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Mothers' Neural Activation to Own and Other Children's Faces: Examining the Association with Emotional Availability and Caregiving History

Supervisor Professor Paola Rigo

> *Candidate:* Kaisa Schiffer *Student ID number:* 2040682

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Abstract

The functional magnetic resonance imaging (fMRI) research investigating postpartum mothers' neural activation to infant stimuli has established a specific group of brain areas implicated in the global caregiving network (Abraham, 2018; Rigo et al., 2019; Swain, 2008). The present study aimed to extend the findings to lesbian mothers (n = 8) in a later stage of parenting by examining their brain activations in response to their own versus other children's neutral visual stimuli. Stimuli presented were video clips of their own and unfamiliar preschool-aged children with neutral facial expressions. Furthermore, it was explored how the recorded neural activations can be affected by parents' emotional availability assessed through the Emotional Availability Scale (EAS) and parents' personal history with caregivers assessed by the Parental Acceptance and Rejection Scale (PARQ). The results showed activation in some of the areas hypothesized and novel deactivations that previous research has not reported. Moreover, the results indicated that mothers who perceived their own mothers as hostile and aggressive were less sensitive and more intrusive in interactions with their own children. Yet, no interaction was found between PARO: Father and EAS. Lastly, the higher the levels of mothers' structuring measured by EAS, the lower the activation in the parahippocampal area extended to the amygdala when the mother was viewing their own child's neutral face compared to the unfamiliar child. These findings represent the importance of perceived maternal acceptance on the child's future caregiving behaviors, suggesting an intergenerational transmission of sensitivity.

Keywords: parental brain, child faces, fMRI, EAS

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Introduction

Undeniably, the most critical relationship for a child is the one they build with their parents. Children's internal world and how they approach future relationships are shaped by the first emotional bonds created between them and their main caregivers. Therefore, parents are faced with the responsibility of providing their child with sensitive care in correspondence with the changing needs of a growing human who needs to learn how to communicate, be independent, sensitive to others, and kind to themselves. However, a parent's sensitivity and emotional availability depend not only on their internal will to be caring but also on their past experiences. These internalized experiences with their own parents might even interact with the neural areas established through evolution, which activate to provide care for an offspring.

Research, up until now, has tried to demonstrate critical areas in a parent's brain that activate in response to their own infant and how these brain areas differ in sensitive and insensitive parents. Yet, studies conducted have been slow to catch up with the changes in society and have not yet analyzed the neurobiology of lesbian parents. Furthermore, there seems to be a lack of investigation on mothers with preschool and school-aged children and how the maternal brain might have changed to care for a child in the middle phases of childhood. The factors concluded from previous research that might influence the past parental experience of lesbian caregivers, their emotional availability, brain responses to their own preschool or school-aged child, and the associations that might exist between these variables will be thoroughly addressed in the coming chapters.

To provide context for the current study, the first chapter will provide relevant historical background on sensitive parenting, how it has developed into an emotional availability framework, the importance of considering the emotional relationship of a preschool or school-aged child and their parent, and why it is important to consider lesbian mothers. The second

chapter will give an understanding of the current parental brain research and the stimuli used, and finally, the last chapter will bring together behavior and biology to understand how experiences and behaviors might interact with each other and how they might associate with the neural activations of parents in response to their own child.

CHAPTER 1. Behavioral background

An infant cannot survive by itself, but even as the child grows, parental care is still highly needed and helps them build capacities to become progressively more independent. This unique parental bond is the foundation for a child's healthy development and well-being in adulthood, so it is not surprising that researchers have been persistently trying to determine the factors that can make or break a caregiver-child relationship. Nevertheless, given the changing social environment and developments in brain imaging techniques, a lot is yet to be unraveled.

1.1. Attachment theory

The attachment framework was one of the first theories to shed light on the profound nature of the parent-infant bond while also considering the motivations, social influences, and emotional connection between the parent and the child. Considering our current knowledge, it might seem logical that a child's separation from their mother has harmful consequences on the child's emotional state, future behaviors, and mental health. However, John Bowlby was not taken seriously when he first proposed the idea of separation anxiety to fellow psychoanalysts based on his wartime observations ("Conclusions," 2011). Only when Bowlby turned to ethology did he find support for his theories, leading to the development of an attachment framework (Bowlby, 1969).

The attachment theory, further refined by Ainsworth (Ainsworth & Bell, 1970), quickly became the cornerstone of parenting research, maintaining its relevance even today. The Attachment Theory draws heavily from the concepts of natural selection and the drive for maximal survival and is a straightforward way to practically measure infants' predisposition to direct attention toward their primary caregiver (Rholes & Simpson, 2004). Starting with a more theoretical focus on the infant-parent bond, but later enriched by the Strange Situation studies

conducted by Ainsworth, the attachment framework connected theory with observable exploratory behaviors elicited by infants as they were separated from their primary caregiver. In the Strange Situation Paradigm, infants aged 9 to 18 months are briefly left alone in a room without their mother (Ainsworth & Bell, 1970). The scenario was designed to be unique enough to encourage exploratory behavior while not being so strange as to elicit fear and heighten attachment behavior right away (Ainsworth & Bell, 1970). This study by Ainsworth and Bell (1970) revealed that infants are more likely to explore an unfamiliar situation if they are in reasonable proximity to their attachment figure. In other words, the securely attached children used their mother as a secure base to explore the strange situation.

Based on further research, Ainsworth and Bell described attachment as an enduring affectionate bond between two people and identified three consistent attachment patterns: secure, insecureavoidant, and insecure-resistant (Ainsworth et al., 1979). Later, research by Main and Solomon (1986) added the fourth pattern, insecure-disorganized. Around the same time, the Adult Attachment Interview was developed to predict the quality of parent-infant interactions and their attachment relationships based on autobiographical memories and evaluation of the parent based on their current perspective (George et al., 1985). Coded from their freeform answers, parents can be classified as *secure* when they have had either supportive childhood experiences or when they come from a difficult background but are coherent in their interpretation of these experiences (van IJzendoorn et al., 1995). Parents are categorized as *dismissive* when their descriptions of the relationship with their mother are contradicting; parents are classified as *dismissive, preoccupied* when they show confusion and anger towards their attachment figure, or *unresolved/disorganized* when the relationship has been potentially traumatic (van IJzendoorn, 1995). Although classifying attachment behaviors into distinct patterns can be helpful in some cases, contemporary research analyzing adult attachment questionnaires has shown that people do not usually match with a specific pattern of behavior. Therefore, a dimensional approach to attachment can better describe the multifaceted nature of the infant-parent bond (Stein et al., 2002). Even though the approach to attachment has evolved, the notion of a secure bond remains at the core of different theories. Ainsworth's extensive home observation conducted in Uganda (Ainsworth, 1967) and later in the United States suggested that attachment security is determined by variations in parenting and, more specifically, by the parent's sensitivity towards the child (Ainsworth, 2015). Sensitive caregiving is a parent's capacity to quickly and appropriately notice, interpret, and respond to their infant's behavioral, verbal, and emotional signs (Ainsworth et al., 1974).

Deeply rooted in the attachment theory, sensitivity can also be considered a continuum rather than a categorization, with low sensitivity on one end and high sensitivity on the other (Cooke et al., 2022). Another way to visualize parental sensitivity is a U-shaped curve suggesting that too little neural reactivity and excessive reactivity to infant needs can be problematic in parenting (Young et al., 2017). Finding a balance between the two is sought after, but it can be difficult in reality.

1.2. Emotional availability

Emotions are a core feature of human interactions and an essential regulator of how parents react to their children and, in return, how children feel about the communication they have with their caregivers (Bornstein et al., 2012). During these mutually fulfilling family interactions, the child learns how to regulate their own emotions, balance their arousal, and build and hold close relationships in the future (Bornstein et al., 2012). Therefore, only considering attachment, which has more of a biological basis and does not focus much on emotions and

reciprocal relations between the two parties, can limit the understanding of how secure bonds develop and affect children's future outcomes and close relationships. The Strange Situation Paradigm, for example, focuses on a stressful situation and the psychological unavailability of the parent and does not account for all the complex interactions in everyday life (Erickson & Egeland, 2019; Saunders et al., 2015). Expanding on the concept of attachment while also considering parental sensitivity, the complexity of everyday interaction, and the dyadic relationship between the parent and the child has led to the development of the emotional availability framework (Biringen et al., 2014).

For instance, everyday dyadic interactions can be as simple as a smile shared between the child and parent, one person pointing at an object and the other looking at it, or when a parent offers help after the child cannot find the right puzzle piece. Another common interaction could be bedtime, when a child needs to get into a comfortable and restful state to fall asleep and sleep separately from parents throughout the night (Kim et al., 2014). These examples can directly assess emotional availability and show positive dimensions of parental presence presented by parental involvement, pleasure of the interaction, and appropriate responses to infant cues (Ziv et al., 2000). Suppose a parent approaches their child in this positive manner. In that case, the child also feels inclined to interact with the caregiver and enjoy this mutual communication, facilitating similar situations where both parties feel happy and rewarded. On the other hand, Kim et al. (2014) showed, based on the bedtime example, that if a parent is emotionally unavailable during bedtime, the child will most likely have less adaptive frustration regulation strategies. In addition, the examples of everyday interactions are clearly dyadic since a caregiver and a child are analyzed in relation to each other, meaning that the emotional availability theory shifted the focus from only the parent as an active agent to the emotional reactions of both the caregiver and the child (Biringen et al., 1991).

Biringen et al. (1991) were the first ones to introduce direct measurement of emotional availability in the context of infant-parent research. Soon after introducing the concept, the Emotional Availability Scales (EAS) were developed, making it feasible to assess emotional availability directly (Biringen et al., 1998). EAS presents a multidimensional set of features, including scales measuring the child's and caregiver's affect and behavior during a dyadic interaction (Biringen et al., 2014).

Maternal emotional availability has been shown to be a stable construct that generally is carried from infant to preschool age (Célia et al., 2018) and probably even further. For instance, Célia and others (2018) demonstrated through a longitudinal study that it is expected that a mother who is emotionally unavailable during a child's infancy will stay emotionally unavailable later in childhood. Therefore, studying a child-caregiver interaction during a child's preschool age will give us an understanding of the pattern of emotional availability that has most likely been there from infancy.

1.3. Emotional development of children

Emotional interactions with an adult are the first indicator of how the infant is feeling. They give us an understanding of the child's emotional state even before they can learn the language and say what they feel in words (Malik & Marwaha, 2023). The child's physical development goes hand in hand with their emotional development. A preschool-aged child starts to test the limits of what emotional reactions and behaviors are acceptable by showing more initiative and testing their autonomy over situations (Malik & Marwaha, 2023). According to Kochanska's (2001) longitudinal study, children aged around 3 years with a secure relationship with their parents were able to show appropriate emotional reactions in positive situations. In contrast, children with an insecure relationship with their parents got angry or upset even if their situation was positive. Through the ages of 5 to 10 the relationship with friends becomes

increasingly important, more complex emotional coping skills are learnt during this time, and the child learns to be independent and responsible without depending so much on their parents (Malik & Marwaha, 2023). Still, building self-confidence and self-assurance requires developing a positive, caring connection with a caregiver that includes praise, love, and setting up a healthy balance between freedom and home rules.

As children's understanding of emotions deepens, they also start grasping the more detailed variations in their parent's feelings and how these feelings relate to themselves (Denham et al., 2009). Easterbrooks and colleagues (2012) showed that maternal sensitivity, measured by the Emotional Availability Scales when the child was seven, was associated with children's functioning in middle school. Teachers described children with more insensitive mothers as more behaviorally problematic and children with passive parents as having more externalizing problems. However, children who had more insensitive mothers also reported more internalizing problems like depressive feelings about themselves, which went unnoticed by teachers.

The study by Easterbrooks and others (2012) was one of the few studies exploring how the emotional availability of mothers in their child's middle school age is related to the behaviors and feelings of school-aged children. Nevertheless, it is an important area of research to cover since school-aged children start understanding the nuances of their parent's emotions around this age, and based on this emotional relationship, they can also develop behavioral and internalizing problems that can easily go unnoticed. Gaining more insight into the emotional availability of mothers during a child's school age might lead to quicker recognition of children at risk, who may need psychological interventions or support from school staff to deal with their inner world of emotions.

1.4. Changes in family structures

Studies exploring the parent-child bond have been conducted with different types of families but mainly focused on the bond between the mother and child. Recent research, however, is trying to refrain from only focusing on the mother-child bond. This dynamic does not represent all the different family structures and can limit understanding of the relationships and variables that can affect the children's future outcomes.

