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Long-term behavioural neurocognitive outcomes after exposure to early psychosocial deprivation in rhesus macaques : a longitudinal study : a longitudinal study

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Alice Massera

**Effets neurocognitifs comportementaux à long terme après
l'exposition à une privation psychosociale précoce chez les
macaques rhésus: une étude longitudinale**

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**Long-term behavioural neurocognitive outcomes after
exposure to early psychosocial deprivation in rhesus
macaques: a longitudinal study**

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Résumé

L'objectif de cette thèse de doctorat était d'étudier les effets à long terme de la privation psychosociale précoce sur l'autorégulation chez les singes macaques, en mettant l'accent sur la fonction exécutive et ses corrélats neuraux sous-jacents. Les principaux résultats suggèrent que la privation psychosociale précoce est associée à des déficiences de la fonction exécutive des macaques à l'adolescence, et que ces déficiences persistent au début de l'âge adulte. La fonction exécutive était liée au développement structurel du cortex préfrontal et à sa connectivité avec les structures cérébrales pariétales et striatales. Le développement atypique du cortex préfrontal et de sa connectivité était lié à des aspects spécifiques de la fonction exécutive médiocre chez les macaques exposés à une privation précoce, avec un certain soutien fourni pour un mécanisme compensatoire favorisant certains aspects de la fonction exécutive chez ces animaux en termes de développement cérébral structurel, mais au détriment du développement ultérieur et des résultats connexes. D'autres résultats démontrent également un traitement anormal de la menace, une dysrégulation des émotions et un risque accru d'anxiété dans le contexte d'une privation psychosociale précoce chez les macaques.

Les résultats ont été interprétés à la lumière des théories actuelles sur les trajectoires de développement après une exposition à l'adversité sociale précoce. Les modèles de stress et de plasticité sont examinés, y compris les modèles dimensionnels qui mettent en évidence la menace par rapport à la privation comme des éléments clés conduisant à des résultats différents après une privation psychosociale précoce. Des modèles plus récents suggèrent que l'imprévisibilité de l'environnement et d'autres aspects de l'adversité, au-delà de la menace et de la privation, jouent un rôle important, et que de nombreuses formes d'adversité sociale précoce impliquent un mélange de menace, de privation et/ou d'imprévisibilité, ainsi que des mécanismes distincts et superposés liant l'adversité précoce à un développement neurocognitif atypique. Les résultats de cette thèse soutiennent cette dernière théorie.

Les points forts et les limites de la recherche actuelle sont également explorés. Parmi les points forts, citons la conception de la recherche longitudinale qui inclut un point de temps adolescent (c'est-à-dire une période centrale de risque de mauvais résultats liés à la privation psychosociale précoce, tels que les troubles de l'humeur et les problèmes d'extériorisation) ;

l'utilisation de mesures objectives pour évaluer le comportement ; le suivi simultané du développement cérébral et comportemental dans le temps ; l'utilisation d'une approche multidisciplinaire impliquant des domaines tels que l'éthologie, les neurosciences et la psychologie ; et l'utilisation de techniques multimodales, notamment l'imagerie par résonance magnétique, l'observation comportementale et la modélisation informatique. Toutefois, les limites de la recherche, telles que la petite taille de l'échantillon, l'absence de points d'évaluation antérieurs ou postérieurs et l'absence d'examen du mécanisme d'apprentissage spécifique (par exemple, associatif ou de renforcement) lié au développement atypique, ont également été reconnues.

Plusieurs orientations en termes d'étapes futures pour le projet plus large présenté ici et le domaine de la recherche en général sont proposées dans le dernier chapitre. Dans le cadre du projet actuel, l'analyse de mesures cérébrales supplémentaires, y compris la myélinisation et l'IRM de l'état de repos, l'étude du système dopaminergique et l'exploration de la manière dont les aspects cognitifs et émotionnels de l'autorégulation interagissent pour prédire des résultats tels que l'anxiété ou les symptômes de type TDAH seront réalisés. Dans les recherches futures, il sera important de mener des études longitudinales sur un plus grand nombre de stades de développement, d'étudier les mécanismes d'apprentissage spécifiques affectés par la privation psychosociale précoce et de comparer les effets de différents types d'adversité précoce.

En conclusion, les principaux résultats présentés dans cette thèse fournissent des informations nouvelles et précieuses sur les effets à long terme de la privation psychosociale précoce sur la fonction exécutive et ses mécanismes neuronaux sous-jacents chez les macaques. Des recherches supplémentaires sont maintenant nécessaires pour mieux comprendre les différentes trajectoires de développement après une adversité sociale précoce, et les liens avec des résultats sains ou pathologiques. Cela sera essentiel pour la conception future de traitements et d'interventions visant à améliorer et à prévenir les mauvais résultats dans le contexte de l'adversité sociale précoce, et donc à améliorer la qualité de vie des personnes concernées.

Abstract

The aim of this PhD thesis was to investigate the long-term effects of early psychosocial deprivation on self-regulation in macaque monkeys, with a particular focus on executive function and its underlying neural correlates. The main results suggest that early psychosocial deprivation is associated with impairments in macaque executive function in adolescence, and that such impairments persist into early adulthood. Executive function was related to structural development of the prefrontal cortex and its connectivity with parietal and striatal brain structures. Atypical development of prefrontal cortex and its connectivity was related to specific aspects of poor executive function in macaques exposed to early deprivation, with some support provided for a compensatory mechanism promoting some aspects of executive function in these animals in terms of structural brain development, but at the expense of later development and related outcomes. Additional findings also demonstrate abnormal threat processing, emotion dysregulation, and increased risk for anxiety in the context of early psychosocial deprivation in macaques.

The results were interpreted in light of current theories on developmental trajectories after exposure to early social adversity. Models of stress and plasticity are discussed, including dimensional models that highlight threat versus deprivation as key components leading to different outcomes after early psychosocial deprivation. More recent models suggest that environmental unpredictability and other aspects of adversity beyond threat and deprivation play an important role, and that many forms of early social adversity involve a mix of threat, deprivation, and/or unpredictability, as well as both distinct and overlapping mechanisms linking early adversity to atypical neurocognitive development. Findings from this thesis support this latter theory.

Strengths and limitations of the current research are also explored. Key strengths include the longitudinal research design that included an adolescent time-point (i.e. a core period of risk for poor outcomes linked to early psychosocial deprivation, such as mood disorders and externalizing problems); use of objective measures to assess behaviour; simultaneous tracking of brain and behavioural development across time; the use of a multidisciplinary approach involving areas such as, ethology, neuroscience, and psychology; and the use of multimodal techniques including magnetic resonance imaging, behavioural observation, and

computational modelling. However, limitations of the research such as small sample size, no earlier or later assessment time-points, and lack of examination of the specific learning mechanism (e.g. associative or reinforcement) linked to atypical development were also acknowledged.

Several directions in terms of future steps for the wider project presented here and the research field in general are proposed in the final chapter. For the current project, analysis of additional brain measures, including myelination and resting-state MRI, investigation into the dopaminergic system, and exploration of how cognitive and emotional aspects of self-regulation interact to predict outcomes such as anxiety or ADHD-like symptoms will be conducted. In future research, it will be important to conduct longitudinal studies across more stages in development, to investigate specific learning mechanisms affected by early psychosocial deprivation, and to compare effect of different types of early adversity.

In conclusion, the principal findings presented in this thesis provide novel and valuable insights into the long-term effects of early psychosocial deprivation on executive function and its underlying neural mechanisms in macaques. Further research is now needed to more fully understand the different trajectories of development after early social adversity, and links to healthy versus pathological outcomes. This will be essential for the future design of treatments and interventions to ameliorate and prevent poor outcomes in the context of early social adversity, and thus improve the quality of life in affected individuals.

Preface

In this Ph.D. thesis, I present my work on the longitudinal effects of early psychosocial deprivation on neurocognitive and behavioural development in rhesus macaques (*Macaca Mulatta*). I completed this research at the Institut des Sciences Cognitives – Marc Jeannerod (ISC-MJ) CNRS, in the Laboratory of Social Neuroscience and Comparative Development led by Pier Francesco Ferrari. Dr Ferrari served as my main supervisor, with Dr Holly Rayson, a postdoctoral researcher in my team, and Dr James Bonaiuto, who heads the Decision, Action, and Neural Computation lab at the ISC-MJ, also giving additional supervision. All the magnetic resonance imaging (MRI) work presented in my thesis was conducted in collaboration with Drs Bassem Hiba and Suliann Ben Hamed, who are also based at the ISC-MJ, and their research teams.

In **Chapter I**, a literature review is given. This includes information on the historical bases of research on the effects of early social experience on development, on current theories of how early psychosocial adversity may lead to long-term poor outcomes in many developmental domains, and details about current evidence in support of these theories and outstanding questions. In **Chapter II**, methods utilized in the wider project of which the research presented in this thesis is part is described, including information about the project design, the subjects, and all assessments conducted. In **Chapter III**, longitudinal effects of early psychosocial deprivation on macaque executive function are explored, both in adolescence and early adulthood, and in **Chapter IV**, aspects of structural brain development via which poor executive function may arise are investigated. In **Chapter V**, the main findings presented in the previous chapters are discussed, as well as future steps for the wider project and for the research field in general. In **Appendices A, B, and C**, additional work related to the wider longitudinal project is presented, including results concerning emotion regulation and anxiety, brain development across all three assessment time-points, and posters based on work from this project that were presented at conferences.

