyseal connection" or "hypothalamo hypophyseal system" or "hypothalamo hypophyseal systems" or "hypothalamo hypophyseal tract" or "hypothalamo neuropituitary system" or "hypothalamo neuropituitary systems" or "hypothalamo pituitary axis" or "hypothalamo pituitary system" or "hypothalamo pituitary systems" or "hypothalamohypophyseal axis" or "hypothalamohypophyseal neurosecretory system" or "hypothalamohypophyseal neurosecretory systems" or "hypothalamohypophyseal system" or "hypothalamohypophyseal systems" or "hypothalamo-hypophyseal system" or "hypothalamo-hypophyseal systems" or "hypothalamohypophysis axis" or "hypothalamohypophysis	304

	system" or "hypothalamohypophysis systems" or "hypothalamoneurohypophyseal system" or "hypothalamoneurohypophyseal systems" or "hypothalamopituitary axis" or "hypothalamopituitary function" or "hypothalamopituitary system" or "hypothalamopituitary systems" or "hypothalamus hypophyseal axis" or "hypothalamus hypophyseal system" or "hypothalamus hypophyseal systems" or "hypothalamus hypophysis" or "hypothalamus neurohypophysis system" or "hypothalamus neurohypophysis systems" or "hypothalamus neuropituitary system" or "hypothalamus neuropituitary systems" or "hypothalamus pituitary gland axis" or "hypothalamus pituitary system" or "hypothalamus pituitary systems").tw.	
18	"respiratory sinus arrhythmia".tw.	1033
19	Human Biological Rhythms/ or ("circadian clock" or "circadian clocks" or "circadian cycle" or "circadian fluctuation" or "circadian periodicity" or "circadian rhythmicity" or "circadian variation" or "day night rhythm" or "diurnal cycle" or "diurnal fluctuation" or "diurnal pattern" or "diurnal rhythm" or "diurnal rhythmicity" or "diurnal variation" or "diurnal variations" or "nychtohemeral" or "circadian rhythm" or "circadian rhythms" or "diurnal rhythm" or "diurnal rhythms" or "nycthemeral rhythm" or "nycthemeral rhythms" or "nyctohemeral rhythm" or "nyctohemeral rhythms" or "twenty four hour rhythm" or "twenty-four hour rhythm" or "twenty-four hour rhythms" or "biological rhythm" or "biological rhythms").tw.	13403
20	vagus nerve/ or ("cranial nerve x" or "nerve x" or "nerve xs" or "pneumogastric nerve" or "pneumogastric nerves" or "tenth cranial nerve" or "tenth cranial nerves" or "regeneration nervi vagi" or "tenth cranial nerve" or "vagal nerve" or "vagal receptor" or "vagosympathetic trunk" or vagus).tw.	2469
21	homeostasis/ or (autoregulation or homeostasis or "homeostatic equilibrium" or "homeostatic mechanism" or homoeostasis or homoiostasis).tw.	10212
22	("adrenal hypophyseal axis" or "adrenohypophyseal axis" or "diencephalon adrenal axis" or "hypophyseal adrenal axis" or "hypophyseal adrenal system" or "hypophyseal adrenal systems" or "hypophyseal adrenocortical system" or "hypophyseal adrenocortical systems" or "hypophyseoadrenocortical system" or "hypophyseoadrenocortical systems" or "hypophysis adrenal axis" or "hypophysis adrenal cortex system" or "hypophysis adrenal cortex systems" or "hypophysis adrenal function" or "hypophysis adrenal gland" or "hypophysis adrenal gland system" or "hypophysis adrenal gland systems" or "hypophysoadrenal axis" or "pituitary adrenal axis" or "pituitary adrenal function" or "pituitary adrenal system" or "pituitary adrenal systems" or "pituitary adrenocortical function" or "pituitary adrenocortical regulation" or "pituitary adrenocortical system" or "pituitary adrenocortical systems" or "pituitary gland adrenal gland axis" or "pituitary-adrenal system" or "pituitary-adrenal systems").tw.	5259

23	exp electroencephalography/ or ("alpha activit*" or "alpha rhythm" or "alpha rhythms" or "alpha wave" or "alpha waves" or "beta activit*" or "beta rhythm" or "beta rhythms" or "beta wave" or "beta waves" or "brain activity" or "brain electric activit*" or "brain electrical activit*" or "brain wave" or "brain waves" or "brainwave" or "brainwaves" or "delta activit*" or "delta rhythm" or "delta rhythms" or "delta wave" or "delta waves" or "e.e.g." or EEG or electroencephalogram or electroencephalograms or electroencephalography or "electric encephalography" or "electrical encephalography" or "electro encephalography" or "electric encephalogram" or "electrical encephalogram" or "electro encephalogram" or "gamma activit*" or "gamma rhythm" or "gamma rhythms" or "gemma wave" or "gemma waves" or "theta activit*" or "theta rhythm" or "theta rhythms" or "theta wave" or "theta waves").tw.	54864
24	("cortical synchronisation" or "cortical synchronization").tw.	73
25	or/7-24	129729
26	5 and 25	2899
27	(infan* or newborn* or "new born*" or baby* or babies or toddler* or minors* or "under* age*" or pediatric* or paediatric* or peadiatric* or prematur* or pre-term or preterm*).mp. or (child* or pediat*or paediat*).jn.	212440
28	26 and 27	1423