Lamb (2012) has discussed in his meta-analysis that there is no clear evidence that one type of family style leads to better developmental outcomes than others, suggesting that other psychological and societal factors are more likely to affect the child's future than the fact that the caregivers are in a same-sex union for example. This is supported by recent studies conducted in Italy that have not shown differences between emotional regulation, children's well-being, and adjustment from same-sex and different-sex families (Baiocco et al., 2015, 2018). However, these results have not been associated with parental measurements like parents' emotional availability or emotional regulation skills. The only measures used on parents have assessed the self-reported competence in parenting and the quality of the relationship between the parents without considering measures that are not reported by the parents themselves.

Lesbian couples have different possibilities for becoming a parent or caregiver through a previous relationship, artificial insemination, surrogacy, or adoption. All these options imply that at least one of the caregivers is not going to be a biological parent of the child. A sensitive caregiver, regardless of sex or genetic relatedness, is someone who has good synchrony with their child (Abraham, 2018). Recent research suggests that the quality of the parent-child relationship may be more determined by the presence and participation of the caregiver in the infant's everyday life rather than by the caregiver's sex (Abraham, 2018).

CHAPTER 2. Biological background

Understanding the meaning of infant cues is essential for a caregiver to stay synchronous with their child's needs. A human baby cannot survive independently and relies entirely on an adult, who decodes the child's needs based on their emotional reactions. For this reason, mammalian brains show changes in the neurobiology of the brain during the postpartum period and adjust to caring for a child (Cohen & Mizrahi, 2015). Recent research findings show that these changes in brain structures are not exclusive to birth mothers, and increased attention to their infant cues might be modulated by the direct childcare experiences of the biological or non-biological caregiver (Abraham, 2018).

Infant cues serve two fundamental functions in building a bond between an infant and their caregiver. Firstly, these cues offer the adult caregiver valuable insights into the infant's physiological and emotional condition (Young et al., 2017). When considered alongside other contextual factors, these signals aid in determining suitable caregiving actions. Secondly, starting just days after birth, parents and their infants begin to mimic each other's emotional expressions, building synchrony between the caregiver and the child (Young et al., 2017), suggesting that parents have predetermined attentional prioritization of their infant's cues.

2.1. The salience of facial stimuli

Sensitive and trusting caregiver-child interactions depend on how well both parties in the dyad understand and engage with each other's needs (Bornstein, 2013). An essential part of this dynamic is the caregiver's ability to understand the vital information a child's face conveys and engage with these emotional cues to help, comfort, or be happy with them. However, the existing body of research in this area has primarily focused on the significance of infants' facial cues during the early postpartum period, leaving a notable gap in our understanding of parental responses to children's cues as they mature into later stages of childhood. Moreover, a significant limitation in the existing literature is its narrow focus on biological mothers, with non-biological caregiver populations receiving relatively little attention. This imbalance underscores the need for a more comprehensive examination of how various caregivers respond to the facial cues of their children.

At the core of the discussion on child facial cues has been the concept of the baby schema (*Kindchenschema*) developed by Lorenz (1943), which describes features of a young child's face that are found across species and are highly salient for adults. These features include a high rounded forehead, big eyes, and chubby cheeks (Glocker et al., 2009; Lorenz, 1943). This face pattern elicits caregiving behaviors in adults and draws more attention than a grown-up's face (Gemignani et al., 2023), highlighting the importance of specific facial attributes (Brosch et al., 2007).

It is vital for caregivers to adapt to their child's facial expressions to offer sensitive care and emotional support aligned with the child's needs. Caregivers' reactions are critical in assisting babies in managing their emotions and behaviors (Sroufe et al., 2009). As the infant develops, caregiver-child dyads establish unique regulatory patterns with ongoing interactions throughout childhood (Abraham, 2018; Sroufe et al., 2009). Therefore, it would be logical to assume that dyads involving school-aged children and their mothers, who have had extensive interactions with each other through the years, are more attuned to each other's emotional and behavioral needs than an infant-caregiver dyad.

Attuning to one's own child's emotions during everyday interactions means that the parent sees their child as more salient than others, regardless of facial expression. The mother does not need to react only when their child is in obvious distress or shows strong signs of happiness. Neutral expressions are as relevant in everyday actions as any other emotion. A mother should be attentive to their own child's neutral expression since it is more ambivalent than smiling or crying and can be interpreted differently depending on the context.

A neutral face can express boredom when engaging with content that is not particularly interesting for the child. It can express disengagement when others are having fun, but there is no smile on one child's face, or it can mean that everything is normal when just sitting on the sofa and reading a not particularly emotional book. Since the small variations that a neutral expression can show are more likely noticed in one's own child, using neutral stimuli of one's own versus another child's neutral expression in a neural recording of the mothers' brains may give a better understanding of differences between the brain activation to own versus other child faces without the confound of a clear emotional reaction.

2.2. Functional imaging of the brain

The primary technique for examining the parental brain in response to one's own or other infants' faces is non-invasive blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) (for review see Rigo et al., 2019; Swain et al., 2014). fMRI enables the acquisition of functional and structural data while providing an optimal spatial resolution. The brain activity is indirectly assessed by measuring differences in regional blood oxygenation in the caregiver's brain while infant cues are presented. Therefore, fMRI does not create images based on direct firing of neurons. Instead, it generates images of the physiological changes in blood flow associated with neural activity (Huettel et al., 2014).

The vascular system provides energy to active neurons through oxygen extracted from oxygenated hemoglobin and glucose (Huettel et al., 2014). These changes in the active area of the brain provide the basis for fMRI, which is dependent on the variations in the local concentration of deoxygenated hemoglobin (Huettel et al., 2014). The hemodynamic response

to an activation of a brain area consists of a short onset delay, a rise to a peak within seconds, a return to baseline, and a prolonged undershoot (Huettel et al., 2014). It is important to consider that changes in blood flow are delayed over seconds, whereas the neural activation in response to stimuli might be instantaneous.

Often, the short visual stimuli presented are pictures, not videos, of the own vs. the other child. Rigo et al. (2019) suggested in their meta-analysis that considering one stimulus format over studies (image or video) can give us a better general understanding of the changes happening in the parental brain independent of the stimulus type. Considering the ecological validity of the stimuli, presenting video clips of one's own and other children might be a more realistic representation of an everyday parenting situation. However, studies until now have rarely used video clips of just the child as a stimulus.

In addition, brain responses are not a direct assessment of parenting behaviors. This limitation can be partly addressed by correlating the activations recorded in the brain to psychological or behavioral parental care measures like questionnaires or observations (Swain et al., 2014). Newer studies have started to take this into account and always compare brain responses to actual parental care behavior. In the current study, parental brain responses between two conditions (own and other child's face) will be correlated with emotional availability and the attachment history of the mother.

2.3. Networks and brain areas implicated in the parental brain

After years of studying caregivers' and mainly the mother's brains, research has established areas that are usually activated when looking at one's own child versus another child. The areas vary a little between studies, but the networks activated tend to stay the same, suggesting that the parental brain activates a so-called *global caregiving* network (Abraham, 2018) or *the*

parental brain (Swain et al., 2014) when attending to salient infant cues. Based on four recent and relevant meta-analyses (for review see Abraham, 2018; Abraham et al., 2016; Rigo et al., 2019; Swain et al., 2014) focusing on the fMRI studies on the parental brain, I have summarized the networks that are most often activated when the caregiver is attending to infant visual cues or one's own infant cues, with different emotional valences including sad, happy, and neutral faces (see Figure 1 (pp. 15)).

To swiftly pick up on visual infant cues, the brain mainly activates (1) *emotional processing*, (2) *motivation and reward* areas, and (3) *sensory and motor* areas (see Figure 1; for review, see Abraham, 2018; Abraham et al., 2016; Rigo et al., 2019; Swain et al., 2014). The three networks are interconnected and support different interaction processes, even having multiple shared brain areas (Abraham, 2018). In addition, the emotional processing areas have strong connections with areas implicated in the (1.1.) *empathy-* and (1.2.) *mentalizing* networks (Abraham, 2018). Importantly, emotional availability is fostered by emotion regulation processes, allowing mothers to interact with their children in an emotionally flexible way by attending to a range of children's emotions and needs (Rigo et al., 2019).

The motivation and reward areas mainly include mesocorticolimbic dopamine reward system areas and the frontostriatal brain regions (Abraham, 2018; Abraham et al., 2016; Swain et al., 2014). As mothers are exposed to cues from their own infants, the initial feeling of pleasure and the activation of reward/motivation brain circuits improve the salience detection of their infant's signals (Swain et al., 2014). This, in turn, encourages increased attention and bond formation between the caregiver and the child, ensuring continued commitment to sensitive parenting (Swain et al., 2014).

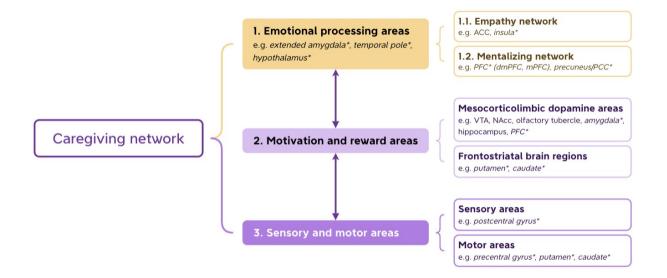


Figure 1. *The main areas and networks implicated in the parental brain or the global caregiving network.* Note: The arrows represent that all areas work together to attend to a child, and the areas in italics with an asterisk are most often activated when looking at one's own child's neutral (or all emotional valences compared together) face versus an unfamiliar infant's face. Abbreviations: ACC - anterior cingulate cortex, PFC - prefrontal cortex, dmPFC - dorsomedial prefrontal cortex, mPFC - medial prefrontal cortex, PCC - posterior cingulate cortex, VTA - ventral tegmental area, NAcc - nucleus accumbens.

2.3.1. Emotional processing network

The foundation of parental care relies on the *emotional processing* network mainly based on different brain areas implicated in two strongly associated networks and the extended amygdala, temporal pole, and hypothalamus that cannot be fully categorized in one of the subdividing systems and are also connected to the reward processing. The two networks can be named (1) the *empathy* network, including the anterior cingulate cortex (ACC) and the insula, and (2) the *mentalizing* network, including the prefrontal cortex (PFC) areas like dorsomedial prefrontal cortex (dmPFC) and medial prefrontal cortex (mPFC), and precuneus/posterior cingulate cortex (PCC) (see Figure 1; for review, see Abraham, 2018; Rigo et al., 2019; Swain et al., 2014). These two systems connect limbic and cortical areas via multiple ascending and descending projections to support future-directedness and flexibility of caregiving through understanding the beliefs and thoughts of others in relation to ourselves (Abraham, 2018).

The (1.1) *empathy network* enables the caregiver to recognize infant facial cues effectively and, with the help of *mentalizing*, sensitively respond to them, fostering the motivation to prioritize the child's well-being driven by these emotional signals (Decety, 2015). These mechanisms are rooted in the evolved capabilities of humans and their ancestors to detect and react to social signals crucial for survival, reproduction, and overall welfare (Decety, 2015). The emotional, motivational, and cognitive aspects of empathy depend on distinct mechanisms and brain areas correlated with these behaviors (Decety, 2015). A system that corresponds to the *empathy network* is the mirror neuron system, which is well-suited to monitor the evolving intentions of others (Frith & Frith, 2006). The main brain areas in the *empathy network* are the insula and the cingulate cortex, especially the ACC (Abraham, 2018; Rigo et al., 2019).

The second network, known as the (2) *mentalizing network*, enables parents to mentally grasp infant states, comprehend nonverbal cues, anticipate their needs, and make future caregiving plans (Abraham, 2018). The networks of *mentalizing* and *empathy* are intertwined. However, the *mentalizing network* has an added dimension of providing insight into the stable attitudes of others and planning one's behaviors according to one's own and others' needs (Frith & Frith, 2006). Empathy towards others can be considered the first step in the mentalization process (Frith & Frith, 2006). The PFC with dmPFC, mPFC, and precuneus/PCC are mentalizing areas frequently active when looking at infants' faces (for review, see Abraham, 2018; Abraham et al., 2016; Swain et al., 2014).