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CHAPTER 1. INTRODUCTION

Brain Plasticity and Critical Periods

Early life experience plays a crucial role in shaping the neural pathways that underlie cognitive, emotional, and perceptual abilities, with the early foundations of brain architecture laid down via ongoing interactions between genetic and environmental factors. In the 1940s, Donald Hebb posited that the cellular basis of learning and memory arises from increased synaptic efficacy through the repeated and persistent stimulation of a postsynaptic cell by a presynaptic cell (Hebb, 1949). This theory was later extended to any situation involving a long-lasting modification of synaptic connectivity, including brain circuit development (Brown, 2020; Lazari, 2022). According to this theory, reinforced synapses, strengthened by developmental activity, will be retained and tend to expand their connections, while unused ones will be eliminated. As a result, the formation and growth of synapses and neurons will be influenced by postnatal experience. From birth to adolescence, such neural changes coincide with the acquisition of sensory and motor skills, social interaction capacities, and increasingly sophisticated cognitive abilities (Martin, 2002; Ryan, 2015; Johansen, 2014; Fisher, 2017; Keyzers, 2004; McDonald, 2020). Thus, it seems likely that the relationship between behaviour and postnatal, experience-dependent changes in synapses plays a key role in shaping the brain's anatomo-functional architecture and ability to navigate a complex and dynamic environment.

'Critical periods' are specific periods in which the impact of neural activity and the acquisition and execution of a particular behavior is maximized. For example, newborns who have a lens deficit (e.g. cataracts) or eye misalignment (strabismus), which results in the loss of visual input from one eye to the visual system, can develop a form of functional blindness called amblyopia. Research by Hubel and Wiesel with cats and monkeys showed that this occurs because the cortical neurons related to the impaired eye are unable to establish normal connections with thalamic inputs (Hubel, 1965; Hubel, Wiesel, & Levay, 1977). Crucially, if these eye abnormalities are corrected early in development, vision impairment can be prevented, but if corrected later, vision problems persist (Hubel, Wiesel, 2000; Huberman, 2002; Hensch, 2004). Evidence for critical periods of early brain plasticity also comes from patients who sustained brain damage. If sustained early on in life, such damage is less debilitating than brain damage later in life, a generalization known as the Kennard Principle, based on research with monkeys by Margaret Kennard (Kennard, 1940).

The concept of "imprinting" also highlights the importance of critical periods for the emergence of particular behaviors or abilities. Imprinting is a type of rapid learning that occurs early in the life of social animals, which establishes a behavior pattern such as recognition of and attraction to its own species or a surrogate. This was first described by Douglas Spalding in 1870 and then later studied by Konrad Lorenz with ducks. Lorenz found that ducks identify their mother as the first moving object or person experienced in a visual, auditory, or tactile way immediately after birth (Lorenz, 1935). He also discovered that the time frame for imprinting in ducks is less than one day; if newborn ducks do not receive an appropriate stimulus within this time frame, they are unable to establish an adequate parental relationship. Subsequent research on other animals, including rats, birds, and sheep, has shown that this type of bonding can also occur in a bidirectional way between mothers and their offspring (Immelmann, 1972; Marr & Lilliston, 1969; Poindron, Lévy, & Keller, 2007).

Early Social Experience and Attachment

Early findings concerning critical periods of brain development and imprinting influenced the subsequent work of Harry Harlow and his colleagues at the University of Wisconsin in the 1950s on infant attachment (Harlow, 1959). These researchers separated rhesus macaque (*Macaca mulatta*) infants from their biological mothers a few hours after birth, and raised them without any human substitute parent. In one well-known experiment, the infant monkeys were placed in the presence of two different inanimate 'maternal' surrogates: one was a simple wire and wood construction, and the other was covered in foam rubber and soft cloth material. The infants were assigned to one of two conditions. In the first, the wire mother had a milk bottle and the cloth mother did not; in the second, the cloth mother had food while the wire mother had none. In both conditions, Harlow found that the infant monkeys spent significantly more time with the cloth mother than they did the wire mother. When only the wire mother had food, the infant came to the wire mother to feed then immediately returned to cling to the cloth surrogate. Harlow also compared socially deprived infants to non-isolated monkeys (Harlow & Zimmermann, 1959). He observed that while the non-isolated monkeys developed into healthy adults, the monkeys reared in isolation with the surrogate mothers all displayed dysfunctional adult behavior (e.g., more aggression, more fear, and more motor stereotypies). Therefore, Harlow's research was the first to demonstrate that maternal care

was emotional, not just physiological, and that early social experiences were essential for healthy behavioral development. These studies provided novel empirical evidence for the centrality of the parent-child relationship and the importance of maternal touch for infant development.



Figure 1. Infant rhesus monkey with cloth and wire mother surrogates (Harlow, 1959)

John Bowlby, a British psychologist, later developed a theory which integrated these empirical findings into a model based on the concept of 'attachment; that is, the emotional processes through which a caregiver regulates their child's arousal levels (Bowlby, 1958). Attachment can be defined as a deep and enduring emotional bond that connects one person to another across time and space (Ainsworth, 1979; Bowlby, 1969), and a "lasting psychological connectedness between human beings" (Bowlby, 1969). Attachment theory posits that infants have a universal need to seek close proximity with their caregiver when under stress or threatened, with the expectation of receiving protection and emotional support. Bowlby, working as a psychiatrist alongside James Robertson (1952) in the 1930s, observed that children experienced intense distress when separated from their mothers, and even being fed by other caregivers did not alleviate the child's distress. These findings, along with Harlow's research, challenged the dominant theory of attachment at the time, which stated that children become attached to their mothers only because they are being fed (Dollard & Miller, 1950).

Bowlby's attachment theory is grounded in evolutionary theory and the concept of sensitive periods (i.e. periods of development during which the brain is particularly receptive to certain types of stimuli or experiences and is more capable of adapting and reorganizing in response to experience) and experience-dependent mechanisms. It posits that infants are biologically

pre-programmed to form attachments with others in order to increase their chances of survival (Bowlby, 1958). According to John Bowlby's attachment theory, infants form attachments with primary caregivers during a period between 0 and 30 months (2.5 years) that serves as a template for their future attachments, known as "internal working models." While this period was originally referred to as a critical period, it is now most often described as a sensitive period (Goldberg, 2000; Rutter, 1991). These attachments play a crucial role in shaping an individual's adult relationships and can have long-lasting effects on an individual's emotional and social development.

Mary Ainsworth, a developmental psychologist who conducted extensive research on attachment and attachment styles, supported and expanded upon John Bowlby's attachment theory (Ainsworth, 1967). Ainsworth's research focused on the interactions between infants and their primary caregivers, developing the "Strange Situation" protocol to observe and classify attachment styles in infants (Ainsworth, 1978). Different interactions between infants and their primary caregivers can result in three types of attachment: secure, anxious-ambivalent, or anxious-avoidant (Ainsworth, 1991). Securely attached infants feel confident in their caregivers' availability and responsiveness and enjoy exploring their environment. Anxiously-ambivalent infants are uncertain about their caregivers' availability and responsiveness and become anxious when their caregiver leaves. Anxiously-avoidant infants tend to dismiss their caregiver and may avoid or resist physical contact and interaction. Mary Ainsworth's work had a lasting impact on the field of psychology, providing a foundation for understanding the role of attachment in the development of social and emotional skills and resilience in the face of adversity, and having deepened our understanding of the importance of early social experience in shaping an individual's development.

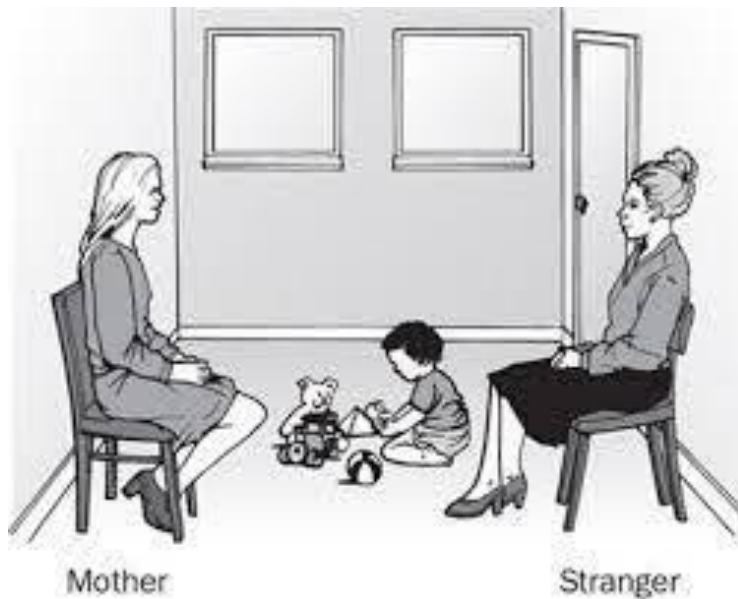


Figure 2. Strange situation (Mary Ainsworth, 1978).

Modern Approaches to Studying the Effects of Early Social Experience on Development

The literature reviewed so far sparked a keen interest into how the early environment, particularly early social experience, can influence specific aspects of development in primates (Frankenhuis, 2020, Cicchetti, 2006; Thompson, 1998; Masten, 2001, Morison, 2017). While genetics clearly play a vital role in such early development (Cloninger, 2019; Coyne, 2016), it is important to recognize that many environmental factors can also shape future structure and function during the first years of life in crucial ways (Tremblay, 2006; Fox, 2010). A wealth of evidence now highlights the role of various factors, such as attachment and parenting practices in shaping an individual's social and emotional development (Cicchetti, 2006; Denham, 2010). The "expectable environment" during early development includes the emotional support and familiarity of a caregiver (Nelson, 2007; Fox, 2010; Kim, 2019; Masten, 2017; McLaughlin, 2017; Fox, 2017), with the study of critical or sensitive periods for socio-cognitive and emotional development vital for better understanding the impact of early social experience on specific developmental outcomes (Fox, 2010; Frankenhuis, 2020).