Appendix C

12-, 18-, and 24-month Within Vaccination Timelines with Measurement Epochs



Note. HF-HRV = high frequency heart rate variability, HR = heart rate, FLACC = Face, Legs, Activity, Cry and Consolability scale

Appendix D

Research Consent Form (12-month example)

Title of Research Project:
Physiological and Behavioural Regulatory Processes in Recovering from Distress: Developmental and Contextual Dimensions in Infancy

Investigators:
Primary Investigator: Dr. Rebecca Pillai Riddell, CPsych, PhD
Room 2038 Sherman Health Sciences Research Centre, York University, (416) 736-2100 x20177

Project Co-Investigators:
Dr. Louis Schmidt, PhD, McMaster University
Dr. Karen Mathewson, PhD, McMaster University
Dr. Hartley Garfield
Dr. Dan Flanders, MD
Dr. Eitan Weinberg, MD

Purpose of the Research:
Infants undergo immunization injections, or 'shots' as part of routine medical care. Immunizations are given with a needle, which can be painful. We are doing this study to understand ways that we can help infants when they are experiencing pain. We are interested in how this pain behaviour and physical reaction to pain develops over the second year of life (12, 15, 18, and 24 months), and how it relates to developmental outcomes (cognitive, communication, physical, social/emotional, adaptive) at the end of the second year of life (24 months). We are also interested in how moms' well-being impacts the development of these outcomes. We have already videotaped 2000 immunizations over the first year of life and look forward to learning more about the second year of life.

Description of the Research:
You will be asked to participate at 4 appointments over the course of the year. At your child's 12-month, 15-month, 18-month, and 24-month appointment:

1. We will be videotaping you and your child during their immunizations.
2. Parents will be asked to fill out questionnaires to help us better understand the parent and their child (10-15 minutes) over the phone within one week that is convenient for the parent.
3. We will be connecting your child to equipment that measures their heart rate and stress. Heart rate will be monitored using baby-friendly stickers put on their shoulder blade and lower ribs.
4. Parents will be connected to equipment that measures their heart rate and stress. Heart rate will be monitored using the same baby-friendly stickers put on below their shoulder blade and lower ribs.
5. We will conduct a chart review of your child's health record at your pediatrician's clinic

Videotapes will be viewed later by research assistants that do not know the identity of the children or parents to score their behaviours. Videotaping infants is not part of standard care, however, it does not interfere with the procedures being conducted, and is the usual way we measure pain in research studies.

I agree for my child and myself to be videotaped by research team: □ AGREE □ DISAGREE
I agree for my child's medical chart to be reviewed by research team: □ AGREE □ DISAGREE

In addition, I agree or consent for this immunization tape(s)/photograph(s) to be used for:
□ Other studies on the same topic
□ Teaching and demonstration at York University
□ Teaching and demonstration at meetings outside York University
□ Not to be used for anything else.

Risks and Potential Discomforts or Inconvenience:
The baby-friendly stickers used to measure heart rate and emotional arousal may cause minor discomfort to you or your child's skin. In pilot testing, no children were distressed by removal of the baby-friendly stickers. Additionally, filling out the well-being questionnaires will take about 20 minutes of your time outside of the clinic appointment.

Physiological data will be analyzed later by research assistants that do not know the identity of the parents or children. Putting baby friendly stickers on parents and infants to measure their physical reaction is not part of standard care, however, it does not interfere with the procedures being conducted, and is a common way we measure pain in research studies.

Potential Benefits:
You will receive an electronic copy of your child's immunization, a $10 gift certificate after each immunization, and a $5 gift certificate after each phone interview.

Potential Benefits to Society:
In the future, society may benefit from the results of this study because it will teach us about how regulating from distress during infancy relates to later child development.

Alternatives to Participation:
Participation in research is voluntary. You can choose not to participate or you may withdraw at any time without affecting your child's medical care.