2.3.2. Motivation and reward network

The *motivation and reward* network cannot be considered separately from the *emotional processing* network. Both systems are strongly connected; sometimes, the *motivation and*

reward network has even been categorized as part of the more extensive *emotional processing* system (Abraham, 2018). The main areas implicated in the *motivation and reward network* are related to the mesocorticolimbic dopamine areas, including the ventral tegmental area (VTA), nucleus accumbens (NAcc), and the connections with the amygdala, hippocampus, oxytocin-producing hypothalamus, and the orbitofrontal cortex (OFC) (see Figure 1 (pp. 15); for review see Abraham, 2018; Abraham & Feldman, 2022; Arias-Carrión et al., 2010).

In the adult brain, dopaminergic neurons reside in different areas, yet most are localized in the ventral part of the mesencephalon (Arias-Carrión et al., 2010). The mesolimbic part of the mesocorticolimbic dopaminergic system involves the VTA, which sends projections to NAcc and olfactory tubercle innervating the amygdala and hippocampus. In contrast, the mesocortical part of the system starts with VTA as well, but the fibers extend to the prefrontal cortex (Arias-Carrión et al., 2010). Once a connection has been formed between an infant cue and its rewarding value, the dopamine system facilitates the motivation to attend to the stimulus repeatedly (Arias-Carrión et al., 2010).

Infant cues, for example, facial cues, have been primed by pregnancy hormones like oxytocin, estradiol, and prolactin to be salient and highly rewarding for a parent, so an adult would protect the infant against harm and give the baby the attention and care they need to grow up to be healthy (Abraham, 2018). Previous EEG findings on foster parents have found a positive correlation between the oxytocin levels of the nonbiological caregivers and a greater parietal maximal P3 amplitude, which indicates the mesocorticolimbic reward circuit activation in response to the pictures of their infant (Abraham, 2018; Bick & Dozier, 2013). These results suggest that the heightened neural processing related to motivation, reward, and attention in response to infant cues may not depend on genetic relatedness (Abraham, 2018).

2.3.3. Neural activations when viewing infant faces

Brain imaging studies in parenting literature use contrasts to compare neural activation between multiple conditions of interest. Comparing two conditions between each other means that we are subtracting general brain activation in one condition from the general activation in the other condition. For example, suppose we want to compare how adults' pattern of neural activation to infants' faces differs from the neural activation to adults' faces. In that case, we should make these two comparisons: (1) activity to infant faces minus activity to adult faces to see which areas are more active when looking at infants' faces (infant > adult), and (2) activity of adult faces minus activity of infant faces to see which areas are more active when looking at adults' faces (adult > infant).

Studies conducted with non-parents show that some areas in a human adult's brain have a heightened neural response to infant stimuli compared to adult stimuli in emotional processing and reward networks, even when the adult is not a child's caregiver. Therefore, there are areas in these networks that might not be specific to the *global caregiving network* and reflect the evolutionary need of humans to help their species survive. An fMRI study by Caria and others (2012) investigated non-parent male and female adults. The non-parents were presented with neutrally valenced pictures of infant and adult human or animal faces, which they had to rate according to how positively they made them feel. In line with their expectations, human infant faces were the most salient stimuli among the four conditions. Compared to animal infants, the human infant faces showed more robust activation in the right middle frontal/precentral gyrus, right fusiform gyrus, right supplementary motor area, left middle occipital gyrus, left superior temporal pole/anterior insula, and middle cingulate cortex (MCC). Similarly, compared to adult humans, infant human faces elicited increased activation in the right supplementary motor area, fusiform gyrus, precentral gyrus, MCC, cerebellum, left anterior insula, and thalamus (Caria et

al., 2012). Participants also presented a more positive affect towards infant's faces than adults from both species.

All areas activated in response to a human infant versus a human adult and animal infant faces, besides the MCC and thalamus (Caria et al., 2012), are the main areas of the mental visualizing network that allows us to imagine a picture in our head and connect these mental visuals to our past experiences (Spagna et al., 2021). The primary purpose of connecting imagery to our already existing knowledge base is to allow us to generate precise predictions about the future (Moulton & Kosslyn, 2009). According to embodied simulation theory, visual imagination reactivates the sensorimotor experiences, and mental simulation based on past experiences reactivates the associated neural patterns. This reactivation can be seen as a preparation for future action. It may suggest that human adults give more attention to human baby faces and are implicitly ready to react to infants based on their visual cues, and this has a clear adaptive value for the survival of helpless infants' and the whole human species (Caria et al., 2012).

Similar findings were recorded in a study by Bos and others (2018), who analyzed data from 26 childless young women. The stimuli in this study were inspired by Glocker and colleagues (2009). Infant faces of 3-month-olds to 1.5-year-olds presented to the adults were either with a neutral unmanipulated baby schema or manipulated baby schema (Bos et al., 2018). In the manipulated condition, the baby schema was changed to be more or less distinctive than the regular face. In accordance with the study by Caria et al. (2012), infants' faces compared to the control condition showed activation in the mental visualizing network, and additionally, some areas related to the *motivation and reward* network like the hippocampus, thalamus, putamen, ACC, VTA, and amygdala.

Likewise, the latest study on the distinctiveness of baby schema confirmed the previous results (Endendijk et al., 2020). The stimuli were the same as in Bos et al. (Bos et al., 2018). However,

this time, the participants were 23 mothers of children of various ages, from infants to preschool-aged children, viewing pictures of unfamiliar babies (Endendijk et al., 2020). Neither the study from Endendijk et al. (2020) nor from Bos et al. (2018) showed a significant effect of the distinctiveness of baby schema on neural activation, giving a basis to expect that mentioned visual mentalization and *motivation and reward* areas would activate in the caregiver's brain even if they are looking at a preschool-aged child, whose baby schema is not as distinctive anymore as it was in infancy. Moreover, the results demonstrate that the adult does not have to be from a particular gender or a biological parent of the child to show increased attention to baby faces to activate future preparation and reward and motivation areas.

Notably, all three studies mentioned found an activation in the left or left anterior insula. The overall significance of the insula lies in its involvement in different networks. Insula is considered to be linked with action representation by mirror neurons in the *empathy* network and mental visualizing network, as well as *reward and motivation* in response to stress and threats within the limbic system (Abraham, 2018). Particular attention can be given to the anterior insula, which has been activated in parents visualizing their coparent's and infant's interaction (Abraham et al., 2017), in adults looking at unknown infant faces (Caria et al., 2012), and in mothers watching video clips of their own infant (Noriuchi et al., 2008).

The anterior insula is mainly associated with subjective feelings of any emotions activating often together with the ACC ((Bud) Craig, 2009). These areas are also important in salience detection, empathy, and connecting emotions with cognitions ((Bud) Craig, 2009). Due to the anterior insula's many functions, it will likely activate whenever a caregiver views a highly salient stimulus like a baby's face, indifferent to the child's personal significance to the adult.

2.3.4. Neural activations when viewing own vs unfamiliar child's face

Areas implicated in non-caregiver adults and mothers, when looking at unfamiliar babies' faces compared to grown-ups or animals, might not be the same ones that are active when viewing one's own child. The *global caregiving* model suggests that there are brain areas that activate only when caregivers perceive their own child's cues (Abraham, 2018). Viewing one's own child's visual stimuli compared to another child's visual stimuli should eliminate the activations we can see when comparing child vs. adult or child vs animal faces, which would only show the preference to any offspring of our species. Furthermore, faces comparing neutral emotional stimuli of infants will likely eliminate the confounding factor of emotion salience, leaving only the difference between one's own child versus another child. For example, the condition: own infant's neutral face > other infant's neutral face would eliminate the areas that are similarly activated in both, which could be areas that just activate when looking at any face or any salient emotional state, no matter the age, or at any infant's face, no matter if they are one's own or unfamiliar child.

One fMRI study comparing own versus other baby faces was conducted by Strathearn and others (2008). Novel face images of their own 6- to 10-month-old infants and matched unknown control infant faces were shown to 28 first-time mothers from Texas. The study included conditions with different emotional valences and a control condition without a face. As expected, comparing neutral infant faces to the no face condition activated face areas like the ventral visual pathway, including the fusiform gyrus with fusiform face area. However, contrasting own to other infant faces (all own > all other) presented activations in areas of *motivation and reward* (left amygdala, OFC, anterior insula, hippocampus, hypothalamus), *mentalizing* and *empathy* (ACC, mPFC, PCC), executive function (dIPFC), movement (caudate, putamen, primary motor cortex), *emotional processing* (temporal pole) and thalamus.

Neural activations to own and other baby faces were also recorded in a study by Noriuchi and others (2008), who scanned 13 mothers of 16-month-old infants in Tokyo. The mothers were shown video clips of their own infants or other infants, where the baby was either interacting with their mother and smiling, or the mother had left and the baby was crying. Furthermore, a behavioral scale assessed the mothers' subjective emotions while looking at the pictures. The mother felt more happy, motherly, joyful, and warm when viewing their own child versus an unfamiliar child. According to Noriuchi and others (2008) the brain areas that were more activated when looking at own child's face versus another child's face (all own > all other) included areas that were also active in the study by Strathearn and colleagues (2008) like the OFC, anterior insula, hypothalamus implicated in *motivation and reward* network, posterior cingulate gyrus/PCC, dmPFC of *mentalizing* and *empathy*, middle temporal gyrus/temporal pole of *emotional processing*, putamen and primary motor cortex of movement, and thalamus. Additional activations were found only in frontal gyri and periaqueductal gray (PAG), meaning nine areas were similarly activated in the previous two studies.

Moreover, four of the areas, including putamen, anterior insula, thalamus, and primary motor cortex from two previously mentioned studies, were also activated when non-parents or parents were looking at unfamiliar faces compared to adult or animal faces, implying that these areas are essential in viewing a child's face in general, but have heightened activity when looking at one's own child's face. This can also be confirmed by the fMRI data collected by Atzil and colleagues (2011) from 23 mothers in Tel Aviv who were videotaped interacting with their 4-to 6-month-old infants. These short video clips, where the infant was playing alone or with a mother, were shown to the parents in the scanner, and comparisons were made between brain areas activated with their own infant and other infant stimuli. In line with the idea of a *global caregiving* network, mostly the same areas were activated as in the previous two studies, except for a few areas, including enhanced activation in the nucleus accumbens (NAcc), which has

also been activated when parents are viewing unfamiliar infant faces compared to no face condition (Glocker et al., 2009). NAcc is a part of the ventral striatum and is implicated in the *motivation and reward* network. According to animal models, it is a region that reinforces maternal motivation and social interaction (Abraham, 2018; Hoekzema et al., 2017; Lonstein et al., 2015).

A review by Rigo and colleagues (2019) that took into comparison neutral own versus other faces provided the same brain areas as the previously mentioned three studies. However, after reviewing twelve articles they also found that there tends to be an activation in the amygdala. The amygdala plays a role in many functions, however, some of the most relevant ones for parenting are salience detection, maternal memory, and responsiveness assuring the continued motivation to respond to one's own child (Meurisse et al., 2009; Phelps & LeDoux, 2005).

Interestingly, not many studies have contrasted non-emotionally valenced own infant faces to other infant faces (either all own > all other or neutral own > neutral other). Studies that have added a covariate to the analysis do not usually present the results without the effect of the covariation. However, based on the three mentioned studies comparing these groups (Atzil et al., 2011; Noriuchi et al., 2008; Strathearn et al., 2008), and a review by Rigo and others (Rigo et al., 2019), six areas have shown heightened activity to neutral or all infants faces. These areas are the precuneus/PCC, middle temporal gyrus/temporal pole, insula (left or anterior insula), primary motor cortex, amygdala, and thalamus. Another five areas were activated in multiple studies, but not all, including putamen, hypothalamus, caudate, postcentral gyrus, and the PFC (mPFC and dIPFC). Three areas have been active in all currently summarized studies: insula, thalamus, and precentral gyrus (Atzil et al., 2011; Bos et al., 2018; Caria et al., 2012; Endendijk et al., 2020; Noriuchi et al., 2008; Strathearn et al., 2008). All the important areas

are represented by the network in Figure 1 (pp. 15) and according to their position in the brain in Figure 2.