The integration of social neuroscience with other fields such as developmental psychology, ethology, psychoanalysis, and psychiatry has greatly enhanced our understanding of the neural circuits underlying the development of emotional, cognitive, and social behavior in

primates. For example, researchers have used techniques such as functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) to examine functional brain activity during attachment behaviors, leading to the identification of specific brain regions and neural pathways involved, including the amygdala and ventral striatum (James, 2017; Ein-Dor, 2018). Research has also illuminated the neural basis of other key social behaviors in primates, such as empathy, aggression, and social decision-making (Paradiso, 2021; Zhu, 2019; Gangopadhyay, 2021). By combining insights from social neuroscience with those from other disciplines, researchers have been able to more fully comprehend the intricate relationship between genetics, environmental influences, and brain function in the development of these behaviors. ***However, many questions remain regarding the mechanisms connecting specific aspects of early social experience with specific neurocognitive outcomes, particularly in terms of typical versus atypical developmental trajectories and risk versus resilience.***

Early Social Adversity

Over the past few decades, research concerning effects of early social experience on development has increasingly focused on how early social adversity (ESA) impacts both neural and psychological outcomes. ESA encompasses a range of experiences such as neglect and physical, sexual, or emotional abuse. These experiences involve violations in the early expectable environment (McLaughlin, 2016; Wade, 2022; Holz, 2022; Malave, 2022), including different levels of threat and/or deprivation. For instance, physical abuse or exposure to violence in the community both involve a degree of threat to the child, and institutional rearing or other forms of parental absence can involve a degree of deprivation in terms of social and cognitive stimulation.

ESA in humans has been linked to later poor academic performance, occupational functioning, and social competence (Oeri, 2022; Miller, 2012), as well as increased risk for psychiatric disorders such as mood and substance use disorders (Zhang, 2017; Carp, 2016; Nelson, 2008; Humphreys, 2019; Zeanah, 2016). Furthermore, increased prevalence of the neurodevelopmental disorder attention deficit/ hyperactivity disorder (ADHD) has been observed (Tibu, 2016; Golm, 2020; Boss, 2011; Humphreys, 2019), as well as quasi autistic-like behaviours (Levin, 2015; Imai, 2021). Growing evidence suggests that these poor outcomes

arise via atypical development in related brain networks (e.g. McLaughlin, 2014; Holz, 2022; Tottenham, 2021), but much is still unknown about the actual links between these outcomes and changes in the brain across development. This especially the case in terms of neurocognitive mechanisms across different stages of development and in the context of specific forms of adversity.

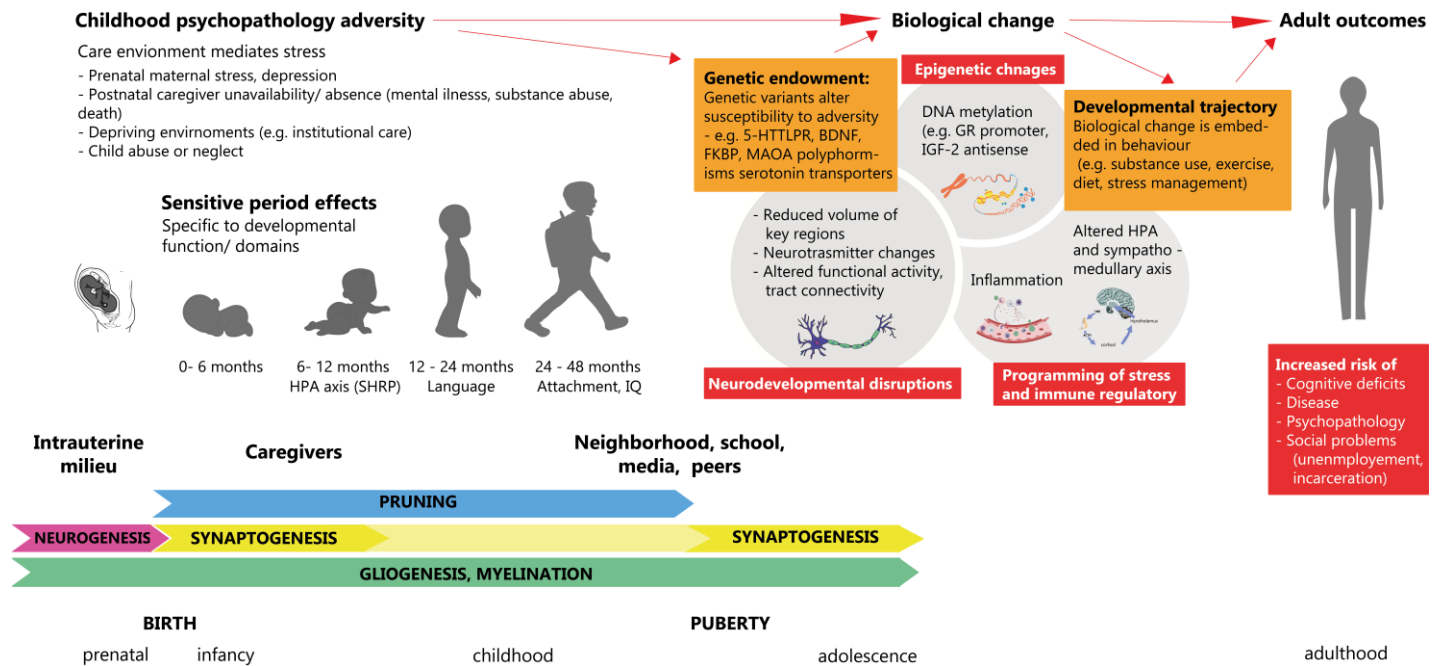


Figure 3. Adapted from Nelson 2020 and Ho 2021.

Neurocognitive Mechanisms Underlying the Relationships between ESA and Poor Outcomes

There are several theories that have attempted to explain how ESA may affect neurocognitive development. ‘Stress response theories’ suggests that ESA can lead to dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, a bodily system responsible for regulating the stress response (Gunnar & Quevedo, 2007; Loman, 2010). Chronic exposure to stress or trauma during early development can lead to changes in the way the HPA-axis functions, resulting in an overactive or underactive stress response (McEwen & Seeman, 1999). The stress response is a series of physiological reactions involving activation of the HPA-axis that occur when exposed to a stressor, such as physical injury, infection, or psychological stress. Once activated, the HPA-axis releases a cascade of glucocorticoid hormones, including corticotropin-releasing hormone (CRH) from the hypothalamus, adrenocorticotrophic hormone

(ACTH) from the pituitary gland, and cortisol from the adrenal glands. Cortisol, also known as the 'stress hormone', plays a central role in the body's response to stress. It helps to increase blood sugar levels, suppress the immune system, and regulate blood pressure, among other functions., which leads to the production of glucocorticoids. Glucocorticoids such as cortisol can easily cross the blood-brain barrier and bind to receptors in the hippocampus, prefrontal cortex, and amygdala. In particular, cortisol can bind to receptors in the prefrontal cortex, and high levels of cortisol have been associated with impairments in prefrontal cortex-dependent tasks, such as memory and decision-making (McEwen, 2002).

Chronic activation of the HPA axis and elevated cortisol levels have also been linked to structural and functional changes in the prefrontal cortex, including reductions in the size and density of neurons (Gudsnuk et al., 2015). However, these three brain structures (hippocampus, prefrontal cortex, and amygdala) do not develop at the same rate in humans, and exposure to stress at different times during brain development may increase the risk of certain mental health disorders linked to particular brain circuitry (Raymond, 2018). Dysregulation of the HPA axis has been linked to increased risk for various mental health problems involving emotion dysregulation, including depression, anxiety, and post-traumatic stress disorder (Gunnar & Quevedo, 2007). There are two main theories within the 'stress response' approach that attempt to explain the relationship between early adversity, HPA-axis dysfunction, and poor psychiatric outcomes later on. The 'stress sensitization model' posits that early adversity leads to heightened reactivity to future stressors, increasing the risk of negative outcomes (McLaughlin, 2010). The 'stress inoculation model', instead suggests that moderate levels of adversity may enhance resilience and protect against negative consequences, while extreme levels of adversity may increase later vulnerability (Parker, 2006). In such models, chronic activation of the stress response system due to adversity may increase the risk of psychiatric disorders through different mechanisms such as allostatic load (Danese, 2012; Guidi, 2021) and accelerated maturation (Callaghan, 2016). Allostatic load, first described by McEwen in 1993, is the cumulative impact on the body of chronic stress, which can lead to allostatic overload when the body's coping mechanisms are exceeded. Accelerated maturation refers to the speeded-up development of brain circuits that control emotion, often observed as an adaptive response to early life caregiving stress. This accelerated development can manifest in the premature activation and advancement of neural structures and molecular

compositions in brain regions that control emotion expression, learning and memory. While this may provide a short-term adaptive advantage, studies have shown that it could also have negative long-term consequences on the brain's circuit integrity and functioning, which probably has negative effects on other later developing circuits, and increase the risk of developing psychiatric disorders and behavioural problems in adulthood (Herzberg, 2021; Hanson, 2010).