Confidentiality:
We will respect your privacy. No information about who you are will be given to anyone or be published without your permission, unless the law requires us to do this. For example, the law requires Dr. Pillai Riddell to give information about you if a child has been abused, if you have an illness that could spread to others, if you or someone else talks seriously about suicide (killing themselves), or if the court orders us to give them the study papers. You will be contacted if any of these issues have arisen. York University Research Monitors may see your health record to

check on the study to ensure we are being compliant. By signing this consent form, you agree to let these people look at your records. We will give you a copy of this research consent form for your files.

The data produced from this study will be stored in a secure, locked location. Only members of the research team (and maybe those individuals described above) will have access to the data. This could include external research team members. Following completion of the research study, the data will be kept as long as required and then destroyed as required by York University policy. Published study results will not reveal your identity. The supervisors of this project are located at York University.

Participation:
It is your choice to take part in this study. You can stop at any time. The care you get at your doctor's office will not be affected in any way whether or not you take part in this study.
New information that we get while we are doing this study may affect your decision to take part in this study. If this happens, we will tell you about this new information. And we will ask you again if you still want to be in the study.
During this study we may create new tests, new medicines, or other things that may be worth some money. Although we may make money from these findings, we cannot give you any of this money now or in the future because you took part in this study.

Sponsorship:
This study is paid for through funds allocated to Dr. Pillai Riddell from the Natural Sciences and Engineering Research Council.

Conflict of Interest:
The research team members have no conflict of interest to declare.

Consent:
By signing this form, I agree that:
1) You have explained this study to me. You have answered all my questions.
2) You have explained the possible harms and benefits (if any) of this study.
3) I know what I could do instead of taking part in this study. I understand that I have the right not to take part in the study and the right to stop at any time. My decision about taking part in the study will not affect my health care.
4) I am free now, and in the future, to ask questions about the study.
5) I have been told that my medical records will be kept private except as described to me.
6) I understand that no information about who I am will be given to anyone or be published without first asking my permission.
7) I have read and understood pages 1-4 of this form. I agree, or consent, to take part in this study.

If you have any questions about this study, please call Dr. Rebecca Pillai Riddell at the number on the first page of the consent form. You can also contact the Project Coordinator at ouchlab@yorku.ca. This research has been reviewed by the Human Participants Review Committee in accordance with York's Senate Policy on Research Ethics (York University). This study conforms to the standards of the Canadian Tri-Council Research Ethics guidelines. If you

have any questions about this process or about your rights as a participant in the study, please contact Ms. Alison Collins-Mrakas, Manager, Research Ethics, Office of Research Ethics, 5th Floor, York Research Tower, York University (telephone 416-736-5914 or e-mail at acollins@yorku.ca).

Contact Information (RA must check legibility):

Parent Name: _____
Child's Name: _____

Preferred Telephone Number for scheduling appointments (with parent who spends most time with child): _____
Alternative Telephone Number: _____

Best Days and Time to Contact Parent (Please give an approximate 1-2 hour window e.g. 3 to 7pm)?

Monday to Friday_____
Saturday and Sunday_____

Home Mailing Address (To mail Tim Card after phone interview):

_____

_____

_____

_____

Email Address:

_____

Please print clearly!

Future Contact Specifications:

The OUCH lab is often conducting new studies where parents and their children can get involved. We would like to ask you to participate but you can always choose not to participate and ask us to stop contacting you. We would never contact you more than once or twice a year for a new study.

I consent for researchers to contact me via mail, email or phone regarding *(check which statement applies)*:

☐ No further contact (aside from contact directly related to participation in this current study).
☐ Further contact for results of this study.
☐ Further contact for results of this study and opportunities for participation in new future studies.

Video Use in the Media:

- ☐ I consent for my videos to be used for media coverage of the study, which may include print articles, news videos, or publications on websites. We will never release you or your child's identity and will notify you in advance if your video is chosen to be used and which media outlet the video will be using your video. You will always be given the opportunity to withdraw consent.

Appendix E

Participant Information Sheet (12-month example)

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 Mother & Father Other _____

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)	_____	□Heart disease	_____
□Seizure	_____	□Asthma	_____
□Allergy	_____	□Bleeding problems	_____
□Hay Fever	_____	□Eczema or hives	_____
□Injuries to head	_____	□Paralysis	_____
□Broken bones	_____	□Stomach pumped	_____
□Hospitalization *(please specify reason)*	_____	□Thrush	_____
□Operations *(please specify)*	_____	□Circumcision	_____
□Otitis media	_____	□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) graduate
   4. Trade School/Community College
   5. High School Graduate
   6. Some high school
   7. Junior high school
   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) graduate
   4. Trade School/Community College
   5. High School Graduate
   6. Some high school
   7. Junior high school
   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:_____

3. Vaccines in needle #3:_____

4. Vaccines in needle #4:_____

Name(s) of physician or resident who gave needle(s):

_____