One of the areas activated in most of the mentioned studies is the precentral gyrus. The precentral gyrus is the location of the premotor and primary motor cortex responsible for voluntary movement of the body in the contralateral side (Banker & Tadi, 2023; Witteman et al., 2019). According to Caria et al. (2012), the activation in the premotor cortex might reflect the unconscious preparation to respond to infant faces. Moreover, the premotor cortex is also a part of the dual visual pathways, specifically the dorsal stream that contributes to visual attention (Sheth & Young, 2016; Witteman et al., 2019). The medial temporal gyrus/ temporal pole is part of the same system of pathways, but in this case, the ventral stream (Sheth & Young, 2016). Problems in the ventral stream underlie the inability to recognize faces in patients with prosopagnosia (Marotta et al., 2001). More specifically, the temporal pole is responsible for facial emotional recognition and emotional responses to faces (Ranote et al., 2004).

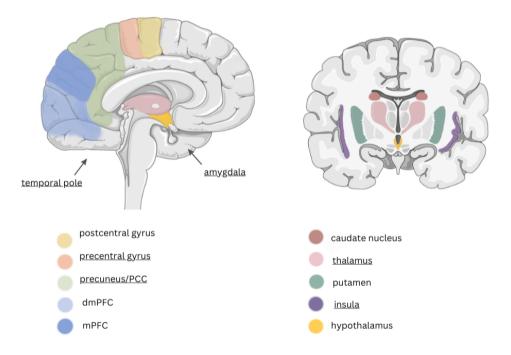


Figure 2. Brain areas that are most often active in a mother's brain when looking at her own vs other *infant's faces.* Note: The underlined areas have been active in all of the studies reviewed in the current

section. The temporal pole and amygdala cannot be viewed in the presented sagittal and coronal sections of the brain and therefore the arrows point to an estimated area. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

Precuneus and PCC hold a significant position among the cortical midline structures of the brain. For instance, the precuneus is recognized for processing highly integrated and associative information (Valadez et al., 2020). It likely maintains representations of self and others across diverse domains and directly connects with the mirror neuron system, essential for mentalizing others' actions and attitudes and planning future behaviors (Valadez et al., 2020).

The studies that have compared non-emotionally valenced own vs. other child faces have done it usually with infants aged 4-16 months, reflecting the *global caregiving* activations only in the earlier postpartum period. Secondly, the research has mainly focused on mothers from heterosexual couples, leaving out the population of lesbian mothers. More data should be collected from healthy parents to generalize *the parental brain* or *global caregiving* network to more populations and to understand or extend the model. Therefore, the current study will add to the literature by extending the previous findings on the neural activation to own versus other neutral infant faces in the lesbian population without the variation of emotional valence.

CHAPTER 3. Interaction with child cues

3.1. Neural activations in association with emotional availability

Several fMRI studies have focused on the parental response to different visual infant stimuli and correlated results with behavioral findings. However, only a handful of studies have explored the association of attachment with parents' fMRI neural activity in hopes of finding the areas implicated in a sensitive parent's brain. Furthermore, "sensitivity" is a broad construct that can be measured in various ways, making it hard to generalize the construct over multiple studies. In their recent review, Clark and colleagues (2021) suggested using *Emotional Availability Scales (EAS)* to understand various behavioral aspects of the parents that influence their caregiving. The *EAS* not only measures the parent's sensitivity but also investigates the various other aspects that make up an attentive and warm parent, including structuring, nonintrusiveness, and non-hostility (Saunders et al., 2017). Although the use of EAS is seeing growing interest in neuroimaging studies, to the best of my knowledge, only two studies utilizing fMRI (Kim et al., 2017; Olsavsky et al., 2019) have used the measure of Emotional Availability (EAS) to correlate the dimensions of sensitivity with parental brain activity in response to infant faces.

It is surprising that there are only a few studies investigating this relationship since cultivating emotionally available relationships between the parent and the child is essential for the healthy brain development of the latter. Parents presenting higher emotional availability are receptive to their child's signals, and this trusting and attentive environment underpins a sensitive and secure parent-child attachment relationship (Rossen et al., 2018). As the EAS is a multidimensional approach to assessing caregiving behaviors that work together to create a sensitive parent, it is generally better to focus on multiple subscales of EAS without taking an interest in only one (Clark et al., 2021; Rossen et al., 2018). Multidimensionality of EAS goes

beyond the construct of attachment by also considering important relational aspects that are more representative of the quality of everyday interactions (e.g., regulating one's emotions, creating an emotionally positive and warm environment, setting healthy boundaries, promoting autonomy, and supporting child's exploration) (Saunders et al., 2017).

Human neurobiology research is only beginning to investigate the influence of the environment on healthy caregiving since many studies have currently focused on children and parents who have lived in extreme circumstances or are going through a psychological disorder (Clark et al., 2021). One example of the latter is an fMRI study on child maltreatment-exposed mothers (Olsavsky et al., 2019). This preliminary research found that increased amygdala activity to unfamiliar infant faces was associated with a higher level of maternal sensitivity during motherinfant interaction in maltreatment-exposed mothers. In contrast, mothers with no exposure to maltreatment showed the opposite relationship. Additionally, the exploratory analysis suggested that changes in face processing in maltreatment-exposed mothers reflect less attentiveness to neutral and positive infant facial cues, which, in return, might have represented how emotionally available and responsive the mother is.

Kim and others (2017) suggested that a lower income-to-needs ratio, which predicts a higher likelihood of childhood struggles, might be associated with how intrusive the mother is. Intrusive mothers exhibited less activity in the amygdala in response to positive infant faces, but they also found elevated activation in response to negative infant faces. Therefore, a higher likelihood of childhood adversity is indirectly related to growing up in a low-income family, and this suggests that the mother presents more intrusive behaviors with their own child. Childhood adversity in a general context might also mean sexual orientation-based discrimination from the family, which will be further discussed in the next chapter. In conclusion, the only two studies that correlated EAS to fMRI neural activations during an infant face-viewing task highlight the importance of the amygdala. More specifically, the amygdala activity is thought to be higher in parents with lower levels of emotional availability, which can be caused by some adverse experiences in childhood.

Behavioral research has also shown that EA is a correlate or a predictor of attachment. Children whose parents are more sensitive and emotionally available usually have more secure attachments (Almeida et al., 2022). Strathearn and others (2009) compared securely and insecurely/dismissively attached mothers in their brain responses to own versus other infant faces. Secure mothers showed increased activation in mesocorticolimbic *reward and motivation* regions, when looking at their own infant's smiling face in contrast to mothers with insecure/dismissive attachment. These results confirmed a previous similar study with adult faces (Vrticka et al., 2008), where insecure/dismissive mothers showed lack of activation in the *reward* system especially the ventral striatum, which was also implicated in the study by Strathearn and others (2009). Strathearn and others (2009) suggested that the link between ventral striatum and secure attachment may mean that emotional infant facial cues represent the importance of salient infant facial cues in reinforcing and motivating responsive and emotionally sensitive maternal care.

Although brain research with fMRI does not give a lot of insight into emotional availability in parenting, the effect of the EA has been studied extensively in behavioral developmental research. In relevance to the current study, a four-wave longitudinal study followed mothers and children from at-risk and disadvantaged families during infancy and preschool years (Célia et al., 2018). Even though children's emotional availability changed over time, mothers' EA was found to be stable. These results align with previous studies that have shown stability and continuity of EA across years of parenting (Bornstein et al., 2006, 2006). Therefore, it is likely that the brain activity shown by emotionally sensitive mothers during a child's infancy to own

baby visual infant stimuli is similar to the one they present when later looking at their own preschool-aged child's facial cues.

3.2. Perceived quality of parental care in mothers' childhood

Parents' emotional reactions to a child at any age cannot be undermined. It is important to remember that how parents communicate with their children during their development shapes the child's internal beliefs and how they interact with people in the future (Almeida et al., 2022). Therefore, we will also look at the parents' caregiving history in the current study to see if mothers' perceived caregiving experience with their parents has affected their emotional availability and brain responses to their children.

Considering that the mothers in our study are lesbian, it is especially relevant to assess their perceived parental acceptance and rejection through a dedicated questionnaire (*Parental Acceptance and Rejection Questionnaire - PARQ*, Rohner, 2005) and the corresponding theory (*Parental Acceptance and Rejection Theory - PARTheory*, Rohner et al., 2005). Acceptance from the parent implies that the child feels their parents expressing warmth, affection, nurturance, and love towards them (Rohner, 2005). In contrast, rejection is characterized by the child feeling that their parent is hostile, aggressive, indifferent, or shows them a lack of attention (Rohner, 2005). Even if the parent tries to be accepting, the child might not always perceive it that way (Bowlen, 2023). For example, a caregiver may fear that other people will be prejudiced and judgmental against their lesbian daughter and, therefore, try to protect them by preventing the daughter from coming out or expressing affection towards their same-sex partner (Bowlen, 2023). This, in return, might be perceived by the lesbian or other minority individual as rejection from their parents (Bowlen, 2023).

The Minority Stress Theory postulated by Meyer (2003) suggests that people who identify as lesbian or as other sexual minorities have an increased risk for mental health struggles due to the discrimination and stigmatization they experience. According to the theory, sexual minority individuals are subject to external stressors from society and internal stressors that threaten their identity, like fear of rejection or hiding their identity (Meyer, 2003). On the other hand, these stressors have less of an impact on the individual if they find positive coping strategies like responding to themselves with compassion and kindness (Bowlen, 2023). According to Bowlen (2023), general acceptance from the family is associated with less psychological distress, self-coldness, and more kindness towards oneself. Therefore, approval from the parent might make the lesbian youth less scared of rejection from society.

It is more likely for sexual minority individuals to form negative representations about themselves if they do not have the protective effect of the perceived support from their parents during their youth and childhood (Meyer, 2003). These negative patterns constitute the forming of pessimistic internal working models (Almeida et al., 2022). Children who build internal working models through experience with their parent, who is perceived as unresponsive to their needs, tend to seek confirmation of their negative internal representations from the outside world (Steele et al., 2009). Furthermore, if not changed early on, the internal working model will be engrained and hard to change, affecting all the attachment relationships built from thereon (Steele et al., 2009).

3.3. Perceived care associated with emotional availability

Studies with attachment theory suggest that parents' current mental representations of their past attachment relationships with their caregiver influence the secure bond they will develop with their own children (De Wolff & van IJzendoorn, 1997; Main & Solomon, 1986; van IJzendoorn et al., 1995). These studies have shown that sensitive parenting is a mediator between the

parents' past relationship experiences with their mother and the attachment pattern of their children. Cassibba et al. (2012) supported the intergenerational transmission of a secure bond between the parent and the child. Mothers with a more secure attachment representation had more emotionally available mother-infant interactions as measured by EAS and, therefore, a more secure attachment relationship with their infant (Cassibba et al., 2012). This means that emotional availability is an important mediator between mothers' mental representations of their past attachment relationships and how secure is the bond they develop with their own children.

Similarly, intergenerational transmission can be seen with regard to emotion-related parenting practices (Buckholdt et al., 2014). Parents' accepting responses to children's emotions have a high chance of making them feel comforted and develop skills to understand and express their own emotions through regulating negative ones (Buckholdt et al., 2014). Emotion regulation is vital since it mediates between parents' retrospective reports of their own experiences with caregivers' parenting practices and maladaptive behaviors in adulthood (Buckholdt et al., 2009, 2010). Emotion regulation is an essential part of sensitive caregiving and emotional availability. An emotionally available parent can also understand their child's emotions and regulate their own to respond appropriately to their child (Saunders et al., 2017).

3.4. Neural activation in association with perceived care

Perceived retrospective caregiving experiences might affect not only the transmission of emotion regulation and emotional availability but also the neural activations of mothers to their own children's visual facial cues. Until now, no study has looked at the effect of perceived care experiences of mothers on their neural reactions to visual own versus other child stimuli. Behavioral studies have found the effect of one's own parental relationship with their mother on the way non-parents attentionally react to infant versus adult stimuli (Jia et al., 2017; Long

et al., 2021). To the best of my knowledge, only one study (Gemignani et al., 2023) has explored how parents' reaction to infant faces changes in relation to perceived parental acceptance and rejection.

Gemignani and others (2023) did not explore this topic through brain imaging. However, they did see how parents' attention to infant stimuli compared to adult stimuli is influenced by their history with their own caregivers measured with PARQ. They found that in a Go/No-Go task, parents presented slower reaction times in the presence of infant visual stimulus compared to adult faces. The authors suggested that this attentional bias for infant faces might mean that parents have difficulty disengaging from the faces that provide so much information about the infant's needs. Prioritization of infants' faces is likely one of the foundations for sensitive parenting and a building block of an emotionally secure infant-child bond.