Another theory suggests that early social adversity can lead to changes in the way the brain forms and maintains connections between neurons; i.e. neural plasticity (Shonkoff et al., 2012; Ho, 2021; Miskolczi, 2019). This is important for learning and memory, and is thought to be influenced by genetics, environment, and experience. When neural plasticity is disrupted by early social adversity such as abuse, neglect, or chronic stress, it may lead to differences in the way the brain processes and integrates information, resulting in cognitive problems such as difficulties with learning, memory, and problem-solving (Shonkoff et al., 2012). One mechanism through which early social adversity may affect neural plasticity is by disrupting the process of synaptic pruning, which involves the elimination of unnecessary or weak connections between neurons (Shaw et al., 2008). This process plays a key role in the development of functional brain connectivity and the emergence of complex cognitive abilities. Abnormal patterns of synaptic pruning may contribute to the development of cognitive and behavioral problems, and have been linked to differences in the thickness and surface area of cortical brain regions such as the prefrontal cortex (PFC) (McLaughlin et al., 2014; Hodel, 2015), the temporal cortex (Mackes, 2020; Mclaughlin, 2014), the parietal cortex (Mclaughlin, 2014), and subcortical structures such as the hippocampus and the amygdala (Mehta, 2009; Hodel, 2015; Hanson, 2015; Tottenham, 2010; Sheridan, 2012).

Recently, several researchers have tried to integrate different theorized mechanisms linking ESA to poor outcomes in order to better understand the complex interactions between several factors that may be central for the developing brain. ESA can have long-lasting effects on an individual's behavior, stress responses, and brain function, particularly if it occurs during sensitive periods of development when crucial neural connections are being formed (Smith, 2020; Malave, 2022). These effects may be caused by changes in several processes including the functioning of the HPA axis, neurotransmitter systems, and processes such as neurogenesis, myelination, synaptogenesis, and synaptic pruning, and modifications to stress

regulatory genes (Marsh, 2008; Ho, 2021; Malave, 2022; Holz, 2022). These changes can impact brain regions and circuitry involved in emotion processing, memory, and learning, and may underlie negative social, cognitive and emotional outcomes. The timing of adversity within sensitive periods of development may also affect whether it influences experience-expectant or experience-dependent mechanisms, with infancy and early toddlerhood often being cited as particularly vulnerable periods (Nelson, 2020; Gabard-Durnam, 2020). Nevertheless, more research is now needed to elucidate more clearly the actual neural mechanisms linking ESA to increased risk for psychiatric and behavioural difficulties (Smith, 2020; Holz, 2022). This will be crucial for identifying the best targets for early intervention after exposure to ESA, and developing better treatments for psychopathologies that have their roots in early life (Malave, 2022).

ESA and Early Institutionalization: Effects of Psychosocial Deprivation on Development

Psychosocial deprivation in the form of early institutionalization is one model of ESA that has been of particular interest in previous literature. Institutionalization refers to the long-term care of children in institutions, such as orphanages, rather than in family settings. Institutionalization has been associated with negative outcomes in numerous areas of development, including lower levels of physical growth, cognitive and language development (Kornilov, 2019), and increased rates of attachment disorders and other mental health problems such as mood disorders (van IJzendoorn, 2020).

The Bucharest Early Intervention Project (BEIP) is a randomized controlled trial that is investigating the effectiveness of foster care as an intervention for abandoned children placed in institutions as infants in Bucharest, Romania (Zeanah, 2003; Nelson, 2014; Fox, 2017). The ongoing study includes comprehensive longitudinal assessments of 136 institutionalized children and their caregiving environments, with half being randomly assigned to high-quality foster care and the other half remaining in institutional care. The average age of foster care placement was 22 months, with follow-up assessments occurring at 30, 42, 54 months, 8, 12, and 16 years of age, with assessments at later ages to come. Results so far show that, compared to a control group of never-institutionalized children, children raised in institutions exhibit significant delays in cognitive and motor development, language, and socio-emotional

behaviors, as well as higher rates of psychiatric disorders (Bos, 2011; Nelson, 2019; Wade, 2022; Humphreys, 2020; Wade, 2019; Bick, 2017; Lamm, 2018). Foster care was found to produce notable improvements in certain areas, but children in foster homes still lagged behind the control group in various aspects of development (Nelson, 2014; Humphreys et al. 2022).

To examine the neural underpinnings of how early social deprivation may have led to such poor socio-cognitive, emotional, and behavioural outcomes in institutionalized children, the project has utilized electroencephalography (EEG) and magnetic resonance imaging (MRI) to investigate differences in brain development among the Romanian orphans. At the baseline assessment at 22 months, using EEG, institutionalized children were found to have higher theta power but lower alpha and beta power compared to a never institutionalized control group (Marshall, 2004). This pattern of brain activity has been associated with ADHD, learning disorders, and other negative developmental outcomes (McLaughlin, 2010). At 12 years, previously institutionalized children also showed worse inhibition of planned action, which was associated with a larger P2 event-related potential (ERP) component and a smaller N2 component; the degree of neural activation moderated the association between ESA and externalizing symptoms (Lamm, 2018). Notably, alpha power in the foster care group and never institutionalized control group were comparable, whereas the institutionalized children continued to display reduced alpha power and greater theta power compared to the other groups even at 16 years (Vanderwert, 2010, 2016; Debnath, 2020). Reduced alpha power and elevated theta power suggest a deficit in cortical maturation in institutionalized children (Debnath, 2020). Reduced alpha power predicted worse executive function in adolescence (Wade, 2019). In addition, by age 16, adolescents who had been in institutional care but were later placed in foster care showed greater mediofrontal theta cortical activity and better ability to adapt their behavior after making mistakes compared to those who had experienced prolonged institutionalization (Buzzell, 2020). These findings suggest that high-quality foster care and a stable caregiving environment are important for the long-term optimal brain development of children who have been previously institutionalized (Debnath, 2020; Buzzell, 2020).

The use of MRI in this project also revealed that institutionalization can also lead to alterations in brain structure, including cortical thickness, white matter volume, and structural

connectivity (Zeanah, 2017). Children who had been institutionalized had smaller brains overall, with reduced grey matter and white matter volume compared to controls (Kelly et al., 2013; McLaughlin et al., 2014; Sheridan et al., 2012; Tendolkar et al., 2018). A study by Sheridan et al. (2012) found that between the ages of 8 and 11 years, white matter in the cortex was reduced in children who had been institutionalized compared to those who had not. In a study using diffusion MRI to investigate structural connectivity, Bick et al. (2017) found that at 8 years, children who had been institutionalized had higher levels of depression and anxiety compared to non-neglected children, which was not affected by early intervention. However, at 12 years, girls who received early intervention via foster care had reduced levels of internalizing symptoms, while boys did not show similar improvement (Bick, 2017). Importantly, depression and anxiety symptoms at 8 and 12 years were linked to lower integrity in certain white matter tracts in the brain, and the integrity of these tracts indirectly affected the relationship between early rearing status and levels of internalizing symptoms. Specifically, the integrity of the external capsule and body of the corpus callosum were found to significantly moderate the link between early rearing status and internalizing symptoms.

Recently, Sheridan et al. (2022) found that from age 9 to 16, children from this project raised in families from birth had thinner cortex in certain areas of the PFC (i.e. medial (mPFC), inferior frontal gyrus (IFG), temporal pole) compared to those exposed to institutional care, suggesting a long-term impact of institutional care on cortical development. Thinning in these areas was associated with externalizing psychopathology and cognitive ability. Institutionalization, randomization to foster care, and timing of placement were not related to subcortical volumes for any structure. The study also showed that the timing of placement into foster care and the length of time spent in institutional care were important factors in cortical development. In particular, children placed in foster care before 24 months of age had relatively thicker cortex in the middle frontal gyrus (MFG) in adolescence compared to those placed later, and a thinner cortex in the anterior cingulate cortex (ACC) and IFG at 16 years. In addition, children exposed to institutional care had decreased white matter integrity in the superior longitudinal fasciculus (SLF), a tract that connects the lateral PFC to the parietal cortex which supports executive function, compared to those in foster care. In contrast, the foster care group had greater white matter integrity in the uncinate fasciculus, a tract that connects the inferior PFC with the amygdala and anterior temporal cortex, and is linked to emotion regulation.

The English and Romanian Adoptee (ERA) study is another longitudinal project examining the development of children adopted from Romania and placed in the UK in the 1990s (Rutter, Sonuga-Barke, 2017). According to current findings from this study, Romanian adoptees who spent less than six months in an institution had similar rates of ADHD, autism spectrum disorders (ASD), cognitive impairment and excessive friendliness compared to UK control groups across most ages and outcomes. However, those who spent more than six months in an institution consistently had higher rates of behaviours characteristic of ASD, disinhibited social engagement, and inattention and hyperactivity compared to control participants. Additionally, the group who spent more than six months in an institution had initially higher rates of cognitive impairment compared to controls, but these rates normalized in young adulthood. In contrast, self-rated emotional symptoms showed minimal differences compared to controls during childhood and adolescence, but increased significantly by young adulthood (Sonuga-Barke, 2017). Another study found that early institutionalization was linked to impairment in various neuropsychological domains, including IQ, prospective memory, proactive inhibition, decision-making, and emotion recognition, in young adulthood (Golm, 2020). The study also found that the quality of early post-adoption home environment moderated the association between institutional deprivation and neuropsychological outcomes in adulthood, with better post-adoption environments leading to better outcomes. Finally, this study also demonstrated that children with a history of deprivation had a higher prevalence of mood disorders, with the severity of these disorders being linked to the duration of deprivation (Golm, 2020). Mackes et al. (2020) followed-up this finding, showing that being exposed to early deprivation during the first years of life was related to significantly smaller total brain volumes and was negatively associated with the duration of deprivation in adulthood. The reduction in total brain volume mediated the relationship between institutionalization and lower IQ and higher ADHD symptoms. Deprived adult adoptees had lower right inferior frontal cortex surface area and volume, but higher right inferior temporal lobe thickness, surface area, and volume compared to the control group. The increase in right inferior temporal volume in deprived individuals was associated with lower levels of ADHD symptoms. Deprivation duration was positively associated with right mPFC volume and surface area. They found no effects of deprivation on subcortical brain structures in the adult sample (e.g. amygdala and hippocampus). These findings suggest that limited severe deprivation during the early years of life is associated with lasting changes in adult brain

structure, even when there is extended enrichment in adoptive homes later in childhood (Mackes, 2020).

