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**GROWING EMOTION REGULATION:
DEVELOPMENTAL ORIGIN OF SOCIO-EMOTIONAL
COMPETENCE IN TODDLERS VIA AN
ETHOLOGICAL APPROACH**

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ABSTRACT

Even though emotion self-regulation has been widely studied over the last several decades, much of the research on this topic has neglected to frame specific regulatory strategies across development, particularly during infancy and toddlerhood. It is during these stages of life that such behaviors emerge and increase in complexity. Scientific literature has shown that these early emotion regulation abilities are easily shaped by external factors, not only through intimate interactions with primary caregivers but also through progressive exchanges with the broader environment.

To address this gap, the current dissertation investigates emotion self-regulation behaviors at two time points in infants' ontogeny: 12 and 22 months. The research employs experimental tasks designed to elicit increasing frustration, utilizing data from a larger longitudinal study involving mother-child dyads. As part of the study, mothers attended follow-up appointments where their infants participated in structured tasks. These laboratory tasks were coded for infants' emotion regulation strategies using a novel ethological approach, favoring a bias-free and objective measurement of behaviors over traditional, inference-biased methods. Furthermore, positive emotional expressions were analyzed through cutting-edge software to examine whether and how smiling varies with purpose and context, even at this young age. Results suggest that early social experiences, including childcare attendance, play a pivotal role in shaping emotional responses and regulatory strategies.

The implications of these findings and key directions for future work are discussed.

CHAPTER 1

GENERAL INTRODUCTION

During early childhood, individuals acquire and continuously refine self-regulatory skills to facilitate adaptive functioning in everyday life (Calkins, 2007; Kopp, 1982). Self-regulation (SR), especially during early development, has been examined in relation to various other constructs, including effortful control (Kochanska et al., 2000; Rothbart et al., 2004) and **emotional self-regulation** (ER) (Eisenberg et al., 2003), both discussed later in the present study. At the same time, many different definitions and measurements of SR have been implemented to understand how it evolves throughout childhood (Karreman et al., 2006). While it is extensively recognized that individual SR abilities emerge during infancy (Kopp, 1982; Posner & Rothbart, 2000), there is a consistent gap regarding the timing and process of SR across ontogeny and therefore, its ever-changing definition. Kopp (1982) theoretically outlines the development of SR in early childhood as a transition from externally regulated behavior toward internally regulated behavior; a process relying on the maturation of attentional and cognitive circuits, alongside parental socialization. Specifically, between 12 and 18 months of age, children begin to recognize social demands and how to comply with them through parental requests. At this stage, SR has been defined as directed compliance (Calkins, 2007; Kopp, 1982). Gradually, as children learn how to internalize parental values, they regulate their behavior without the need for external support (Kochanska et al., 2000). Then, approximately when turning 3, children start to exhibit internally motivated self-regulatory behaviors, when they effectively shift from simple compliance to SR, becoming independent in guiding their actions. In recent years, the interest in infant SR has tremendously increased, likely because of its connection to a broad variety of developmental outcomes, such as refined social competence (Calkins et al., 1999; Spinrad et al., 2007) and few behavioral problems (Eisenberg et al., 2009; Willoughby & Hamza, 2011). Over the past few decades, SR in all its declinations has become the focus of considerable number of theoretical and empirical studies (for reviews, see Aldao et al., 2010; Baumeister et al., 2007; Webb et al., 2012). While researchers are in agreement about the long-term positive effect of a good ability to self-regulate, they diverge on how to measure it in infants. This discussion has led to a plethora of

tasks, designed to induce emotional responses while managing frustration stimuli (see Chapter 2 to know which of them have been employed in this work). Finally, when considering key areas of psychological development, SR plays a fundamental role as an organizing construct, whose correct development is linked to a wide range of both short and long-term effects.

In the following sections, an overview of SR, specifically in its emotional dimension, is presented. Particular attention is given to the significance of studying the early development of emotional SR abilities. Two key variables are analyzed in depth: the first concerns the effects of the growth environment on the development of self-regulatory behaviors, and the second examines the motor manifestations of emotional responses, particularly through facial expressions. These topics are the primary focus of two out of the three studies included in this work (**Chapter 3, Chapter 4**). Finally, special emphasis is placed on the **ethological approach**, which constitutes the focal point of **Chapter 5**. Chapter 2 will provide a detailed exploration of the methods used to induce regulatory responses in infancy and, most importantly, the methodology applied in the present work.

Emotion regulation as a feature of self-regulation. SR can be defined as the deliberate control of emotional and cognitive responses to stimuli, aimed at putting into effect actions that have a defined goal (Blair et al., 2012). For instance, individuals exhibit SR when they inhibit impulsive behaviors or adjust emotional and cognitive responses to advance positive social exchanges (Kochanska et al., 2000). Individuals also display SR when they consciously/automatically modulate their arousal levels (i.e. by decreasing them when perturbed) to successfully engage with the environment (Blair & Ursache, 2011). When it comes to investigating SR in early childhood, the topic often revolves around **effortful control**, an aspect of temperament. The reason lies in the fact that effortful control can be defined as a blend of emotional and cognitive components of SR. Behaviorally, effortful control refers to the ability to sustain attention on a task, also for extended periods; eventually to transit smoothly between tasks, and plan or wait before entering new activities (Rothbart et al., 2003). From a cognitive perspective, effortful control involves the ability to also intentionally monitor and regulate attention in the presence of conflicting information, with the purpose of managing emotions (Posner & Rothbart, 2000). Thus, following these definitions, effortful control includes both automatic processes related to behavioral and socio-emotional regulation, as well as intentional control of attention.

Nonetheless, ER is, by definition, a control process as well. In fact, it falls within the broader class of processes through which individuals exert control over their own behavior. The

regulation of emotional responses involves mitigating, upholding, inhibiting or busting the degree of intensity or the valence of these responses, encompassing all the circuits where this process can occur, i.e. biologically, behaviorally or socially (Cole et al., 2004; Gross & Thompson & Meyer, 2007; Perry & Calkins, 2018; Thompson, 1994). To explain the control mechanisms underlying ER, psychologists have suggested that it can be considered as a form of self-regulated, goal-directed behavior (Erber & Erber, 2000; Tice & Bratslavsky, 2000), highlighting the profound connection between SR and ER.

Emotion regulation as a predictive tool in human development. At birth, infants display emotional responses and, more in general, a range of affective expressions as a reaction to environmental events (Tronick & Cohn, 1989). ER starts at the earliest stages of life, becoming increasingly more complex as the infant develops. In the first 12–24 months of life regulating emotions basically means increasing one emotion while decreasing another. While, especially in infancy, this is often quite unconscious and can happen without deliberate intention (Cole et al., 1994), these emotional experiences and the related attempts to regulate them have been proved to be adaptive, serving developmental functions (Abe & Izard, 1999). The first emotional regulatory pattern occurs at birth, involving self-soothing behaviors, and around 6 months of age attentional control begins to emerge. By 6 months, infants can shift the attentional orientation toward objects of interest (Posner & Petersen, 1990), allowing them to intentionally disengage attention from stimuli that may be stressful to them and move it towards neutral or less distressing stimuli. These early mechanisms seem to foster a learning process in terms of regulating frustration; it has been in fact reported that infants who use self-soothing behaviors demonstrate decreased negative affect during a fear paradigm (Crockenberg & Leerkes, 2004) and frustration paradigms (Stifter & Braungart, 1995) at 6 months. In the same way, infants who are better able to purposefully orient the attention have been shown to display less distress to novel stimuli (Crockenberg & Leerkes, 2004) or frustrating environments (Stifter & Braungart, 1995). Therefore, developing ER is a functional accomplishment of early development, and it has been proved to be useful as a predictive tool for later wellbeing (Cole et al., 2004; Posner & Rothbart, 2000; Sroufe, 1997). The association between ER skills and infancy **social and emotional competence** has been thoroughly investigated and supported by a wide body of scientific literature over the past few years (just to cite few: Bailey et al., 2016; Eisenberg et al., 2010; Perry & Calkins, 2018). Extending beyond infancy, ER competencies also predict better childhood mental health outcomes, including decreased anxiety, more prosocial attitude and better academic accomplishments even during adolescence (Aldao et al.,

2010; Compas et al., 2017; Daniel et al., 2020; Jacobs & Gross, 2014; Lonigan et al., 2017; Schäfer et al., 2017). Even in adulthood, work success and healthy relationships have been correlated with better regulatory capacities (Doerwald et al., 2016; Kotsou et al., 2011).

Emotion regulation and dysregulation. Being such a key concept, it is reasonable to state that if ER develops in a maladaptive manner, the infant will most likely have difficulties with emotional adjustments through life. ER has been a predominant topic in scientific literature for more than 20 years, while emotion dysregulation (ED) has been less extensively studied (Thompson, 2019). Infant ED has been linked to difficult temperament, irritability and high intensity of outbursts which represent risk factors for later psychopathology in childhood and adolescence (Althoff et al., 2010; Biederman et al., 2012; Earls & Jung, 1987; Guerin et al., 1997; Keenan, 2000; Teerikangas et al., 1998). Toddlers and preschoolers struggling with ED may also have academic problems, such as lower test scores in preschool and kindergarten (Graziano et al., 2007; Howse et al., 2003) and in the social competence domain, for instance in maintaining healthy relationships with peers (Keane & Calkins, 2004; Penela et al., 2012). Additionally, ER deficits have been suggested to correlate with later depression, anxiety, aggressive behavior, eating disorders, borderline personality disorder and substance use disorders (Aldao et al., 2010; Berking & Wupperman, 2012; McLaughlin et al., 2011). To date, works on ED are not only rare but also characterized by inconsistencies in its definition among young or very young subjects. Instead of forcing ED in a univocal concept, it may be more accurately depicted as a multi-dimensional system of developmental processes (Thompson, 2019). Keenan (2000) described infant ED as ‘an inability to respond to stimuli with well-maintained control’ (p. 420). Cole et al. (1994) referred to infant ED as ‘the failure to meet the developmental tasks of emotional development’ (p. 77). Finally, Ostlund et al. (2019) offered a more intriguing perspective, by suggesting that we would not be born equipped with ED and we do not grow it during infancy *per se*; most likely individual differences in newborn neurobehavior may hold vulnerabilities that, if reinforced over early childhood, can contribute to later ED (Beauchaine, 2015). This approach makes ER and ED deeply intertwined, representing two facets of the same overarching domain. Not by chance, ED has been named also poor, abnormal or maladaptive ER, lack of regulatory strategies or emotional competence (Adrian et al., 2011). The boundaries of discrete distinction between ER and ED become even more blurry when it comes to defining these dimensions during infancy and childhood, where it can be challenging or even detrimental to separate the two concepts. Therefore, ER and ED

are often studied complementarily: methods and reasoning should be designed with the common goal to unveil emotion regulatory skills as a whole.

Regulating emotions through the face. Another variable notably affecting early self-regulatory growth in human development is the progressive control over **emotional responses**. Individual's developmental stage and his/her ER strategies influence the emotional experience and its expressions (Izard, 1977; Izard & Malatesta, 1987). Although a substantial body of research has investigated emotional expressions, including studies that demonstrate consistent relationships between expression and emotional experience, the exact role of expressive behavior in shaping emotional experience remains debated. Buck (1985) suggested that expressions function as the mere reflections of underlying emotional states, without influencing internal experiences. However, it seems reasonable to hypothesize that emotional expressions have a role in regulating the emotional experience. Spontaneous, self-driven, and self-regulated expressions are actions that the individual is motivated to perform for a specific goal. Consequently, self-initiated expression plays a crucial role in goal-oriented behavior. The objective of such expressive behavior may be consciously selected, with the belief that it will assist in addressing the challenges of a particular situation. Alternatively, the goal may be embedded within unconscious cognitive processes (Kihlstrom, 1987).

It is widely acknowledged that a particular emotion expression can be simulated even when the expressor is experiencing a contrary emotion feeling (Ekman & Friesen, 1975; Izard, 1977). Connections between voluntary expressions and emotional feeling are subject to learning and SR. There are many learned connections or associations between voluntarily produced expressions and emotion experiences. In few circumstances the expression shown is incongruent with the ongoing subjective feeling, and this learned dissimulation facilitates ER.

The impact of social environment on growing regulatory skills. In addition to the transition from external to internal motivation, the **context in which the infant develops** is a crucial feature in the maturing of SR (McClelland & Cameron, 2011). During the preschool period, children find themselves managing progressively complex social and emotional situations that need SR. Therefore, assessing the developmental maturation of SR across childhood cannot be disentangled from the context (McClelland & Cameron, 2011). Russell and colleagues (2013) highlight a shift in psychological views on personality and behavior, moving away from the belief in broad, stable personality traits that lead to consistent behavior across situations. Instead, the focus should be on how behavior is shaped by the interaction between an

individual's inherent qualities and the contextual factors of specific situations. This emphasis on context led to many studies on the impact that young children's early care environments have on self-regulatory cognitive and affective domains. Research in the cognitive domain frequently focuses on areas such as attention and language, while studies in the affective domain typically explore ER within the framework of social interactions involving peers, or caregivers in general (i.e. parents or teachers) (Eigsti et al., 2006; McClelland et al., 2007; NICHD ECCRN, 2003). Aside from the aforementioned indicators of SR as predictors of long-term success, contextual factors, such as the quality of early caregiving relationships and the presence of key figures who foster SR within social interactions, promote the development of adaptive ability to regulate emotions (Eisenberg et al., 2010; Rodriguez et al., 2005).

Indeed, the first social context experienced after birth is the infant-caregiver interaction, and this early socialization provides the foundation for the development of SR (Feldman & Klein, 2003). Caregivers respond to infant distress by providing comfort and soothing, helping to return infants to a state of rewarding calm (Tronick, 2007). Consequently, a child who learns to regulate positive and negative affect within the parent-child relationship is also likely to utilize these skills as he/she develops social-emotional competence in future interactions with peers and other-than-parents adults. Thus, effective SR is built upon optimal co-regulation by caregivers, who initially manage the child's emotional states.

Numerous studies have demonstrated significant connections between proper **social-emotional competence** throughout childhood and adaptive early experiences within the social environment. When a sensitive and responsive parent engages with his/her infant, the baby is likely developing mature competencies as an interactive partner, discovering that dyadic exchanges are rewarding, and learning to trust that others would respond appropriately to their communicative attempts. This sort of continuity for adaptive regulation across the self-and-other domains was suggested by Fuertes and colleagues (2009) and supported by Raikes and colleagues (2007), who showed that infants with more effective SR skills are more likely to cultivate positive social relationships as they grow. Nevertheless, the same continuity can be found between dis-regulatory competence in infancy to subsequent mismatched social interactions. This is why parental style is an influential factor that cannot be overlooked when studying SR and ER. Even though attachment styles are discussed in the present work (see Chapter 5), the infant-parent relationship quality has not been considered as a variable. The lack of this kind of data in the present work represents one of the major limitations.

However, apart from proximal influences, environmental impact can be distal and eventually influence children's emotional experiences in other-than-familiar contexts. For example, whether an emotional expression is compatible with a social context or whether a child can effectively use a strategy to modulate emotion, is something that can be learned through socializing with peers or other adults. Keeping in mind the stages of SR development, it is essential to recognize that infants can display social competence in interactions with others. Therefore, selected variables that may impact the development of children's social skills, starting as early as one year, should be considered when studying emotion-SR.

1.1 Emotion regulation and temperamental dimensions

It has been suggested that ER can be considered an aspect of temperament (Rothbart, 2007). Following the definition of temperament by Rothbart and colleagues, temperament encompasses individual differences in reactivity and SR, which are influenced by genetics, development, and experience (see 'the psychobiological model of temperament'; Rothbart et al., 2000; Rothbart & Derryberry, 1981). Therefore, through the study of temperament, the individual's spontaneous emotional reactivity to familiar and unfamiliar stimuli is measurable in terms of behavior and physiology. Some children have naturally lower thresholds for emotional experiences than others which, in turn, affects their ability to employ top-down emotion regulatory strategies to effectively modulate reactive emotional processes. Regarding the second aspect of the psychobiological model of temperament's definition, i.e. SR, refers to one's ability to modulate emotional reactions, expressions, and behavior. The term, in its broad definition, also includes ER, as previously explained in this chapter. Buss and Goldsmith (1998) observed that infants employed a range of ER strategies during frustrating experiences to mitigate negative affect. Calkins and Johnson (1998) demonstrated a negative correlation between the propensity to exhibit distress in frustrating situations and certain regulatory behaviors. In a similar vein, Nigg and colleagues (2002) posited that a negative temperament characterized by frustrated reactivity may hinder the development of appropriate regulatory behaviors. In 2002, Calkins and colleagues measured frustration during three separate tasks, where infants were classified as easily and less easily frustrated. The regulatory strategies examined included behaviors such as self-comforting, distraction, and seeking maternal proximity. Nigg et al. (2004) argued that some of these regulatory behaviors are contingent upon attention, as conceptualized within the framework of effortful control. Therefore, attention processes and self-soothing behaviors are proved to be reliable indicators when

measuring infants' regulatory capabilities. Moreover, researchers have recently begun to emphasize the significance of considering context and individuals' goals within that context, when studying ER (Aldao, 2013; Bridges et al., 2004; Campos et al., 1994; Raver, 2004; Thompson & Meyer, 2007).

In Chapter 3 the role of intrinsic factors, such as sex, and extrinsic factors, including social functioning through childcare attendance, in the development of self-regulatory strategies in infants have been investigated. An adapted impossible task paradigm was employed, where infants interacted with a toy under varying conditions of accessibility, in the presence of both the mother and an experimenter. The hypotheses posited that females would exhibit greater self-regulatory behaviors and reduced reliance (measured through attention processes) on maternal cues compared to males, particularly during stressful situations. Results confirmed that females engaged in more self-regulatory behaviors than males and gazed longer at their mothers during stress, indicating their dependency on maternal support.

Furthermore, infants attending childcare displayed a strategic shift in attention, looking at their mothers later and the experimenter sooner, suggesting that prior social experiences shape their regulatory responses. These findings underscore the importance of both intrinsic and extrinsic factors in early ER and social competence, highlighting the dynamic interplay between individual temperament and social environment.

1.2 Detecting positive emotional states: what smiles can reveal about developing emotion self-regulation

Most past research on ER has focused on the regulation of negative emotional states, probably also due to the most common tasks employed in this domain which, being designed to induce frustration, bring with them a predictable negative valence. However, a broad conceptualization of ER includes the regulation of positive state as well.

'Discrete emotion' theory posits that a limited number of fundamental emotions exist, underpinned by neurophysiological mechanisms and signaled by distinct facial expressions, which manifest early in infancy (Izard & Malatesta, 1987). According to this perspective, smiling reflects a single positive emotion, i.e. enjoyment. Thus, these variants would represent different levels of intensity of that emotion. For instance, lip corner retraction with an open mouth would indicate a higher intensity of enjoyment than lip corner retraction with a closed mouth. Conversely, theories such as functionalist, dynamic systems, and social process models of emotion (Barrett, 1998; Camras, 1992; Dickson et al., 1998; Fogel et al., 1997; Frijda, 1986;

Messinger et al., 1997) argue against a fixed set of basic emotions. Instead, they propose that emotions form groups, emerging as much from ongoing interactions with the environment as from neurophysiological processes. In these frameworks, the same expression, such as a smile, may represent different emotions depending on the communicative context.

Between the neonatal period and the second year of life, smiles develop to intentionally shared positive messages (Jones & Hong, 2001; Sroufe & Waters, 1976). During the next months, smiles are most likely when infants are stimulated by the human voice (Wolff, 1987). Between one and two months, infants become more reactive towards the surrounding and start to smile toward a human face (Spitz, 1946). It is in this period that the social smile develops (Anisfeld, 1982) and from this stage onward, smiling primarily serves as a social-emotional expression. During the first six months of life, face-to-face interactions are a significant part of the infant's time with the caregiver, and smiles play a key role in these interactions (Weinberg & Tronick, 1994). As infants grow older, they increasingly smile while looking at their mother, rather than when looking away (Kaye & Fogel, 1980). By around nine months of age, smiling becomes integrated into communicative exchanges about objects (Adamson & Bakeman, 1985; Messinger & Fogel, 1998). At this stage, infants use smiles, along with gestures and eye contact, to draw the caregiver's attention both to their own actions and to the objects around them (Morales et al., 2000). As 1 year of age approaches, infants begin to share their emotional experiences with their caregivers by smiling and looking at them (Jones & Raag, 1989).

The developmental trajectory of smiling is well known, but contextually to the present work, the most compelling issue is that exploring this path can provide clues to the meanings of different types of smiles. All kinds of smiles in fact rise with age in early infancy, as indicated by literature reporting that certain types of smiling become more likely than others in specific periods of development (Fogel et al., 2000; Messinger et al., 2001). Nevertheless, different variants are basically indistinguishable in this ontogenetic route, if the contexts in which the smiles occur is overlooked (Messinger et al., 1999). If it is true that not all infant smiles uniformly reflect joy, the significance of the various types of infant smiles remains an open question. Another consequent key inference is that different kinds of smiles may convey qualitatively distinct connotations.

In Chapter 4, the meanings of different types of smiles were examined from a morphological perspective, without disregarding the contextual framework. 1-year-old tested subjects smiled even during the most stressful phase of the experimental task (the same task as the one in Chapter 3). Smiles, in this case, were directed towards the experimenter (social context), yet in a setting entirely devoid of interaction (as the experimenter wore a mask and refrained from

communicating either verbally or non-verbally). This scenario emerged as an ideal context for investigating smiles that did not signify genuine happiness, but likely fulfilled an alternative function. To conduct this analysis, the Baby FaceReader software (see Chapter 2 for detailed description and functioning of the software) was utilized, ensuring full compliance with the objectivity standards dictated by the ethological approach. By limiting the investigation to the facial musculature activated in the infants' smiles during the test, it was revealed that the smiles which did not correspond to authentic joy were those typically described in the literature as non-spontaneous (lacking the activation of the cheek muscles), thus suggesting the potential for a manipulative use of facial expressions, whether consciously or not. The capacity to modulate facial activity for goal-oriented actions that such behavior necessitates indicates a sophisticated level of SR. Furthermore, it is noteworthy that this ability is already observable at the age of one year, even though it remains uncertain whether it is intentional or not. This phenomenon also implies a certain degree of contextual awareness, as well as an understanding of the role of the interlocutor, who during the stress phase is the sole figure in complete control of the situation. Nevertheless, the most compelling aspect for further speculation remains the intrinsic function of these varied smiles. In Chapter 4 the Ansfield (2007) 's perspective has been adopted, suggesting that smiles can have a self-regulatory function of coping with negative emotional experiences. If this is true, the recruitment of different facial muscles is a key element, employed to shape smiles in different contexts and for different purposes. Among these purposes, it is reasonable to hypothesize a strategical use of facial expressions.

1.3 Emotion regulation and attachment styles

Along with behavioral and emotional expressions, parental style determines the development of SR in early stages of life. Among many aspects and measurements of this variable, the parent-child attachment relationship is well known to provide the sets needed to develop a range of adaptive or maladaptive patterns of socioemotional and behavioral functioning across childhood (Ainsworth, 1979; Bowlby 1969, 1973; Carlson & Sroufe, 1995). Attachment is expected to exert a wide-ranging influence, as it is considered a fundamental basis for the development of children's ability to effectively identify and manage their emotions, as well as to cope with stressful experiences in an adaptive manner (i.e. SR and ER). The Strange Situation Procedure (SSP; Ainsworth et al., 1978) is the most widely utilized method for assessing attachment in early childhood (Bowlby, 1969, 1973, 1980). The SSP is a standardized observational procedure (Ainsworth & Wittig, 1969) designed to elicit and intensify a child's

attachment behaviors towards their parent by placing the child in a moderately, yet progressively stressful situation. It involves the presence of an unfamiliar adult as well as two episodes of separation and reunion with the mother. The coding of the Strange Situation Procedure (SSP) is grounded in the observation of the overall organization of a child's attachment behavior and involves two levels of assessment. The first level utilizes graduated ordinal scales, measured on a Likert scale, which capture specific behavioral sequences that the child may exhibit across the different episodes of the procedure (for more detailed description, see Chapter 5). A significant challenge for the most recent literature and the present work is that fully capturing the behavioral patterns observed in the SSP likely requires far more than just two dimensions and four scales. Conversely, a multiple dimensions system should encompass the range of behaviors exhibited in this context. While many factors (such as distance interaction, passivity, quality of exploration, preferential treatment of the caregiver, affective sharing, search behavior during separations, wariness, and greeting behavior) are not included in the formal coding system for various reasons, some function as binary indicators (either present or absent), while others, though dimensional in nature, have proven difficult to scale effectively. Nonetheless, in her early work, Ainsworth placed particular emphasis on the importance of the ethological method, as evidenced by her focus on detailed behavioral observations and the development of measures based on the actual behaviors of mothers and infants (Waters & Beauchaine, 2003). In Chapter 5, a continuous rather than discrete measurement of behaviors during SSP has been implemented, as many other researchers previously did (see Fraley & Spieker, 2003; Sroufe, 2003). Even though the debate between types and dimensions was not a central concern for Bowlby (1969), as the understanding of the role attachment processes play in shaping social and emotional development deepens it becomes increasingly important to ensure that the most accurate and effective measurement models is available. Measuring children solely on a security dimension fails to capture the nuanced distinctions between different insecure strategies. Based on this, in Chapter 5 a novel ethological method is proposed for the analysis of SSP in contrast to the classic categorical approach. From an established ethogram, specific behaviors that align with Ainsworth's four primary attachment categories were continuously measured, allowing consideration of their frequency and duration. This methodological approach provides a reliable and quantifiable assessment of the child's attachment behavior, as it enables a comprehensive understanding of behavioral patterns during the SSP. By utilizing video-analysis coding systems, objectivity is ensured and individual biases minimized, ultimately offering a robust framework for evaluating the attachment as a feature contextual to development.

It is, however, essential to report that Chapter 5 remains incomplete, pending the final validation of the new method by a subject-matter expert. This expert will conduct the analysis using the traditional methodology on the same subjects. The alignment, or any potential discrepancies between the two approaches, will provide critical insights into the reliability of the new method, while also potentially highlighting the weaknesses or limitations of the traditional approach.