Interestingly, parents who prioritized infant facial cues more also reported their mothers to have been more sensitively accepting during their childhood, supporting the idea of intergenerational transmission of emotional sensitivity. On the other hand, when mothers of the parents are perceived as more rejecting, neglecting, and hostile, this attentional sensitivity to infant faces might be weakened. Therefore, their attachment to their own mothers is most likely insecure (Gemignani et al., 2023).

3.5. Purpose of the study

3.5.1. Aims and importance

The current study aims to expand on our existing knowledge of the neural *caregiving areas* discussed in Chapter 3 (pp. 26-32) by providing insight into the brain activity presented in lesbian mothers looking at their own versus unfamiliar preschool or school-aged children's facial stimuli. The previous literature's significant limitation is its narrow focus on biological

mothers, with non-biological caregiver populations receiving relatively little attention. This imbalance underscores the need for a more comprehensive examination of how various caregivers respond to the facial cues of their children.

Activation will be examined with BOLD fMRI, and a face-viewing task using neutral child faces (for further explanation, see pp. 11-12). Secondly, this neural activation will be correlated with the dyadic and emotional qualities of adult–child relationships (*Emotional Availability Scales, EAS*) since it is expected that the level of activation in the amygdala and ventral striatum might change in interaction with the level of emotional availability of mothers. Moreover, the correlation between the parental perceived caregiving history (*Parental Acceptance and Rejection Questionnaire, PARQ*) and the neural activations and *EAS* (see Figure 3) will be investigated to see how maternal acceptance and rejection can be associated with the activation in the ventral striatum and amygdala and the score of emotional availability in mothers.

It is important to explore the neural activations of mothers in response to their children and to see how behavioral aspects can be linked to these activations to help parents most in need. Humans are not able to see what others are thinking and feeling and how it affects how they act. Therefore, it's important to start noticing factors and asking questions that might reveal if a family needs psychological support, more encouragement, or if the child needs to hear more acceptance from their parents or adults around them.

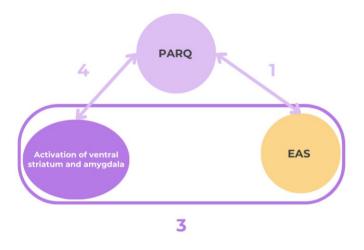


Figure 3. A visual representation of the variables and their expected relation to each other. Note: The arrows show that if *PARQ* correlates with *EAS* and neural activations of the ventral striatum and amygdala similarly to *EAS*, then the *PARQ* might also indirectly affect the correlation between the activation of the ventral striatum and amygdala and *EAS*. The numbers present the order of the corresponding research questions and hypotheses.

3.5.2. Research questions and hypotheses

• Research questions (for a visual representation, refer to Figure 3):

- 1. Is there a significant correlation between the maternal measures of the short version of the *Parental Acceptance and Rejection Questionnaire* and the maternal *Emotional Availability Scales*?
- 2. Which brain areas will be more activated when mothers are viewing their own child's neutral face stimuli compared to an unfamiliar child's neutral face stimuli?
- 3. Which brain areas are associated with different levels of maternal emotional availability measured by the *Emotional Availability Scales*?
- 4. Which brain areas are associated with different levels of paternal measures of the short version of the *Parental Acceptance and Rejection Questionnaire*?

- Hypotheses (for a visual representation, refer to Figure 3):
 - 1. The higher the perceived parental rejection, the lower the score in the maternal *Emotional Availability Scales*.
 - The mothers' neural activation recorded by BOLD fMRI in response to neutral own compared to neutral other child faces (own > other) will present activation in the following groups of areas: (1) emotional processing networks including empathy and mentalizing network (including the amygdala, temporal pole, hypothalamus, insula, PFC with mPFC and dmPFC, precuneus/PCC); (2) motivation and reward network (including amygdala, PFC, putamen, caudate); (3) sensory and motor areas (including postcentral gyrus, precentral gyrus, putamen, caudate), and thalamus.
 - 3. The higher the mothers' amygdala and the lower the activation in the ventral striatum activity, the lower their score in the maternal *Emotional Availability Scales*.
 - 4. The higher the perceived parental rejection measured with the *Parental Acceptance and Rejection Questionnaire,* the lower the activation in the ventral striatum and the higher the amygdala activation of mothers.

CHAPTER 4. Methodology

4.1. The broader research project

The data were obtained from a more extensive research project called "bRAINBOW". The aims of the broader project of "bRAINBOW" are threefold. Firstly, to compare the quality of parenting, caregiver-child relationships, and child adjustment between same-sex and different-sex parent families. Secondly, understanding how or if a child's attachment security is affected by the parent's sexual orientation, gender, genetic relatedness, reflective functioning, emotional availability, and sensitivity. Thirdly, the project is investigating neurobiological correlates of the caregiver-child relationship and how the caregiver responds to their own and other infant cues in general, considering if the parent has a primary or secondary caregiving role and the attachment history of the parent. The neurobiological correlates are acquired through electroencephalography (EEG) and functional magnetic resonance imaging (fMRI). In my thesis, I will analyze the neurobiological correlates of lesbian parents who participated in an experiment using visual infant stimuli. The brain data was gathered through fMRI and compared to behavioral data and questionnaires, which will be further explained in the Measures section.

4.2. Ethical considerations

The data used in this study were collected through the "bRAINBOW" project. All the participants gave informed consent to participate in the study. The form of informed consent followed the guidelines of the consent of the Department of Psychology and Cognitive Sciences (DipSCo) from the University of Trento, and all the procedures conducted were approved by the ethical committee of the University of Trento (prot. #2020-034), following the declaration

of Helsinki. The data were saved and used anonymously using an individual code assigned for each participant.

4.3. Participants

Participants comprised a subsample of 8 lesbian mothers between the ages of 39 to 55 (M = 45.4, SD = 5.32), from whom two were couples (4 mothers, 50% of all mothers) and four were not coupled in the current subsample. This means that two children had both parents in the current sample used. Therefore, the child sample consisted of 6 children between the ages of 5 and 11.5 (M = 6.67, SD = 2.48). Mothers were recruited through a collaboration with a parent association called "Rainbow Families" ("*Famiglie Arcobaleno*"), who shared the participation info with the individuals in their mailing list. The eligibility criteria for the study were as follows: neither the mother nor the child had any diagnosed neurological or psychological disorders, the family resided in Italy, and the mothers had been in a relationship at least since the conception of the child.

4.4. Procedure

The procedure consisted of four phases. In Phase 1, the children were recorded during a Zoom meeting watching funny, neutral, and sad short films to elicit spontaneous emotions. The emotional short films used come from an ad hoc database; all films were previously evaluated for their emotional value by an independent sample of children (n = 23) aged 3 to 11 years. Children's facial expressions were videotaped to create the experimental stimuli to use during mothers' fMRI recording. In Phase 2, the mother-child interactions were recorded during a Zoom meeting. Mothers and children were asked to do a puzzle together for about 15 minutes. In Phase 3, the mothers visited the fMRI laboratory, preferably within 2-3 weeks of collecting

the stimuli (child's face recording). In Phase 4, mothers were asked to complete self-reported measures through the online platform Qualtrics.

4.5. Measures

4.5.1. Emotional availability

Emotional availability was assessed through the *Emotional Availability Scales (EAS*, 4th Edition, Biringen, 2008). The observation was done based on a recorded video call, where the mother and child and the task they were completing were clearly visible. Each family was sent the same puzzle the child was trying to assemble with their mother's help. The mother was instructed to help their child as they usually would but not to put together the puzzle by themselves. These interactions lasted for 15-20 minutes. After the puzzle task, the mother was instructed not to interact with their child for 5 minutes while the child was still trying to figure out the puzzle. Since this study focuses on maternal emotional availability, only the four adult scales are relevant. Based on the two previously mentioned dyadic interactions, all four subscales were rated on a seven-point scale from 1 to 7; the higher the score, the more of that quality the parent presented.

The four scales used were *adult sensitivity, structuring, non-intrusiveness,* and *non-hostility*. Biringen and Easterbrooks (2012) have described these scales as follows. *Adult sensitivity* refers to positive and appropriate interactions between the mother and the child. It also considers the accuracy of emotional perception, responsiveness, timing understanding, and how the mother handles difficulties. The focus is on emotional changes, no matter their valance, in the dyad. The higher scores represent optimal sensitivity, and the lower the score, the more emotional detachment can be seen from the mother's way of interacting. *Adult structuring* highlights the extent to which the mother appropriately guides their child's task by setting limits if needed. The higher scores represent adequate and consistent guidance that is not excessive, supporting the child's autonomy. The lower scores mean that the mothers showed a lack of structuring either by not giving any suggestions at all or guiding too much, so the child lost their autonomy. *Adult non-intrusiveness* means that the mother is not overly directing, interfering, or protective and, therefore, lets the child have autonomy while also considering what is appropriate for their age. The high score shows the optimal level of non-intrusiveness, and the low score indicates physical intrusions that are inappropriate in this situation.

Adult non-hostility refers to the parents' lack of hostility, including raising their voices, making demeaning comments, and showing impatience or boredom. The high scores signify a lack of hostile reactions, and the low scores represent the visible hostility of the mother. The video-recorded interactions were coded by one researcher, whom the *EAS* system approved after a comprehensive training period. The researcher was blind to any descriptive information and results of the behavioral questionnaires of the participants. Regarding psychometrics, the *EAS* has demonstrated acceptable internal reliability ranging from .79 for *nonhostility* and .92 for *sensitivity* (Bornstein et al., 2006) and good construct validity (Biringen et al., 2014).

4.5.2. Parental perceived caregiving history

The validated Italian short-form version (Senese et al., 2016) of the *Parental Acceptance and Rejection Questionnaire (PARQ)* (Rohner, 2005; Rohner & Ali, 2016) was used in the current study to assess mothers' retrospective remembrances of parental warmth that they experienced in their childhood. The warmth is measured through two dimensions: rejection and acceptance (Rohner & Khaleque, 2012). Perceiving the parent as accepting means that the child feels cared for, appreciated, and loved (Rohner & Ali, 2016). Whereas a parent perceived as rejecting can be described as cold and lacking affection, the child might feel that they are not loved or wanted (Rohner & Ali, 2016).

The Italian short-form version has two scales, one for assessing the experience with the mother and one for the father. Based on the aim of the current project, only the maternal scale was used. The maternal scale has 24 items that are organized into four dimensions including *warmth and affection* (8 items; e.g., My mother makes me feel wanted and needed), *hostility and aggression* (6 items; e.g., My mother treated me harshly), *indifference and neglect* (6 items; e.g., My mother paid no attention to me as long as I did nothing to bother her), and *undifferentiated rejection* (4 items; e.g., My mother saw me as a big nuisance) (R. Rohner & Ali, 2016; Senese et al., 2016). Each participant had to answer on a four-point Likert scale (from 4 = almost always true to 1 = almost never true), how well the statement represents the experience they had with their own mother during childhood. The total score is calculated by adding up all the individual ratings. The total score can range from 24 (highest perceived acceptance) to 96 (highest perceived rejection). In terms of psychometrics, the Italian version of short-form PARQ has presented acceptable internal reliability (C_a >.72) and invariance of the measure across different countries' populations (Senese et al., 2016).

4.5.3. fMRI paradigm

During functional scanning, participants viewed videos of children's faces and were asked to attend to all stimuli. Stimuli were backprojected onto a screen 32" LCD for visual stimuli (NNL). During the fMRI session, 30 videos (each video lasted 3 sec.) of their own child (10 happy, 10 neutral, and 10 sad facial expressions) and 30 videos (each video lasted 3 sec.) of unfamiliar children (10 happy, 10 neutral and 10 sad facial expressions) appeared in a randomized order (see Figure 4). Each trial consisted of a 5-7-s jittered inter-stimulus interval (ISI) followed by a 6-s (same video repeated two times) video presentation. During each ISI, participants were presented with a grey fixation. A 2 (Levels: own, unfamiliar) \times 3 (Levels: happy, neutral, sad) within factorial design has been implemented.