The International Adoption Project in the United States, launched in 1999 by Megan Gunnar and her team, also focused on the impact of early institutionalization on development. The project involved analysis of a registry of 6,000 children adopted by families in the USA from institutions abroad. Post-institutionalized children were found to struggle with executive function such as cognitive flexibility, inhibitory control, and working memory, and perhaps experienced delays in theory of mind development; i.e. the ability to understand the mental states of others (Gunnar et al., 2001; Gunnar & Van Dulmen, 2007). Certain differences in brain development, including a reduction in total brain volume and changes in the development of the PFC and hippocampus (Hodel et al., 2015) were proposed to underlie these difficulties.

Although a number of studies from the projects above failed to find effects of early institutionalization on subcortical structures, other research suggests that psychosocial deprivation does affect subcortical development. A longitudinal study by VanTieghem (2021) found that institutionalization was associated with reduced hippocampal volume and reduced growth rate of the amygdala in children, as well as smaller hippocampal and amygdala volume in adolescence. Tottenham (2011) found increased amygdala activity measured fMRI during an emotional Go-No-Go task only in post-institutionalized children, which was inversely related to the amount of eye-contact made during the task, with Callaghan (2019) showing decreased amygdala reactivity to parental cues which predicted reduced anxiety in previously institutionalized adolescents. Silvers (2016) found that the engagement of a broader prefrontal-subcortical circuit in post-institutionalized older children and adolescents contributed to their aversive learning compared to a control group, and increased connectivity between the hippocampus and the ventromedial prefrontal cortex was related to improvements in anxiety in the post-institutionalized group. Interestingly, Silvers (2017) found that although individual differences in vigilant behavior were positively correlated with amygdala responses to fearful and happy faces in all youths, the degree to which amygdala responses were stronger for fearful versus happy faces was associated with greater anxiety in previously institutionalized youths only. However, other studies found no differences between never institutionalized and institutionalized children in terms of emotional face

processing (Jeon, 2010), including neural correlates of facial emotion processing (Young, 2017). Finally, Fareri (2017) found that stronger coupling between the ventral striatum and the medial prefrontal cortex was associated with parent-reported social problems in previously institutionalized adolescents, and differences in resting-state connectivity between these two brain regions increasingly affected social behaviors, particularly during adolescence.

Some recent theories concerning the effects of ESA on brain development have attributed more focus to the striatum than previous ones. Along with amygdala and hippocampus, which form important components of the stress system, the striatum is being posited as part of a regulatory mechanism that can also be disrupted by psychosocial adversity (Fareri, 2017) and interacts with the stress system. In particular, it is hypothesized that changes in fronto-striatal circuitry following psychosocial deprivation may have an important role in the emergence of poor emotional regulation and executive function (Eluvathingal, 2006; Fareri, 2017). The little research connected so far has demonstrated effects of early institutionalization on both functional (Fareri, 2017) and structural connectivity (Bick et al., 2017) of the striatum. However, only few studies so far have considered the role of the striatum, and more research is needed to elucidate how this subcortical structure and its connectivity may contribute to negative outcomes after exposure to psychosocial deprivation.

To summarize, studies of psychosocial deprivation have consistently revealed effects of early and prolonged exposure to deprivation, in the form of institutionalization, on the development of the PFC and other cortical regions using different measurements of brain structure, including cortical thickness and surface area. These changes in the PFC are linked to negative outcomes, including impaired executive function and increased risk for psychopathology. However, there is inconsistency in findings concerning the effects of institutionalization on subcortical development, such as the amygdala, hippocampus, and striatum, though a number of studies have linked connectivity of these regions to institutionalization. Additionally, few studies thus far have looked at functional or structural connectivity, most studies focus on only one age of assessment, and measures are often not looked at concurrently. ***Altogether, this limits our understanding of the aspects of neural development linking institutionalization to specific poor outcomes.***

Early Institutionalization and Self-Regulation

Critically, many of the adverse psychological and behavior outcomes discussed so far in the context of psychosocial deprivation are linked to problems of 'self-regulation'. Self-regulation is the flexible regulation of cognition, behavior, and emotion (Calkins, 2016), with poor self-regulation linked to poor academic and social functioning, criminal behavior (Bridgett, 2015; Graziano, 2010; Eisenberg, 2010), and several psychiatric and neurodevelopmental disorders (Nigg, 2017; Forbush, 2015; Lochman, 2006). For example, research has shown that difficulties with self-regulation in childhood can lead to lower income and educational attainment by adulthood (McClelland, 2013). Self-regulation can be posited to be comprised of two distinct, but interacting components: i) "top-down" self-regulation, which reflects processes that require more effortful and executive control, helping to resolve conflict or prepare for an anticipated goal; and ii) "bottom-up" self-regulation, which reflects processes that are more automatic and stimulus driven. "Top-down" self-regulation has also been further divided into behavioral and emotional self-regulation, while "bottom-up" self-regulation has been divided into behavioral inhibition/fear and impulsivity (Bridgett, 2015).

In terms of top-down processes, behavioral constructs of self-regulation include effortful control, cognitive control and executive function, which although often discussed in the literature as distinct entities, are starting to be viewed increasingly as similar or overlapping processes, at both conceptual and behavioral levels (Bridgett, 2015). These involve the modification of attention, memory, or working memory to try and enhance adaptation or achieve a goal, thus accounting for top-down aspects of self-regulation at the cognitive level (Nigg, 2017). These self-regulation constructs also overlap at the neurobiological level, with several regions of the brain such as the dorsal ACC, dorsolateral prefrontal cortex (dlPFC), ventrolateral prefrontal cortex (vlPFC), orbitofrontal cortex (OFC), ventromedial prefrontal cortex (vmPFC) being associated with these various self-regulation processes (Posner, 2012; Wager, 2014; Borst, 2014). For clarity, from now on, the term executive function will be used in this thesis to refer to the cognitive dimension of the self-regulation construct. Emotional self-regulation has been studied primarily in the context of specific emotion regulation strategies such as reappraisal and suppression (Ellis, 2018; Kelley, 2019; Lopez, 2020). Reappraisal involves cognitive reframing of emotions, while suppression reduces outward expression of emotions (Gross, 2008). Neurobiological studies show that reappraisal and

suppression are associated with activation of the dlPFC, vlPFC, dorsal mPFC, ACC, and other areas in the frontal lobes (Buhle, 2014).

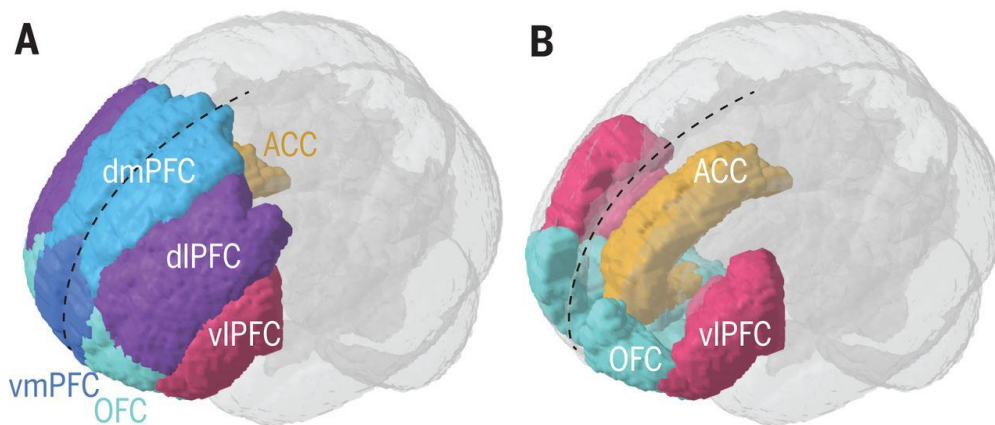


Figure 7. PFC structural organization (Carlen 2017).

In terms of bottom-up processes, behavioral inhibition/fear/avoidance is a reactive and "over-controlled" type of self-regulation, where individuals tend to be more cautious and reserved in novel situations, and are more likely to be fearful and/or shy. Neurobiological studies have found that the subcortical structures most consistently associated with behavioral inhibition/fear are the amygdala and hippocampus (Kennis, 2013), but also the striatum (Clauss, 2014). Impulsivity, on the other hand, is a reactive, undercontrolled type of self-regulation, characterized by acting without much forethought or consideration of the long-term implications of behaviour (Eisenberger, 2013). The neural areas often associated with impulsive behaviors include the ventral striatum, specifically the caudate nucleus, nucleus accumbens, and putamen (Besson, 2010; Whelan, 2012).