METHODS OF INVESTIGATION

2.1 Tested subjects: a cohort from the Life-MILCH project

The subjects included in the present study are part of a cohort drawn from a larger longitudinal project with broader research objectives.

The European Commission Life Program in 2019 financed the **Life-MILCH Project titled “Mother and Infant Dyads: Reducing the Impact of Endocrine Disrupting Chemicals in Milk for a Healthy Life.”** This Italian pilot study aims to ascertain the relationship between levels of maternal milk contamination/exposure to endocrine disrupting chemicals (EDCs) and the neurobehavioral development and health of infants. The Life-MILCH project is a quinquennial, multicenter investigation that involves three distinct hospital centers across two Italian regions: Emilia-Romagna (Parma and Reggio Emilia hospitals) and Sardinia (Cagliari hospital). Furthermore, the Pept-Lab at the University of Florence is tasked with conducting the chemical analyses (<https://lifemilch.eu/en/index.html>).

The Life-MILCH project represents a pioneering study in Europe that seeks to correlate EDC exposure in mother-infant dyads with subsequent health ramifications for infants in three selected city hospitals within two different Italian regions. Emilia-Romagna is a highly anthropogenic area characterized by intensive agriculture and farming practices. In contrast, Sardinia is a relatively rural island situated in the Mediterranean Sea, featuring comparatively low levels of urbanization. These differing geographical contexts facilitate the evaluation of potential variations in exposure between urban and rural settings in Italy, thereby enabling the development of a risk assessment model for each surveyed exposure.

The primary objective of this project is to assess the impact of EDCs on human health in order to formulate specific strategies aimed at reducing EDC exposure. This investigation focuses on mother-infant dyads, which are particularly susceptible to contamination, and considers breast milk as a vital route of exposure that is crucial for promoting optimal development and future health outcomes for infants. Specifically, the aims are: (i) to measure EDC levels in breast milk and assess EDC exposure in infants during their first year of life; (ii) to correlate EDC concentrations in breast milk with infant growth, physiological and reproductive development,

including neurobehavioral progress within the first year; (iii) to devise a statistical risk model linking exposure sources to various geographic areas to inform public health prevention policies; and (iv) to evaluate the effectiveness of prevention and educational campaigns targeting informed women.

The activities of the project are designed to enhance understanding of the relationship between EDC exposure and health outcomes, with the goal of mitigating the impact of these chemicals on infant health. Specifically, the principal actions of the project include: (1) an initial screening to assess EDC levels in various biological samples and evaluate infants' health status to develop a risk assessment model; (2) the creation of a statistical and computational risk model, along with specific recommendations for stakeholders, with a particular focus on young women; (3) the initiation of information and prevention campaigns aimed at young women of childbearing age, as well as pregnant and lactating women; and (4) a subsequent screening to evaluate the effectiveness of the short-term prevention and awareness campaign among pregnant women by comparing the results from the first and second screenings. Now, the Life-MILCH project is running phase (2) and (3).

During the first screening 689 women across the three involved centers have been recruited in the third trimester of pregnancy and the collection of clinical-anamnestic evaluations, lifestyle factors, and nutritional habits through specific questionnaires based on the previous Life-PERSUADED Project (La Rocca et al., 2018). The purpose of these questionnaires is to describe behaviors, lifestyle choices, and nutritional practices that may expose mothers and infants to EDCs during pregnancy and the postnatal period at 1-, 3-, 6-, and 12-months following delivery. Additionally, EDC exposure is being assessed through biological samples, including serum, maternal and infant urine, and breast milk.

Following the initial phase, the project has been extended to include a follow-up study examining the effects of pre- and postnatal exposure to EDCs on child development during the second year of life, specifically at 20 to 24 months of age. This extension spans from February 2023 to February 2024. The follow-up evaluated emotional responses, social attachment, cognitive and psychomotor development, adiposity, and pubertal stages. Additionally, hormonal responses were assessed through saliva samples collected before and after behavioral testing. For this purpose, 80 mothers and their children, who were previously recruited for the Life-MILCH project, have been invited back for assessment. The children have been examined at 20 months of age, through the Strange Situation Procedure (see Chapter 5).

Inclusion and exclusion criteria of tested subjects. The Life-MILCH study focuses on mother-child dyads during the first year of the child's life, starting with mothers in the final month of pregnancy. Pregnant women were recruited at Parma Hospital between the 36th and 41st weeks of gestation.

Inclusion criteria consisted in healthy pregnancies in women of any ethnic background, between the 36th and 41st weeks of gestation; comprehension of the study's objectives and willingness to participate in follow-up for 12 months after birth; full-term pregnancies with an obstetric risk score of 0.

Exclusion criteria consisted in diagnosis of fetal anomalies or gestational pathologies; likelihood of relocation outside the region within the following 12 months; presence of chronic or genetic diseases; preeclampsia, hypertension, or gestational diabetes; medical indications or personal choice to discontinue breastfeeding.

2.2 Experimental procedures

Observational methods, where individuals are placed in either structured or unstructured settings to provoke specific reactions, are commonly applied to infants, toddlers, and young children, but less frequently to older children, adolescents, or adults (Adrian et al., 2011). These observations can take place at home, offering a naturalistic perspective on interactions and behaviors, or in a lab setting, which allows for more controlled and uniform tasks (Cole et al., 2004). Researchers can choose from a variety of unstructured, semi-structured, or fully structured tasks based on the focus of their study and available resources (e.g., space, time, stimuli). Since young children depend on external support to regulate their emotions, tasks aiming to assess their independent ever skills typically involve placing children in situations designed to induce distress, while caregivers are instructed to "remain neutral" or avoid intervening (Parritz, 1996).

These tasks generally fall into two categories depending on their primary focus: delay tasks and emotion-eliciting tasks. Delay tasks typically involve presenting a desirable object (such as a toy, treat, or gift) and requiring the child to wait for a certain period (using self-regulatory strategies) before receiving it, known as delay of gratification (e.g., Cole et al., 2003; Grolnick et al., 1996; Grolnick et al., 1998). A variation of the delay task requires children to occupy themselves while their caregiver completes another task (e.g., filling out questionnaires; Diener et al., 2002). In all delay tasks, researchers can decide whether caregivers should remain

passive or actively engage in regulating the child's behavior, depending on the construct being measured.

Emotion-eliciting tasks, on the other hand, assess children's ability to regulate emotions in response to specific emotional stimuli, such as frustration (Calkins et al., 2002; Calkins & Johnson, 1998; Little & Carter, 2005), fear (Braungart-Rieker et al., 2010; Buss & Goldsmith, 1998; Feldman et al., 2009), sadness or disappointment (Cole et al., 1994; Morris et al., 2011; Saarni, 1979), and positive emotions (Carlson & Wang, 2007). These tasks allow researchers to explore how children react emotionally and the regulatory strategies they employ. For instance, Buss & Goldsmith (1998) observed differences in how effective certain regulatory strategies were in managing frustration and fear during behavioral tasks. A specific type of emotion-eliciting task involves exposing infants or young children to unfamiliar social interactions with their caregivers or strangers, as seen in the Still-face paradigm (Tronick et al., 1978), the SSP (Braungart & Stifter, 1991; Feng et al., 2008), and the Stranger Approach task (Goldsmith & Rothbart, 1999; Parritz, 1996).

In the present study, the observational method applied on a frustration paradigm has been preferred. Depending on the paradigms, infants and mothers were video recorded in a consultation room located at the Parma Hospital or in a playroom at the Unit of Developmental Psychology at University of Parma. The entire procedures were video recorded.

The Barrier Task paradigm at 12 months. At 12 months mother-infant pairs were recalled for two different follow-up visits: the standard follow-up like and the neurodevelopmental assessment as at 6 months of life. The standard visit occurred at ± 30 days of the first year of life at the Breastfeeding Department at the Parma Hospital in the presence of a neonatologist and a psychologist. During the visit, the mother filled out the questionnaire to collect data about infant's lifestyle and nutritional habits in the last six months of life. The neonatologist assessed the infant growth parameters. The psychological assessment involved the administration of the Barrier Task procedure (Goldsmith & Rothbart, 1999). The procedure is described in detail in Chapter 3 and Chapter 4.

The Strange Situation procedure at 20 months. At 20 months mother-infant pairs were recalled for two different follow-up visits: the standard follow-up like and the neurodevelopmental assessment as at 6 and 12 months of life. The standard visit occurred at the Breastfeeding Department at the Parma Hospital in the presence of a neonatologist and a psychologist. The experimental procedure occurred at the Unit of Developmental Psychology, University of

Parma. On this occasion, the dyads were kept in a playroom for a 20-minute habituation period. During this time, mothers were asked to complete questionnaires regarding perceived attachment (ASQ – Attachment Style Questionnaire, not included in the present study) and dyadic habits (focused on circadian rhythms and general characteristics potentially linked to cortisol levels). Afterward, saliva samples were collected from both the child and the mother using swabs for cortisol analysis (baseline sample). Next, the dyad was accompanied to another room, where the mother was briefly informed about the Strange Situation procedure. General guidelines were provided regarding her behavior to ensure her responses remained natural (e.g., she was instructed on where to sit and where to place the child).

Once the Strange Situation procedure (described in detail in Chapter 5) was completed, the experimenter who initially greeted the dyad escorted them back to the original room. There, they waited for another 20 minutes while the child had access to toys and the mother completed a questionnaire on perceived temperament (QUIT – Italian Questionnaire on Temperament, not included in the present study). Finally, a second saliva swab was collected before the procedure concluded. Cortisol, a key product of the hypothalamic-pituitary-adrenocortical axis, is typically released in response to stress. Spangler & Schieche (1998) explored the link between negative emotional expressions (crying, fussing, sad faces) and cortisol reactivity during the Strange Situation Procedure (SSP) in 12-month-old infants. They found a positive correlation between negative emotional expressions and cortisol levels in insecure infants, but not in secure ones, who showed no such link. The authors explained this difference by stating that, in secure infants, negative emotional expressions likely serve as coping mechanisms, preventing a physiological stress response. In insecure infants, however, these behaviors reflected a more general stress response due to a lack of effective coping strategies. Spangler & Schieche (1998) did not explore other regulatory behaviors or cortisol recovery, leaving gaps in understanding how stress is managed or resolved. For the present work the cortisol analysis has not been considered. The procedure is described in detail in Chapter 5.

2.3 The ethological approach on data analysis

Research on infant ER and ED generally employ a variety of methodological approaches, always consistently framed within the developmental milieu (Thompson, 2019; Wakschlag et al., 2010). The observational method is the most widely used in infancy to measure ED and it has a miscellaneous nature.

In contemporary behavioral sciences, the focus on survey methods has often overshadowed the foundational role of observation in the scientific process. Within psychology, observational

methods have fluctuated in prominence. Initially, observation was critical as psychologists sought to affirm their field's scientific legitimacy by relying on tangible, observable evidence. However, shifts toward cognitive approaches, with an emphasis on internal states and self-reported data, led to a decline in the use of observational methods. Despite this trend, the significance of observational research remains clear. Simple observation can challenge prevailing assumptions, and they have proved to be crucial when studying populations incapable of self-reporting, such as infants or individuals with cognitive impairments, where accurate behavioral measurement is essential. Importantly, observational research enhances external validity by ensuring robustness, representativeness, and relevance, particularly in person-environment research.

For all these reasons, an ethological and inference-free approach has been prioritized in the present work for data analysis.

Scoring infants' behavior. Coding observations involves systematically categorizing observed behaviors, in this case on recorded material. The coding process is crucial for ensuring that observational studies are replicable and maintain scientific rigor. Developing and implementing data codes need to be categorical, mutually exclusive, and exhaustive, meaning each behavior of interest is assigned a distinct and singular code. Clear and specific definitions of observation codes or behavior categories are essential to ensure consistency and accuracy throughout the study. The first step in developing these codes is to review existing codes from similar research and adapt them for the current study. Researchers must thoroughly pre-test and refine the coding system with the target population before the formal study begins. Codes should be based on the theoretical framework, research question, and observed behaviors. When uncertain, the general rule suggests to code more behaviors rather than fewer, as codes can later be consolidated into more meaningful categories if needed. This is particularly useful when behaviors are infrequent, as categories may need to be collapsed for statistical analysis. Throughout this process, broad new ethograms were designed, therefore the final coding system aligns with the research questions and provides sufficient information to answer them accurately. For the studies reported in Chapters 3, 4, 5 a frame-by-frame video analysis for each subject was conducted via Pot Player®, with the accuracy of 1 sec time window. Following the ethogram, coding consisted of manually entering the starting time (and ending time, when lasting >1sec) and the corresponding code, anytime a behavior occurred, into an Excel© sheet (see Appendix for an example of the work sheet -1- and the ethograms implemented -2, 3).

Intercoder reliability. Coding observations inevitably involves some degree of subjectivity, making it vulnerable to observer bias. To mitigate this, multiple observers should independently code the same observations, allowing the comparison of their agreement to assess the reliability of the coding system. High inter-observer agreement (or inter-rater reliability) suggests that the behaviors are clearly defined and easily identifiable, while low agreement indicates potential ambiguity or bias. In such cases, the problematic measures may need to be refined or excluded from the analysis, which could result in significant loss of effort. To avoid such issues, significant time has been invested in training observers (students) for ensuring a reliable coding system. Before conducting any statistical analyses, inter-observer agreement has been calculated. Observational studies typically require at least two observers with a minimum of 50% overlapping observations, though this guideline can vary depending on the context. Cohen's Kappa has been used for the present work (Cohen, 1960). The percentage of agreement has been reported in Chapters 3, 4, 5 in the Methods sections.

Scoring infants selected facial expressions: Baby FACS and Baby FaceReader. The systematic analysis and classification of facial expressions during infant-caregiver interactions are crucial for investigating early socio-cognitive and socio-emotional development. However, manual coding of these expressions is labor-intensive, and differences in methodologies, combined with potential subjective biases, can compromise the consistency of findings across various infant research labs. Recent advances in computer vision have greatly enhanced the accuracy of automated facial expression recognition in adults, offering a promising, more efficient alternative to manual coding (e.g., Ertugrul et al., 2019; Niinuma et al., 2019; Yang et al., 2021). Implementing these automated techniques in infant facial expression analysis can renew the process as well as improve the reproducibility of findings across studies. This would enable researchers to apply objective metrics to larger datasets, overcoming the limitations posed by manual coding methods. The most comprehensive framework for coding infant facial expressions currently available is the **Baby Facial Action Coding System** (Baby FACS; Oster, 2006). Like the adult version, it categorizes facial movements by analyzing the anatomical basis of specific facial muscle Action Units (AUs) in infants, capturing both the frequency and duration of these movements. The combinations and intensities of these AUs are used to infer emotional states, while also accounting for the anatomical differences between infant and adult facial structures (Oster & Ekman, 1978). Unlike other approaches, Baby FACS derives emotional interpretations externally, based on the coding rather than integrated within it, thereby minimizing subjective bias and enhancing the replicability of results (Cohn et al., 2007;

Oster et al., 1992). BabyFACS can be used alongside strategies to test *a priori* hypotheses about specific discrete emotional expressions in infants and young children, particularly those derived from adult universals. Its ability to differentiate subtle facial movements offers advantages over other coding systems. Importantly, BabyFACS can empirically detect nuanced variations in infants' emotional expressions across diverse situations, regardless of whether these resemble typical adult expressions.

In addition to studying emotional development, BabyFACS is well-suited for ethological research on facial signaling in infants. As a comprehensive coding system, it identifies structured patterns of facial expressions within specific age groups (Oster & Ekman, 1978). The significance of these expressions, along with their communicative functions, can be assessed by examining their contexts, behavioral correlates, and impact on others. An ethological perspective enhances our understanding of facial expressions (Smith, 1977), suggesting they convey not only emotional states but also insights into the infant's cognitive processes, behavioral tendencies, and emotional regulation efforts. This framework is particularly valuable for interpreting these expressions.

However, Baby FACS requires coders to undergo rigorous training and certification, which limits its widespread use and restricts the number of studies that can employ this method. This limitation can impede the ability to conduct more extensive assessments of affective facial expressions in developmental research. For this study, it was important to identify a software solution that met these specific requirements for analyzing infants' facial expressions using the FACS method.

Baby FaceReader 9 (Noldus, 2022) is a commercially available tool designed for automated analysis of infant facial expressions. It features a user-friendly interface, making it accessible to research teams with limited technical expertise. By employing a deep learning-based approach (Zafeiriou et al., 2015), Baby FaceReader can accurately locate a face within an image. The output provides continuous intensity measurements for individual AUs, as defined in Oster's Baby FACS (for detailed information regarding model architecture and training, see Noldus, 2021), achieving a maximum accuracy of 15 frames *per second*. Zaharieva et al. (2024) provided a detailed performance evaluation of the Baby FaceReader 9, comparing it to manual coding of affective facial expressions in a longitudinal dataset of infants at 4 and 8 months of age during naturalistic face-to-face interactions. They found that the global emotional valence formula of Baby FaceReader 9 exhibited acceptable classification accuracy in distinguishing manually coded positive facial expressions from negative or neutral ones. Overall, results bolster the argument for employing selected automatically detected facial actions to evaluate

positive and negative emotions in young infants, while casting doubt on the dependability of complex a priori formulas.

The software was acquired by the University of Parma in 2024 and utilized for the purposes of Chapter 4. In this case, *a priori* formulas have not been considered. The AUs constituting the prototypical expression of positive affect in infancy (i.e. smile) have been taken into account as present (contracted muscles) or not.

Assigning discrete behaviors to attachment variables. Given that Chapter 5 is entirely focused on the methodology, the details are not reiterated here. However, it is important to highlight that the methodological approach aims to integrate traditional practices with innovative elements. The Strange Situation classifications primarily rely on “interactive behaviors” exhibited toward the mother during the two reunion episodes (Episode 5 and 8 in the procedure). The term “interactive behavior” encompasses actions traditionally evaluated on four 7-point scales: Proximity seeking, Contact maintaining, Avoidance of proximity and contact, and Resistance to contact and comforting. These behavioral macrocategories have been the starting point for the method implemented in the present work. While the reunion episodes are emphasized, scoring should always involve a thorough review of the entire procedure, which has been historically overlooked. Chapter 5 tries to primarily fills this gap.

2.4 The statistical approach

For the present work, **Generalized Linear Mixed Models** (GLMMs) have been used to analyze the collected data. The reason relies on the potentiality of this approach on ethological observations. For instance, Pekàr and Brabec (2018) provide an overview of mixed models, underscoring their relevance in studying animal behavior. Moreover, Harrison et al. (2018) offers practical considerations for mixed effects modelling and multi-model inference and their application in ecological and behavioral research.

GLMMs are indeed powerful statistical tools in ethological research, offering several advantages that enhance the analysis of individual behavior. One of the primary benefits of GLMMs is their ability to account for the non-independence of data, which is common in studies involving repeated measures or hierarchical data structures. By incorporating random effects, GLMMs can effectively model variability arising from individual differences or nested data, thus providing a more accurate representation of the factors influencing behavior (Bolker et al., 2009). By modelling individual variability and utilizing all available data, these models

yield more accurate estimates of effects, enhancing the statistical power of analyses compared to traditional methods that may overlook complexities inherent in the data.

A noteworthy aspect is the specific methodology employed in the study of FACS in Chapter 4. Studies on facial signals have often adopted a binary perspective, asserting that specific combinations of facial AUs are directly linked to distinct emotions, such as fear or anger (Ekman & Oster, 1979; Matsumoto et al., 2008). However, this view is problematic since many AUs are shared across different emotional expressions, some AUs are not utilized in any basic emotion, and facial signals exhibit significant dynamic characteristics (Jack et al., 2014; Krumhuber et al., 2013). Consequently, the significance of AUs and their combinations may be more probabilistic than deterministic (Crivelli & Fridlund, 2018). The emphasis on a limited range of stereotypical facial expressions has hindered a deeper understanding of the potential for variability in facial signals. The advent of automated FACS coding provides an opportunity for analyzing extensive datasets of diverse facial expressions (Lewinski et al., 2014). However, the tools for analyzing this data have not kept pace with advancements in coding software. Unfortunately, the proliferation of different methodologies has led to reduced reproducibility. FACS datasets possess unique features that render traditional statistical models inadequate. FACS data intertwine spatial and temporal AU combinations, with temporal factors influencing interpretation (Krumhuber et al., 2013). Moreover, different facial signals may occur in sequences, often intertwined with speech or gestures (Kessous et al., 2010). At a fundamental level, FACS coding categorizes AUs as present or absent, with intensity measures available. Traditional analyses, such as analysis of variance (ANOVA), examine the mean usage of AUs across conditions. However, given the binomial and categorical nature of FACS data, these methods violate certain distributional assumptions (Harris & Alvarado, 2005). Additionally, AUs do not necessarily follow identical distributions; for instance, certain AUs might only appear in subsets of individuals or be combined with others to convey different meanings, resulting in bimodal distributions. Shared participant characteristics (e.g., sex, origin, age) can also influence facial expressions (Jack et al., 2012). Addressing these levels of dependence is crucial to prevent pseudoreplication (Hurlbert, 1984; Waller et al., 2013). Nonetheless, AUs themselves are rarely isolated: facial expressions typically involve multiple AUs working in concert (Krumhuber & Scherer, 2011). This suggests that the combination of AUs may be more significant for facial communication than their individual occurrences. In the present study, **NetFACS**, an R package developed by Mielke et al., 2022, was employed for the analysis. Network approaches provide a highly flexible framework for examining communicative units, such as AUs, treating them as "nodes" interconnected by "edges" that represent various types

of interactions. This methodology facilitates the investigation of the interdependencies among AUs, the reconstruction of stereotypical facial signals across different contexts, and the identification of clusters of AUs without prior knowledge of the underlying contexts.

AIM OF THE STUDY

This study investigates the development of emotional regulation in infants during the first year of life, a critical period for establishing the foundation of future emotional well-being. Utilizing an ethological approach, we analyze emotional behaviors and social interactions in naturalistic settings, allowing for the observation of how 1-year and 21-months-old infants express and manage their emotions in response to environmental and social stimuli, during frustration-inducing procedures. Through observational methodologies, we identify key emerging mechanisms of emotional regulation, integrating traditional approaches with newly developed, purpose-driven measures.

This research contributes to a deeper understanding of emotion regulation in infancy, emphasizing the importance of socio-emotional competence in fostering healthy emotional development. Moreover, the innovative approach chosen to analyze and interpret these data brings a fresh perspective to developmental psychology, a field that often leans away from inference-free, objective methodologies. The present work may represent a first step toward more evidence-based, objective approaches in the study of early childhood development.

To be submitted

EARLY CHILDHOOD SELF-REGULATION: INSIGHTS FROM AN ETHOLOGICAL PERSPECTIVE

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Abstract

Regulatory abilities developed during toddlerhood are critical for fostering social skills and emotional intelligence. Pro-social behaviors and self-control are key elements of social competence. This study extends understanding of regulatory patterns during the first year of life, focusing on how variables such as sex and social context influence emotional responses in stressful situations. An ethological approach was employed, diverging from traditional bottom-up frameworks.

Twenty-nine 1-year-old toddlers (15 ♂, 14 ♀) participated in the Barrier Task, an adapted impossible-task paradigm. The child played with a toy for 2 minutes (P); the toy was then placed behind a transparent barrier, visible but out of reach (B). After 2 minutes, the toy was returned to the child for another play period (R).

During the stressful B phase, toddlers exhibited longer self-regulatory behaviors, with females demonstrating significantly more than males, supporting the hypothesis that females better manage negative emotions. Males showed greater dependence on their mothers, gazing at them longer during P and B conditions, while females showed no such effect.

Childcare attendance emerged as a significant factor. Toddlers attending childcare gazed at their mothers later than non-attending peers, reflecting a shift toward opportunistic environmental orientation. In the B condition, non-attending toddlers gazed longer at their

mothers, while attending toddlers displayed consistent gaze patterns, suggesting reduced maternal reliance during stress.

This study highlights early self-regulation as a marker of emotional competence and suggests that childcare experiences may foster adaptive stress management strategies, contributing to our understanding of emotional regulation development in toddlers.

Keywords: other-oriented behavior - toy-removal task - emotional cognition - human ethology
– *Homo sapiens*

1. Introduction

1.1. General framework

Developing social-emotional competence is crucial for setting up appropriate relationships throughout childhood, and this process substantially depends on children's early social experiences. Interactions with primary caregivers begin at birth and provide toddlers with resources that enable them to handle internal and external stimuli in a secure and continuously regulated environment. The ability to regulate attention, arousal, and emotion starts developing at early stages of life and establishes the groundwork for assembling individual functioning over lifetime (Sameroff & Emde, 1989; Sroufe, 1996). Indeed, during the first months of life, caregivers work as an external source of baby behavior regulation. For example, soothing a baby or moving his/her attention away from distressing stimuli are common practices for managing negative affective states (Harmann et al., 1997). As the child grows, he/she gradually develops greater ability to regulate emotional and behavioral responses, with control becoming increasingly internalized (Rothbart & Rueda, 2005). Individual variations in reactivity, whether emotional, motor, or attentional, constitute the basis of temperament, along with self-regulation (Rothbart & Derryberry, 1981; 2002). The term reactivity refers to motor, emotional, and attentional responses to both internal and external stimuli, while all those processes modulating these responses fall under the name regulation (Putnam et al., 2001; Rothbart & Bates, 2006). Emotional self-regulation regards the specific capacity to control emotional experiencing and its expression (Calkins & Hill, 2007). Rothbart & Derryberry (1981) outlined the reactivity-regulation framework for temperament, which has been functional in developing methods to assess it across lifespan. Although temperament has been demonstrated fairly consistent across various situations and stable over time, developmental changes seem to affect this domain (Rothbart & Bates, 2006). In this model and those evolved from its refinement (Rothbart, 1989a, 1989b; Rothbart & Ahadi, 1994; Rothbart & Bates, 1998; Rothbart et al., 2000), the ability to suppress a dominant response and engage in a subdominant one is referred to as effortful control, which is implicated in a wide range of self-regulatory outcomes, including various forms of emotion regulation, from toddlerhood to early school age (Kochanska et al., 1996; 1997; 2000). The effortful control is of particular interest since it encompasses the voluntary ability to shift, focus, and sustain attention and behavioral regulation, which involves both inhibitory (i.e. the ability to intentionally suppress behavior) and activation control (i.e., the capacity to act despite a strong tendency to avoid it). By the end of the first year of life, such control mechanisms start emerging. At this stage, the maturation of the frontal lobe, along with its connections to parietal structures involved in attentional orientation, promotes a

gradual enhancement of goal-directed and intentional focus. Concurrently, the transition from relying only on parents in emotion regulation to displaying self-regulatory strategies (e.g., gaze aversion, self-soothing, escaping, attention-seeking) starts to occur (Kopp & Neufield, 2003). According to Planalp et al. (2017) there is a clear distinction between temperament and emotional reactions, which refer to immediate, short-term responses to specific stimuli. Emotional traits design the individual temperament being patterns of emotional responses that remain consistent across different situations. Rothbart & Derryberry (1981)'s temperament model provides limited specificity regarding the regulatory behaviors modulating various types of emotional responses (Stifter et al., 1999). Additional factors such as sex differences and social experiences also play a significant role at this age in shaping the emergence of regulatory patterns.