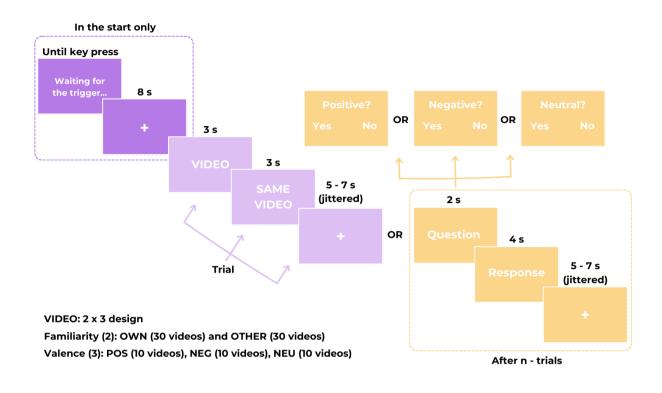


Figure 4. *Behavioral task presented during fMRI scanning.* Note: Dark purple represents the starting block that appeared once, light purple represents the trial block, and yellow represents the question block that appeared in between a random number of trials. Abbreviations: s – seconds, "n – trials" – random number of trials, OWN – own child's face stimuli, OTHER – unfamiliar child's face stimuli, POS – positive, NEG – negative, NEU – neutral.

4.5.4. fMRI data acquisition

The functional magnetic resonance imaging data were acquired using a 3 Tesla Brucker MedSpec 30/100 full-size whole-body MRI scanner (Bruker and Ettlingen, Germany) and Siemens Sonata Gradients at 40mT/m, a system with 8 channel head coil, interleaved slice acquisition and standard T2*-weighted echo planar imaging (EPI) sequence with the field of view 192x192 mm², 64x64 matrix, voxel size 3x3x3 mm³, slice thickness 3 mm, echo time (TE) 33 ms, flip angle α =75°, repetition time (TR) 2000 ms per volume. For each run, 374 whole-brain scans were obtained [AM1] in an average time of 792 seconds, which is

approximately 13 minutes. Following the functional scan, a T1-weighted high-resolution structural scan using 3D magnetization-prepared rapid gradient-echo (MPRAGE) protocol with 176 axial slices, slice thickness 1 mm, a field of view 256x256 mm², inversion time of 1020 ms, flip angle α =7°, repetition time (TR) 2700 ms, echo time (TE) of 4.18 ms were acquired for all participants.

4.6. Analyses

4.6.1. Behavioral ratings

The four scales from *maternal EAS* (*adult sensitivity, structuring, non-intrusiveness, and non-hostility*), *PARQ: Mother* (based on experiences with the mother), *PARQ: Father* (based on experiences with the father) and beta-values of the ROIs were compared using Jamovi Version 2.3 (The jamovi project, 2022; R Core Team, 2021). Significance was set to p < .05. Bonferroni correction was used in the correlational analysis, changing the p-value to $p_{adj} < .01$.

Behavioral exploratory analyses were conducted to check for in group differences between perceived experiences with the father of the mother (*PARQ: Father*) versus the mother of the mother (*PARQ: Mother*). Since the groups were dependent the paired samples t-test was used, and assumption checks were conducted with Shapiro-Wilk normality test and Q-Q plots. Shapiro-Wilk test shows that data are significantly deviated from the normal distribution when the p < .05 and Q-Q plots probability distribution suggests that data are normally distributed, when the points follow a linear pattern. Based on the results from the assumption checks either a parametric Student's t-test or non-parametric Wilcoxon Signed-Rank Test was used to see if there might be any significant difference between scores of *PARQ: Mother* and *PARQ: Father*.

4.6.2. fMRI preprocessing

The software used for preprocessing and fMRI data analysis was Statistical Parametric Mapping 12 (SPM 12, Wellcome Department of Cognitive Neurology, London, UK) running on MATLAB R2023a (Mathworks Inc, Sherborn, MA, USA). The preprocessing started with converting *dicom* images to *nii*, and from there onwards, the default SPM12 pipeline was followed. This included realigning functional images, coregistration, normalizing anatomical and functional images, and smoothing with a kernel size of 8 mm³.

During realignment, images acquired from the same subject are spatially matched through estimating and reslicing (*Chapter 1: Realigning and Unwarping the Data — Andy's Brain Book 1.0 Documentation*, n.d., pp. 1) . In estimation, each volume is compared to the reference volume to understand how it is positioned in comparison to the reference, and reslicing means that these estimates of position will be used to match each volume with the reference volume in terms of their spatial position. The next step is coregistration, where we make sure that each voxel for the subjects corresponds to the right part of the brain (*Chapter 3: Coregistration — Andy's Brain Book 1.0 Documentation*, n.d., pp. 3). Therefore, functional and anatomical images are aligned for every participant. From there, spatial normalization will map the anatomical and functional brain images to a reference brain in order to allow comparisons between subjects (*Spatial Normalization - an Overview | ScienceDirect Topics*, n.d.). Lastly, the functional data will go through smoothing, where the signal of each voxel is replaced with the weighted average of neighboring voxels with a goal of normalizing the brains of all subjects to a template brain with standardized coordinates to allow more accurate comparisons between participants (*Chapter 5: Smoothing — Andy's Brain Book 1.0 Documentation*, n.d., pp. 5).

4.6.3. fMRI data analysis

Firstly, fitted time series were created for each participant so we could use estimated beta weights later in the group analysis. In other words, the first-level analysis has to be conducted to make the neural data of each participant comparable to all the other subjects in the study. An analytical design matrix was constructed for each participant, modeling one regressor for each condition (total of 6 conditions), one regressor for the first-time derivative (one for each condition regressor), and six regressors of head motion correction parameters. The first level analysis comprised of creating a General Linear Model (GLM) for each participant, estimation of beta weights for each condition, and creating contrasts. The GLM puts together the design matrix representing the experimental conditions with hemodynamic response function (HRF). After the GLM has been created, beta weights can be estimated, which are parameters that minimize the difference between the predicted and observed BOLD signal. GLM and estimation allow the identification of specific brain regions that are activated for each condition for each participant. Lastly, contrasts of interest were created to compare conditions of interest. We specified three contrast models of interest (neutral own > neutral other and neutral other < neutral own; positive own > positive other and positive other < positive own; negative own > negative other and negative other < negative own).

Secondly, we ran a group-level (second-level) analysis implementing the Random Effects Analysis (RFX). RFX takes into account variability between individual subjects and treats it as random effects, which may provide a more realistic representation of natural variability in a population without overfitting the data into the model. By modeling this variability, we can generalize the findings over our group of interest, providing how a specific condition of interest affected the whole group. For the purpose of the present thesis, we focused on one contrast model of interest (delta values of neutral_own > neutral_other and neutral_other < neutral own) and tested the hypotheses through a one-sample t-test. A one-sample t-test was

chosen because we wanted to determine the within-group differences of our contrast model of interest. The parametric maps were calculated for each model considering an uncorrected p < .0005 and cluster size of at leats 10 voxels. We did not use corrections because of the really small sample size. The beta maps were then viewed using the XjView toolbox (XjView Toolbox, 2019) in Matlab. Lastly, regions of interest (ROI) were extracted through the MarsBaR toolbox (Brett et al., 2002) used in Matlab.

CHAPTER 5. Results

5.1. Behavioral measures: PARQ and EAS

5.1.1. Descriptive statistics

The eight homosexual mothers presented *PARQ: Father* total scores ranging from 25 to 80 (M = 49.0, SD = 19.6) and *PARQ: Mother* total scores ranging from 25 to 63 (M = 42.6, SD = 16.9). The descriptive statistics of subscales from *PARQ: Father* are shown in Table 1 and the descriptive statistics of subscales from *PARQ: Mother* in Table 2. The *maternal EAS* descriptive statistics can be viewed in Table 3.

Table 1.

Descriptives						
	Warmth/affection _father	Hostility/aggression _father	Indifference/neglect _father	Undifferentiated_rejection _father		
Ν	8	8	8	8		
Mean	19.0	11.5	12.6	5.88		
Median	20.5	10.0	13.0	4.50		
Standard deviation	7.52	5.35	5.68	2.42		
Minimum	9	6	6	4		
Maximum	29	18	23	10		

Descriptive statistics of PARQ: Father

Table 2.

Descriptive statistics of PARQ: Mother

Descriptives

	Warmth/affection _mother	Hostility/aggression _mother	Indifference/neglect _mother	Undifferentiated_rejection _mother
N	8	8	8	8
Mean	16.0	10.0	10.8	5.88

	Warmth/affection _mother	······································		Undifferentiated_rejection _mother
Median	17.5	7.50	10.0	4.00
Standard deviation	6.41	5.10	4.56	2.80
Minimum	8	6	6	4
Maximum	23	19	18	11

Descriptives

Table 3.

Descriptive statistics of the maternal EAS

Desc	

	EA_sensitivity	EA_structuring	EA_non-intrusiveness	EA_nonhostility
N	8	8	8	8
Mean	5.50	5.63	6.19	6.63
Median	5.50	5.75	6.50	7.00
Standard deviation	0.926	0.876	1.07	0.694
Minimum	4	4.00	4.00	5.00
Maximum	7	7.00	7.00	7.00

5.1.2. Correlations between EAS and PARQ

EAS and *PARQ: Father*. The correlation matrix with all the correlations between four *maternal EAS* subscales, four *PARQ: Father* subscales, and *PARQ: Father* total score is presented in Appendix A (pp. 79). There were no statistically significant correlations between *maternal EAS* subscales and *PARQ*: Father subscales or total score with or without Bonferroni correction.

EAS and *PARQ: Mother*. The correlation matrix with all the correlations between four *maternal EAS* subscales, four *PARQ: Mother* subscales, and *PARQ: Mother* total score is presented in Appendix B (pp. 80). Only two statistically significant correlations remained after

adjusting the p-value with the Bonferroni correction ($p_{adj} < .01$). The subscale called *hostility* and aggression from the *PARQ: Mother* was negatively correlated with maternal EAS subscales sensitivity (r = -.85, $p_{adj} < .01$) and non-intrusiveness (r = -.84, $p_{adj} < .01$). Therefore, the mothers' who perceived their own mothers' as hostile and aggressive were less sensitive and more intrusive in interactions with their own children.

5.1.3. Exploratory comparison between PARQ: Mother and PARQ: Father

The Shapiro-Wilk normality check showed that the subscales *indifference and neglect of* mother and *indifference and neglect* of father had a significant deviation from normality (W = 0.76, p < .05), however other subscales and the total score did not show evidence of non-normality. The Q-Q plots presented non-linear distribution only in the subscales: *indifference and neglect* of mother and father and *undifferentiated rejection* of mother and father. These two subscales did not pass the assumption checks and, therefore, were not considered in the parametric Student's t-test that the other scales (*total score of PARQ, warmth and affection, hostility and aggression*) between *PARQ: Mother* and *PARQ: Father* were compared with (see Table 4 for results). All the scales compared with the Student's t-test presented a statistically significant difference between the mother and the father scores. To compare *indifference and neglect* of mother and father, and *undifferentiated rejection* of mother and father, a non-parametric Wilcoxon Signed-Rank Test was used (see Table 5 for results). However, no statistically significant results were found.

Table 4.

Student's t-test comparing total score of PARQ, warmth and affection, and hostility and aggression between mothers' perceived acceptance and rejection to mother or father.

Paired Samples T-Test					
			Statistic	df	р
TOT_PARQ_mother	TOT_PARQ_father	Student's t	-2.89	7.00	0.012
Warmth/affection_mother	Warmth/affection_father	Student's t	-3.74	7.00	0.004
Hostility/aggression_mother	Hostility/aggression_father	Student's t	-1.77	7.00	0.060

Note. Ha μ Measure 1 - Measure 2 < 0

Table 5.

Wilcoxon Signed-Rank Test comparing indifference and neglect and undifferentiated

rejection between mothers' perceived acceptance and rejection to mother or father.

Paired Samples T-Test				
			Statistic	р
Indifference/neglect_mother	Indifference/neglect_father	Wilcoxon W	1.00 ^a	0.099
Undifferentiated_rejection_mother	Undifferentiated_rejection_ father	Wilcoxon W	5.00 a	0.579

Note. Ha μ Measure 1 - Measure 2 < 0

^a 4 pair(s) of values were tied

5.2. fMRI contrasts

The contrast comparing neural activation to neutral own child faces and other child faces ($p_{unc} < .0005$) revealed increased activity to own child faces, in left anterior lobe of cerebellum, left cuneus and cerebrum, right subgyral region and hippocampus, left lingual gyrus, left fusiform gyrus, right PCC and lingual cortex, left precuneus and left cerebrum and lateral ventricle. Decreased activation to own child neutral faces ($p_{unc} < .0005$) or in other words increased

activation to other child neutral faces was seen in left superior frontal gyrus, right temporal pole, left parahippocampal area extended to amygdala, and in the midbrain. All the activation peaks can be viewed in the Table 4.