Previously, top-down and bottom regulation processes have been viewed rather separately, with top-down self-regulation processes appearing to originate largely in neural structures of the frontal lobes and ACC, and bottom-up self-regulation processes apparently served more by subcortical structures. However, increasing attention has been given to integration across different aspects of self-regulation. Specifically, the distinction between bottom-up and top-down self-regulation is no longer commonly viewed as absolute but rather on a continuum (Nigg, 2017). These two systems are not mutually exclusive, but instead interact through successive reciprocal neural feedback loops. For example, top-down systems can affect bottom-up responses by activating, suppressing, or biasing them, while bottom-up systems can also activate goal-related behavior through priming or other effects, which in turn can

limit the effects of top-down processes (Verbruggen, 2016, Avital-Cohen, 2016). This highlights the complex and dynamic nature of self-regulation and its underlying neural mechanisms, and the importance of considering brain connectivity and interactions between different circuits rather than neural single structures only.

Previous research framing differences in development in terms of self-regulation has shown that adverse early social experience is associated with deficits in self-regulation during childhood (Evans, 2013; Bridgett, 2015; Hostinar, 2012), which are then linked to outcomes such as indiscriminate friendliness and problems in regulating affect in young adults who experienced early institutionalization (Kennedy, 2017). Additionally, much evidence has been provided linking early psychosocial deprivation to increased risk for symptoms of ADHD (Loman, 2013; Pollak, 2010; Roy, 2004; Rutter, 2001; Sheridan, 2010; Wiik et al., 2011), which is characterized by self-regulatory difficulties. However, findings are again not entirely consistent across studies, with the common use of teacher or parental reports, questionnaires, and different ages of assessment likely contributing to this. Furthermore, very few studies have assessed differences in both psychological and brain development or their relationship. Recent theories have proposed that stress physiology may act as a primary mechanism linking early experiences of adversity to reduced self-regulatory abilities (Raymond, 2018), with adversity-related alterations in brain regions such as the hippocampus, amygdala, and prefrontal cortex (PFC) following ESA (McCrory, 2010; Lupien, 2009). Therefore, impairments in the development of PFC, which plays a crucial role in the control of behavior, cognition, and emotion, supporting self-regulation mechanisms, may underlie deficits in children's self-regulation abilities (Miller, 2001; Nigg, 2017). Notably, recently research has also considered how poor in executive function may also contribute to risk factors associated with self-regulation difficulties in the context of early psychosocial deprivation (Hofmann, 2012), with PFC and PFC-subcortical networks also playing a central role in executive function (Diamond, 2002; Friedman, 2022).

It is now essential to integrate research on emotional and cognitive processes involved in self-regulation in order to gain a more comprehensive understanding of the complex mechanisms underlying this (Nigg, 2017; Pruessner, 2020). This is especially true in the context of ESA, where many poor outcomes may arise from effects of emotional and cognitive processes, and their interactions and overlapping features. For example, studies have shown that a higher

level of executive function is associated with successful emotion regulation, and that the implementation of emotion regulation strategies correlate with individual differences in executive function, including the ability to inhibit prepotent responses, update information in working memory, and shift mental sets (Gross, 2015a; Joormann, 2015). ***However, research on these two dimensions are very rarely studies of these processes jointly, and research focused on the role of cognitive factors in poor outcomes seen in the context of ESA is currently lagging behind that of emotional processing generally.***

Adolescence as a Sensitive Period of Development after Psychosocial Deprivation

Sensitive periods, i.e. stages in development when an organism can most rapidly acquire a particular skill or characteristic, can occur at various points in an individual's life. The timing and duration of sensitive periods is likely influenced by evolutionary constraints, stress, and attachment style (Opendak, 2017). Sensitive periods in infancy have been linked to changes in HPA-axis function and brain plasticity (i.e. plasticity linked to periods of prolific synaptic pruning and myelination), and are commonly associated with effects on development of the amygdala, hippocampus, and PFC (Ho, 2021). Although much research on sensitive periods has focused on infancy and early childhood, interest in potential sensitive beyond the earliest stages of development is growing. In particular, adolescence is being considered increasingly as a particularly important sensitive period later in development (Fuhrmann, 2015; Larsen, 2018 ;Frankenhuis, 2020). This period is marked by significant physical, cognitive, and emotional changes, including substantial refinement of self-regulatory processes (Nigg, 2017; Casey, 2015; Cohen et al., 2016), and especially marked development in the PFC (Spear, 2000). As well as changes in PFC regions, important modifications in connectivity of the PFC take place during adolescence. This includes development in fronto-striatal circuits (Ohja, 2022), which are thought to play a significant role in the refinement of executive function and emotion regulation abilities (Ohja, 2022; Darki, 2015; Baker, 2022-preprint). Relatedly, adolescence is also a time of increased risk-taking and experimentation, which may be exacerbated by effects of ESA on emotional and social development (Steinberg, 2004).

The concept of " sleeper effects" in development refers to the delayed manifestation of early experiential impact on development, and may contribute to poor outcomes emerging in adolescence after exposure to ESA. This phenomenon, also referred to as an "incubation period" (Lupien, 2009), suggests that the early experiences in infancy and early childhood may

not affect certain aspects of development until later on in life, and may interact with prolonged stressors that accumulate over time (Sapolsky, 1996; Sapolsky, 2000). Evidence of sleeper effects has been found in a variety of domains, including face processing (Maurer, 2007), visual processing (Maurer, 2007), behavioral disturbance, executive function, and social-emotional development (Merz, 2010). For example, in the context of ESA, individuals who experienced maltreatment in childhood may not demonstrate a negative impact of this experience until later on in life in the form of reduced hippocampal volume (which has a protracted developmental time-course) linked to externalizing psychopathology (Woon, 2008; Zeanah, 2009; Zeanah, 2014; McGoron, 2013). This underscores the importance of considering later sensitive periods and sleeper effects in future longitudinal studies of ESA, especially during adolescence, with further research now needed to fully understand the mechanisms underlying these phenomena.

Despite psychosocial deprivation being a form of ESA that appears most closely connected to atypical aspects of self-regulation such as executive function (Johnson, 2021), and evidence suggesting that adolescence may be a sensitive period for the EF development (Thompson, 2020), ***very little is known about the trajectory of EF development across adolescence after exposure to early deprivation.*** In a rare longitudinal study (Wade et al., 2019) examining the impact of institutionalization on cognitive development in children and adolescents, early-emerging deficits in attention, short-term visual memory, spatial planning, and problem solving were apparent in children who had been institutionalized versus those who had not. These differences remained consistent throughout adolescence, with the gap in spatial working memory between ever- and never-institutionalized children actually increasing by adolescence. However, the study also revealed that other early difficulties in visual-spatial memory displayed by children in foster care were mitigated by adolescence. Such findings suggest that early psychosocial deprivation may have varying effects on cognitive development depending on the specific skill being evaluated and whether or not they were later placed in a supportive family environment.

Macaque Model of Early Psychosocial deprivation

Macaques have often been used as a non-human primate model for studying the effects of early social adversity on development because they share many similarities with humans in terms of their social, cognitive, and emotional development, and physiological and genetic

characteristics (Wang, 2020; Xue, 2016; Phillips, 2014). Like humans, macaques are highly social animals and their social relationships can have a significant impact on their development and well-being (Cooper, 2022). Self-regulatory related behaviours are also clearly demonstrated by macaques including executive function and affective regulation (Machado, 2003; Miller, 2019). In addition, macaques have a similar brain organization and development to that of humans, making them a good model for studying effects of social experience on brain development (Kovacs-Balint, 2021; Schumann, 2019; Kim, 2020).

Another advantage of the macaque model is that researchers can use a variety of more objective methods to assess developmental outcomes (e.g., via behavioral observations); in humans, often only self-report measures (e.g., questionnaires) are utilized. Another advantage of using a macaque model is the possibility to better control variables in the early social environment, which allows for more precise experimental control than is possible in human studies. This makes it easier to identify the specific effects of early social adversity on various aspects of development and behavior. Finally, macaques have a shorter developmental time-frame than humans, allowing researchers to conduct longitudinal studies in a relatively shorter time period compared to humans, and assessment of various aspects of brain development is more feasible.

Several studies have investigated the effects of early psychosocial deprivation in macaques on behavioural development. These studies have exposed macaques to rearing conditions involving caregiver absence, such as being separated from their mothers for extended periods of time or reared by human experimenters in a nursery of peers (Sanchez, 2001; Barrett, 2009; Rutter, 2004; Suomi, 1991). Early psychosocial deprivation appears to have a long-term impact on many aspects of macaque development, similar to humans, including increased sensitivity to threat, anxiety, aggression, impaired social relationships, and motor function (Houser, 2013; Karere, 2009; Rayson et al., 2021). Most research has focused on earlier stages of macaque development, though the little that has focused on the later juvenile period or adulthood does suggest long-lasting effects of early deprivation (Corcoran, 2012; Bauer, 2016; Dettmer and Suomi, 2014).

There have not many studies on the effects of early psychosocial deprivation on brain development and related poor outcomes in macaques, but there is evidence that this is associated with changes in brain structure and function. This includes altered dendritic arborization and synaptic signaling in the PFC, hippocampus, and amygdala (Meaney, 1996; Houser, 2013; Spinelli, 2009). These brain areas are an important part of the fear circuitry, and as previously noted, play a crucial role in HPA axis regulation (Mckeon, 2022; Kalin, 2007; Raper, 2014). A few EEG studies also suggest that early deprivation is associated with abnormal cortical activity in attention and sensorimotor regions in response to social stimuli (Vanderwert et al., 2015; Festante et al., 2021). These changes may contribute to the behavioral and cognitive changes observed in macaques exposed to early psychosocial deprivation, however, like in humans, more research is needed to elucidate the neurocognitive mechanisms linking early deprivation to specific poor outcomes later in life.