Daily situations provide salient occasions for acquiring emotional regulation skills, by modulating behaviors which toddlers already possess. Opportunities for training emotional and behavioral regulation in infancy may be closely associated with experiencing frustration. Toddlers frequently encounter frustrating events in their routines, such as waiting for food or sitting in a car seat. For this reason, experimental procedures to study early regulatory behaviors often imply distress-inducing episodes. The technique involves placing the infant in a frustrating or restrictive situation to assess the strategies they use to cope with it (Buss and Goldsmith, 1998).

1.2. The goal of the study

The overarching goal of this study is to evaluate which factors affect emotional responses in 1-year-old toddlers under a frustrating situation. We applied an induced-frustration experimental paradigm (the Barrier Task) and measured the behavioral reactions to provide an ethological description of toddlers' responses, offering an authentic snapshot of the maturation of early regulatory capacities at a very specific point in human development. During the Barrier Task, the infant participates in three consecutive two-minute episodes while being held by the mothers: i) a toy play session, ii) a period where the toy is made inaccessible by an experimenter, and iii) a final phase where the toy is returned for free play.

1.2.1 Hypothesis 1

While sex differences in temperament are rarely found in infancy, early regulation differences between sexes may be observed (Weinberg et al., 1999). Females showed more emotion regulation strategies than males (Else-Quest et al., 2006). Accordingly, emotional

dysregulation seems to occur more frequently in males than in females (Herndon et al., 2013). If infant males are less equipped to face frustrating situations, we predict that they i) engage in more attentive behaviors towards the mother (*Prediction 1*) especially during the administration of stressful stimuli and ii) females show more self-regulatory behaviors than males (*Prediction 2*), especially when the toy is made inaccessible by the experimenter.

1.2.2 Hypothesis 2

The progress of emotional competence across life is also due to social experiences beyond the familiar setting. An expanding body of research has examined the construct of emotion regulation in relation to infant social functioning (Halle & Darling-Churchill, 2016). For instance, children manifesting an emotionally positive mood are preferred by their peers than emotionally negative children, thus improving social acceptance (Hubbard & Coie, 1994; Putallaz & Gottman, 1981). If social experiences affect socio-emotional competence, we expect that toddlers having daily opportunities to interact with people beyond the familiar setting (i.e., attending childcare) i) are less mother-oriented (*Prediction 3*) and ii) express more self-regulatory behaviors than toddlers not attending childcare during the Barrier Task (*Prediction 4*), especially when the toy is made inaccessible by the experimenter.

2. Methods

2.1 Participants

The sample comprises 29 caregivers and their 12-months-old toddlers (15 males, 14 females), recruited as a part of a longitudinal investigation led by the University of Parma. All mothers were recruited between 36th-41st week of gestation, at the Parma Hospital, Italy. Recruited toddlers were all healthy full-term toddlers with routine pre- and postnatal medical histories. Specific questionnaires have been administered to the caregivers with the purpose of describing lifestyle and habits at 1,3,6 and 12 months after delivery. From these questionnaires we got information about childcare attendance and composition of the families: 54.5% of toddlers participating in the present study attend childcare from 6 or 12 months of age, 51.7% of them are first child. Mothers' age is 33.4±4.07 mean ±SD years. Data have been collected between April 2022 and January 2023.

2.2 Experimental procedure

Toddlers and caregivers were observed in a consultation room located at the Parma Hospital, Italy. Via two cameras (Sony Handycam® CX405), one offering a full-frontal view of the infant's face and one offering a frontal, enlarged view of the dyads' faces, we were able to record the entire scene.

The Barrier Task procedure. The Laboratory Temperament Assessment Battery (LAB-TAB, Goldsmith & Rothbart, 1996) assessing infant behavioural responses to stimuli in a controlled setting, consists in 20 tasks. Among these tasks, we selected the Barrier Task procedure to induce frustration in the toddlers, by preventing them to reach a desired object (i.e. a toy). The test was administered before the paediatric examination, to avoid previous distress to the toddlers. The assessment was conducted only if they were awake and in an alert and relaxing state. During the Barrier Task procedure, the infant is engaged in three two-minute episodes: (1) Play episode (P) during which the infant is allowed to manipulate and play with the toy chosen before the procedure; (2) Barrier episode (B) during which the experimenter removed the toy from the infant's hands and placed it behind a transparent plastic barrier, thus creating an out-of-reach situation lasting 2 minutes, or until 20 seconds of hard crying; (3) Return episode (R) during which the experimenter removed the plastic barrier and returned the toy to the infant, for another session of free play (Figure S.1a,b,c in Supplementary Materials). During the Barrier episode the mothers were asked not to interact with their infant. The same experimenter (C.S.) conducted all the tests. Across all the episodes, both mothers and experimenter always wear a white face mask.

2.3 Scoring toddlers' behavior and operational definitions

Frame-by-frame video analysis for each subject was conducted via Pot Player®, with the accuracy of 1 sec time window. Following the purpose-build ethogram, coding consisted of manually entering the starting time (ending time, when lasting >1sec) and the corresponding code, anytime a behavior occurred, into an Excel© sheet. Behavioral categories included in the complete ethogram are: gaze and attentive patterns, vocalizations, facial expressions, self/other-oriented displays, engagement with the toy, engagement with the barrier, gestures, postures. To investigate specific aspects of early self-regulation, duration and latency of specific behaviors have been calculated in secs for the analysis (Table S.1 in Supplementary Materials).

2.4 Intercoder reliability

29 videos were analyzed by an assistant (coder#1). Before starting the video analyses, inter-observer reliability was checked between coder#1 and a senior coder (C.S., coder#2) on about 10% of total recording, covering all three experimental conditions and considering all behavioral categories. The values of Cohen's kappa coefficient (κ) (Cohen, 1960) between the coders were higher than 70% for each behavioral category (mean $\kappa = 0.84$).

2.5 Statistical analysis

To test our hypotheses, we ran a Generalized Linear Mixed Model (GLMM; glmmTMB package in R; Brooks et al., 2017; R Core Team, 2020; version 1.4.1717).

In the Model 1, we set as response variable the latency of the first gaze at the mother (Poisson error distribution). The fixed factors were the interaction between the sex and the condition (sex*condition), the interaction between childcare attendance and the condition (childcare*condition) and the age of the mother. Fixed factors showed no collinearity: $VIF_{\max}=1.31$, $VIF_{\min}=1.00$.

In the Model 2, we set as response variable the latency of the first gaze at the experimenter (Poisson error distribution). The fixed factors and the random factors were the same as model 1. Fixed factors showed no collinearity: $VIF_{\max}=1.49$, $VIF_{\min}=1.00$.

In the Model 3, we set as the response variable the duration of gazing at the mother (Poisson error distribution). The fixed factors and the random factors were the same as model 1. Fixed factors showed no collinearity: $VIF_{\max}=1.40$, $VIF_{\min}=1.00$. The subject identity and the date of the experiment were included as random factors.

In the Model 4, we set as the response variable the duration of gazing at the experimenter (Poisson error distribution). The fixed factors and the random factors were the same as model 1. Fixed factors showed no collinearity: $VIF_{\max}=1.12$, $VIF_{\min}=1.00$.

In the Model 5, we set as the response variable the duration of the self-regulatory behaviors (Poisson error distribution). The fixed factors and the random factors were the same as model 1. Fixed factors showed no collinearity: $VIF_{\max}=1.11$, $VIF_{\min}=1.00$.

By using a likelihood ratio test (LRT, anova with argument test 'Chisq'; Dobson, 2002) to test the significance of the full model (Forstmeier & Schielzeth, 2011), we compared the full model against a control model comprising only the random factor and the fixed factor "age of the mother". Then, the P values for the individual predictor were calculated on the basis of the LRT between the full and the null model by using the R function 'Anova' (car package, Fox & Weisberg, 2019). We performed all pairwise comparisons for the levels of the multilevel factor with the Tukey test (Bretz et al., 2010) by using the R package emmeans (Lenth et al., 2023).

3. Results

3.1. The first gazing

Model 1 (*at the mother*) - The full model was significantly different from the control model (Likelihood Ratio Test: $\chi^2_8 = 100.39$, $p < 0.001$, Table 1). The fixed factors condition, childcare attendance and the interactions condition*sex and condition*childcare attendance had a significant effect on the latency of the first gaze at the mother (response variable). The shortest latency of gazing at the mother occurred in the B, compared to both P (z-ratio=-7.165; df = inf; $p < 0.001$) and R conditions (z-ratio=-8.210; df = inf; $p < 0.001$). No differences were found between P and R condition (z-ratio=-1.068; df = inf; $p = 0.534$). Toddlers attending childcare gazed at the mother significantly later compared to those not attending childcare (Figure 1a). Males gazed at their mothers faster in B than R condition (z-ratio=-5.120; df = inf; $p < 0.001$), and in P than R condition (z-ratio=-3.043; df = inf; $p = 0.028$). No differences were found between P and B conditions (z-ratio=-2.096; df = inf; $p = 0.289$). Females gazed at their mothers faster in B than in P (z-ratio=-7.993; df = inf; $p < 0.001$) and R condition (z-ratio=-6.636; df = inf; $p < 0.001$). There were no differences between P and R conditions (z-ratio=1.392; df = inf; $p = 0.732$). Toddlers not attending childcare gazed at their mothers faster in B than P (z-ratio=-4.235; df = inf; $p < 0.001$) and R condition (z-ratio=-4.684; df = inf; $p < 0.001$). No differences were found between P and R conditions (z-ratio=-0.451; df = inf; $p = 0.998$). Toddlers attending childcare gazed at their mothers faster in B than in P (z-ratio=-5.800; df = inf; $p < 0.001$) and R condition (z-ratio=-6.792; df = inf; $p < 0.001$). No differences were found between P and R conditions (z-ratio=-1.006; df = inf; $p = 0.916$; Figure S.2 in Supplementary Materials).

Model 2 (*at the experimenter*) - The full model was significantly different from the control model (LRT: $\chi^2_8 = 624.48$, $p < 0.001$, Table 1). The fixed factors condition, childcare attendance and the interaction condition*sex had a significant effect on the latency of the first gazed at the experimenter (response variable). In B toddlers gazed at the experimenter faster than in P (z-ratio= -7.251; df= inf; $p < 0.0001$) and R condition (z-ratio= -15.232; df= inf; $p < 0.0001$). In P toddlers gaze faster at the experimenter compared to R condition (z-ratio= -12.761; df= inf; $p < 0.0001$; (Figure 2).

Toddlers attending childcare immediately gazed at the experimenter compared to those not attending childcare (Figure 1b). Females gazed at the experimenter faster in B and P than in R condition (B vs R, z-ratio= -11.551; df = inf; $p < 0.0001$; P vs R, z-ratio= -11.439; df= inf; $p < 0.0001$). No differences were found between P and B conditions (z-ratio= -1.786; df= inf; $p = 0.475$). Males gazed at the experimenter faster in B condition than in P (z-ratio=-8.618; df

= inf; $p < 0.0001$) and R condition (z-ratio=-11.839; df = inf; $p < 0.0001$). In P the latency was shorter than in R condition (z-ratio= -5.917; df= inf; $p < 0.0001$). In P condition, females gazed faster at the experimenter than males (z-ratio=4.024; df = inf; $p < 0.0001$).

3.2. Gaze duration

Model 3 (at the mother) – The full model was significantly different from the control model (LRT: $\chi^2_8 = 105.38$, $p < 0.001$, Table 1). The fixed factors condition, condition*sex, and condition*childcare attendance had a significant effect on the time toddlers spent gazing at their mothers (response variable). Toddlers gazed longer at their mothers in B compared to R condition (z-ratio=2.500; df= inf; $p = 0.033$). No differences were found between B and P (z-ratio= 1.140; df= inf; $p = 0.490$) and P and R conditions (z-ratio= 1.562; df= inf; $p = 0.262$). Specifically, males gazed at the mother for longer in B than in R condition (z-ratio=5.395; df = inf; $p < 0.001$) and in P than R condition (z-ratio=3.399; df = inf; $p = 0.009$). No differences were found between B and P conditions (z-ratio=2.390; df = inf; $p = 0.160$). Females gazed at the mother for shorter time in B than in R condition (z-ratio=-2.995; df = inf; $p = 0.033$). No differences were found between B and P conditions (z-ratio=-0.675; df = inf; $p = 0.985$) and between P and R conditions (z-ratio=-2.435; df = inf; $p = 0.148$; Figure S.3 in Supplementary Materials). Toddlers not attending childcare gazed for longer toward the mother in B than in P (z-ratio=5.628; df = inf; $p < 0.0001$) and R condition (z-ratio=5.654; df = inf; $p < 0.0001$). No differences were found between P and R conditions (z-ratio=0.372; df = inf; $p = 0.999$; Figure S.4 in Supplementary Materials). Toddlers attending childcare showed no differences across the conditions.

Model 4 (at the experimenter) – The full model was significantly different from the control model (LRT: $\chi^2_8 = 171.36$, $p < 0.001$, Table 1). The fixed factors condition, condition*sex and condition*childcare attendance had a significant effect on the time toddlers spent gazing at the experimenter (response variable). Toddlers gazed for shorter time at the experimenter in the R than P (z-ratio=7.854; df = inf; $p < 0.001$) and B condition (z-ratio=7.273; df = inf; $p < 0.001$). No differences were found between P and B conditions (z-ratio=-0.843; df = inf; $p = 0.676$). Males gazed for shorter time at the experimenter during R compared to both P (z-ratio=6.460; df = inf; $p < 0.001$) and B conditions (z-ratio=4.507; df = inf; $p < 0.001$). No difference between P and B conditions (z-ratio=-2.241; df = inf; $p = 0.219$). Females gazed for shorter time at the experimenter during R compared to both P (z-ratio=5.508; df = inf; $p < 0.001$) and B conditions (z-ratio=5.861; df = inf; $p < 0.001$). No difference between P and B conditions (z-ratio=0.636; df = inf; $p = 0.988$; Figure S.5 in Supplementary Materials). Toddlers not attending childcare

gazed for shorter time at the experimenter during R compared to both P (z-ratio=8.641; df = inf; p<0.001) and B conditions (z-ratio=8.777; df = inf; p<0.001). No difference between P and B conditions (z-ratio=0.310; df = inf; p=1.00). Toddlers attending childcare gazed shorter in R compared to P (z-ratio=3.315; df = inf; p=0.012), whereas no difference between B and R (z-ratio=2.253; df = inf; p=0.214) and P and B conditions (z-ratio=-1.175; df = inf; p=0.849; Figure S.6 in Supplementary Materials) was found.

3.3. Self-regulatory behaviors

Model 5 – The full model was significantly different from the control model (LRT: $\chi^2 = 259.72$, p<0.001, Table 1). The fixed factors condition, sex and the interactions condition*sex and condition*childcare attendance had a significant effect on the time toddlers spent performing self-regulatory behaviors (response variable).

Toddlers spent more time performing self-regulatory behaviors in B than P (z-ratio=11.031; df = inf; p<0.001) and R condition (z-ratio=6.463; df = inf; p<0.001) and in R compared to P condition (z-ratio=-6.665; df = inf; p<0.001; Figure 3a). Females spent more time in performing self-regulatory behaviors than males (Figure 3b).

Males spent less time in self-regulatory behaviors in P than in B (z-ratio=9.422; df = inf; p<0.001) and R condition (z-ratio=-4.034; df = inf; p<0.001). During the B condition, they performed self-regulatory behaviors for longer compared to the R condition (z-ratio=8.197; df = inf; p<0.001). Females performed self-regulatory behaviors for shorter time in P than in B (z-ratio=6.463; df = inf; p<0.001) and R conditions (z-ratio=-6.877; df = inf; p<0.001). No differences were found between the B and the R conditions (z-ratio=-0.562; df = inf; p=0.993). In R (z-ratio=-4.349; df = inf; p<0.001) and P conditions (z-ratio=-3.944; df = inf; p<0.001), males performed shorter self-regulatory behaviors than females. No sex difference was found in B condition (z-ratio=-0.871; df = inf; p=0.953) (Figure 3c).

Toddlers not attending childcare performed self-regulatory behaviors for shorter time in P than in B (z-ratio=8.368; df = inf; p<0.001) and R conditions (z-ratio=-3.869; df = inf; p=0.002). During the B condition, they performed self-regulatory behaviors for longer compared to the R condition (z-ratio=5.619; df = inf; p<0.001). Toddlers attending childcare performed self-regulatory behaviors for shorter time in the P compared to both B (z-ratio=8.441; df = inf; p<0.001) and R conditions (z-ratio=-6.0673; df = inf; p<0.00). During the B condition, they performed self-regulatory behaviors for longer compared to the R condition (z-ratio=3.914; df = inf; p=0.001; Figure S.7 in Supplementary Materials).

4. Discussion

During early infancy, when voluntary forms of self-regulation are not yet fixed, temperamental regulation is primarily associated with attentional orientation. As voluntary control gradually develops toward the end of the first year of life, individual differences in effortful control begin to emerge (Posner et al., 2016). Interest or persistence to gaze at something/someone reflects the motivational system on which attentional processes rely, and individual differences in their expression are often considered part of broader dimensions of effortful control (Rothbart & Goldsmith, 1985). Despite their potential importance in predicting key developmental achievements (Rothbart et al., 2000; Auerbach et al., 2004), infant gaze-related attentional processes have been often overlooked. Our results show a faster initial gaze at both the mother and the experimenter during the stressful condition (B) compared to the other phases (Table 1, Figure 2). This short time latency indicates that toddlers are actively seeking information and reassurance from adults. This explanation is also held by results emerging from the analysis of the amount of time toddlers spend gazing at the two adults across the experimental paradigm. Toddlers gazed at their mothers significantly longer during the most stressful episode (B condition), compared to the last phase (R), since they do not need any help. Complementary, the time spent gazing at the experimenter did not vary between B and P (Table 1). As a whole, these findings reinforce the idea that toddlers are engaging in emotional regulation in response to challenging situations by differentiating the roles of caregivers.

The overall picture becomes even more compelling when considering the impact of childcare attendance. Attentional shifting can rely on previous social experiences, which can shape specific skills to navigate the social world (Bakeman et al., 1984). Accordingly, whatever the experimental condition, toddlers attending childcare gazed at the mother significantly later compared to those not attending childcare (Table 1, Figure 1a). The opposite situation occurs when considering the latency of gazing at the experimenter (*Prediction 3* supported; Table 1, Figure 1b). Moreover, when considering the interaction between childcare attendance and experimental conditions, we found that toddlers not attending childcare gazed longer at their mothers in the frustration condition (B) compared to the other ones, while toddlers attending childcare did not show any difference across the experimental conditions, proving to be less mother-oriented (*Prediction 3* supported, Table 1). While enhancing social development, attending childcare seems to determine the difference to be oriented to the primary caregiver or opportunistically oriented to the person controlling the situation (the toy holder). Due to modern working patterns, children are frequently asked to rely on different caregivers for

several hours a day. Since toddlers directly or indirectly know from their parents that other adults are responsible for their safety, these individuals are, in every respect, caregivers (Howes & Ritchie, 1999). Therefore, in terms of opportunities for interaction with other-than-parent adults, childcare can be viewed as a context for socialization (Feldman & Klein, 2003). In these settings where also peers are hosted, not just toys but also the adult attention must be shared with others, most of the time. Therefore, social competence, defined as behaviors reflecting successful social functioning, begins in caregiving environments where toddlers have many occasions to refine social communicative skills to achieve their own goals (in this case, regain a desired object).

An additional major topic explored in our study is the behavioral dimension of early self-regulation. Toddlers exhibited significantly longer duration of self-regulatory behaviors during the most stressful moment of the test (the B condition). Looking at the whole test, moreover, it is interesting to note that the lowest performance of these behaviors occurred in the baseline (P) (Table 1, Figure 3a). The increase of stress-regulatory behaviors during B indicates that such behaviors are reliable outputs linked to the frustration experienced by the toddlers when deprived of the toy. The relation between regulation and negative effects obtained under controlled conditions has been examined in several studies (Calkins, 1994; Calkins & Fox, 1992; Stifter & Fox, 1990). Specific stress-regulatory behaviors that toddlers display under frustrating or constraining situations appear to reduce negative affect. Thus, the idea that certain behaviors serve to minimize the affective negative state has gained clear support (Buss and Goldsmith, 1998). Past research measuring regulation in toddlers and young children already considered self-comforting and auto-manipulative patterns as regulation strategies to be monitored under stress tests (Calkins, 1997; Calkins & Johnson, 1998; Stifter & Braungart, 1995). However, to our knowledge, this is the first study providing information about enacting self-comforting behaviors to cope with a negative situation (the B condition). Such self-directed strategy seems to favor the restoration of the homeostasis disrupted by toy deprivation, as they decreased in R compared to B although not reaching the low duration scores recorded in P. Such distribution across the three conditions indicates that probably toddlers required more time to fully recover from the emotional perturbation.

While childcare attendance does not seem to affect self-regulatory behaviors (*Prediction 4* not supported, Figure S.4 in Supplementary Materials), sex seems to modulate them with females engaging in longer self-regulating behaviors than males (*Prediction 2* supported; Table 1, Figure 3b). The effect of the interaction between sexes and experimental conditions goes in the same direction. Males show a peak in duration of self-regulatory behaviors during B, while

females maintain such peak both in B and R thus expressing the necessity of longer recovery time (*Prediction 2* partially supported, Table 1, Figure 3c).

Overall, literature confirms that females demonstrate significantly better self-regulatory skills than males (Cadime et al., 2018; Kochanska et al., 2000; Veijalainen et al., 2017). A recent EEG study in preschool children has shown that the increased prefrontal activation recorded in girls during resting periods may contribute to their generally stronger self-regulation and emotional control compared to boys (Toffoli et al., 2024). Weinberg et al. (1999) hypothesized that since males would show greater difficulty than females in affective regulation, it is also expected that they would be more dependent on their mothers to engage in such regulation. Our study provides empirical support to such expectations. During both P (a new situation) and B condition (toy deprivation) males spent more time gazing at their mother compared to R condition. The female scenario appears more nuanced with an apparent random distribution of gazing at the mother across the three conditions (*Prediction 1* supported, Table 1, Figure S.2 in Supplementary Materials).

In conclusion, our findings, taken together, contribute to a more nuanced and complex picture compared to what is currently available in the literature. The high degree of individual variability, the lack of data regarding caretaker responsiveness propensity, and the relatively small sample size, may have played a role in providing less clear-cut results. Nonetheless, the use of standardized behavioral analysis—a notable strength of this study—may have been instrumental in uncovering such variability.

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Ethical Approval

This study was conducted in accordance with the World Medical Association Declaration of Helsinki and the ICH Harmonized Tripartite Guideline for Good Clinical Practice. The study

protocol was approved by the Ethical Committee of the Area Vasta Emilia Nord, Local Secretariat of Parma, and the University of Parma (approval date: [insert date]; reference number: [insert number]).

All procedures were performed in compliance with relevant laws and institutional guidelines. Written informed consent was obtained from all participants' legal guardians prior to their involvement in the study, ensuring the privacy rights of human subjects observed.