Table 4.

The anatomical	l areas activatea	l in the contrast	OWN > OTHER c	and OWN < OTHER

					Tailarach coordinates		
Anatomical area	BA	Side	z-score	Cluster size	x	У	Ζ
Neutral OWN > OTHER							
Anterior lobe of cerebellum		L	4.47	14	-26	-58	-34
Cuneus	18	L	4.27	63	-4	-90	16
Subgyral region and hippocampus		R	4.27	71	42	-40	2
Lingual gyrus		L	3.86	20	-24	-66	0
Fusiform gyrus	18	L	3.83	19	-22	-94	-22
PCC/ lingual cortex	30	R	3.72	48	10	-62	4
Precuneus	7	L	3.68	23	0	-72	46
Lateral ventricle		L	3.68	14	-22	-46	12
Neutral OWN < OTHER							
Superior frontal gyrus	8	L	4.38	26	-16	48	1
Temporal pole	38	R	4.35	20	28	12	-36
Parahippocampal area extended to amygdala	28	L	4.19	30	-22	2	-30
Midbrain	4	R	3.54	12	8	-28	-22

p < .0005 (uncorrected), BA – Broadmann area, PCC – posterior cingulate cortex

5.3. Neural activations correlated to EAS and PARQ

Correlations were only conducted with one ROI of parahippocampal area extended to the amygdala since the ventral striatum is not significantly activated in mothers, when looking at own and other child's neutral faces. The correlation matrix with four *maternal EAS* subscales

and the ROI of parahippocampal area extended to amygdala (Appendix C (pp. 81)) showed one statistically significant correlation before and after adjusting the p-value with Bonferroni correction. The deactivation to neutral own child's faces in the parahippocampal area extended to amygdala is negatively correlated to *structuring* subscale of the *maternal EAS* (r = -.85, p_{adj} < .01, for scatterplot see Figure 5). Therefore, the higher the activation in the parahippocampal area extended to amygdala, when a mother is looking at neutral other child's face, the lower is the mother's score in the *structuring* subscale of *EAS*. However, *PARQ: Father* (Appendix D (pp. 82)) and *PARQ: Mother* (Appendix E (pp. 83)) correlations matrices showed no statistically significant correlations with the deactivation of parahippocampal area extended to amygdala, when mothers were looking at one's own neutral child's face before and after adjusting the p-value.

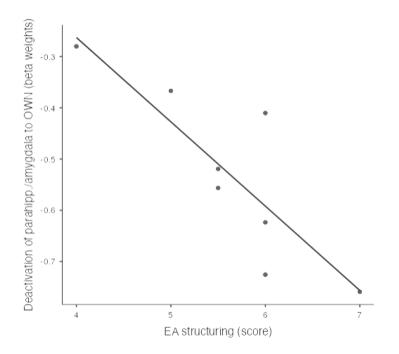


Figure 5. Scatterplot presenting the correlation between structuring subscale of maternal EAS and deactivation of parahippocampal area extended to amygdala, when looking at one's own child's face (beta weights). Abbreviations: EA – emotional availability, parahipp. – parahippocampal area, OWN – refers to own child's face.

CHAPTER 6. Discussion

The current study aimed to expand or confirm the previous fMRI neural findings on the parental brain in a lesbian population when the mothers are viewing neutral own child's faces compared to unfamiliar children. Furthermore, we investigated how these neural findings are associated with the mothers' own retrospective perceived experiences with their mother and father and maternal emotional availability towards their preschool or school-aged child. Also, we explored the interactions between the behavioral measures (*PARQ* and *EAS*) to gain further insight into how parental sensitivity might be shaped through caregiving history and current behaviors. The findings will be interpreted based on the proposed hypotheses.

6.1. Behavioral findings

The first null hypothesis, suggesting that there is no negative correlation between *PARQ* scores and *maternal EAS*, cannot be entirely rejected since a significant negative interaction was found between the *PARQ*: *Mother* subscale called *hostility and aggression* and two maternal *EAS* subscales of *sensitivity* and *intrusiveness*, indicating that the mothers' who perceived their own mothers' as hostile and aggressive were less sensitive and more intrusive in interactions with their own children.

These strong correlations are in line with previous findings on the transmission of attachment that the concept of emotional availability and parental acceptance and rejection are closely related, since emotionally available parents are more likely to form secure bonds with their children (Almeida et al., 2022) and *PARQ* addresses the perceptions of acceptance and rejection based on the mothers' parental attachment figures (Ali et al., 2019). Multiple authors (De Wolff & van IJzendoorn, 1997; Main & Solomon, 1986; van IJzendoorn, 1995) have shown that mothers with insecure past attachment representations related to their own parents will also have a more insecure attachment bond with their children and this relationship can be

moderated by maternal sensitivity (Buckholdt et al., 2009, 2010). The results from Cassibba and colleagues (2012) found that more secure maternal attachment representations predicted more emotional availability in mothers' interaction with their own children. In return, mothers' emotional availability predicted a more secure infant-mother bond. However, to the best of my knowledge, no study has specifically measured perceived past attachment relationship quality with *PARQ* and correlated the results of this measurement to *maternal EAS*, which seem to show a similar pattern of correlations as suggested by studies on intergenerational transmission of attachment.

Furthermore, the current findings on the limited sample size show that perceived maternal acceptance or rejection affected the mother's emotional availability more than that of the paternal scale. Again, these findings can be related to research on attachment that has demonstrated a lack of association between the attachment representations reported by a father and their children's attachment among preschoolers from two-parent families (Miljkovitch et al., 2004, 2012). On the other hand, Miljkovitch and others (2012) found a significant correlation between the father's attachment and the child's attachment when the father was the child's sole caregiver and, consequently, the primary caregiver. Therefore, it might be possible that in the current study, we found no interaction between *maternal EAS* and *PARQ: Father* because the fathers were not the participants' primary caregivers when they were children - affecting the mothers' emotional availability less.

Studies have also investigated the effect of acceptance and rejection on other psychological outcomes of children. Mothers' rejection has consistently shown a more substantial negative impact on the child's mental health than the father's rejection, whether the child is from a sexual minority group or not (Ali et al., 2019; Davis & Anderson, 2021; Reyes et al., 2015; Ulu-Yalçınkaya & Demir, 2018). This effect tends to be less prominent yet still existent in

adults who have built multiple other attachment relationships through the years, which can also affect their attachment to their parents. However, even in adults, the rejection they felt from their parents can still affect them in adulthood (for review, see Ali et al., 2019). Especially significant in previous studies has been the correlation between perceived undifferentiated rejection from the mother and adult maladjustment (for review, see Ali et al., 2019). This interaction with *undifferentiated rejection* of a mother was not present in our study, indicating that there might be a difference in how *PARQ: Mother* and *PARQ: Father* affects the emotional availability of mothers in interaction with their children compared to how these scales affect their psychological maladjustment.

Interestingly, although no relationship was found between paternal acceptance and rejection on mothers' emotional availability, the exploratory analyses indicated that fathers of the mothers were perceived as significantly less warm and affectionate and more rejecting overall compared to the mothers of the mothers. Past research on non-minority adults in Italy has not shown any significant differences between the acceptance and rejection of mothers compared to fathers (Senese et al., 2016). However, when *PARQ* has been used in sexual orientation, minority young adult fathers have presented more perceived *undifferentiated rejection* towards their child compared to the mother (Davis & Anderson, 2021). Even though the rejection from fathers in our study was perceived as significantly higher, there was no correlation between fathers' rejection and emotional unavailability. This might again indicate that the primary caregiver is more important in shaping how emotionally available the children are to their children in the future.

6.2. Neural activation findings

The second null hypothesis indicating that the mothers' neural activation recorded by BOLD fMRI in response to neutral own versus neutral other child faces (own > other) would present

no activations in the following groups of areas: (1) emotional processing networks, including empathy and mentalizing network (including the amygdala, temporal pole, hypothalamus, insula, PFC with mPFC and dmPFC, precuneus/PCC); (2) motivation and reward network (including the amygdala, PFC, putamen, caudate); (3) sensory and motor areas (including postcentral gyrus, precentral gyrus, putamen, caudate), and thalamus cannot be entirely rejected since two areas were found to be active when looking at own child's neutral face in the current study. These areas were from the mentalizing network: PCC/lingual cortex and precuneus.

Furthermore, activation was seen in other networks mentioned in the hypotheses but not the specific areas. Yet, these findings are not considered as support for the hypotheses since many areas could be grouped into these particular networks. In comparison to unfamiliar neutral child's faces activations were recorded when looking at one's own neutral child's face in the motivation network with subgyral region and hippocampus, sensory and motor areas like left fusiform gyrus, left lingual gyrus, cuneus, and left anterior lobe of cerebellum. An outlier activation was also found in the lateral ventricle.

The activation in PCC/lingual cortex and precuneus is consistent with previous findings that recorded activation of these areas in response to neutral or all valence conditions together to own infant's faces compared to other infant (Atzil et al., 2011; Noriuchi et al., 2008; Rigo et al., 2019; Strathearn et al., 2008). PCC and precuneus are essential areas in the mentalizing network. Valadez and others' (2020) research indicated that precuneus has an important function of maintaining representations of self and others, connecting them with the mirror system. Therefore, these functions are essential for understanding the actions and attitudes of one own child and using this knowledge to plan caregiving actions or reactions.

Although not hypothesized in the current study, the hippocampus which was activated in response to neutral own child faces, has also shown heightened activation in multiple previous

studies on the parental brain (Abraham, 2018; Bos et al., 2018; Strathearn et al., 2008). The hippocampus is part of the dopaminergic mesocorticolimbic reward network (Arias-Carrión et al., 2010). It is also essential in declarative memory formation, binding different information together to create complex representations based on our perceptions and memories (Yonelinas, 2013), processing of contextual cues, learning (Kim & Lee, 2011), and anxiety regulation (Leuner et al., 2010). Stark and others '(2020) research indicated that the hippocampus is part of the brain network that encodes learning of infant emotionality with the orbitofrontal cortex and amygdala. Therefore, the hippocampus and related network might be necessary for connecting incoming sensory information to the memories of the parent's past experiences and expectations, even when a neutral infant face is presented (Stark et al., 2020).

In the current study, mothers also presented heightened activation to their own child neutral faces in sensory and motor areas like the left fusiform gyrus, left lingual gyrus, cuneus, and left anterior lobe of the cerebellum. Fusiform gyrus that includes the fusiform face area has mainly been shown to be active when looking at an infant face compared to an adult face (Caria et al., 2012) or when compared to a no-face control condition (Strathearn et al., 2008). The present study indicated a heightened activation in the fusiform gyrus and face area even when two child faces were compared, suggesting that the fusiform face area might have a particular reactivity to one's own child faces. Caria and colleagues (2012) also recorded the activation of the cerebellum; however, the current research found activation specifically in the left anterior cerebellum. The anterior lobe of the cerebellum is the part of the cerebellum where the face representation and eye movement control area are localized (Nitschke et al., 1996). Furthermore, damage in the cerebellum has been associated with deficits in emotion attribution (Hoche et al., 2016). The lingual gyrus (also shown to be activated in Bos et al., 2018; Strathearn et al., 2008) and cuneus indicate imaginative abilities (Olivetti Belardinelli et al., 2009) and lingual gyrus in complex visual processing (Bogousslavsky et al., 1987).

In conclusion, all the areas that were activated in the contrast "own > other", were also seen to be activated in previous studies in parenting, infant face viewing, or visualizing. However, areas deactivated in the present study, when looking at own neutral child faces compared to other child faces, were somewhat surprising in the context of previous studies. Deactivations to own child neutral faces were found in the left superior frontal gyrus, right temporal pole, left parahippocampal area extended to the amygdala and right midbrain.

In previous research left superior frontal gyrus has been shown to be more active to own infant faces in mothers with secure attachment without considering the valence (Strathearn et al., 2009); temporal pole has shown a consistent activation in many studies comparing own > other infant faces (Atzil et al., 2011; Noriuchi et al., 2008; Rigo et al., 2019; Strathearn et al., 2008), left amygdala has been also activated in parents and non-parents looking viewing infant faces (Bos et al., 2018; Strathearn et al., 2008). Some studies have also reported the activation of the midbrain areas like the ventral tegmental area (Bos et al., 2018; Strathearn et al., 2008). Furthermore, dopamine is mainly produced in the ventral tegmental area (VTA; Nakamura et al., 2020), and the mesolimbic projections to other regions of the mesocorticolimbic reward system are sent out from VTA. As an essential area of the dopaminergic reward network, it would be expected that the midbrain activated in response to one's own child's neutral visual stimuli since one's own child has been previously reported to be more rewarding and salient for the parent (for review Abraham, 2018; Rigo et al., 2019; Swain et al., 2014).