Using another macaque model of ESA, maternal abuse, a couple of studies have investigated the effects of infant maltreatment in rhesus monkeys on development of brain white matter tracts involved in socioemotional behavior using diffusion MRI (Howell, 2019). Early maternal abuse was associated with reduced structural integrity in certain white matter regions, including the corpus callosum, occipital white matter, external medullary lamina, and brainstem in adolescent macaques (Howell, 2013). These changes were suggested to be linked to reduced myelin, and were also associated with elevated cortisol levels and increased social aggression. In another study, the same group found that white matter integrity increased longitudinally from the infant to early juvenile period, consistent with human findings and other primate work on DTI (Dubois, 2014; Kubicki, 2018; Kim, 2020). In addition, they found a decrease in the structural integrity of the middle longitudinal fasciculus and the inferior longitudinal fasciculus in maltreated animals, with these tracts thought to be associated with motivational valence, social learning, and social interactions (Howell, 2019).

Very few studies have examined the effects of psychosocial deprivation on cognitive functioning in macaques, despite this likely playing a crucial role in risk or resilience for many adverse outcomes seen in the context of deprivation. One study by Sanchez et al. (1998) demonstrated that infant macaques reared individually in a nursery showed impaired cognitive functioning (i.e. spatial learning and memory) compared to infant macaques reared in normal conditions. However, another study by Murphy et al. (2020) found that infant

macaques reared in a nursery of peers did not show differences in reward association, cognitive flexibility, or impulsivity compared to macaques reared in normal conditions. These inconsistent findings suggest that the effects of early psychosocial deprivation on cognitive functioning in macaques may be complex, and that findings likely depend on the measures of cognitive functioning used, and we do not know about cognitive development later on. Overall, these studies provide strong evidence that early psychosocial deprivation in macaques likely impacts many aspects of social, emotional, and cognitive development. However, ***much is still unknown about the neural mechanisms linking early deprivation to specific outcomes in macaques or different trajectories of development after ESA exposure, especially in terms of cognitive development.***

This Thesis

Clearly, many questions remain about the neurocognitive mechanisms linking early psychosocial deprivation to specific poor outcomes across different periods of development. Notably, adolescence is a time where huge developments in executive function take place, which has been identified as a key predictor of academic, socio-emotional, and occupational success, and psychiatric health. This is proposed to rely on a sensitive period of brain development, characterized by significant maturation of the PFC, as well as its connectivity with other regions such as the striatum. By studying the effects of early psychosocial deprivation in macaques, a nonhuman primate species with a neurocognitive structure and function similar to humans, we can gain a better understanding of these mechanisms and inform the development of interventions and prevention strategies for individuals who have experienced early psychosocial deprivation. This research has the potential to not only improve our understanding of the impact of early life experiences on brain development, but also to promote healthy development and prevent negative outcomes in later life after exposure to ESA.

Based on some of the knowledge gaps highlighted here, ***this thesis*** aimed to address the following ***research questions: a) how does early psychosocial deprivation influence executive function in macaques across adolescence; and b) what are the neural mechanisms linking early social experience to executive function in macaques?*** To answer these, the two ***core objectives***

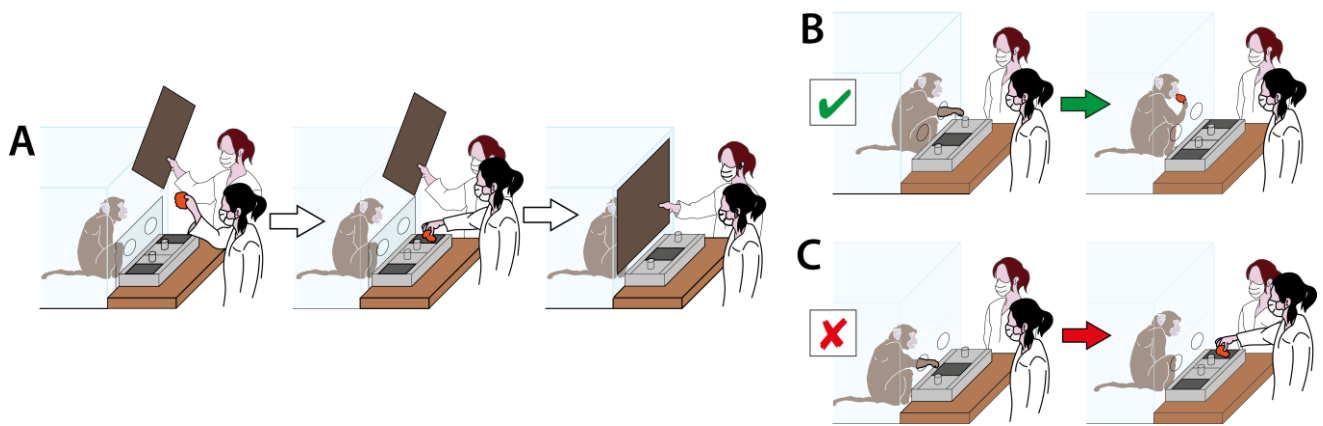
were: *i) to investigate the longitudinal and long-term effects of psychosocial deprivation on macaque executive function, as well as working memory and inhibitory control components of executive function specifically;* and *ii) identify aspects of structural brain development that may underlie differences in executive function during this period.*

To achieve these objectives, a standardized behavioural assessment was used to assess executive function at two time-points corresponding to adolescence and adulthood in macaques. At the same time-points, MRI (anatomical and diffusion) were acquired in order to assess white-matter integrity in executive function-related brain circuitry. **Results are presented in Chapters 3 and 4**, which are outlined briefly below. The studies presented in these chapters are part of a wider project investigating the effects of early psychosocial deprivation on multiple aspects of macaque development. This is the first longitudinal project looking at the effect of early psychosocial deprivation on the development of both brain and behaviour in rhesus macaques across adolescence. The novel sample is comprised of two groups, one that was raised by their biological mothers (mother-reared) and one that was raised by human experimenters in a nursery of peers (peer-reared; i.e. exposed to early psychosocial deprivation). The **methodology of this wider project is described in Chapter 2**.

Study 1: Chapter 3

This chapter is comprised of a paper that has been submitted to a peer-reviewed journal (Massera et al., *under review*) in which we asked what the longitudinal effects of early psychosocial deprivation are on executive function in rhesus macaques across adolescence into early adulthood, assessed behaviourally using an 'A-not-B' task (Holmboe, 2021; Diamond, 1989). The use of this task allowed for the assessment of both general executive function performance, and via the fitting of a computational model of decision-making, the assessment of specific components of executive function.

It was **hypothesized** that: *i) early psychosocial deprivation will be negatively associated with general executive function;* *ii) that negative effects of early deprivation on executive function will persist across adolescence into adulthood;* and *iii) animals exposed to early deprivation will show specific deficits in working memory and inhibitory control.*



Study 2: Chapter 4

This chapter is comprised of work that will be included in a paper about the longitudinal effects of early psychosocial deprivation on structural brain development implicated in macaque executive function. Anatomical and diffusion MRI/DTI are utilized here to investigate development in structural connectivity (i.e. white matter microstructure) between brain regions involved in executive function circuitry, as well as grey matter thickness in PFC. MRI data was acquired at the same two time-points as the 'A not B' task presented in Chapter 3, and therefore MRI and behavioural measures could be compared across adolescence and adulthood.

It was ***hypothesized*** that: ***i)*** cortical thickness in PFC regions (vlPFC and dlPFC) would decrease over time, but would be greater in the peer-reared versus mother-reared group; ***ii)*** integrity in fronto-parietal and fronto-striatal white matter tracts would increase over time, but would be lower in in the peer-reared versus mother-reared group; ***iii)*** EF measures would be related to cortical thickness in dlPFC and vlPFC and white matter integrity in fronto-parietal and front-striatal tracts, with better EF linked to thinner cortex and higher integrity, respectively; ***iv)*** working memory and response inhibition would be related to both overlapping (e.g. superior longitudinal fasciculus) and distinct patterns of structural connectivity and cortical thickness in these different regions and circuitry; and ***v)*** cortical thickness and white matter integrity would modulate the relationship between psychosocial deprivation and EF across time, with fronto-striatal tracts having greater effects in adulthood compared to adolescence.

CHAPTER 2. METHODS

The work presented in this thesis is part of a wider project investigating the longitudinal effects of early psychosocial deprivation on neurocognitive development in rhesus macaques from pre-adolescence to early adulthood. This project aims to elucidate the mechanisms linking early psychosocial deprivation to specific poor outcomes such as risk for anxiety and symptoms of ADHD, with a focus on self-regulation related processes (e.g. emotion regulation and executive function). To achieve these aims, the project is utilizing a macaque model of early social deprivation, with macaques representing a nonhuman primate species that has similar neurocognitive structure and function to humans, as well as early social organization. A number of different techniques are being used to behavioural and brain development, including naturalistic behavioural observation, standardized behavioural assessments, magnetic resonance imaging (MRI; anatomical, diffusion, and resting-state functional), and genetic and hormonal measures are being collected.