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FIGURES

Figure 1a: Model 1 – latency of the first look towards the mother, effect of childcare attendance (0=not attending, 1=attending)

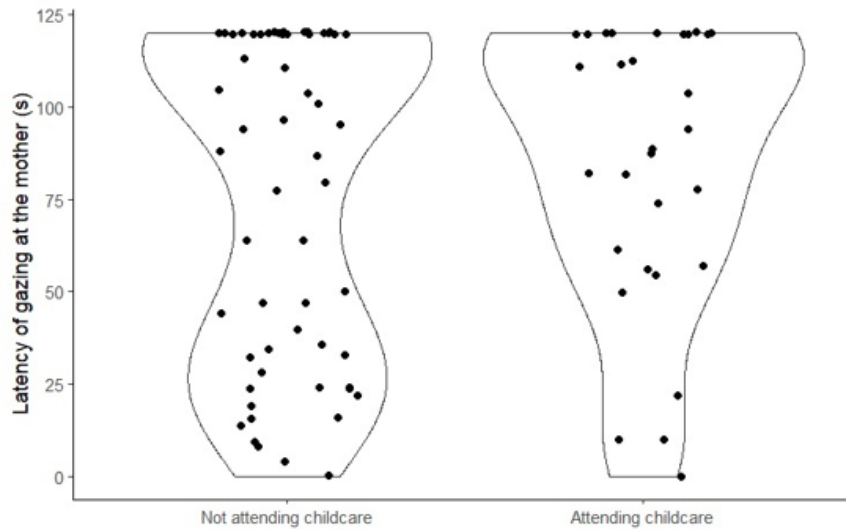


Figure 2: Model 2 – latency of the first look towards the experimenter, effect of condition

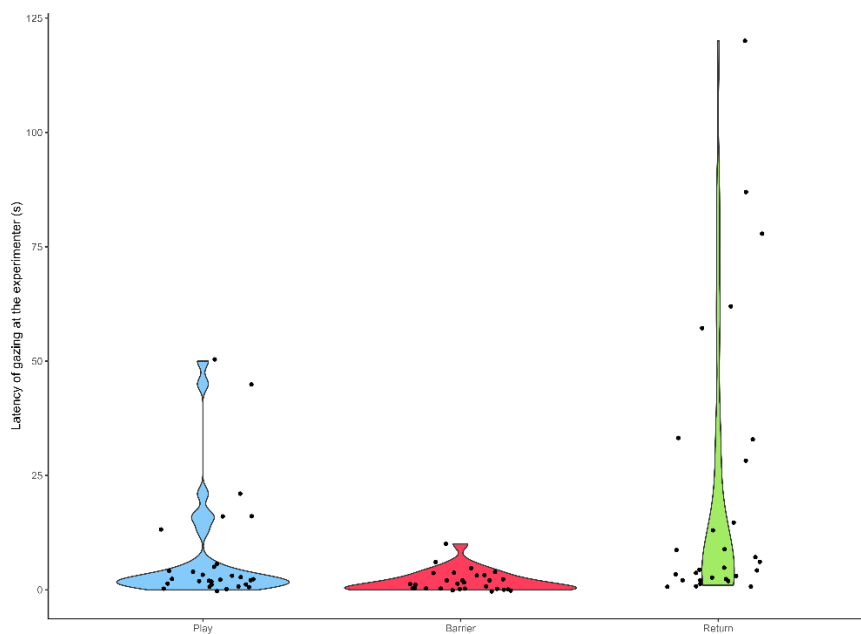


Figure 1b: Model 2 – latency of the first look towards the experimenter, effect of childcare attendance

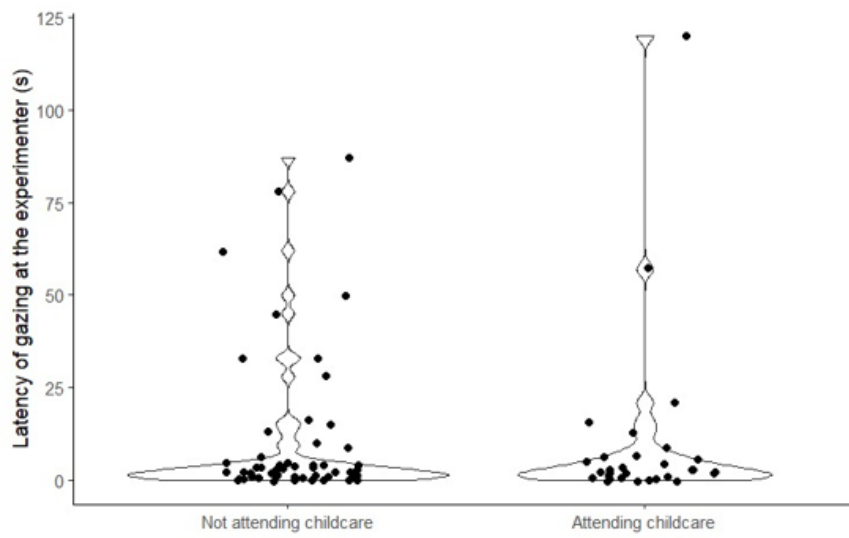


Figure 3a: Model 5 – duration of the displaying of self-regulatory behaviors, effect of condition

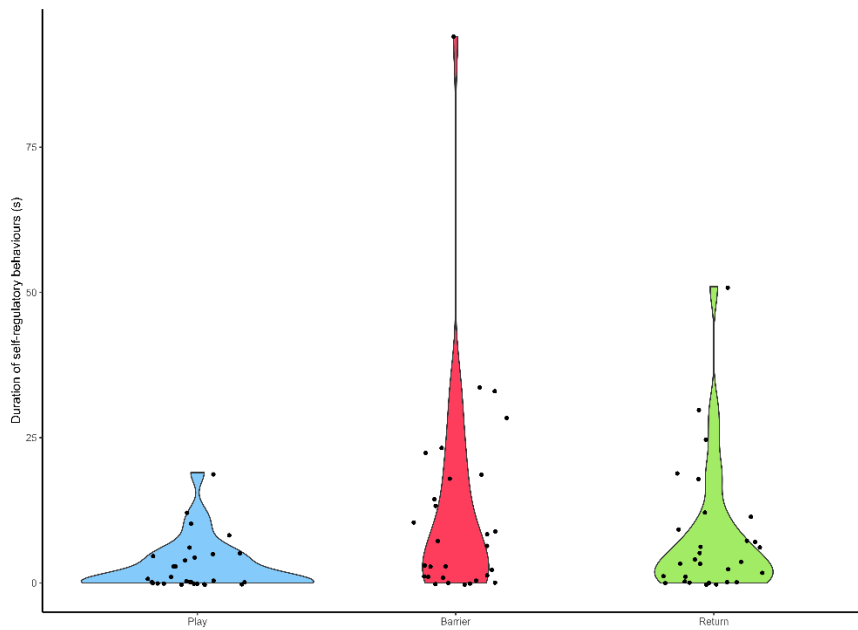


Figure 3b: Model 5 – duration of the displaying of self-regulatory behaviors, effect of sex

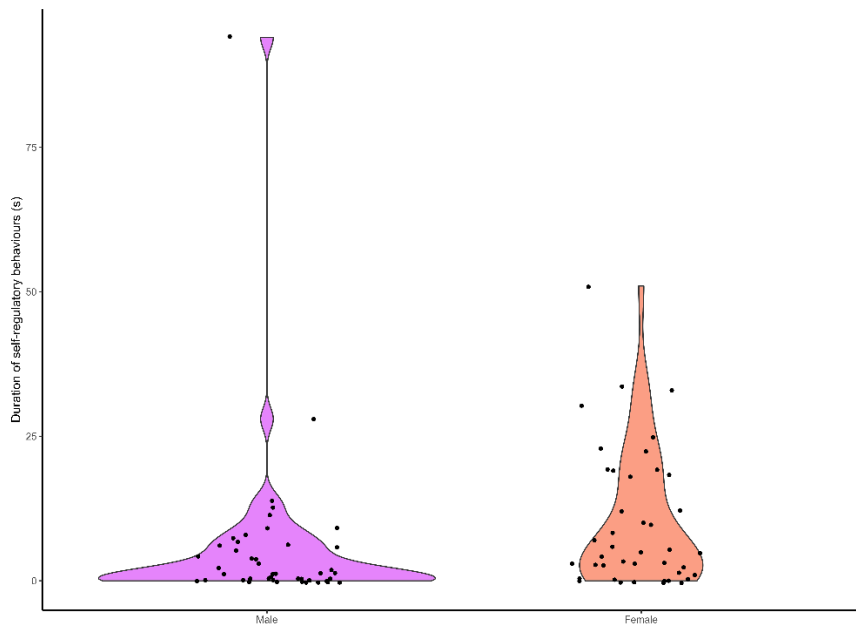


Figure 3c: Model 5 – duration of the displaying of self-regulatory behaviors, effect of condition*sex

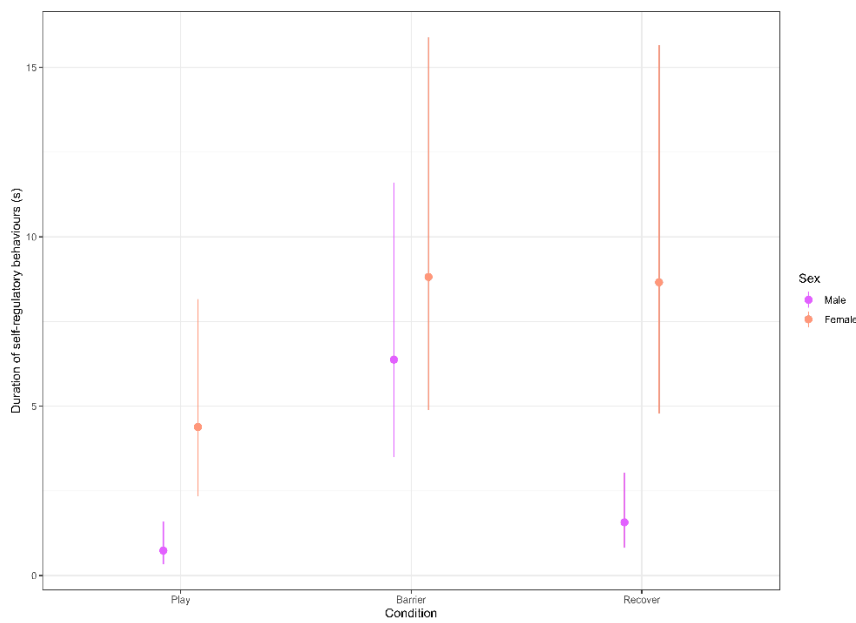


Table 2 – Estimated parameters (Coeff), Standard Error (SE), and results of the Likelihood Ratio Tests (χ^2) of the models. Significant P values are shown in bold; df= degree of freedom; n/a = not applicable

<i>1 - GLMM – Model 1 – Response Variable: latency of the first look toward the mother</i>					
Fixed Effects	Coeff	SE	χ^2	df	P
Intercept	4.168	0.729	n/a	n/a	n/a
Tested variables					
Sex	-0.261	0.130	1.042	1	0.307
Condition			65.258	2	<0.001
Condition _[PLAY]	0.023	0.048			
Condition _[RETURN]	0.130	0.047			
Childcare	0.087	0.147	1.871	1	0.171
Sex*Condition			22.039	2	<0.001
Sex*Condition _[PLAY]	0.280	0.061			
Sex*Condition _[RETURN]	0.099	0.061			
Childcare*Condition			7.264	2	0.027
Childcare*Condition _[PLAY]	0.132	0.064			
Childcare*Condition _[RETURN]	0.163	0.063			
Control variable					
Mother's age	0.001	0.020	0.002	1	0.961

N_{observations}=87, N_{ID}=29, N_{dateexp}=21 Variance for the random factor: ID=0.051±0.226SD Variance for the random factor: DATE EXP=0.107±0.327SD

2 - GLMM – Model 2 – Response Variable: latency of the first look toward the experimenter

Fixed Effects	Coeff	SE	χ^2	df	P
Intercept	4.273	1.770	n/a	n/a	n/a
Tested variables					
Sex	0.430	0.413	1.400	1	0.237
Condition			354.047	2	< 0.001
Condition _[PLAY]	1.913	0.241			
Condition _[RETURN]	2.580	0.232			
Childcare	-1.707	0.580	5.580	1	0.018
Sex*Condition			47.381	2	< 0.001
Sex*Condition _[PLAY]	-1.815	0.335			
Sex*Condition _[RETURN]	-0.623	0.299			
Childcare*Condition			3.370	2	0.185
Childcare*Condition _[PLAY]	0.649	0.358			
Childcare*Condition _[RETURN]	0.567	0.330			
Control variable					
Mother's age	-0.115	0.048	5.635	1	0.018

N_{observations}=87, N_{ID}=29, N_{dateexp}=21 Variance for the random factor: ID=0.185±0.430SD Variance for the random factor: DATE EXP=1.114±1.056SD

3 - GLMM – Model 3 – Response Variable: time spent in looking at their mothers

Fixed Effects	Coeff	SE	χ^2	df	P
Intercept	2.516	2.694	n/a	n/a	n/a
Tested variables					
Sex	-0.556	0.514	0.0007	1	0.979
Condition			22.136	2	< 0.001
Condition _[PLAY]	-1.117	0.186			
Condition _[RETURN]	-1.833	0.242			
Childcare	-0.996	0.624	0.153	1	0.695
Sex*Condition			44.385	2	< 0.001
Sex*Condition _[PLAY]	0.587	0.251			
Sex*Condition _[RETURN]	1.885	0.283			
Childcare*Condition			21.342	2	< 0.001
Childcare*Condition _[PLAY]	1.316	0.290			
Childcare*Condition _[RETURN]	0.956	0.303			
Control variable					
Mother's age	-0.024	0.074	0.102	1	0.750

N_{observations}=87, N_{ID}=29, N_{dateexp}=21 Variance for the random factor: ID=0.051±0.226SD Variance for the random factor: DATE EXP=0.107±0.327SD

4 - GLMM – Model 4 – Response Variable: time spent in looking at their experimenter

Fixed Effects	Coeff	SE	χ^2	df	P
Intercept	2.182	2.692	n/a	n/a	n/a
Tested variables					
Sex	-0.366	0.586	1.283	1	0.257
Condition			63.414	2	<0.001
Condition[PLAY]	-1.169	0.126			
Condition[RETURN]	-1.655	0.230			
Childcare	-0.167	0.649	0.072	1	0.789
Sex*Condition			10.290	2	0.006
Sex*Condition[PLAY]	-0.410	0.203			
Sex*Condition[RETURN]	-1.114	0.377			
Childcare*Condition			27.069	2	<0.001
Childcare*Condition[PLAY]	0.266	0.225			
Childcare*Condition[RETURN]	1.654	0.323			
Control variable					
Mother's age	-0.025	0.077	0.108	1	0.743

N_{observations}=87, N_{ID}=29, N_{dateexp}=21 Variance for the random factor: ID=2.120±1.456SD Variance for the random factor: DATE EXP=1.826e⁻⁴±0.0002SD

5 - GLMM – Model 5 – Response Variable: duration self-directed behaviors

Fixed Effects	Coeff	SE	χ^2	df	P
Intercept	-0.937	1.772	n/a	n/a	n/a
Tested variables					
Sex	0.324	0.372	5.243	1	0.022
Condition			111.961	2	<0.001
Condition[PLAY]	-2.157	0.279			
Condition[RETURN]	-1.400	0.187			
Childcare	0.084	0.411	0.032	1	0.857
Sex*Condition			62.634	2	<0.001
Sex*Condition[PLAY]	1.458	0.299			
Sex*Condition[RETURN]	1.382	0.196			
Childcare*Condition			8.513	2	0.014
Childcare*Condition[PLAY]	-0.686	0.284			
Childcare*Condition[RETURN]	0.160	0.182			
Control variable					
Mother's age	0.085	0.050	2.831	1	0.093

N_{observations}=87, N_{ID}=29, N_{dateexp}=21 Variance for the random factor: ID=0.852±0.923SD Variance for the random factor: DATE EXP=8.349e⁻⁸±2.890e⁻⁴SD

SUPPLEMENTARY MATERIALS

Figure S.1: The Barrier Task procedure: 1a) Play condition 1b) Barrier condition 1c) Return condition.



Table S.1: Behaviors considered in the two macro-categories conceived for the analysis. The operational definition for each behavior is reported.

Category	Behaviors comprised	Operational definitions
Attentive behaviors	<i>looking at mom</i>	The infant looks toward the face of the mother, seeking eye contact.
	<i>looking at the experimenter</i>	The gaze is oriented towards the experimenter. Use this code also when the gaze is oriented towards the experimenter' hands (because she is holding the barrier).
Self-regulatory behaviors	<i>finger/hand suckling</i>	The baby sucks one finger or the entire hand, or part of it.
	<i>finger/hand in mouth</i>	Finger(s) thumb, or hand(s) are in contact with the mouth or around the mouth region, but not engaged with the mouth.
	<i>mouthing/tongue protrusion/chewing</i>	The infant moves the tongue, i.e. protruding it from its resting position beyond the inner margin of the red part of the lips; any other movements of mouth or lips, i.e. lip smacking, blowing bubbles, making raspberries, vacuum chewing.
	<i>yawning</i>	Inhaling deeply through the open mouth and exhaling.
	<i>self-touching</i>	One or both hands or one or more fingers are used to statically touch any part of the body or the face (i.e. eyes, hands, belly, ecc..).
	<i>self-rubbing</i>	One or both hands or one or more fingers are used to rub the eye/eyes, the belly, or any parts of the body or the face.

	<i>self-scratching</i>	The infant scratches her/himself. To code this, it should occur with more than one rubbing action.
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Figure S.2: Model 1 – latency of the first look towards the mother, effect of condition*childcare attendance

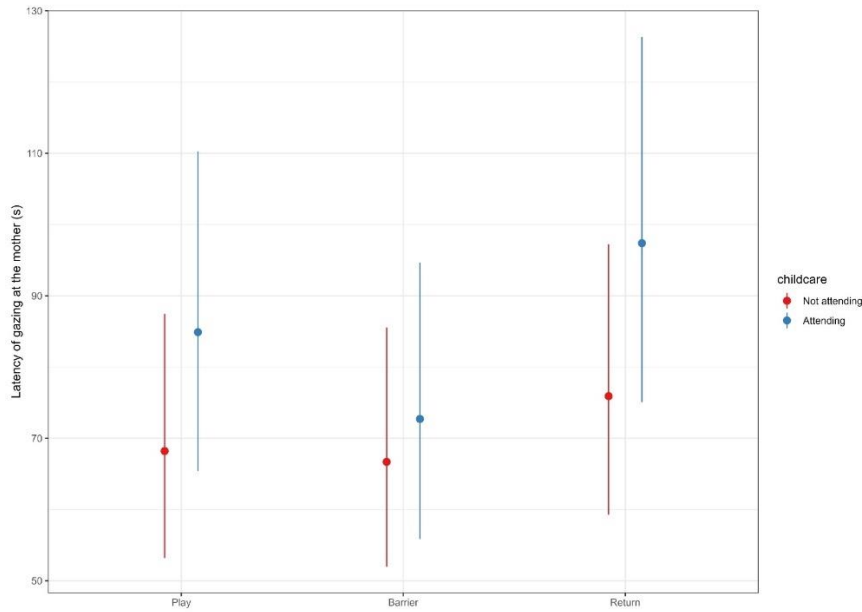


Figure S.3: Model 3 – duration of looking at the mother, effect of condition* sex (0=males, 1=females)

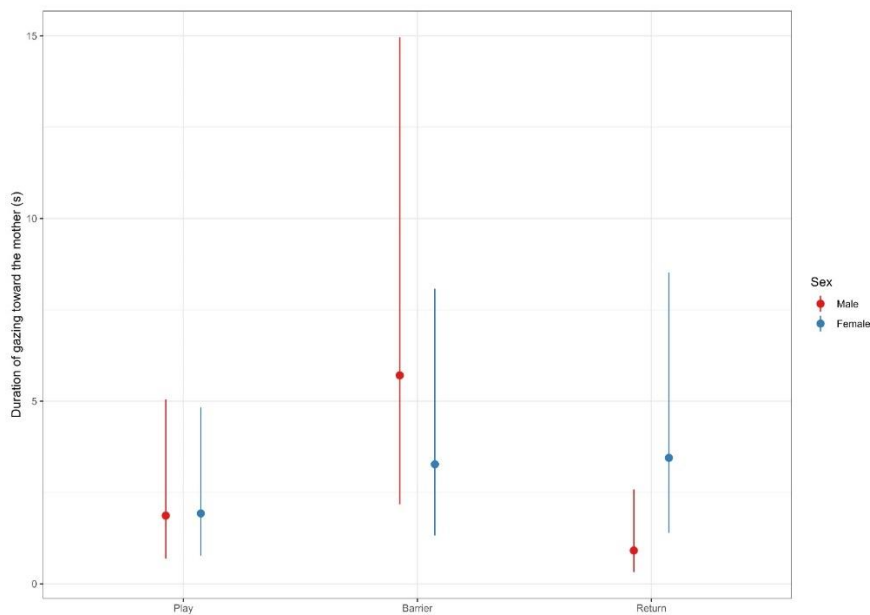


Figure S.4: Model 3 – duration of looking at the mother, effect of condition* childcare attendance

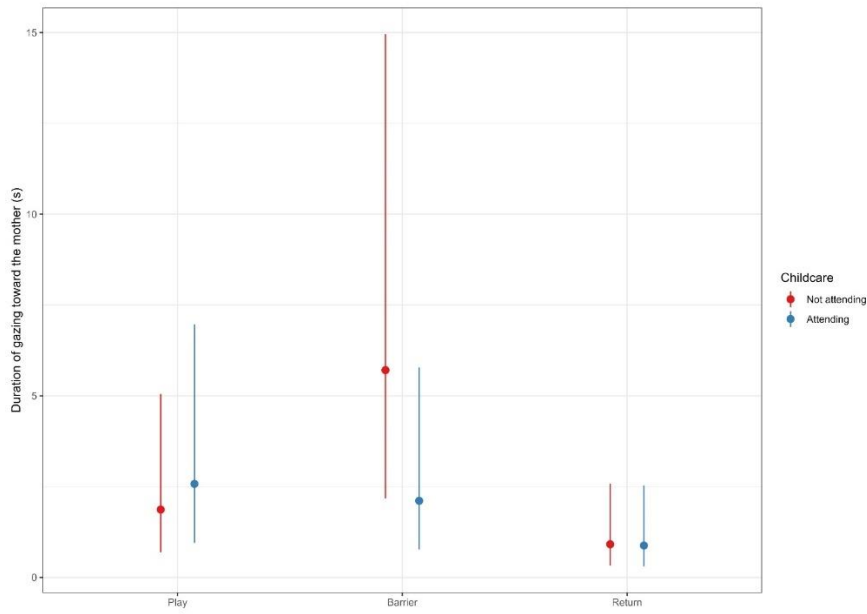


Figure S.5: Model 4 – duration of looking at the experimenter, effect of condition*sex

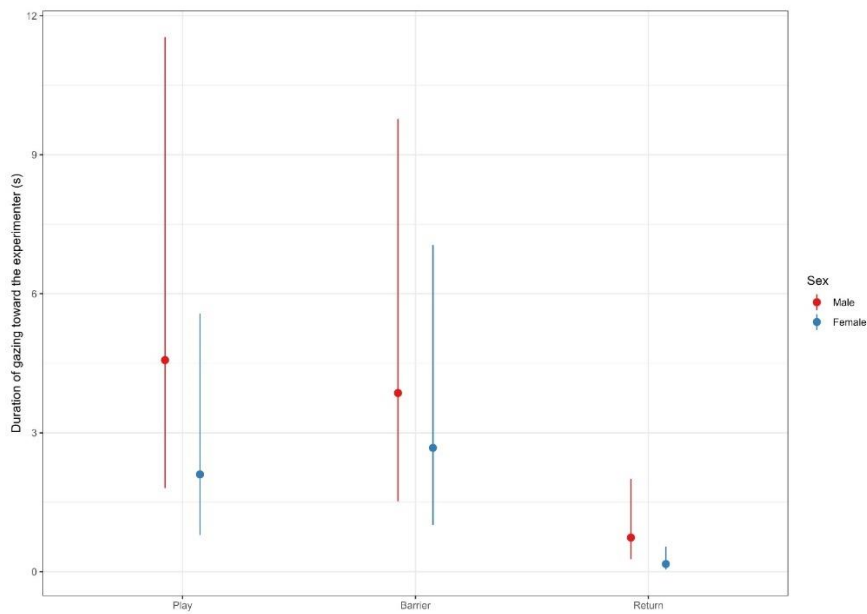


Figure S.6: Model 4 – duration of looking at the experimenter, effect of condition*childcare attendance

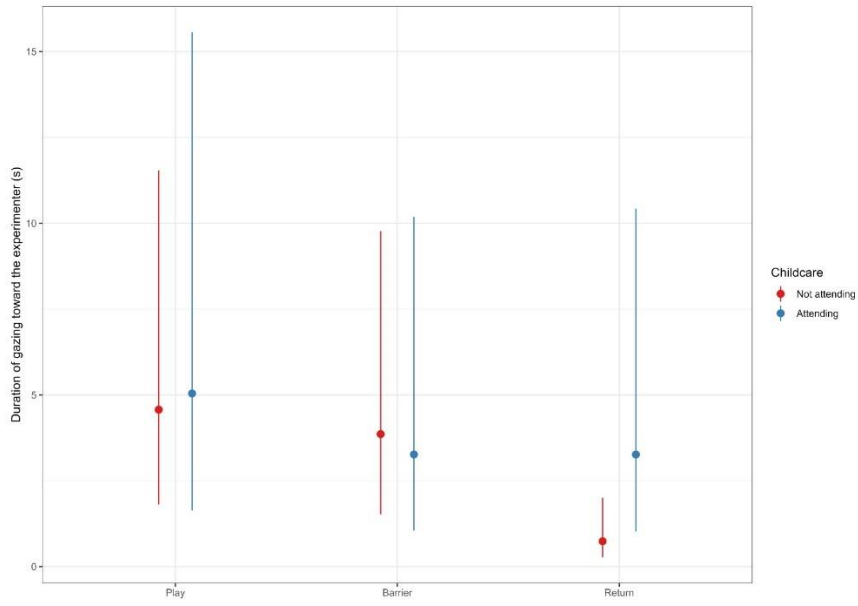
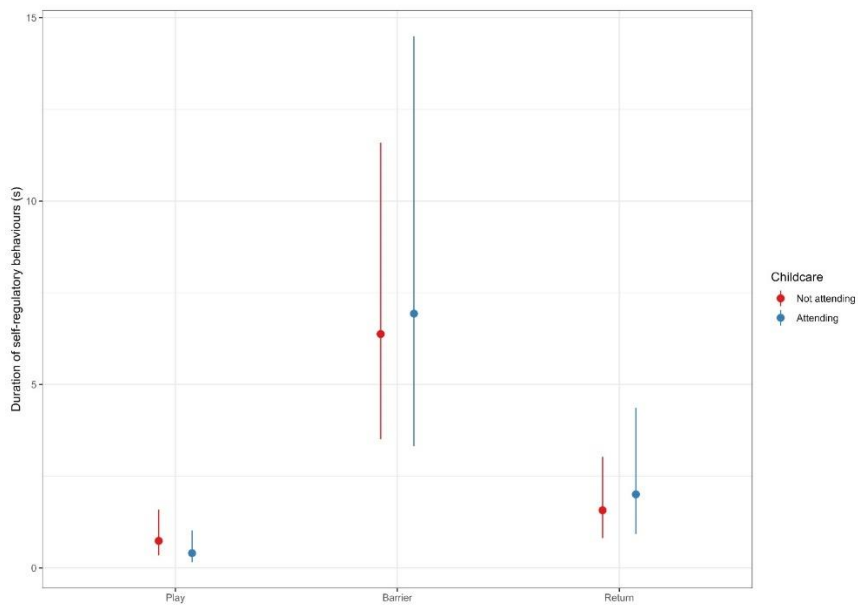


Figure S.7: Model 5 – duration of the displaying of self-regulatory behaviors, effect of condition*childcare attendance



To be submitted

FAKE IT TILL YOU MAKE IT: DEVELOPMENTAL ORIGIN OF SOCIAL REGULATION OVER SPONTANEOUS SMILING IN INFANTS

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Abstract

This study investigates the role of smiling as an emotional expression in 12-month-old infants, exploring how different types of smiles are used in varying contexts. Drawing on the notion that positive emotions motivate interactions and facilitate caregiver bonding, we hypothesized that infants would display non-Duchenne smiles during stressful situations to regulate emotions, while Duchenne smiles would be more prevalent during a session of free toy-play. A sample of 29 infants was observed during the Barrier Task, which involved a play episode followed by a frustration episode where access to a toy was denied. Using the BabyFACS coding system, we pinpointed positive emotional facial expressions across the task and then analyzed them via Baby FaceReader 9.0 to delineate the exact facial muscles recruitment. Results indicated that infants displayed non-Duchenne smiles (absence of AU6, cheek raiser) during the frustrating condition and Duchenne smiles (presence of AU6) during the baseline play condition, supporting our hypothesis. Additionally, AU26 (jaw dropping) was activated in the frustration condition, suggesting that more pronounced expressions were used as affiliative signals to elicit support from the audience. Contrary to expectations based on literature about non-spontaneous smiles, there was no significant difference in the duration of

smiles between conditions. These findings underscore the developmental plasticity of emotional expressions in infants and highlight the adaptive function of smiles. Our results also indicate the potential for early identification of social development issues, emphasizing the importance of studying emotional expressions in understanding infant emotional regulation and socialization processes.