These deactivations are hard to interpret since most previous studies comparing own versus other infant faces with different emotional valences or without the consideration of valence have not reported deactivations, but only activations in response to own child faces (for review see Rigo et al., 2019). Further research should start presenting all the areas activated and deactivated in response to own versus other child or infant faces in different populations to

further the research and understand the complexities of activations in the neural networks as well as deactivations, which might be as important. Current study has only a limited number of participants and therefore it would be implausible to make any reliable conclusions on these preliminary deactivation findings till they are not replicated in the bigger research project called "bRAINBOW".

6.3. Interactions between the neural activations and behavior

The last hypotheses focused on correlating behavioral measures to activations in ventral striatum and the amygdala. No significant correlation was found with *PARQ* in either of these areas. Yet, a statistically significant association was found between the *maternal EAS* subscale *structuring* and the deactivation in the parahippocampal area extended to amygdala, when mothers were viewing their own child's faces compared to other children's faces, explaining what one of the deactivations might relate to. Given this strong significant correlation we cannot fully reject the third null hypothesis.

Recent research has also reported the association of higher parental emotional availability to less activation in the amygdala, when attending to infant faces (Kim et al., 2017; Olsavsky et al., 2019). Current study expanded on these findings, since the correlation was recorded in lesbian mothers, who were viewing neutral children's faces instead of infants' faces. Showing that similar correlations can be seen in populations with different sexual orientations, with older children, and with neutral emotional valence. As amygdala has a significant role in salience detection and responsiveness to one's own child (Meurisse et al., 2009; Phelps & LeDoux, 2005), when there is a lack of activation in the area, these functions could be impaired in some way. Therefore, making it harder to also stay highly emotionally available. This however does not explain why deactivation to own child's neutral cues was found in amygdala in a healthy population.

6.4. Limitations and future perspectives

One of the main limitations of the current study was its small sample size, since the data collection for the project is still ongoing. Also one of the novel aims, which was expanding the caregiving network to lesbian parents could not be fully explored since there was no comparison group of either gay parents, heterosexual parents or a non-parent control group to contrast the brain activations to. The limitation of comparison groups will be addressed in the bigger research project "bRAINBOW". Furthermore, although neutral facial expressions of infant's were presented through small videoclips that were showing a real-life interaction, it cannot be undermined that the situation of viewing child faces in an fMRI scanner is an artificial way of measuring parental reactions to children. This means that any of interpretations of the study or attempts to apply the findings to the real word should consider this aspect.

Conclusion

The present dissertation investigated the activations in the maternal brain in response to neutral preschool and school-aged own child's faces compared to other children. These activations were recorded in a population of lesbian mothers to see if the previous findings on the caregiving network could be expanded to non-heteronormative parents. Although only two brain areas from the hypothesized regions of interest were activated, most other activations had been previously reported in parental brain studies in response to neutral infant faces. However, some surprising deactivations that should be addressed in future studies with a bigger sample size and comparison groups were recorded.

One of the other aims of the study was to explore how maternal emotional availability and mothers' retrospective remembrances of caregiving experience with their own mother and father are associated with each other and how these behavioral measures relate to the brain responses recorded. Interestingly, only *hostility and aggression* of the mother of the

participants was related to more *intrusiveness* and less *sensitivity* in mothers' interactions with their own children, even though the fathers of the participants were found to be more rejecting compared to the mothers of the participants. Therefore, it could be speculated that the relationship with primary caregivers, who have usually been the mothers, have a stronger impact on how emotionally available the child will be with their own children in the future. Lastly, a strong correlation was found between the maternal *structuring* abilities and deactivation in the parahippocampal area extended to amygdala, which is reflective of the previous findings that have shown deactivation of amygdala in parents, who are more emotionally available.

In conclusion, the findings seem to be relevant to both children and parents, since internalized patterns of interaction and how supportive one's parents were in childhood can have a significant impact on how the child interacts with their children in the future. If not addressed in childhood the internalized patterns of interacting can be transmitted to the next generation. Being mindful of the possible transmission of negative behaviors through generations could impact the way mothers address their own mental and emotional health to have a more sensitive and caring relationship with their children. Especially mindful of their reactions should be the parents of sexual minority children since the children might be in an especially vulnerable situation. In these situations, where a child is facing this societal pressures, parental acceptance could be a possible positive and emotionally comforting shelter, where the child can always find needed support and care.

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Appendix A. Correlation matrix: *maternal EAS* and *PARQ: Father*

Correlation Matrix

		EA_sensitivity	EA_structuring	EA_non-intrusiveness	EA_nonhostility	Warmth/affection_father	Hostility/aggression_father	Indifference/neglect_father	Undifferentiated_rejection_father	TOT_PARQ_fathe
EA_sensitivity	Pearson's r	_								
	df	_								
	p-value	_								
EA_structuring	Pearson's r	0.880 **	_							
	df	6	_							
	p-value	0.004	-							
EA_non-intrusiveness	Pearson's r	0.904 **	0.812 *	_						
	df	6	6	_						
	p-value	0.002	0.014	_						
EA_nonhostility	Pearson's r	0.778 *	0.734 *	0.928 ***	_					
	df	6	6	6	_					
	p-value	0.023	0.038	< .001	_					
Warmth/affection_father	Pearson's r	-0.615	-0.368	-0.409	-0.178	_				
	df	6	6	6	6	_				
	p-value	0.104	0.369	0.314	0.674	_				
Hostility/aggression_father	Pearson's r	-0.779 *	-0.564	-0.683	-0.520	0.831 *	_			
	df	6	6	6	6	6	_			
	p-value	0.023	0.145	0.062	0.187	0.011	_			
Indifference/neglect_father	Pearson's r	-0.475	-0.362	-0.222	-0.023	0.856 **	0.741 *	_		
	df	6	6	6	6	6	6	—		
	p-value	0.234	0.378	0.596	0.958	0.007	0.035	_		
Undifferentiated_rejection_father	Pearson's r	-0.670	-0.565	-0.544	-0.372	0.762 *	0.946 ***	0.829 *	_	
	df	6	6	6	6	6	6	6	—	
	p-value	0.069	0.144	0.164	0.363	0.028	< .001	0.011	_	
TOT_PARQ_father	Pearson's r	-0.670	-0.471	-0.475	-0.263	0.954 ***	0.924 **	0.924 **	0.915 **	_
	df	6	6	6	6	6	6	6	6	_
	p-value	0.069	0.239	0.234	0.530	< .001	0.001	0.001	0.001	_

Correlation Matrix

		EA_sensitivity	EA_structuring	EA_non-intrusiveness	EA_nonhostility	Warmth/affection_mother	Hostility/aggression_mother	Indifference/neglect_mother	Undifferentiated_rejection_mother	TOT_PARQ_moth
EA_sensitivity	Pearson's r	_								
	df	_								
	p-value	_								
EA_structuring	Pearson's r	0.880 **	_							
	df	6	—							
	p-value	0.004	_							
A_non-intrusiveness	Pearson's r	0.904 **	0.812 *	_						
	df	6	6	_						
	p-value	0.002	0.014	_						
EA_nonhostility	Pearson's r	0.778 *	0.734 *	0.928 ***	_					
	df	6	6	6	_					
	p-value	0.023	0.038	< .001	_					
Narmth/affection_mother	Pearson's r	-0.601	-0.356	-0.428	-0.257	_				
	df	6	6	6	6	—				
	p-value	0.115	0.387	0.290	0.540	_				
Hostility/aggression_mother	Pearson's r	-0.847 **	-0.671	-0.840 **	-0.807 *	0.611	_			
	df	6	6	6	6	6	—			
	p-value	0.008	0.068	0.009	0.015	0.107	_			
ndifference/neglect_mother	Pearson's r	-0.711 *	-0.492	-0.532	-0.305	0.860 **	0.756 *	_		
	df	6	6	6	6	6	6	_		
	p-value	0.048	0.216	0.174	0.463	0.006	0.030	_		
Undifferentiated_rejection_mother	Pearson's r	-0.689	-0.662	-0.613	-0.432	0.581	0.770 *	0.870 **	_	
	df	6	6	6	6	6	6	6	—	
	p-value	0.059	0.074	0.106	0.286	0.131	0.025	0.005	_	
TOT_PARQ_mother	Pearson's r	-0.789 *	-0.579	-0.660	-0.494	0.891 **	0.864 **	0.967 ***	0.852 **	_
	df	6	6	6	6	6	6	6	6	_
	p-value	0.020	0.132	0.075	0.213	0.003	0.006	< .001	0.007	_

		EA_sensitivity	EA structuring	EA_non- intrusiveness	EA_nonhostility	Deactivation of parahipp./amygdala to OWN (beta weights)
EA_sensitivity	Pearson's r	_				
	df	—				
	p-value	_				
EA structuring	Pearson's r	0.880 **	_			
	df	6	—			
	p-value	0.004	—			
EA_non-intrusiveness	Pearson's r	0.904 **	0.812 *	_		
	df	6	6	—		
	p-value	0.002	0.014	_		
EA_nonhostility	Pearson's r	0.778 *	0.734 *	0.928 ***	_	
	df	6	6	6	—	
	p-value	0.023	0.038	< .001	_	
Deactivation of parahipp./amygdala to OWN (beta weights)	Pearson's r	-0.606	-0.845 **	-0.532	-0.469	_
	df	6	6	6	6	—
	p-value	0.111	0.008	0.174	0.241	_

Appendix C. Correlation matrix: *maternal EAS* and Own < Other ROI

Correlation Matrix

Appendix D. Correlation matrix: *PARQ: Father* and Own < Other ROI

Correlation Matrix

		Warmth/affection_fath er	Hostility/aggression_fath er	Indifference/neglect_father	Undifferentiated_rejection _father	TOT_PARQ_fath er	Deactivation of parahipp./amygda la to OWN (beta weights)
Warmth/affection_father	Pearson's r	_					
	df	—					
	p-value	—					
Hostility/aggression_father	Pearson's r	0.831 *	_				
	df	6	_				
	p-value	0.011	—				
Indifference/neglect_father	Pearson's r	0.856 **	0.741 *	—			
	df	6	6	—			
	p-value	0.007	0.035	—			
Undifferentiated_rejection_fat her	Pearson's r	0.762 *	0.946 ***	0.829 *	_		
	df	6	6	6	_		
	p-value	0.028	<.001	0.011	—		
TOT_PARQ_father	Pearson's r	0.954 ***	0.924 **	0.924 **	0.915 **		
	df	6	6	6	6	_	
	p-value	<.001	0.001	0.001	0.001	_	
Deactivation of parahipp./amygdala to OWN (beta weights)	Pearson's r	-0.398	-0.393	-0.430	-0.457	-0.441	_
	df	6	6	6	6	6	—
	p-value	0.329	0.335	0.287	0.255	0.274	—

Appendix E. Correlation matrix: *PARQ: Mother* and Own < Other ROI

Correlation Matrix

		Warmth/affection_mo ther	Hostility/aggression_mo ther	Indifference/neglect_m other	Undifferentiated_rejection_mo ther	TOT_PARQ_mother	Deactivation of parahipp./amygd ala to OWN (beta weights)
Warmth/affection_mother	Pearson's r	_					
	df	—					
	p-value	—					
Hostility/aggression_mother	Pearson's r	0.611	_				
	df	6	—				
	p-value	0.107	—				
Indifference/neglect_mother	Pearson's r	0.860 **	0.756 *				
	df	6	6	_			
	p-value	0.006	0.030				
Undifferentiated_rejection_mo ther	Pearson's r	0.581	0.770 *	0.870 **	_		
	df	6	6	6	—		
	p-value	0.131	0.025	0.005	—		
TOT_PARQ_mother	Pearson's r	0.891 **	0.864 **	0.967 ***	0.852 **		
	df	6	6	6	6	_	
	p-value	0.003	0.006	<.001	0.007	_	
Deactivation of parahipp./amygdala to OWN (beta weights)	Pearson's r	-0.402	-0.385	-0.374	-0.540	-0.458	_
	df	6	6	6	6	6	
	p-value	0.324	0.347	0.362	0.167	0.253	_