Keywords: early emotion regulation – Duchenne/non-Duchenne smiles – BabyFACS – Baby FaceReader – automated scoring

1. Introduction

In infancy positive emotions are assumed to motivate and organize responses to achieve goals (Blehar et al., 1977; Cohn et al., 1991; Malatesta et al., 1989) and to help fostering beneficial interactions with caregivers (Campos et al., 1994). Spontaneous smile is widely regarded as the symptomatic indicator of positive emotions (Messinger et al., 2001) However, it remains unclear whether in infancy all smiles indicate joy or if this role is limited to a specific type or several distinct types of smiles. It is also uncertain whether these smiles are exclusive to specific contexts or are just representative of certain phases of an individual growth. This further complicates our understanding of the relationship between smiling and emotional expression in early development.

Smiles, beyond the context or the function and regardless the age of the performer, can occur in a variety of forms, all involving the contraction of the *zygomatic major* muscle responsible for pulling the corners of the lips upward and laterally (Ekman & Friesen, 1978). Smiling may also entail cheek elevation, produced by the contraction of the *orbicularis oculi, pars lateralis*. This muscular action raises the cheeks, resulting in a squinting of the eyes and, in adults, the formation of wrinkles at the outer corners of the eyes (Rosenstein & Oster, 1997). Ekman and colleagues have made a pioneering distinction between cheek-raised smiles, known as Duchenne smiles, and other types of smiles (Ekman & Friesen, 1978; Ekman et al., 1990). Smiling with and without cheek elevation seems to underpin different connotations. In this regard, Messinger et al. (1999) found that smiles involving cheek elevation were often accompanied by mouth opening. The open-mouth smile characterized by a dropped jaw is commonly referred to as the play smile. Among adults, it has been proposed that Duchenne smiles are associated with authentic positive emotion. In contrast, smiles without cheek elevation (non-Duchenne smiles) are thought to be useful in facilitating social interactions, potentially concealing uncomfortable or even negative emotions. Subsequent research has named these two types of expressions spontaneous or genuine and posed or deliberate smiles, respectively (Guo et al., 2018). Ekman (1992, 1994) argues that, in adults, posed smiles work as social signals unrelated to happiness. In infants, however, this distinction is more difficult to prove.

Smiling during the first stages of life primarily serves as a social-emotional expression. Shortly after one month of age the social smile emerges (Anisfeld, 1982). Until at least six months of age face-to-face interactions represent a significant aspect of the time infants spend with their caregivers and smiles are a crucial component of these interactions (Weinberg & Tronick,

1994). The frequency and duration of infants' smiles during interactions increase after six months of age (Gewirtz, 1965; Messinger et al., 1999), when infants mainly smile when gazing at their caregivers, especially when they are smiling too (Kaye & Fogel, 1980; Messinger et al., 2001; Weinberg & Tronick, 1994). Therefore, with developmental progress, infants shift from passive to active role in the exchanges that elicit smiles (Sroufe & Waters, 1976), learning how to employ smiles to actively initiate interactions with their caregivers (Kaye & Fogel, 1980). At this stage, smiling functions not only as an expression of genuine positive emotions but also as an affiliative cue that can evoke positive responses from others (Owren & Bachorowski, 2001, 2003). From one to six months, the two types of smiles (the Duchenne and non-Duchenne) are performed, eventually in close temporal proximity (Messinger et al., 1999), suggesting that there is already the muscular plasticity allowing both facial expressions, but that their specific function or the appropriate context in which to display them is an ability still being refined. Through EEG recordings, Fox & Davidson (1988) demonstrated that Duchenne smiles at ten months of age are associated with left frontal brain activation, a neural pattern consistently linked to positive affect in adults. As they approach their first birthday, infants begin to smile and subsequently turn to their caregivers, as if sharing their emotional states (Jones & Raag, 1989; Jones & Hong, 2001). At this age, the open-mouth Duchenne smile becomes an increasingly reliable indicator of positive emotional engagement since it is more likely to occur in the positive periods of social interactions. These patterns suggest that, during the first half of life, smiling behavior is progressively differentiating. Among older infants, a preponderance of certain type of smiling in specific situations has been observed, indicating that smiles may reflect qualitatively different shades of positive emotions (Fogel et al., 2000; Fox & Davidson, 1988; Dickson et al., 1997).

The various typology of smiles has been determined using different methods, but the most widely used is the Facial Action Coding System (FACS) (Ekman & Friesen, 1978; Ekman et al., 2002), which allows to identify the muscle movements involved in facial expressions. FACS provides a description of all possible and visually detectable facial variations in terms of 44 Action Units (AUs). According to FACS, the Duchenne smile consists in the co-occurrence of the 'lip corner puller' (AU 12) produced by the *zygomaticus major* muscles and the 'cheek raiser and lid compressor' (AU 6) produced by the *orbicularis oculi pars lateralis* muscles. It has been demonstrated that most people are unable to intentionally control AU6 (Duchenne, 1990; Ekman et al., 1990); as a result, a genuine smile cannot be faked and probably serves as an honest signal of sincere positive emotions. Oster (2006) developed a version of the FACS manual specifically for infants, known as BabyFACS. While the coding

system is similar to the adult FACS, the baby version accounts for the distinct features of infant faces. According to the Oster's manual, a smile can be coded when AU12 is the leading component of the facial expression. Nonetheless, the anatomical description of a smile alone does not imply its meaning. Instead, an individual's goals in a specific social interaction play a crucial role in shaping the significance of the smile, even in infants, as the same type of smile can convey different meanings depending on the context (Barrett, 1998; Fogel et al., 1992). For instance, Ekman (1992, 1994) claimed that adult smiles without cheek raising have social regulatory functions, such as masking displeasure, that are unrelated to joy. The ability to regulate emotional stress develops early, within the first months of life, when infants begin to develop adaptive behaviors for managing emotional stress through daily interactions with their primary caregivers. These interactions often involve ruptures and repairs, making emotional stress a normal part of the infant's environment.

Based on the developmental trajectory of smiling in infancy and on these assumptions, we here hypothesize that 1-year-old infants are able to display non-Duchenne smiles with regulatory purposes, in those situations in which it is necessary to respond to emotional stress by activating coping behaviors, such as dissimulating negative emotions (*Hypothesis 1*). Smiling is employed as an affiliative signal to prompt responses to others, working as external regulators. We expect to find different recruitment of facial muscles depending on the situation. Specifically in a removal-toy task in which an experimenter impedes the access to a desired toy, we expect infants to display non-Duchenne smiles (AU6 deactivated) toward the experimenter, as a strategy to both cope with the induced frustration and enhance an affiliative response in the counterpart, without however experiencing a genuine positive emotional state (*Prediction 1*). As a control, we expect infants to display Duchenne smiles (AU6 recruited) mostly during the baseline condition when engaging in a free play session, where positive emotions are more likely to be felt (*Prediction 2*).

In the literature it is well documented that posed smiles differ from spontaneous ones not just for the conformation but also for temporal features, intensity and symmetry. The total duration of a genuine smile can range from 500 to 4.000 milliseconds, while posed smiles show a more unstable nature (Ekman & Friesen, 1982). Generally spontaneous smiles tend to last longer than posed ones (Schimdt et al., 2009; Cohn & Schmidt, 2004). Therefore, we hypothesize that if 1-year-old tested infants show different smiles depending on different experienced emotional states, a difference between these two configurations should be found also in terms of duration (*Hypothesis 2*). We expect that posed smiles displayed in the frustration phase would be shorter

than the genuine ones, primarily shown during the baseline phase (*Prediction 3*). Intensity and symmetry are not investigated in the present work.

For these purposes, only selected AUs have been considered to focalize on those facial expressions implying unambiguous emotional states, thus involving the facial muscles commonly composing a smile. Emotional utterances with positive valence include the combinations of lip corner raising (AU12) with other non-random facial actions (Oster & Ekman, 1978), such as cheek raising (AU6), mouth opening (AU25, 26, 27), nose wrinkle (AU9). Each smile exhibited by the infants across the entire procedure was initially coded manually; after that, a dedicated software was employed to spot the recruitment of the selected AUs.

2. Methods

2.1 Participants. The sample comprises of 29 caregivers and their 12-months-old infants (15 males, 14 females), recruited as a part of a longitudinal investigation led by the University of Parma. All mothers were recruited between 36th-41st week of gestation at the Parma Hospital, Italy. Recruited infants were all healthy full-term infants with routine pre- and postnatal medical histories. Data showed in this study have been collected between April 2022 and January 2023, at Parma Hospital, Italy.

2.2 Experimental procedure. Infants and caregivers were observed in a consultation room located at the Parma Hospital. The views from two cameras (Sony Handycam® CX405), one offering a full-frontal view of the infant's face and one offering a frontal, enlarged view of the dyads' faces, were recorded during the entire procedure.

The Barrier Task procedure. The Barrier Task procedure is a paradigm adapted from the Laboratory Temperament Assessment Battery (LAB-TAB, Goldsmith & Rothbart, 1996) that assesses infant responses to stimuli in a controlled setting to measure infant's emotional or behavioural reactivity to stimuli. The LAB-TAB manual describes 20 episodes. To assess the infant response to frustration in term of adaptive regulatory behaviour, we here used the Barrier Task procedure. The assessment was conducted only if the infants were awake and in an alert and relaxing state. During the Barrier Task procedure, the infant is engaged in three two-minute episodes: (1) Play episode (P) during which the infant was allowed to manipulate and play with the toy chosen before to start the procedure; (2) Barrier episode (B) during which the experimenter removed the toy from the infant's hands and placed it behind a clear plastic

barrier, out of reach. The toy remained out of reach for 2 minutes, or until there had been 20 seconds of hard crying; (3) Return episode (R) episode during which the experimenter removed the plastic barrier from the table and returned the toy to the infant who was allowed to play with it. During the Barrier episode the mothers were asked not to interact with their infant. The same experimenter (C.S.) conducted all the tests. Across all the episodes, both mothers and experimenter always wear a white face mask.

2.3 Scoring infants selected facial expressions.

Manual procedure. For each subject smiles have been collected any time they occurred. In finding the onset time of a facial action, coders proceed until the movement peaks, and it is clearly visible. They then move backwards until the movement stops and note the onset time, confirming or adjusting it by moving the record forward and backwards across the estimated onset point. The offset time is obtained exactly in the same manner. This coding procedure based on a frame-by-frame video analysis is known as behavioural micro-analysis. This latter was conducted via Pot Player®, which allows the accuracy of 25 frames *per sec*. Duration has been calculated accordingly, in milliseconds (see Supplementary Material 1).

Automated procedure. BabyFACS coding requires that the face and the onset or the offset of the expression are clearly visible so that it is possible to evaluate the face's appearance changes resulting from muscle activation (Fig. 1, 2 for an example of the software interface). For this reason, a considerable number of instances have been discarded. In this regard, Baby FaceReader automatically calculate the minimum model quality required for a model to be valid (from 0= low quality, to 1= high quality). Only those videos whose quality overcome the 0.9 were included for our analysis. The default threshold of 0.50 on a scale between 0 (low model fit certainty) to 1 (high model fit certainty) was automatically set by the system, indicating the consistency of the deep neural network model. For the same reason, only those facial expressions whose quality overcome the 0.7 were included in the analysis and considered reliable (Fig. 3).

Utilizing a deep learning-based approach (Zafeiriou et al., 2015), Baby FaceReader is able to localize a face within an image. The resulting output provides continuous collection for individual AUs, as defined in Oster's Baby FACS (for detailed information about model architecture and training, Noldus, 2021), with the maximum accuracy of 15 frames per sec. The resultant data were downloaded into an Excel© sheet (see Supplementary Material 2 for

an example of the work sheet). The Baby FaceReader also calculates the intensity of each AU, using a 5-point scale, however the intensity has not been considered in the present study. For the statistical analysis, AUs occurring in each facial expressions were coded as 0 (absent) or 1 (present), respectively, in an Excel© sheet, (see Supplementary Material 1 for an example of the work sheet).

2.4 Intercoder reliability.

The videos were analysed by F.L. (coder#1). Before starting the video analyses, inter-observer reliability was checked between coder#1 and a senior coder (C.S., coder#2) on about 10% of total recording, covering all three experimental conditions and considering all behavioural categories. The values of Cohen's kappa coefficient (κ) (Cohen, 1960) between the coders were higher than 0.80 for each behavioural category (mean $\kappa = 0.82$).

2.5 Statistical analysis

To test *Hypothesis 1*, for each facial display we recorded the combination of the AUs recruited. Then, we created an Excel© sheet in which each row represented a smile performed and each column represented the recruitment of a specific AU (0/1). To investigate which AUs were more likely to be recruited during smile we used the function “element plot” from the R package NetFACS (version 0.5.0; Mielke et al., 2022).

To test *Hypothesis 2*, we ran a Generalized Linear Mixed Model (GLMM; glmmTBM package in R; Brooks et al., 2017; R Core Team, 2020; version 1.4.1717). In Model 1, we set as response variable the duration of each smile performed (Poisson error distribution, $N_{\text{cases}}=89$). The fixed factor was the experimental condition (Play, P or Barrier, B). The random factor was the identity of each subject. By using a likelihood ratio test (LRT, anova with argument test ‘Chisq’; Dobson, 2002) to test the significance of the full model (Forstmeier & Schielzeth, 2011), we compared the full model against a control model comprising only the random factor and the fixed factor “age of the mother”.

3. Results

Smile characterization in Play condition. The element plot revealed that the AU12 (lip corner puller), AU25 (lips part), AU6 (cheek raiser) were recruited significantly more compared to the expected distribution (Figure 3).

Smile characterization in Barrier condition. The element plot revealed that the AU12 (lip corner puller), AU25 (lips part), AU26 (jaw dropping) were recruited significantly more compared to the expected distribution (Figure 4).

Duration of smiles. The full model was not significantly different from the control model (Likelihood Ratio Test: $\chi^2_1 = 0.407$, $p=0.523$).

4. Discussion

Emotional expressions function as significant social cues during infancy, a stage during which verbal communication still holds little relevance (Papousek & Papousek, 2002). Facial affective displays are regarded as pivotal elements in early communication, attachment formation, and social learning processes (e.g., Bowlby, 1969; Stern, 1977; Trevarthen, 1977). These capacities undergo development throughout the first year of life (Campos et al., 1983). In this study, we demonstrate that 1-year-old infants possess the muscular plasticity to modulate their smiles based on the situation, using different types of smiles depending on the intended purpose or the underlying emotion. The tested subjects in this study displayed non-Duchenne smiles during the stressful condition (B) and Duchenne smiles during the baseline condition (P) of the experimental paradigm (*Hypothesis 1* supported). Specifically, the analyses demonstrates that in both the P and the B conditions there was significant recruitment of AU12 (lip corner puller) and AU25 (lips part), but crucially, AU6 (cheek raiser), the typical Duchenne smile marker, was not activated during the frustration phase (B). This aligns with *Prediction 1* positing that infants would deploy non-Duchenne smiles when experiencing emotional stress, with the aim of regulating their emotions and coping with the frustrating situation (Fig. 4).

In the B condition, where the experimenter prevented access to the desired object, AU26 (jaw dropping) was also recruited. In the circumplex models of emotions (Barrett & Russell, 1999; Russell, 1980; Yik et al., 1999) expressions involving mouth opening index increased arousal, independently of the valence. In the present study infants may have employed more pronounced facial expressions as part of their strategy to manage stress situation occurring during the B condition. This smiling conformation, with the opened mouth, likely served as affiliative signals intended to elicit social engagement and support from the experimenter. Such results are consistent with functionalist perspectives on emotional development, which argue that emotions and their expression serve adaptive roles in navigating social relationships (Barrett, 1998; Bretherton et al., 1986). Between 6 and 12 months of age infants undergo rapid growth: during this period, they gain increased muscle tone and improved postural stability,

allowing them to sit more easily, begin crawling, pull themselves to a standing position, and eventually take their first steps. These advancements in motor abilities coincide with significant cognitive and motivational changes, facilitating a broader range of emotional expressions (Izard and Malatesta, 1987). Additionally, enhanced motor and cognitive control enables infants to regulate their emotions more effectively as they approach their first year of age (Thompson, 2001). Infants develop a wider array of emotional expressions in tandem with more refined strategies to regulate emotions more efficiently (Planalp et al., 2017).

The findings also support *Prediction 2*, which proposed that Duchenne smiles (characterized by the activation of AU6) would be prevalent during the free-play baseline condition (P), where positive emotions were expected to be naturally elicited (Fig. 5).

Our results clearly indicate the enactment of both genuine and posed smiles, whose distinction is well established in the literature. Individuals often engage different facial muscles when asked to mimic an emotion compared to when they are genuinely experiencing it (Ekman, 2009). Although posed smiles serve various purposes, their primary aim is to persuade others that one is enjoying themselves when they are not (Ekman & Friesen, 1982; Frank et al., 1993). Distinctions between enjoyment smiles and other types are also based on timing peculiarities. Despite spontaneous smiles have been proved to be relatively slower than posed ones (Schmidt et al. 2009), smiles performed during the B condition did not significantly differ in terms of duration, compared to those displayed during the P condition (*Prediction 3* not supported). It is worth noting that literature does not provide a clear consensus on this issue. For instance, Dibeklioglu et al., (2012) confirmed that duration of spontaneous smiles is longer than posed ones, but also noted that the lip corner movements for posed smiles are faster. In contrast, earlier literature, including Ekman and Friesen's (1982) empirical observations, indicates that emotional expressions have a more limited duration than non-emotional expressions, aligning with Ekman's (1992) theory that emotions are brief, episodic events lasting only a few seconds. This contrasts with findings by Hess and Kleck (1997), suggesting that displays of posed happy emotions were longer than spontaneous happy smiles. Contrasts in the timing of spontaneous *versus* deliberate movements in lip corners or in other parts of the face during smiling suggest the existence of a common control mechanism for spontaneous facial movements that coordinates facial signaling (Schmidt et al., 2009). In the case of the present study, it is reasonable to hypothesize that 1-year old infants are still not equipped with this sophisticated mechanism. Furthermore, the majority, or nearly all, of the literature concerning the duration of spontaneous *versus* posed smiles has been conducted with adult subjects, which are certainly

affected by cultural and social norms regarding the most appropriate display to show in a given situation.

Despite the small sample, our data are in line with the literature indicating that infants, even at this early age, seem to be able to employ smiles to claim attention from the audience (in this case, the experimenter). Generating a specific expression implies a degree of emotional expressions control, partially independent from the felt emotion but somehow subjected to the infant appraisal of the environment (Jones et al., 1991). Here, smiling seems to function as a self-regulatory strategy employed after a disrupting event. The exact age of onset of this ability has been long disputed (Meltzoff & Moore, 1977) and this work may flag the emergence of this skill across development. Finally, findings regarding distinct types of smiles in young infants have the potential to renew interest in the development of positive emotions, a domain that has generally gathered less attention compared to distress and coping mechanisms in this population. These different kinds of smiling prompt significant inquiries into the nature of emotions and their developmental trajectories: Alvari et al. (2021) suggested that the analysis of social smiles can provide essential insights for identifying social development issues as early as 6 months. For example, disparities in spontaneous smiling among infants with Autism Spectrum Disorder (ASD) begin to emerge around 12 months and continue to evolve over time. Therefore, measuring expressive dynamics may represent a considerable promise for improving early detection of infant pathological conditions and, more generally, to assess neurodevelopment.

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Ethical Approval

All the materials used in this study were permitted for scientific use with written informed consent. The study protocol received ethical approval from the Ethical Committee of the Area Vasta Emilia Nord, Local Secretariat of Parma, and the University of Parma. The protocol has been designed and the study will be conducted in accordance with the ethical principles

outlined in the Declaration of Helsinki and the ICH Harmonized Tripartite Guideline for Good Clinical Practice.

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Figure 1. An example of the Baby FaceReader interface, showing how it continuously evaluates the baby's facial expressions. In the upper right segment, the contracted Action Units (AUs) are displayed, indicating the specific facial regions where they are activated. In the lower right segment, their temporal sequence throughout the video is also visible. Different colors represent varying intensities for each AU, data which were not considered in the present study. The colored bar below the video, on the other hand, indicates the model quality. The baby is displaying a non-Duchenne smile.

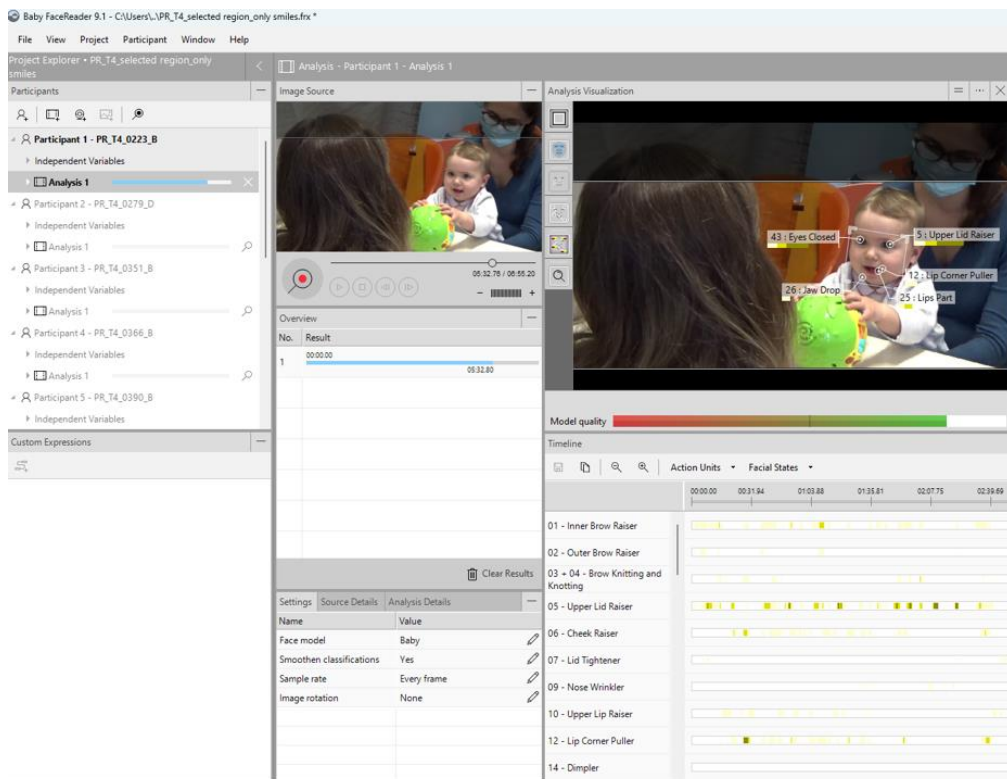


Figure 2. A second example of how Baby FaceReader works. The baby is displaying a Duchenne smile.

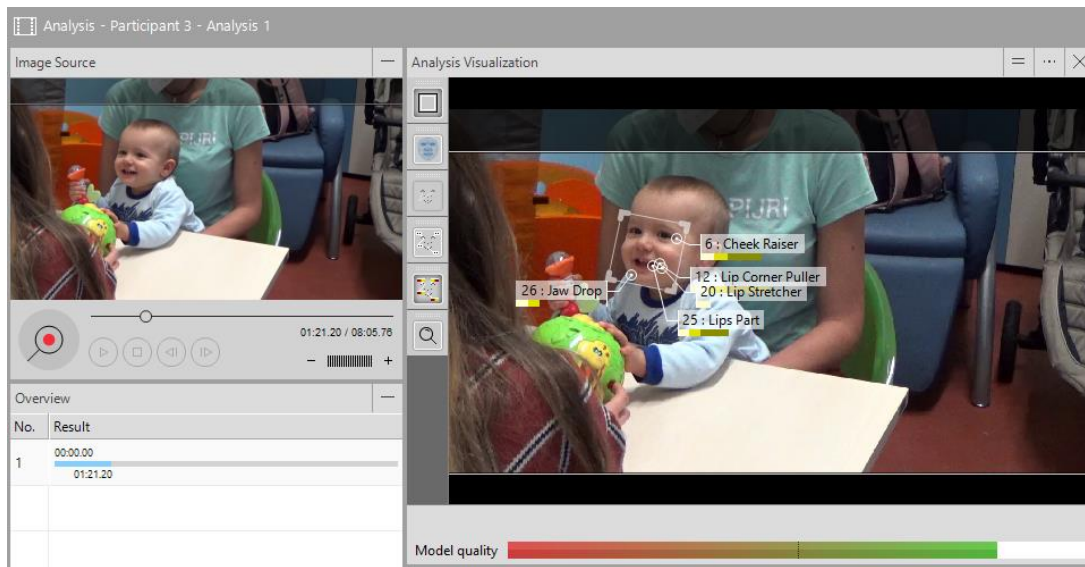


Figure 3. An example of the Baby FaceReader interface when no face could be detected. The bar indicates the model quality, which in this case is below 50 out of 100, and therefore, no facial expression is coded.

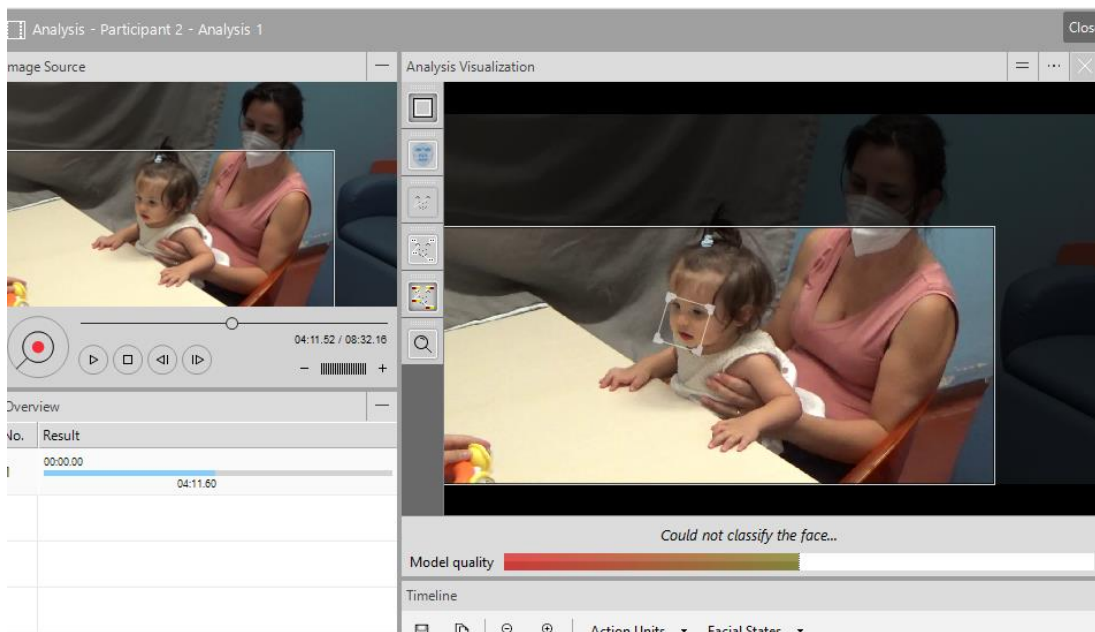


Figure 4. Element plot showing the significant recruitment of AUs during the Barrier condition, taking into account the potential contraction of the selected facial muscles involved in the smile display.

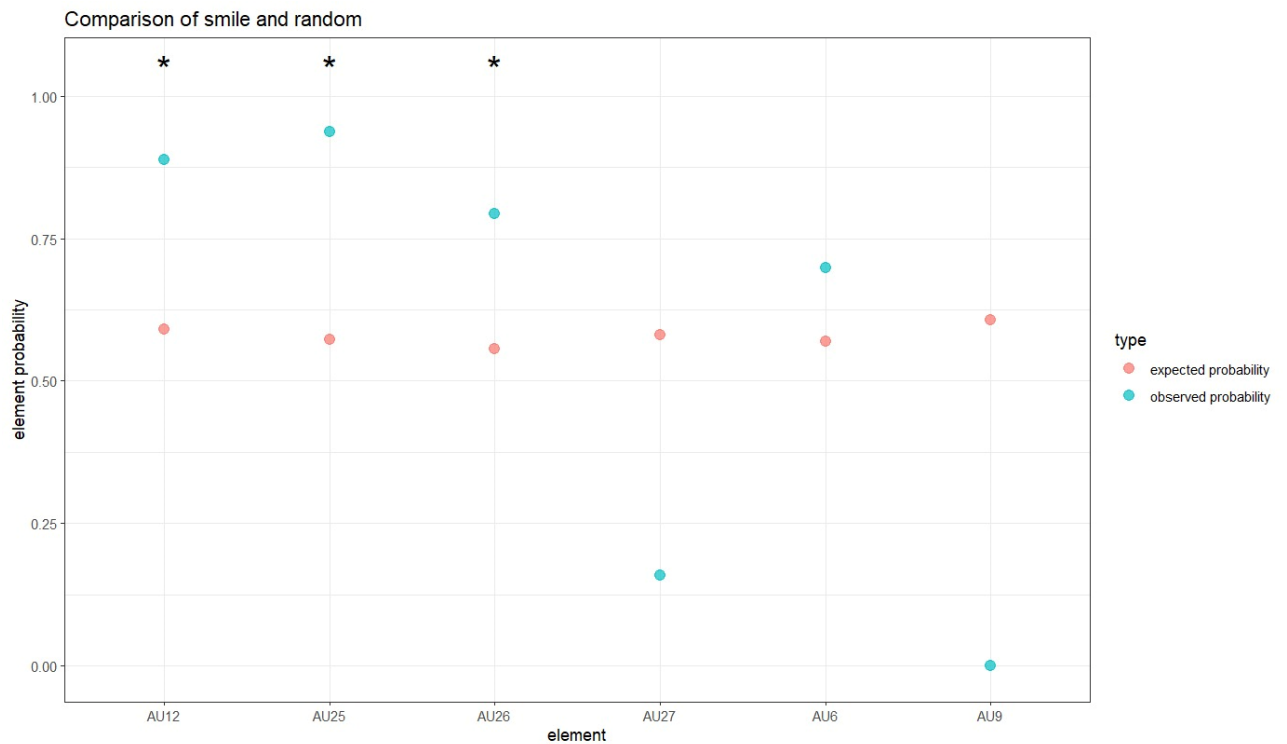
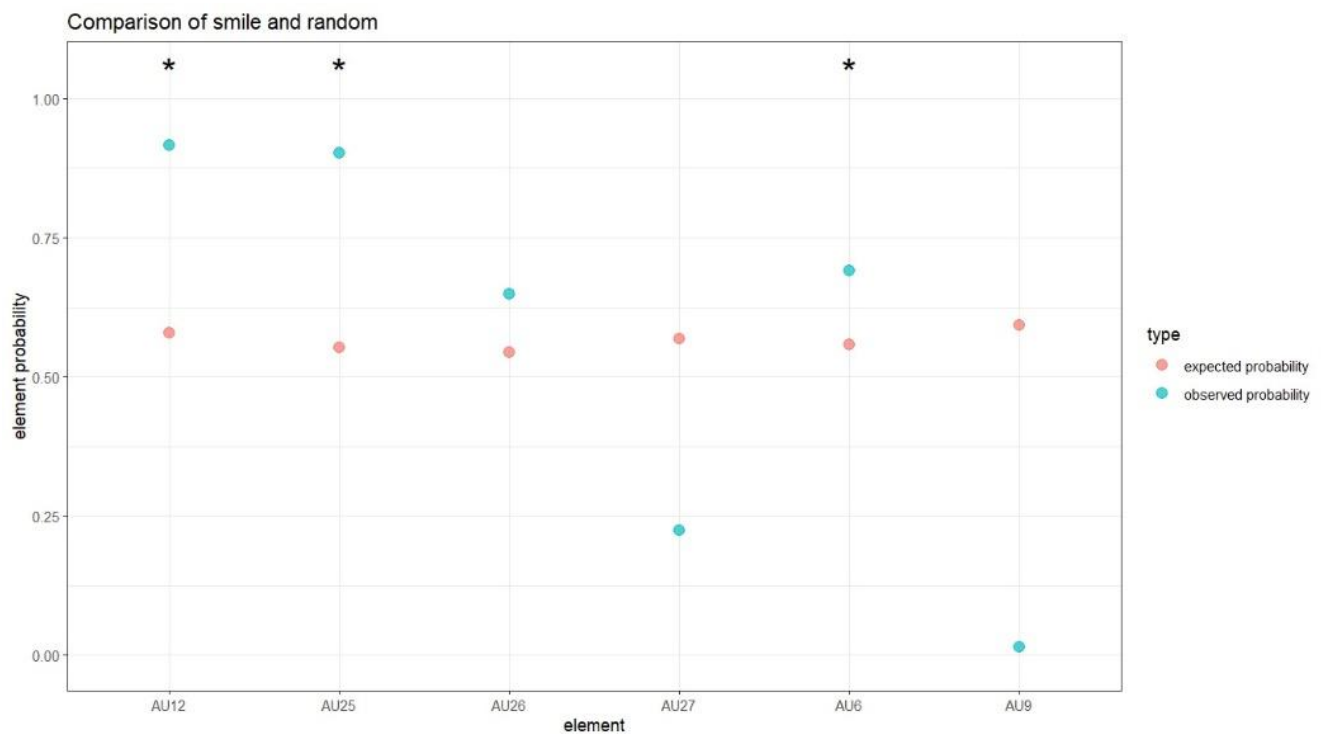


Figure 5. Element plot showing the significant recruitment of AUs during the Play condition, taking into account the potential contraction of the selected facial muscles involved in the smile display.



Supplementary Materials

1. Example of the excel sheet used for the manual scoring and the duration of each smile

video name	sex	exp conditi	toward	start	end	duration	durati	quality model mean	AU6	AU9	AU12	AU25	AU26	AU27	eyes
PR_T4_0203_B	M	P	exp	00:02:20.279	00:02:20.398	00:00:00.119	1	0,75	0	0	1	0	0	0	open
PR_T4_0203_B	M	P	exp	00:02:33.078	00:02:34.959	00:00:01.881	2	0,78	0	0	1	0	0	0	open
PR_T4_0203_B	M	P	exp	00:02:38.919	00:02:39.279	00:00:00.360	1	0,78	0	0	1	1	0	0	open
PR_T4_0203_B	M	P	toy	00:02:39.319	00:02:40.040	00:00:00.721	1	0,81	1	0	1	1	0	0	open
PR_T4_0203_B	M	P	exp	00:02:40.120	00:02:42.470	00:00:02.350	2	0,82	1	0	1	1	0	0	open
PR_T4_0203_B	M	P	toy	00:02:42.598	00:02:46.558	00:00:03.960	4	0,78	1	0	1	1	0	0	open
PR_T4_0203_B	M	B	toy	00:03:26.760	00:03:29.158	00:00:02.398	2	0,73	1	0	1	1	0	0	open
PR_T4_0203_B	M	B	exp	00:03:31.760	00:03:36.720	00:00:04.960	5	0,77	1	0	1	1	1	0	open
PR_T4_0203_B	M	B	exp	00:04:01.400	00:04:02.411	00:00:01.011	1	0,75	0	0	1	1	0	0	open
PR_T4_0203_B	M	B	exp	00:04:11.439	00:04:11.879	00:00:00.440	1	0,74	0	0	1	1	0	0	open

2. Example of Baby FaceReader output

Video Time	Participant Name	Quality	Action Unit 06 - Cheek Raiser	Action Unit 09 - Nose Wrinkler	Action Unit 12 - Lip Corner Puller	Action Unit 25 - Lips Part	Action Unit 26 - Jaw Drop	Action Unit 27 - Mouth
00:01:16.600	PR_T4_0265_D	FIT_FAILED	FIT_FAILED	FIT_FAILED	FIT_FAILED	FIT_FAILED	FIT_FAILED	FIT_FAILED
00:01:16.666	PR_T4_0265_D	FIT_FAILED	FIT_FAILED	FIT_FAILED	FIT_FAILED	FIT_FAILED	FIT_FAILED	FIT_FAILED
00:01:16.733	PR_T4_0265_D	0,7365996	0,1097718	0,07917175	0,118806	0,07737503	0,05987448	0,05231985
00:01:16.800	PR_T4_0265_D	0,7394256	0,1098852	0,0790387	0,1171112	0,07849295	0,05998039	0,05236587
00:01:16.866	PR_T4_0265_D	0,7351266	0,1079891	0,07996426	0,1141114	0,07823201	0,05957508	0,05234299
00:01:16.933	PR_T4_0265_D	0,724336	0,1061726	0,08074273	0,1103229	0,07751058	0,0591149	0,05247779
00:01:17.000	PR_T4_0265_D	0,7217898	0,1052151	0,08008825	0,1098255	0,07654245	0,05878537	0,05276438
00:01:17.066	PR_T4_0265_D	0,7145442	0,1039479	0,07950749	0,1068711	0,07560053	0,05851943	0,0538508
00:01:17.133	PR_T4_0265_D	0,7093043	0,1041425	0,07977141	0,1039053	0,07644848	0,05861544	0,05567819

CHAPTER 5

Ongoing study

To be submitted as brief report

OLD ROOTS NEW BRANCHES: INTEGRATING TRADITIONAL ANALYSIS OF THE STRANGE SITUATION PROCEDURE WITH A NOVEL ETHOLOGICAL APPROACH

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Abstract

This study integrates traditional analysis of the Strange Situation Procedure (SSP) with a novel ethological approach, focusing on multimodal continuous behavioral measurements to enhance the assessment of infant attachment behaviors. By capturing the dynamics of infant-caregiver interactions in naturalistic settings, we aim to address limitations of conventional categorical ratings. Utilizing a sample of 30 typically developing 22-month-old infants and their mothers, this research seeks to provide a richer understanding of how attachment patterns evolve.

Our findings aim to bridge developmental psychology and ethological research, enhancing both methodology and practical applications.

Keywords: attachment patterns – ethological collection – quantitative measures of behaviors – Strange Situation rating

1. Introduction

The Strange Situation (henceforth SSP, for Strange Situation Procedure) is a 20-minute procedure involving a series of episodes designed to increasingly trigger the infant's attachment system: entering an unfamiliar environment, introducing to an unfamiliar person (the stranger), brief separations from the mother, and subsequent reunions with her (Ainsworth et al., 1978). The original aim of this paradigm was to create an experimental tool for assessing the quality of the mother-infant relationship, emphasizing key elements of maternal and infant behaviors that contribute to the formation of secure or insecure attachments. Infants who trust their mother's availability are neither excessively dependent on her nor indifferent to her presence. They are enough confident to explore the environment or play, while occasionally checking the mother's location and adjusting their behavior during separations. Securely attached infants are comfortable with physical contact, initiating and approaching smoothly. They are also relaxed enough to engage in playful exploration of the mother or visually scan their surroundings while being held. This pattern corresponds to Bowlby's model of the direct expression of the attachment behavioral system (1969) and was suggested by Ainsworth and other researchers after her, to reflect an infant's experience of caregiver who responds to the infant's signals of distress with prompt and sensitive contact and soothing. Ainsworth termed the pattern "secure". In contrast, insecurely attached infants can show distress during contact, becoming clingy or fussy; they approach with hesitation, or maintain the distance. These infants may also show conflicting behavior during separation events from the mother, with exploration that appears disorganized or lacks spontaneity. These subjects have been classified as "avoidant" when intentionally orienting their attention away from the caregiver, to focus on toys; most of them show a delayed or suppressed inclination to approach. Ainsworth linked avoidant pattern to those caregivers that frequently decline physical contact. She theorized that infants displaying avoidant style may need to avoid the distress of being rejected (Main 1979; Isabella & Belsky, 1991). Finally, infants displaying anger-like behaviors or passive distress were classified "ambivalent/resistant" and they correspond to a caregiver who, at home, tended to delay responding or whose availability might be unstable. Only later, Main and Solomon (1990) introduced the "disorganized/disoriented" classification for infants displaying conflicted or confused behaviors that disrupted the typical Ainsworth patterns. However, as this classification is considered an additional layer rather than a distinct pattern, we did not take it into account in this study.

Research has demonstrated that infant attachment security reliably predicts a wide range of social competencies during childhood and adolescence, including appropriate responses to social situations and the absence of problematic externalizing or internalizing behaviors (e.g., McCartney et al., 2004). The assessment of attachment security during the SSP requires highly trained raters with a deep understanding of infant-parent interactive behaviors. This proof-of-concept report indicates that a cost-effective set of multimodal sensors can be successfully used to reliably measure attachment behaviors during the SSP. The literature on developmental outcomes consistently highlights the importance of evaluating early attachment patterns, but despite this recognition, the field still lacks a continuous, quantitative analysis of the specific behaviors that underpin early attachment profiles. For the time being, researchers predominantly rely on qualitative descriptions and categorical rating scales to classify attachment types in the SSP, which can limit the precision and depth of behavioral insights. A more nuanced approach could help fill this gap.

The actual method stipulates that trained experts provide summary ratings of four key infant attachment behavior categories, scored during each of the two reunions with the parent. Both infant and parent behaviors during the SSP are summarized using 1-7 Likert scales. These behavioral categories include proximity-seeking, contact-maintenance, resistance to contact and avoidance. The classification process is based on the premise that the infant's motivation to seek, avoid, or resist the parent during the SSP can be inferred from the infant's behavior during reunions. Proximity-seeking ratings reflect the intensity and persistence of the infant's approach to the parent (Waters, 2002). Contact-maintenance measures the infant's persistent efforts to remain in close contact with the parent and the disinclination to interrupt that contact. Resistance ratings capture behaviors like anger or irritability, such as pushing away the parent, kicking or squirming when held, or crying angrily. Lastly, avoidance ratings reflect the extent to which the infant tries to avoid contact with the parent, through actions such as leaning away, turning their head, or ignoring the parent (Waters, 2002). Although the training of expert raters and the classification process is resource-intensive, it does not bear a continuous quantitative description of attachment behaviors. Also, the scoring cannot be totally objective.

In the present study, we attempt to replicate the traditional rating by implementing continuous measurements of specific behaviors later associated to the typical categories defining attachment styles. Our aim is to determine how this hybrid approach can provide insights into attachment patterns during the SSP, offering an objective source of observation. The primary challenge for non-psychologist scientists in this field is not theoretical but methodological. Biologists interested in mother-infant relationships have traditionally operationalized

attachment through discrete ethological behavioral variables, such as time spent in contact or proximity, frequency of approaches, and departures (e.g., Hinde & Spencer-Booth, 1967; Schino & Troisi, 2001). Having a quantitative, objective measure of attachment patterns comparable to traditional measures would help bridge the gap between comparative and developmental psychology, enriching the perspectives of both disciplines (Prince et al., 2021). Many studies suggest that incorporating ethological methods in observational procedures allows for a more nuanced understanding of behaviors by emphasizing their context and natural expressions, rather than relying solely on structured assessments or subjective interpretations. The use of video analysis and computer-assisted coding systems can enhance objectivity by allowing multiple raters to analyze recorded interactions, thereby reducing individual biases. We here propose a novel approach to studying the SSP's coordinates by employing ethological analysis to uncover patterns and subtle variations in the behavior of all participants. The goal is to complement and strengthen the traditional scoring system by validating it with more detailed and refined observations, potentially revealing inherent shades of behavior that may otherwise go unnoticed. Each attachment profile likely encompasses multiple nuances, therefore confining each child to a univocal profile could lead to an oversimplified and rushed definition of the mother-child relationship. Our approach aims to fill existing gaps, often overlooked, and may clarify discrepancies or even correlate with other features that emerge in later developmental stages, which have so far been underexplored.

2. Methods

2.1 Participants

The sample comprises of 30 mothers and their 21-months-old infants (16 males, 15 females), recruited as a part of a longitudinal study. All mothers were enrolled between 36th-41st week of gestation at the Parma Hospital, Italy. Recruited infants were all healthy full-term infants with routine pre- and postnatal medical histories. At the time of SSP administration, their age ranged from 19 to 21 months (mean = 20, SD = 1.03). The mothers' mean age was 34 years (mean = 34.57, SD = 3.80), with a minimum age of 28 and a maximum of 41. Regarding nationality, marital status, education level, and current occupation, the sample can be considered relatively homogeneous, also due to the shared geographic location (Parma or nearby towns). All mothers were of Italian nationality, except for two participants. Two mothers reported being single, while the remaining indicated they were married, in a civil partnership, or cohabiting. 30% of the participants held a high school diploma, while 70% had completed a university degree.

Additionally, the majority of the women reported being employed, while 5 were homemakers, and 1 was pursuing further education. During follow-up assessments, mothers were asked to complete additional questionnaires that explored various aspects such as breastfeeding practices, daycare attendance, and family composition. Based on these questionnaires, the sample is composed as follows: 58% of the children did not attend daycare, while the remaining 42% attended from either 6 or 12 months of age. 45% of the participants had one or more older siblings, while 55% were only children. Finally, 45% of the children were exclusively breastfed, while 55% received a combination of breastfeeding and other forms of feeding or were never breastfed.

2.2 Experimental Protocol

The SSP was conducted following standard procedures (Ainsworth et al., 1978) consisting of 7 three-minute episodes + 1 of 30secs (the first episode), including two separations from the mother, each followed by a reunion (see Table 1 for detailed description). Separations (but not reunions) were curtailed if the infant was highly distressed (e.g., 30 s of crying), which occurred in 42% of cases. The SSP was conducted in a playroom, located in the Unit of Developmental Psychology, University of Parma, from May to September 2023. The room was equipped with a GoPro (HERO5 black) camera while a second camera (Sony Handycam® CX405) simultaneously recorded the video from an associated control room, equipped with a unidirectional window (Fig. 1). Raters mainly used recordings from GoPro for behavioral scoring of the SSP, since these type of action cameras provide a wide-angle lens (fish-eye lens) offering a broad perspective on the entire room. Immediately after the strange situation there was a 30-minute free-play, during which the mother filled out specific caretaker questionnaires not considered in the present study.

Intercoder reliability. Video-recorded events were analyzed via an ethological approach by A.P. (coder#1). Before starting the video analyses, inter-observer reliability was assessed between coder#1 and a senior coder (C.S., coder#2) on about 10% of total recording, covering all three experimental conditions and considering all behavioral categories. The values of Cohen's kappa coefficient (κ) (Cohen, 1960) between the coders were higher than 70% for each behavioral category (mean $\kappa = 0.87$).

2.3 Traditional rating

Following the SSP, an experienced rater (D.R.), reviewed video of the protocol and rated infant attachment behaviors. Ratings are made on a 7-point Likert scale, which includes behavioral examples that anchor specific ratings (Ainsworth et al., 1978; Waters, 2002).

2.4 Ethological rating

Frame-by-frame video analysis for each subject was conducted via Pot Player®, with the accuracy of 1 sec time window. Following the purpose-build ethogram, coding consisted of manually entering the starting time (ending time, when behaviors lasted >1sec) and the corresponding code, anytime a behavior occurred, into an Excel© sheet, for each subject.

Distribution of behaviors coded in each category and calculation of frequency. The frequencies and durations of each behavior were converted into percentages in order to be weighted according to the duration of the episodes considered. Specifically, behaviors directed toward the mother were weighted only for the episodes of the SSP in which the mother was actually present, and not for the entire duration of the procedure. The same was applied to the stranger. Conversely, child-directed behaviors, when aimed at the self (self-directed behaviors), were weighted over the entire duration of the procedure, as they could potentially manifest in every episode. Finally, for each category, the sum of the percentage durations of the associated behaviors was calculated, in order to determine which category exhibited the highest percentage, for each infant. The categories were:

- Proximity and Contact Seeking: includes the infant's behaviors aimed at seeking physical contact or proximity with the caregiver; it should consider also the intensity and persistence of these actions.
- Contact Maintaining: includes the infant's efforts to resist separation and maintain physical contact with the caregiver once established.
- Resistance: all the oppositional behaviors, such as signs of anger, irritation, or aggression. They often occur when the adult attempts to reestablish contact after a separation. These behaviors may be directed toward adults or objects in the environment.
- Avoidance: involves behaviors where the infant ignores or avoids interaction with the adult, even from a distance. During attempts to engage or reunite, the infant may walk away, turn their head or back, hide, or continue their activities while disregarding external stimuli.

According to this method, the category of proximity-seeking and contact-maintaining behaviors was associated with secure attachment style, the category of avoidant behaviors with avoidant attachment style, and the category of resistant behaviors with ambivalent attachment style, following the guidelines of the attachment literature (Waters, 2002).

Based on Waters (2002), the percentage durations of those behaviors not clearly fitting any of the above categories, were adjusted *a posteriori* based on their frequency (see Additional Behaviors in Table 2). The percentage frequencies obtained for each of these behaviors were used to calculate two percentiles (15th and 75th), with the purpose of eventually dividing them into three groups: subjects exhibiting very high percentages of the behavior (above the 75th percentile), subjects with very low frequencies (below the 15th percentile), and those with average frequencies (between the two percentiles). Based on the group they fell into (high, medium, low) and the specific behavior, the frequency percentages were added to the percentages already obtained for the three macro-categories:

- The frequency percentages of behaviors labeled “stress behaviors,” “time spent in proximity of M,” and “looking at M”: when high, they were associated with the category of resistant behaviors; when low, they were added to the category of avoidant behaviors; those between the two percentiles were associated with the category of proximity-seeking behaviors.
- The frequency percentages of the behavior labeled “time spent in proximity of S”: when low, they were associated with the category of resistant behaviors; when high, they were associated with the category of avoidant behaviors; those between the two percentiles were added to the percentages already obtained in the category of proximity-seeking behaviors.

3. Results

3.1 Attachment styles obtained via traditional rating

To be done.

3.2 Attachment styles obtained via ethological rating

As shown in Figure 2, 25 children exhibit a secure attachment style, while a minority of 6 children display an avoidant attachment style. No participants developed a resistant-ambivalent attachment style. The categorization of children in the sample into secure and insecure-avoidant attachment styles is not as rigid as it may seem; many children classified as secure also

exhibited avoidant or ambivalent behaviors. Some children obtained similar percentages in the duration and frequency of behaviors associated with different attachment categories. In contrast, other children display a more pronounced predominance of behaviors related to a specific category. This indicates a degree of variability within the attachment categories.

4. Discussion

The SSP is the most widely used procedure to assess attachment in early childhood (Bowlby 1973, 1980). In her original work Ainsworth et al. (1978) defined the coding as a two-step procedure: first, coders apply a series of observational scales with graduated scores (i.e. proximity seeking behaviors, contact maintaining behaviors, resistant behaviors, avoidant behaviors); only after this, the corresponding descriptive category (i.e. secure, insecure, resistant, avoidant) of attachment was assigned, which somehow would have condensed the child's behavior, which has been already assessed by the aforementioned scales. However, in their review Simonelli et al. (2014) noted that, throughout the literature following Ainsworth's pioneering work, the observational scales are hardly referenced or applied, revealing a tendency for researchers to rely solely on the final categorical classifications, thereby overlooking the detailed behavioral assessment that the scales were intended to capture. This is not the only limit in the applied coding system which we tried to address. Although traditional raters evaluate infant behaviors to assign discrete categories, it is reasonable to hypothesize that attachment styles may not be purely unambiguous. For example, Waters (2002) highlights the importance of a matrix that summarizes the most likely alternative classifications relative to the most predictable one, emphasizing its necessity for thoroughly analyzing each case. Fraley and Spieker (2003) proposed that attachment behaviors can be categorized along two primary dimensions: approach *versus* avoidance behaviors, encompassing proximity-seeking, contact-maintenance, and avoidance scores, and the level of resistance, which includes of course resistance and disorganization scores. In their review about the quality of attachment as a foundation for the emergence and development of children's capacity for emotion regulation, Zimmer-Gembeck and colleagues (2017) pointed out that a more layered assessment of attachment, rather than solely focusing on typical attachment classifications, can play a pivotal role for understanding its connections to developmental features, which is not always straightforward.

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Ethical Approval

All the materials used in this study were permitted for scientific use with written informed consent. The study protocol received ethical approval from the Ethical Committee of the Area Vasta Emilia Nord, Local Secretariat of Parma, and the University of Parma. The protocol has been designed and the study will be conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and the ICH Harmonized Tripartite Guideline for Good Clinical Practice.

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Table 1: The Strange Situation episodes (adapted from Ainsworth et al., 1978). M= mother, I= infant, S= stranger.

Episode	Participants	Short description	Length
1	M, I	The observer introduces M and I into the room, then he/she leaves the room.	30 secs
2	M, I	M does not participate while infant explores. If required by the I, the M can play with him/her by staying seated on the chair.	3 mins
3	M, I, S	S enters and converses with the M. Later, S approaches I and tries to play with him/her. After 3 minutes, M leaves unobtrusively.	3 mins
4	I, S	First separation episode. S behavior is adjusted to that of I, sharing toys or whatever.	3 mins
5	I, M	First reunion episode. M can greet and/or comfort I, then tries to engage him/her in play. M leaves again, saying “bye-bye” or nothing as before.	3 mins
6	I alone	Second separation episode.	3 mins
7	I, S	Continuation of second separation episode. S enters and adjusts behavior to that of infant.	3 mins
8	M, I	Second reunion episode.	3 mins

Table 2: List of behaviors considered for the non-traditional scoring, sorted by the macro-categories defined by the traditional method. M= mother, S= stranger, proximity occurred when the infant was approximately an arm’s length distant from the adult. See Table 1 for the description of each episode.

PROXIMITY SEEKING BEHAVIORS	BEHAVIOR	EPISODES IN WHICH IT CAN OCCUR
	approaching M	2-3-5-8
	being held from M	
	showing, giving, reaching, asking for help to M	
	touching the M + without resistance behaviours	5-8
	approaching S	3-4-7
	being held from S	
	touching the S + without resistance behaviours	
	showing, giving, reaching, asking for help to S	
	calling M + approach, pick-up request, cry/fussy/pouting	all

	fussy/cry/pouting + approach, pick-up request, calling M	
	looking at the door, attention getting, attempt to exit	
	time spent in door space	4-6-7
	time spent chair M	
	request for holding	2-3-4-5-7-8
CONTACT MANTAINING BEHAVIORS	calling M + cry/fussy which stops after being picked-up	
	fussy/cry/pouting + if stops after being picked-up and if starts when the M tries to put down the B	2-3-5-8
	resists to interrupt physical contact with M	
AVOIDANT BEHAVIORS	time spent in door space	2-3-5-8
	looking at the door, attention getting, attempt to exit	2-5-8
	gaze aversion, walking away, shielding M	2-3
	scanning the environment	
	exploration and solitary play	2-3-4-5-7-8
RESISTANT BEHAVIORS	aggressive behaviours toward M	2-3-5-8
	aggressive behaviours toward S	3-4-7
	gaze aversion, walking away, shielding M	5-8
	playing harshly	7-8
	shielding, arching, kicking	
	fussy/cry/pouting + arching, kicking	
	fussy/cry/pouting + in temper tantrum if pairs with rejecting toy, throw toy, shake no/say no/avoid to any play invitation from the adult	2-3-4-5-7
	rejecting the toy, throwing toy, tearing away toy, say no with head or voc + when follows a play invitation from the adult	
	tearing away toy, throwing toy + when it doesn't follow a play invitation from the adult	
ADDITIONAL BEHAVIORS	stress behaviours in absence of physical contact	4-6-7
	time spent in proximity of M	2-3-5-8

	looking at M	2-3-5-8
	time spent in proximity of S	3

Figure 1: The playroom, located in University of Parma, where data was collected.

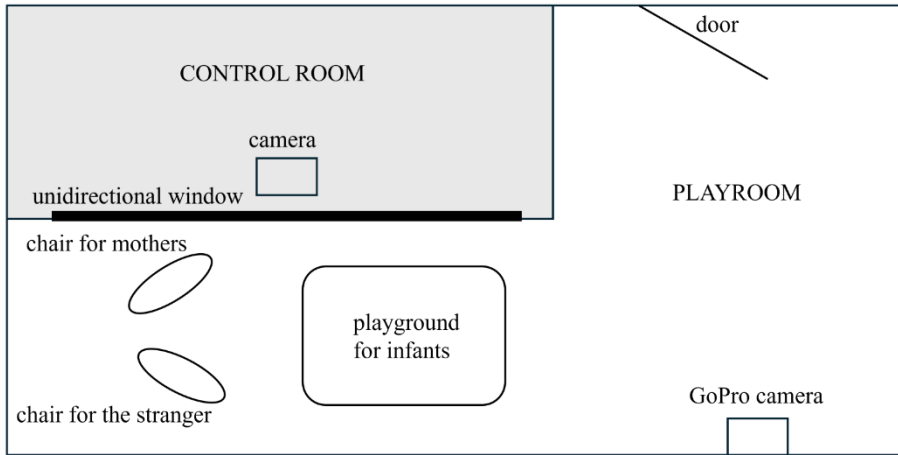
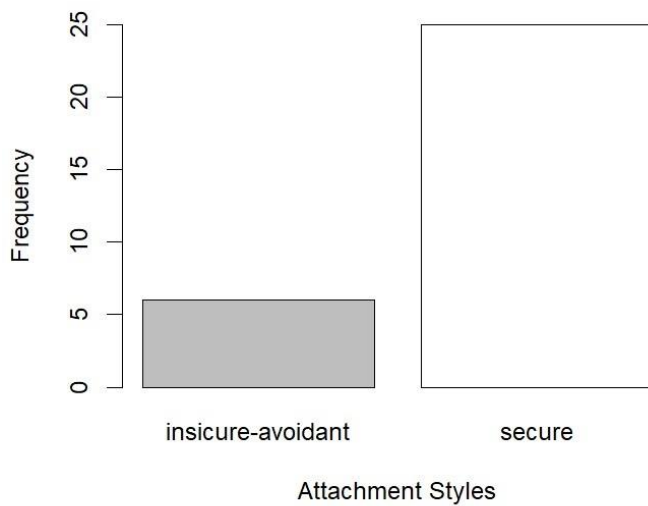


Figure 2: Distribution of attachment styles in our sample



GENERAL DISCUSSION

Emotion regulation (ER) is a fundamental aspect of early development, crucial for fostering children's socioemotional growth (Bronson, 2000; Calkins & Leerkes, 2004; Dodge, 1989; Fabes & Eisenberg, 1992; Kopp, 1989; Thompson, 1994). Difficulty in establishing robust ER skills during infancy and childhood has been strongly associated with a range of challenges, including behavioral (Hill et al., 2006), psychological (Aldao et al., 2010; Berking & Wupperman, 2012; Keenan, 2000; McLaughlin et al., 2011), and socioemotional (Penela et al., 2012) difficulties. A substantial body of research underscores the importance of interactive processes in the acquisition and refinement of self-regulation (SR) skills (McCaslin, 2009; McClelland & Cameron, 2011). However, studies investigating everyday interactions, i.e. contexts in which these regulation skills are applied, required, and reinforced, remain limited. Essentially, SR manifests as adaptive behavior modification. Within everyday learning contexts, SR involves active monitoring, identifying challenges, and adjusting strategies to achieve personal goals and engagement standards (Hadwin, 2013; Winne & Hadwin, 1998). For young children, early SR or metacognitive abilities are reflected in their capacity to observe and adjust their own actions to accomplish intended goals (Bryce et al., 2015; Deák & Narasimham, 2003; McCaslin, 2009).

Gross (1998) defined ER as the processes through which individuals manage felt emotions and their expression. Cole et al. (2004) described emotional SR as the coordinated interaction of behavioral, psychophysiological, attentional, and emotional systems, enabling young children to engage effectively within their social environment. This definition encompasses coping, mood regulation and repair, defense mechanisms, and affect regulation, distinguishing coping as focused specifically on reducing negative emotions. Together, these definitions imply that emotion SR involves not only affective experiences but also cognitive and behavioral mechanisms.

Current research on SR often prioritizes assessments of general regulatory capacities, such as through parent or teacher evaluations (e.g., Howse et al., 2003; Penela et al., 2015) or employs structured observation within laboratory environments (e.g., Spinrad et al., 2007; Duckworth

et al., 2013). This study extends the examination of young infants' emotion SR by focusing on real-life, socio-emotionally challenging scenarios, recognized as common contexts where infants actively practice and develop these skills.

The central theme of this thesis is the analysis of the development of emotional SR, assessed predominantly through an ethological approach. As elaborated in Chapter 2, the assessment through behavioral micro-analysis is particularly valuable because it reveals minute details and specific interactions that might otherwise go unnoticed. This thesis privileges this approach across all the three studies described in Chapters 3, 4 and 5, highlighting its utility in capturing subtle regulatory behaviors in different contexts. Notably, despite the distinct aims and outcomes employed in each study, a unified ethogram was used consistently. This protocol facilitated a focused yet flexible examination of regulation processes, selectively excluding or extending certain macro-categories based on the age of the child.

Moreover, the emphasis on the ethological method has been consistently reflected in the statistical analysis, which is typically applied in the field of behavioral studies. By integrating ethological principles into the statistical evaluation, the analysis remains aligned with the naturalistic observations of emotional SR, thereby enriching the understanding of these processes. This coherence across methodologies reinforces the validity of the findings and highlights the intricate dynamics of emotional SR in early development.

In **Chapter 3** of this thesis, we explored how infants begin to develop and exhibit emotional SR skills through attentional and behavioral responses to challenging situations in the Barrier Task. This experimental paradigm involves three two-minute phases: (1) Play (free play with a toy), (2) Barrier (toy placed out of reach behind a transparent barrier), and (3) Return (toy returned for free play). During the Barrier phase, mothers were instructed not to interact with the infant. Throughout, both mothers and the experimenter wore white face masks. Findings highlighted that, even before fully voluntary SR mechanisms are established, infants used attentional orientation, particularly in response to social partners, as a foundational SR tool. Specifically, infants showed a tendency to direct their attention to caregivers or figures of authority during stressful situations, signaling an early reliance on social cues for emotional guidance. This reliance indicates a developing understanding of adult roles and underscores the interaction between SR and social cognition.

The study also demonstrates that SR strategies, including attention orientation, vary depending on the infants' childcare experience. Infants attending childcare showed delayed attention to the mother and greater engagement with the experimenter in stress-inducing situations,

suggesting that socialization within childcare settings fosters an adaptive flexibility in seeking support beyond primary caregivers. This shift aligns with theories proposing that diverse caregiving environments promote social competence by exposing infants to varied social roles and shared resources, which may include adult attention and assistance in ER.

Behavioral SR responses, such as self-soothing actions, were observed to increase during high-stress conditions, supporting the theory that these behaviors serve as coping mechanisms for ER under frustration. This finding supports previous studies linking self-comforting behaviors with affective regulation during early development. Notably, infants attending childcare showed an increase in self-regulatory behaviors specifically under stress, suggesting that frequent exposure to novel social contexts in childcare may enhance their ability to independently manage frustration.

Further, this study identifies sex differences in SR, with females engaging more in self-regulatory behaviors across various conditions, reflecting existing research on sex differences in SR capacities. Males, however, demonstrated a greater dependence on maternal attention, particularly under stress, which may indicate a different reliance on caregiver presence for SR. These findings align with the literature indicating that females generally display stronger self-regulatory skills and provide empirical support for theories linking males' regulatory behaviors to maternal proximity.

Overall, this study illustrates that early SR development is influenced by both intrinsic factors, such as sex, and extrinsic factors, including socialization through childcare. The observed patterns highlight the potential of ethological perspective on SR, emphasizing how early social experiences shape foundational regulatory behaviors in response to environmental challenges. This study may contribute to understanding the etiology of SR by situating early attentional and behavioral strategies within broader social and developmental contexts.

In **Chapter 4**, the study examined the role of infants' emotional expressions, particularly smiling, as early indicators of social engagement and SR. Findings revealed that even at one year, infants demonstrate a nuanced use of smiles in social contexts, modulating their expressions based on emotional states and situational needs. Specifically, during the experimental baseline session, in which infants could freely play, they exhibited Duchenne smiles involving the raising of the cheeks and marked by the contraction of specific facial muscles composing the Facial Action Unit 6, following the Facial Action Coding System by Ekman et al., 2002. On the contrary, during the experimental condition inducing stress, infants displayed non-Duchenne smiles, the classic conformation of smiles but without raising the

cheeks (AU6 absent). The simultaneous development of motor skills and SR capacities suggests that infants' physical growth supports their ability to manage emotions through increasingly varied expressions across the first year of life. This selective use of smiling suggests that infants are able to employ distinct smile types to navigate social interactions (when the desired object is blocked by an experimenter) and emotional challenges (masking disappointment or discomfort), which may serve as early forms of SR. This aligns with functionalist theories, stating that emotions serve adaptive roles in managing social relationships.

The study also indicates that infants' smiles serve as communicative signals, with distinctions between genuine and posed expressions, an established concept in adult facial expression research. In adults, both muscle activation patterns and additional factors, such as the duration of each expression, often help differentiate between genuine and posed smiles, although findings on duration differences remain debated. In the study described in Chapter 4, smile duration has been examined as a potential differentiating feature. However, no significant duration differences have been found. Nonetheless, 1-year-old infants probably have yet to develop the more sophisticated timing differences characteristic of mature emotional signaling, which may emerge later in development.

This study points out how infants use smiling not merely as a reflexive expression but as an adaptive social tool, signaling distress, drawing support, and regulating emotional states after frustrating events. This exploration of smiles as early SR strategies reinforces the central theme of SR development and contributes to a broader comprehension of emotional and social growth in infancy.

In Chapter 4 a methodological breakthrough is achieved through the integration of Baby FaceReader, an automated facial analysis tool, into infant emotion research. The acquisition and implementation of Baby FaceReader at the Behavioral Biology Lab of the University of Parma have enabled detailed, rapid, and reliable identification of infants' facial expressions (in this case smiles) through an innovative use of machine learning-based AUs detection. This approach complements the traditional, labor-intensive manual coding processes established by Baby FACS, advancing the field by allowing for more consistent, objective, and scalable analyses of emotional responses in infants.

In the manual coding procedure, expert coders identified and coded each infant smile by moving frame-by-frame through the video recording. This high-precision approach, known as behavioral micro-analysis, provided accuracy down to the millisecond. However, the Baby FaceReader automates this process, setting specific quality thresholds (e.g., model quality and

expression reliability) to ensure analytical consistency and reliability. This level of automation, which captures and evaluates AU intensity for smiles and other facial expressions, provides an unparalleled level of data accuracy at up to 15 frames *per second*.

With applications ranging from identifying subtle changes in infants' expressions in real-time to detecting patterns across large datasets, the potential for future explorations via Baby FaceReader is vast. These advancements could provide insights into diverse areas, such as how infants adapt to changes in caregiver responsiveness or respond to different types of emotional stimuli, while also possibly broaden the scope of developmental assessments to include infants at risk for attachment difficulties or neurodevelopmental challenges (Ertugrul et al., 2022).

Chapter 5, despite still being a work in progress, explores ongoing efforts to refine the assessment of attachment in early childhood, particularly within the framework of the Strange Situation Procedure (SSP; Ainsworth et al., 1978). Ainsworth's original methodology involved a two-step coding system, where observational scales for behaviors like proximity seeking, contact maintenance, resistance, and avoidance were applied first, followed by assigning a categorical attachment classification (e.g., secure, insecure, resistant, avoidant). However, subsequent research has tended to focus primarily on the most known categorical outcomes, often overlooking the detailed behavioral assessment that the scales were originally meant to provide (Simonelli et al., 2014).

The research in Chapter 5 aims to address these methodological limitations by exploring a more comprehensive, dimensional approach to coding attachment, moving beyond the rigid classifications of traditional categories. This gap in traditional scoring methods is not new; previous research has already highlighted the potential shortcomings of traditional approaches and has suggested that a dimensional framework could provide a more multifaceted understanding of attachment behaviors. Such a framework may better capture the complex and varied nature of attachment patterns, allowing for a more detailed and flexible interpretation of these early relational behaviors. Furthermore, a layered assessment of attachment can enhance the understanding of the connections between attachment and other developmental processes, such as ER (Zimmer-Gembeck et al., 2017). Thus, going beyond categorical classifications to incorporate behavioral dimensions, may provide a more detailed foundation for exploring how attachment influences the early development of SR. By emphasizing these methodological improvements, this ongoing work seeks to align attachment assessment with a more granular, behavior-oriented approach that better reflects the multifaceted nature of early childhood attachment and its role in shaping emotional development.

6.1 Limitations of the study and perspectives

This work acknowledges some limitations that may impact the findings. Firstly, the immediate stress induction method which has been used in Chapter 3 and Chapter 4, likely affects all participants uniformly, potentially acting as a confounding variable, particularly in the experimental condition where elevated stress responses are anticipated. This could increase the demand for coping strategies, whether externally or self-regulated. Additionally, while significant differences were identified between variables, the magnitude of these differences tends to be small, which aligns with the selection criteria limiting the sample on a population characterized by physiological gestation and normal development. Furthermore, the laboratory setting (which for Chapters 3 and 4 was a room inside the hospital) may introduce factors such as fatigue that could influence data collection, especially as assessments were conducted in the afternoon following childcare or before naps.

The use of the Baby FaceReader in Chapter 4 also presents limitations, as it may result in greater data loss than manual coding, particularly in subjects with significant head pose variations which have been discarded by the software.

Another limitation concerns the application in Chapter 5 of a new method alternative to the traditional one for analyzing the SSP. While potentially more precise, the method has not yet been validated and is time-consuming. It is also worth noting that the integration of this new methodology with self-reported questionnaires or temperament profiles may create imbalances due to the differing nature of the methods employed, necessitating caution in interpretation.

Importantly, the role of parenting style as an influential factor in the development of constructive ER, was not considered in this analysis despite its established impact on behavioral outcomes. In a similar vein, maternal sensitivity serves as a foundational benchmark for a child's SR standards, as infants initially learn to regulate through their mother's responsiveness before developing independent SR. Thus, overlooking these factors may be a missed opportunity.

In perspective it will be necessary to address some of these limitations by expanding the sample size. The sample used in Chapters 3 and 4 could be enlarged by coding additional videos, which was not feasible due to time constraints. Priority was given to dyads for which detailed questionnaire data was available, particularly regarding nursery attendance and other lifestyle factors not examined in this thesis. For Chapter 4, further filtering was necessary due to the video quality required by the Baby FaceReader software. Unfortunately, the need for frontal video footage, and the fact that the initial data collection was not aligned with software needs,

significantly limited sample expansion. For Chapter 5, the limited sample size was based on selecting dyads for which a cortisol saliva sample had also been obtained, allowing for a more comprehensive analysis of physiological responses alongside behavioral coding.

In future studies, an expanded sample size for Chapter 3 could help determine whether the identified factors remain consistently significant and if any interactions become more robust. Additional variables, such as exclusive breastfeeding or the number of siblings per subject, could also be incorporated to explore their impact on the development of SR.

For Chapter 4, it would be beneficial to investigate the effects observed in the third experimental condition, where the toy is returned, and to assess whether the two potential types of smiles cluster differently. Another interesting angle would be to consider the intensity of the AUs engaged, as the software provides this data automatically. However, a key limitation is that the intensity ratings from Baby FaceReader have not yet been validated by the literature, presenting a challenge for interpretation.

Lastly, a significant goal for Chapter 5 is to complete the validation of the new attachment profiling method. Should the expert assessment align with the attachment profiles generated by the new method, this approach would gain validation as a reliable tool. If not, examining the specific discrepancies could be enlightening. The Chapter 5 findings suggest that profiles can sometimes score almost equally across two categories, placing a subject in one category rather than another by a very narrow margin. For example, a child categorized as secure might actually exhibit behaviors aligned with avoidant attachment as well. If discrepancies emerge specifically among these “mashup” profiles, this will reinforce the value of an alternative method that acknowledges such degrees in attachment profiling.

A further advancement and potential application of this research is the re-evaluation of emotional developmental trajectories, particularly concerning prenatal and early postnatal exposure to environmental endocrine-disrupting chemicals, as initially outlined in the Life Milch Project. These findings were not included in this thesis, as the analysis of the collected biological samples (maternal urine, breast milk, and infant urine) was not yet complete at the time of writing. The added value lies in a detailed and objective behavioral analysis, which serves as the interface between the organism and its environment, offering a relevant functional outcome in both healthy and pathological conditions. The ethological approach captures the natural context of behavior, providing novel insights into developmental health.

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APPENDIX

1. Example of an excel file for codification. Valid for Chapter 3 and 4.

Video	Sex	Condition	Behavior	Towards	Starting time	Ending time	Duration	Condition start	Condition end	Condition duration
PR_T4_0984_D_2 0222911	F	P_M	look	toy	0:33		0:01	0:33	2:38	2:05
PR_T4_0984_D_2 0222911	F	P_M	touch	toy	0:33	0:46	0:13			
PR_T4_0984_D_2 0222911	F	P_M	look	exp	0:34	0:36	0:02			
PR_T4_0984_D_2 0222911	F	P_M	mumble		0:34		0:01			
PR_T4_0984_D_2 0222911	F	P_M	look	toy	0:36	0:41	0:05			
PR_T4_0984_D_2 0222911	F	P_M	fac exp		0:40	0:42	0:02			
PR_T4_0984_D_2 0222911	F	P_M	look	exp	0:41	0:45	0:04			
PR_T4_0984_D_2 0222911	F	P_M	voc		0:42		0:01			
PR_T4_0984_D_2 0222911	F	P_M	look	toy	0:45	0:53	0:08			
PR_T4_0984_D_2 0222911	F	P_M	manipul play		0:46	0:53	0:07			
PR_T4_0984_D_2 0222911	F	P_M	smile	toy	0:52	0:55	0:03			
PR_T4_0984_D_2 0222911	F	P_M	look	exp	0:53	0:55	0:02			
PR_T4_0984_D_2 0222911	F	P_M	touch	toy	0:53	0:57	0:04			
PR_T4_0984_D_2 0222911	F	P_M	look	toy	0:55	0:57	0:02			
PR_T4_0984_D_2 0222911	F	P_M	point		0:57	0:59	0:02			
PR_T4_0984_D_2 0222911	F	P_M	look	mom	0:57	0:59	0:02			
PR_T4_0984_D_2 0222911	F	P_M	touch	mom	0:58	1:00	0:02			
PR_T4_0984_D_2 0222911	F	P_M	look	toy	0:59	1:03	0:04			
PR_T4_0984_D_2 0222911	F	P_M	touch	toy	1:02		0:01			
PR_T4_0984_D_2 0222911	F	P_M	manipul play		1:03	1:08	0:05			
PR_T4_0984_D_2 0222911	F	P_M	smile	toy	1:04	1:07	0:03			
PR_T4_0984_D_2 0222911	F	P_M	smile	toy	1:09		0:01			
PR_T4_0984_D_2 0222911	F	P_M	look	toy	1:08	1:10	0:02			
PR_T4_0984_D_2 0222911	F	P_M	touch	toy	1:08	1:19	0:11			
PR_T4_0984_D_2 0222911	F	P_M	look	exp	1:10		0:01			
PR_T4_0984_D_2 0222911	F	P_M	smile	exp	1:10		0:01			
PR_T4_0984_D_2 0222911	F	P_M	look	toy	1:11	1:13	0:02			
PR_T4_0984_D_2 0222911	F	P_M	smile	toy	1:13		0:01			
PR_T4_0984_D_2 0222911	F	P_M	look	exp	1:14		0:01			
PR_T4_0984_D_2 0222911	F	P_M	smile	exp	1:14		0:01			



2. Ethogram implemented for the codification of the Barrier Task procedure

GAZE/ATTENTIVE BEHAVIORS			
label	code	definition	note
looking at the mother	LOOK MOM	when the infants' eyes turn to look at the mother. When coding social interactions, you may want to use this code only when the baby looks toward the face of the mother, seeking eye contact.	ETERO-REGULATION FACS AU69 Looking at a Designated Person
looking at an object	LOOK OBJ	when the infants' eyes turn to look at an object. It is not necessary to specify the object. Code this only if you are sure that the gaze is “stuck” on something for its natural features (for example, mother’s jewelry).	FACS AU69 Looking at a Designated object
looking at the experimenter	LOOK EXP	the gaze is oriented towards the experimenter. When the gaze is oriented to the exp’hands because she is holding the barrier, codify LOOK* EXP and add this particular in the note.	ETERO-REGULATION
scanning the environment	SCAN	it can be used to designate any shifts in gaze away from the midline that are neither directed toward specific targets (look at a designated object) nor strongly averted from these targets (gaze aversion).	FACS AU67 Looking Elsewhere, Searching or Looking Around
looking at the toy	LOOK TOY	the gaze is oriented towards the toy. It is always present during manipulative and functional play.	
looking at the barrier	LOOK BAR	the gaze is oriented towards the barrier.	
gaze aversion*	AVOID	any unambiguous gaze aversion, regardless of the direction of the eye movements. We recommend scoring gaze aversion conservatively, reserving	MOTOR BEHAVIOR for strange sit FACS AU68 Gaze aversion

		AVOID for marked looking away, that is, shifts in gaze away from a target person or object involving effortful eye and/or head movements to the extreme right or left, up into the corner of the room, over the shoulder, behind the infant, etc., where there is no obvious object of interest.	
VOCALIZATIONS			
label	code	definition	note
calling the mother	CALL MOM	intelligible or non-intelligible speech towards the mother often accompanied by indicating/pointing.	It may happen to hear calling dad or others, just write it down in the note
calling the experimenter	CALL EXP	intelligible or non-intelligible speech towards the experimenter, often accompanied by indicating/pointing.	
calling the toy	CALL TOY	non-intelligible speech towards the toy, often accompanied by indicating/pointing.	
mumble / babbling	MUMBLE	non-intelligible speech, such as <i>bababa, dada, oaahh, rrr</i> , typically involves a combination of consonant and vowel sounds. Cooing involves vowels only and can also include sounds that are only consonants (e.g. <i>mmmmm</i>) as long as it is not fussy sound.	MacNeilage & Davis, 1992 It normally lasts longer than a simple vocalization. Indicate duration.
fussy	FUSSY	whining, lower intensity than crying, fussing sounds will often be less broken up and more extended than crying. It can occur in association with a general negative emotional state, boredom, or protest.	SELF-REGULATION, EMOTIONAL STATE
naming toy	NAME TOY	calling the toy with the exact name (for example, picking up a ball saying “ball”) or associating the correct (or	

		similar) sound to the toy (for example, indicating or touching a dog saying “bau” or “dog” or “wolf”).	
general vocalization	VOC	a general vocalized sound which does not fit with any of the previous definitions. It includes vegetative sounds: such as wheezes, sneezes, coughs, hiccups, and clicking sounds; effort sounds: such as grunts.	Hsu et al., 2001 It normally occurs as a single sound.
SELF-REGULATION			
label	code	definition	note
self-rubbing	RUB	one or both hands or one or more fingers are used to rub the eye/eyes, the belly, or any parts of the body or the face. Specify which part in the “toward” column.	specify which part in the note
self-touching	SELF-TOUCH	one or both hands or one or more fingers are used to statically touch any part of the body or the face (i.e. eyes, hands, belly, ecc..).	specify which part in the note
mouthing	MOUTH	movements of tongue and/or lips, lip smacking, blowing bubbles, making raspberries, vacuum chewing.	
arm movements	ARM MOV	repetitive movements of one of both arms or non-repetitive unusual movements of one or both arms.	Baranek, G. T. (1999)
leg movements	LEG MOV	repetitive movements of one or both legs or non-repetitive unusual movements of one or both legs.	Baranek, G. T. (1999)
kicking	KICK	moving the legs and banging the feet against something or the air. decreased at ten months, and then peaked at 13 months of age.	
repeated hand movements	HAND MOV	repeated scratching of the bumper or table, opening and closing of hand, and finger	

		movements. Includes minimal body movements that may indicate tension.	
hands to mouth mov	HAND MOUTH	finger(s) thumb, or hand(s) in contact with the mouth or mouth region, not engaged with the mouth.	
finger suckling	FINGER/HAND SUCK	finger/hand suckling.	
yawning	YAWN	inhaling deeply through the open mouth and exhaling. occurred rarely, and only at three and six months.	
head movement	HEAD MOV	repeated rotation or retroflexion of the head.	
suckling (smt or nothing)	SUCK	this action consists of tongue protrusion and rhythmic movements involving the mandible and the tongue, characterized by constant frequency and duration.	It can be part of MOUTH
sighs	SIGH	breathing in and letting out the breath in a forced exhalation. peaked at six and thirteen months, the two ages of highest motor activity. It can be not related to motor activity.	
scratching	SCR	the infant scratches her/himself. This should occur with more than one rubbing, indicating stress or discomfort	specify which part in the note
tongue exposure	TONGUE	this behavior is coded when the tongue protrudes from its resting position beyond the inner margin of the red part of the lips.	It can be part of MOUTH
arching/escaping	ARCH/ESC	arching or twisting of the back and the neck. The infant seems to make an attempt to get out of the seat or a request to change the position/location.	

gently touching the barrier	TOUCH BAR	the infant touches the barrier (wood or glass) without pushing or slamming it. He/She usually uses one or two fingers (similar to contingent behavior).	If it follows a pointing, code POINT and TOUCH BAR after that.
banging the barrier	BANG BAR	the infant taps, slaps or pushes the barrier, with one or both hands, or with head or tongue/mouth. It seems he/she intentionally wants to tear it down.	
attempt to climb/climb the barrier	CLIMB BAR	the infant tries to climb or actually climbs the barrier using hands and legs.	
rejecting the toy	REJECT TOY	the infant gets the toy away, as he/she played enough with it, peaked at 10 and 13 months.	PLAY STYLES
throwing the toy	THROW TOY	the infant intentionally and/or persistently drops the toy to the floor, peaked at 10 and 13 months. If repeated as a part of the play session collect the duration.	
biting smt or smo	BITE	the infant bites something or someone (mother). If he/she bites a toy, this can be part of manipulative play.	
FACIAL EXPRESSIONS and OTHERS			
label	code	definition	note
pouting, horseshoe mouth, kidney mouth, general distress	POU/DISTRESS	it involves facial muscle actions that oppose the strong, involuntary muscle actions that pull the mouth into a cry face. Even though it occurs either preceding or following a cry face, it is not an intrinsic component of cry faces. Pouts are typically directed to a social partner and are frequently observed in frustrating situations. Also when the mouth corners are not pulled down but the infant looks worried (frown,	 

		knitted brows)/angry/frustrated indicate it as “distress”.	
smiling	SMILE	the principal component is the lips corner obliquely upward raised. It may include varying degrees of mouth opening, contraction of eyes and so cheeks raise. The combination of principal component and others is random. It may be accompanied by nose wrinkles. In the codification sheet SMILE indicates a general manifestation of positive emotional valence.	add “voc” in the note's column when vocalized
crying	CRY	the classic open mouth cry face results from the combined activation of muscles that raise the upper lip (AUs 9,10,11 or in comb), stretch the mouth corners horizontally (AU20) and lower the mandible (AU26-27). This face is accompanied by one or more muscle actions in the brows, cheeks and lower face. In the codification sheet CRY indicates a general manifestation of negative emotional valence.	add “voc” in the note's column when vocalized
facial expressions in general	FAC EXP	other facial expressions which deserve attention and cannot be classified in the previous ones.	Describe it in the note.
BODY CONTACT			
label	code	definition	note
touching the mother	TOUCH MUM	the infant actively seeks physical contact with the mother (try to exclude unintentional touching). contact can be kept for more than a simple touch.	ETERO-REGULATION

pulling down the mask	MASK REMOVING	the infant actively tries to remove the mask from the mother's face in order to see the entire face or just ask for help or require her attention.	
seeking body contact	SEEK CONTACT	the infant tries to be breastfed or twists the body to hug the mother or to engage in a general physical contact (i.e. smack the lips for a kiss).	ETERO-REGULATION
PLAY STYLES/ENGAGEMENT WITH THE TOY			
label	code	definition	note
manipulative play	MANIPUL PLAY	<u>visually guided</u> exploratory manipulation in which the infant treated all the toys as if they were the same (ex: child picks up and visually/manually explores stacking block). It includes <i>mouthing</i> when any toy contacted the infant's lips, tongue, or teeth; <i>fingering</i> when any toy was manipulated in a <u>non-functional</u> manner with one or two digits; <i>waving</i> when a toy moved through an estimated arc of at least 60". It implies looking at the toy.	
touching the toy	TOUCH TOY	keeping a physical contact with the toy <u>without actively playing with it</u> . If the gaze is oriented towards the toy, code LOOK TOY as well.	
dancing	DANCE	the infant swings following the rhythm of the music from the toy (dance) or as anticipation to touch the toy.	
static play	STATIC PLAY	the infant plays with the toy (by finger exploration) but without visually interacting with it. It is basically MANIUPUL PL without visual engagement. <u>The gaze must not be oriented toward the toy.</u>	To be coded as PLAY it must include any motor behavior towards the toy. If not, it is just TOUCH TOY

playing harshly	HARSH PLAY	grasping, rubbing and nervously playing with the toy, can be associated with throwing or rejecting the toy. May include pounding, which is when the infant picks up a toy and bangs it on a support (table, floor..).	If the infant does not look at the toy, code STATIC / HARSH PLAY
retrieving	RETRIEVE	the experimenter struggles to retrieve the toy from the infants' hands. This behavior mainly occurs when shifting from P phase to B phase.	
GESTURES			
label	code	definition	note
pointing	POINT	the infant extends his/her index finger, with the rest of the hand closed and his/her arm partially completely extended, in the direction of an interesting object or event. There are two types of pointing 1. <i>proximal</i> : pointing to things up close that they can touch with their index finger (pointing at things in a book) and 2. <i>distal</i> : pointing to things that are at a distance.	It often precedes TOUCH BAR
showing	SHOW	the infant presents an object, towards the other or towards themselves, observing or passing it from one hand to the other. It is almost always accompanied by looking at mom/exp/str.	
giving	GIVE	the infant places an object in the other person's space.	
reaching	REACH	the infant extends his/her arms towards a desired object to request something from another person (seen at 10 months). May include pointing.	
banging for protest	BANG	the infant slams the table with the open hand or fist, as	

		he/she wants to manifest disappointment.	
POSTURES and LOCOMOTOR/MOTOR BEHAVIORS			
label	code	definition	note
standing up	STAND UP	standing up on mother's lamb or on the table.	
laying	LAY	saying back on caregiver's chest.	

GENERAL RULES

- If not present in the ethogram codify what you see and highlight the record in yellow
- If not visible from the camera but possibly visible from the other one, highlight the record in light blue
- If the infant's behavior is enhanced or promoted by the M or the E (e.g. not spontaneous), highlight the record in grey
- What happens between the end of a condition and the beginning of the next one must not be codified
- For more than one episode of a behavior (such as vocalizations) which happens one at a second, you can choose to code each of the episodes or just the duration of the entire event
- If you are not sure how to codify smt you can add a question mark and write down your doubts in the NOTE column
- If a behavior or a pattern lasts longer than 1 sec, you must add the ending time in the column. This not necessary when it lasts 1 sec.
- If a behavior or a pattern is protracted in the next condition, the behavior stops when the previous condition stops and restarts when the next condition starts.
- If you want the note column to be red, insert * next to the behavior

3. Ethogram implemented for the codification of the Strange Situation Procedure

GAZE/ATTENTIVE BEHAVIORS			
label	code	definition	Note
looking at the mother	LOOK MOM	When the infants' eyes turn to look at the mother. When coding social interactions, you may want to use this code only when the baby looks toward the face of the mother and seems to be making or seeking eye contact.	ETERO-REGULATION, MOTHER-INFANT SYNC FACS AU69 Looking at a Designated Person
Looking at an object	LOOK OBJ	When the infants' eyes turn to look at an object. It is not necessary to specify the object. Code this only if you are sure that the gaze is “stuck” on something for its natural features (for example, mother’s jewelry).	FACS AU69 Looking at a Designated object
scanning the environment	SCAN	it can be used to designate any shifts in gaze away from the midline that are neither directed toward specific targets (look at a designated object) nor strongly averted from these targets (gaze aversion).	FACS AU67 Looking Elsewhere, Searching or Looking Around
exploration	EXPL	Visual exploration of a limited area. This is mutually exclusive with LOOK TOY (ex: in Strange Sit the infant explores the toys on the rug before choosing one or more; in Barrier Task the infant explores the barrier before touching it)	
looking at the toy	LOOK TOY	The gaze is oriented towards the toy. It is always present during manipulative and functional play	
Looking at the door	LOOK DOOR	The gaze is oriented towards the door	
Gaze aversion*	AVOID	Any unambiguous gaze aversion, regardless of the direction of the eye movements. We recommend scoring gaze aversion conservatively, reserving AVOID for marked looking away, that is, shifts in gaze away from a target person or object involving effortful eye and/or head movements	MOTOR BEHAVIOR for strange sit FACS AU68 Gaze aversion

		to the extreme right or left, up into the corner of the room, over the shoulder, behind the infant, etc., where there is no obvious object of interest.	
VOCALIZATIONS			
label	code	definition	note
calling the mother	CALL MOM	intelligible or non-intelligible speech towards the mother often accompanied by indicating/pointing.	it may happen to hear calling dad or others, just write it down in the note
calling the stranger	CALL STR	intelligible or non-intelligible speech towards the stranger, often accompanied by indicating/pointing.	
calling the toy	CALL TOY	non-intelligible speech towards the toy, often accompanied by indicating/pointing.	
mumble	MUMBLE	non-intelligible speech, such as <i>bababa, dada, oaahh, rrr</i> , typically involves a combination of consonant and vowel sounds. Cooing involves vowels only and can also include sounds that are only consonants (e.g. <i>mmmmm</i>) as long as is not fussy sound.	MacNeilage & Davis, 1992 it normally lasts longer than a simple vocalization. Indicate duration.
fussy	FUSSY	whining, lower intensity than crying, fussing sounds will often be less broken up and more extended than crying. It can occur in association with a general negative emotional state, boredom, or protest.	SELF-REGULATION, EMOTIONAL STATE
naming toy	NAME TOY	Calling the toy with the exact name (for example, picking up a ball saying “ball”) or associating the correct (or similar) sound to the toy (for example, indicating or touching a dog saying “dog” or “wolf”).	
play noise	PLAY NOISE	vocalizations made in play, such as <i>brr-brr, peep-peep, wof-wof, meow meow</i> .	Smith & Connolly, 1980
general vocalization	VOC	all those vocalized sounds which cannot be coded in previous categories. It includes vegetative sounds: such as wheezes, sneezes,	Hsu et al., 2001 it normally occurs as a single sound.

		coughs, hiccups, and clicking sounds; effort sounds: such as grunts.	
SELF-REGULATION			
label	code	definition	note
self-rubbing	RUB	one or both hands or one or more fingers are used to rub the eye/eyes, the belly, or any parts of the body or the face. Specify which part in the “toward” column.	
mouthing	MOUTH	movements of tongue and/or lips, lip smacking, blowing bubbles, making raspberries, vacuum chewing.	
arm movements	ARM MOV	repetitive movements of one of both arms or non-repetitive unusual movements of one or both arms.	Baranek, G. T. (1999)
leg movements	LEG MOV	repetitive movements of one or both legs or non-repetitive unusual movements of one or both legs.	Baranek, G. T. (1999)
kicking	KICK	moving the legs and banging the feet against something or the air. decreased at ten months, and then peaked at 13 months of age.	
repeated hand movements	HAND MOV	repeated scratching of the bumper or table, opening and closing of hand, and finger movements. includes minimal body movements that may indicate tension.	
hands to mouth mov	HAND MOUTH	finger(s) thumb, or hand(s) in contact with the mouth or mouth region, not engaged with the mouth.	
finger suckling	FINGER/HAND SUCK	finger/hand suckling.	
yawning	YAWN	inhaling deeply through the open mouth and exhaling. occurred rarely, and only at three and six months.	
head movement	HEAD MOV	repeated rotation, retroflexion of the head.	
suckling (smt or nothing)	SUCK	this action consists of tongue protrusion and rhythmic movements involving the mandible and the tongue, characterized by constant frequency and duration.	it can be part of MOUTH

sighs	SIGH	breathing in and letting out the breath in a forced exhalation. peaked at six and thirteen months, the two ages of highest motor activity. It can be not related to motor activity.	
scratching	SCR	the infant scratches her/himself. This should occur with more than one rubbing, indicating stress or discomfort.	specify which part in the note
tongue exposure	TONGUE	This behavior is coded when the tongue protrudes from its resting position beyond the inner margin of the red part of the lips.	it can be part of MOUTH
arching/escaping	ARCH/ESC	Arching or twisting of the back and the neck. The infant seems to make an attempt to get out of the seat or a request to change the position/location.	
rejecting the toy	REJECT TOY	the infant gets the toy away, as he/she played enough with it, peaked at 10 and 13 months. <u>If the toy is held by the S or the M, indicate it in the notes.</u>	PLAY STYLES
throwing the toy	THROW TOY	the infant intentionally and/or persistently drops the toy to the floor, peaked at 10 and 13 months. If repeated as a part of the play session collect the duration.	PLAY STYLES
biting smt or smo	BITE	the infant bites something or someone (mother). If he/she bites a toy can be manipulative or pretend play.	
FACIAL EXPRESSIONS			
label	code	definition	note
pouting, horseshoe mouth, kidney mouth, general distress	POU/DISTRESS	it involves facial muscle actions that oppose the strong, involuntary muscle actions that pull the mouth into a cry face. Even though it occurs either preceding or following a cry face, it is not an intrinsic component of cry faces. Pouts are typically directed to a social partner and are frequently observed in frustrating situations. Also when the mouth corners are not pulled down but the infant looks worried (frown, knitted	

		brows)/angry/frustrated indicate it as “distress”.	
smiling	SMILE	the principal component is the lips corner obliquely upward raised. It may include varying degrees of mouth opening, contraction of eyes and so cheeks raise. The combination of principal component and others is random. It may be accompanied by nose wrinkles. In the codification sheet SMILE indicates a general manifestation of positive emotional valence.	add “voc” in the note’s column if vocalized
crying	CRY	the classic open mouth cry face results from the combined activation of muscles that raise the upper lip, stretch the mouth corners horizontally and lower the mandible. This face is accompanied by one or more muscle actions in the brows, cheeks and lower face. In the codification sheet CRY indicates a general manifestation of negative emotional valence.	add “voc” in the note’s column if vocalized
facial expressions in general	FAC EXP	other facial expressions which deserve attention and cannot be classified in the previous ones.	describe it in the note.
BODY CONTACT			
label	code	definition	note
approaching the mother	APPR MUM	when the child directly approached the mother by seeking physical/bodily contact or standing close/next to the mother. It can be followed or not by physical contact with her. Approach always includes a locomotion to enter in the mother’s proximity area.	
touching or staying in body contact with the mother	TOUCH MUM	the infant actively seeks physical contact with the mother. The contact can be kept for more than a simple touch.	

touching or staying in body contact with the stranger	TOUCH STR	the infant actively seeks physical contact with the stranger. The contact can be kept for more than a simple touch.	
seeking body contact	SEEK CONTACT	the infant tries to be breastfed or twists the body to hug the mother or to engage in a general physical contact (i.e. smack the lips for a kiss)	ETERO-REGULATION
request for holding	PICK-UP REQUEST	evident request to be held by the mother, can be associated with fussy vocalization or just reaching the arms towards the mother. It can underly the willingness to avoid the stranger.	ETERO-REGULATION
being held	HOLD	sitting in mother's arms. This behavior is mutually exclusive with touching the mother.	ETERO-REGULATION, BODY CONTACT

PLAY STYLES

label	code	definition	note
manipulative play	MANIPUL PLAY	<u>visually guided</u> exploratory manipulation in which the infant treated all the toys as if they were the same (ex: child picks up and visually/manually explores stacking block). It includes <i>mouth</i> ing when any toy contacted the infant's lips, tongue, or teeth; <i>finger</i> ing when any toy was manipulated in a <u>non-functional</u> manner with one or two digits; <i>wav</i> ing when a toy moved through an estimated arc of at least 60°. It implies looking at the toy.	
dancing	DANCE	the infant swings following the rhythm of the music from the toy (dance) or as anticipation to touch the toy.	
static play	STATIC PLAY	the infant plays with the toy (by finger exploration) but without visually interacting with it. It is	to be coded as PLAY it must include any motor behavior towards the toy. If not, it is just TOUCH TOY

		basically MANIUPUL/FUNCT PL without visual engagement. <u>The gaze must not be oriented toward the toy.</u>	
simple relational play	RELAT PLAY	in which the infant intentionally brought together or combined two toys that are commonly seen to be unrelated (ex: child places block on top of ball). It implies looking at the toy.	
functional relational play	FUNCT PLAY	in which the infant brought together two or more objects in a way that was intended to be related (ex: child stacks block on top of another block). Or he/she uses the toy in a way that was intended to be used (ex: reading a book, even though he/she can't). It implies looking at the toy.	
pretend play	PRETEND PLAY	in which pretence was apparent from confirming behaviours, e.g., putting phone receiver to mouth and vocalizing, bringing brush to doll's hair and making brushing-motions. It implies looking at the toy.	
playing harshly	HARSH PLAY	grasping, rubbing and nervously playing with the toy, can be associated with throwing or rejecting the toy. May include pounding, which is when the infant picks up a toy and bangs it on a support (table, floor..). <u>It does not imply looking at the toy; specify where the gaze is oriented.</u>	if the infant does not look at the toy, code STATIC / HARSH PLAY
GESTURES			
label	code	definition	note
pointing	POINT	the infant extends his/her index finger, with the rest of the hand closed and his/her arm partially completely extended, in the direction of an interesting object or event. There are two types of	specify if not contextual

		pointing 1. <i>proximal</i> : pointing to things up close that they can touch with their index finger (pointing at things in a book) and 2. <i>distal</i> : pointing to things that are at a distance.	
showing	SHOW	the infant presents an object, usually with his/her arm extended, towards the other. It can be followed by a playful interaction with the partner, thus functioning as a play invitation. It is almost always accompanied by looking at the partner.	
giving	GIVE	the infant places an object in the other person's space, usually in their hand or lap or he/she throws the toy towards the partner. It can be followed by a playful interaction with the partner, thus functioning as a play invitation.	
reaching	REACH	the infant extends his/her arms towards a desired object to request something from another person (seen at 10 months). May include pointing.	
asking for help	ASK	the infant requests help for solving a task, getting off a chair, and so on. The request can be accompanied by a vocalization (such as CALL MOM) or an extension of the arm or hand; it can also be just a gaze.	
say yes or not with the head/finger or voice	NODDING/SHAKE NO	shaking the head for "no" (emerging between 13 and 15 months) and nodding for "yes" (between 16 and 18 months). Code if the behavior is accompanied by the actual saying yes or no.	
POSTURES and LOCOMOTOR/MOTOR BEHAVIORS			
label	code	definition	note
walking away	WALK AWAY	motor avoidance	
interaction with the chair	CHAIR	any kind of interaction with one of the two chairs; sitting on it or touching it.	specify which chair

stay still near the door	DOOR	remaining in the area close to the door (see the picture).	
shielding	SHIELD	the baby raises a shoulder as she/he wants to put a barrier between she/he and the interlocutor. It appears as if the baby wants to hide part of the face. it often accompanies motor avoidance.	
attempt to exit the room	EXIT	attempts to go out (ex: holding the door handle).	
approaching the mother	APPR MUM	when the child directly approached the mother by seeking physical/bodily contact or standing close/next to the mother. it can be followed or not by physical contact with her. approach always includes a locomotion to enter in the mother's proximity area.	
approaching the stranger	APPR STR	the infant approaches the stranger, it can be followed or not by physical contact with her/him. approach always includes a locomotion to enter in the stranger's proximity area.	
attention getting	ATT GETT	fake cough, knocking or slapping the door (especially when the baby stays alone).	
not encoded	NI	when there are parts of the video that cannot be encoded because they shouldn't have happened (e.g.: The child gets the toy stuck in his mother's hair and the stranger has to help his mother) or the time period between the end of a condition and the beginning of the next one.	
EPISODIC EVENTS			
pat the head	PAT PAT	gently slapping his/her own head.	
hair twisting	HAIR TWIST	twisting hair around fingers.	
posture	POSTURE	the infant assumes a specific posture which is not easy to decode or define, but still evident. Insert note.	

tear away smt from other's hands	TEAR AWAY TOY	if not part of harsh play session, indicate from whose hands the toy has been removed.
drop the toy	DROP TOY	let the toy fall from the hands.

AREA of LOCOMOTION



GENERAL RULES

- If not present in the ethogram codify what you see and highlight the record in yellow
- If not visible from the GoPro but possibly visible from the other camera, highlight the record in light blue
- If the infant's behavior is enhanced or promoted by the M or the S (e.g. not spontaneous), highlight the record in grey
- What happens between the end of a condition and the beginning of the next one must not be codified
- For more than one episode of a behavior (such as vocalizations) which happens one at a second, you can choose to code each of the episodes or just the duration of the entire event
- If you are not sure how to codify smt you can add a question mark and write down your doubts in the NOTE column
- If a behavior or a pattern lasts longer than 1 sec, you must add the ending time in the column. This not necessary when it lasts 1 sec

- If a behavior or a pattern is protracted in the next condition, the behavior stops when the previous condition stops and restarts when the next condition starts.
- If you want the note column to be noted, insert * next to the behavior.

SUGGESTIONS for using POT PLAYER

- Before starting codification, ensure that you are able to switch from 1 sec to the next one just using the left/right arrow key
- If you need to set the time window (sec x sec): Riproduzione > salta a > impostazioni salta al tempo > 1 secondo. Unselect: Salta al frame
- Use D and F to go backwards or forwards to use hundreds of each second
- Use + to zoom the image

Toys Available for Children:

- Tower of Hanoi
- Plush Ball No. 2
- Plush Soccer Ball with Arms and Legs
- Wooden Building Blocks
- Inflatable Snail
- Two Books (Pimpa and a Pirate Tactile Book)
- Two Tambourines
- Rattle
- Basket of Fabric Vegetables (Carrots, Lettuce, Tomatoes, Mushrooms, Garlic, Leeks, Cucumber)
- Rubber Building Sets
- Shape Sorting Wheel with Wooden Shapes (Note: The shapes feature images of animals, e.g., crocodile, lion, hippopotamus, monkey, etc.)