Subjects

The sample is comprised of 21 Rhesus macaque monkeys (*Macaca mulatta*). As infants, half of these animals were reared by their mother (n = 11; 'mother-reared') and half were reared in a nursery of peers (n = 10, 'nursery-reared'); i.e. apart from their mothers and other adults from birth, raised by human caregivers. As such, the nursery-reared group were deprived of typical early psychosocial experiences, which are comprised predominantly of mother-infant interactions in macaques (Maestriperi, 2009; Ferrari et al., 2009). This sample is unique in Europe, and offers an extremely exciting opportunity to look at the effects of early social deprivation in a very well-controlled manner.

As part of the wider project, assessments were made longitudinally at 2.5, 3.5, and 5 years, corresponding approximately to late childhood, adolescence, and early adulthood in macaques (see Fig. 1). We assessed the animals at these ages because they represent key transition points in development when many significant changes related to self-regulation occur. All subjects were housed in a single colony at the Rousset Primatological Station of the CNRS, in semi-free ranging conditions. Groups of 5-6 animals were transferred to the Institut des Sciences Cognitives – Marc Jeannerod (ISC-MJ) in Bron for assessment each year for a number of weeks. At the ISC-MJ, subjects were housed together. All groups contain a balanced mix of mother-reared and peer-reared animals, with groups housed in an indoor enclosure. This enclosure was enriched to allow for meaningful activities and expression of the animals'

full behavioural repertoire. MRI assessments were conducted a minimum of two weeks after this relocation. All subjects returned to the Rousset station after data collection, and all animals were kept alive at the end of the study. All housing and procedures conformed to current guidelines concerning the care and use of laboratory animals (European Community Council Directive No. 86-609), and were approved by our local ethics board, Comité d’Ethique Lyonnais pour les Neurosciences Expérimentales (CELYNE) C2EA #42 (03.10.18), and the French Ministry of Research (10.10.18); project reference APAFIS#15091_2018071014483295_v2. All reporting here conforms to the recommendations in the ARRIVE Guidelines for Reporting Animal Research.

All subjects were born and raised at the *Laboratory of Comparative Ethology at the National Institutes of Health, US*. Peer-reared animals were raised from birth in a nursery with access to same-aged peers; for more details on this rearing protocol see Shannon et al. (1998). At eight months postpartum, mother-reared and peer-reared subjects were placed into a single social group. Rearing procedures were approved by the NICHD and the University of Maryland Animal Care and Use Committee, and adhered to the NIH Guide for the Care and Use of Laboratory Animals. Animals were relocated to the *Rousset Primatological Station, CNRS, France* at two years of age. Although this kind of manipulation of monkeys’ early social experience is being widely prohibited in future research with new infant samples, it was decided that in this case, further study of this particular sample of juvenile animals was the most ethical course of action. Due to the closure of the center in the US where animals were born and lived for the first two years of life, it was agreed that the ISC-MJ would receive these animals rather than allow them to be euthanized for medical research where effects of their early social experiences would not be considered; and consequently, failing to maximize the scientific benefit that could be derived from this existing sample. Every effort has been made to ensure that these animals now live in the most enriching environment possible, including their social environment, and tasks included in the project were designed to be as noninvasive as possible.

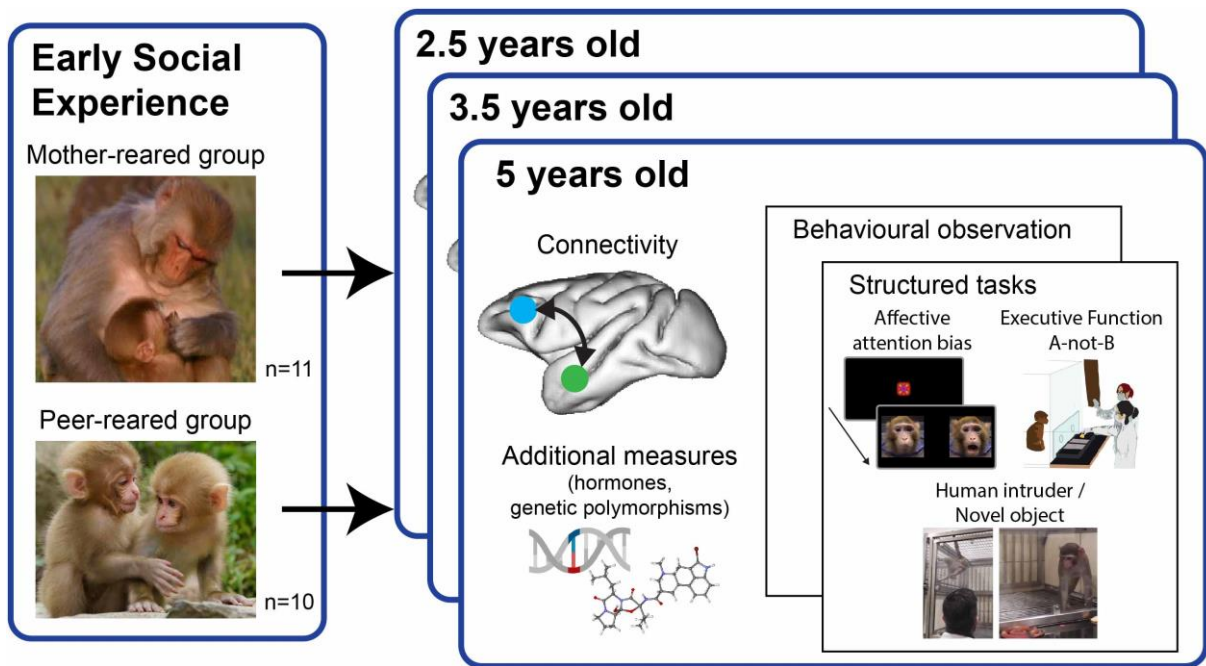


Figure 1: *Project design.* 21 Rhesus macaque monkeys (*Macaca mulatta*) were assessed at three time points (2.5, 3.5, and 5 years) corresponding to late childhood, adolescence, and early adulthood. As infants, half of these animals were exposed to early psychosocial deprivation (i.e. rearing in a nursery of peers by human caregivers) and a half were not (i.e. instead reared by their biological mothers). At each time point, behavioural and biological data were collected.

Behavioural Assessments

Behaviour in the two rearing groups was observed in their everyday social environment over a number of weeks in order to collect data concerning anxiety, social competence, and other relevant behaviours linked to poor outcomes often seen in the context of early psychosocial deprivation (e.g. ‘hyperactivity’). Additionally, the subjects were temporarily separated into a designated area of the home enclosure for several structured behavioral tasks. These tasks were designed to assess the subjects’ responses to affective and potentially threatening stimuli (e.g. an unknown human), and thus differences in emotional reactivity and regulation, as well as executive function.

- 1. Behavioural group observation:** At each of the three project time-points, animals were video recorded (50fps) in their home enclosure (i.e. in the social group) on two days per week (morning and afternoon) for a three-week period (20 minutes per recording). This was for a total of 240 minutes per group. Two cameras were placed in the room

in order to capture a view of the whole enclosure, and recordings from these cameras were synchronized afterwards.

For each animal (i.e. the 'focal animal'), the last 10 minutes of each video were manually coded, with the first 10 minutes constituting a period of habituation to the cameras. A second-by-second coding scheme based on those used in previous studies was used to identify a number of social and non-social behaviours of interest (Pomerantz, 2012; Maestriperi, 2010; Kaburu, 2016). A random 15% of videos were coded by a second researcher at the first time-point to establish reliability. Reliability scores obtained between coders were very strong (i.e. all $\kappa > 0.93$). This included coding of anxiety-like behaviour (more details and related results are presented in Appendix X).

2. Affect-biased attention: At the first time-point (2.5 years), animals were presented with pairs of affective and neutral facial gestures in order to assess attention biases to threat and reward. Each subject was temporarily separated from their social group and placed into the testing area in another section of the room; 87 × 100 x 120cm enclosure with a clear panelled front. Before commencing the task, a widescreen computer monitor (35 × 61cm; 2560 × 1440 resolution) was placed 60cm from the front of the enclosure, and animals were given five minutes to habituate to the enclosure once separated. Note, all animals had already been well familiarized with this process of separation into the testing enclosure and presentation of non-social video stimuli before the day of assessment. Animals were recorded during the task using a webcam (30fps) placed on the top-centre of the monitor.

Animals were presented with pairs of neutral-affective gesture stimuli comprising two conditions: i) Neutral-Threat (five trials per subject); and ii) Neutral-LPS (five trials per subject), i.e. the positive or reward condition. Video pairs were presented for 5s per trial, with condition order and position (left or right) of neutral-affective gesture videos counterbalanced across subjects. Before the stimuli appeared, a moving geometric pattern accompanied by a non-social sound was presented in the centre of the screen to attract the subject's attention, with stimuli presentation then triggered by an

experimenter watching the animal live on a separate monitor (not in view of the subject). Additionally, a calibration procedure was conducted before presentation of experimental stimuli, whereby images of objects (e.g. ball, toy car) were presented on the right, centre, and left of the screen. Each image was jittered up and down slightly to attract attention and was accompanied by a non-social sound. Psychopy v1.90.249 was used for stimulus (calibration and experimental) presentation, with video recording onset and offset automatically triggered at the start and end of each presentation. This sequence and the experimental set-up is illustrated in Fig. 2.

Subjects' gaze (left, right, other, offscreen) was manually coded offline, frame-by-frame, by a researcher blind to the condition being presented and the position of the neutral-gesture stimuli. A random 15% of videos were coded by a second researcher to establish reliability, with very good reliability scores obtained (all $\kappa > 0.84$). More details and related results are presented in Appendix X.

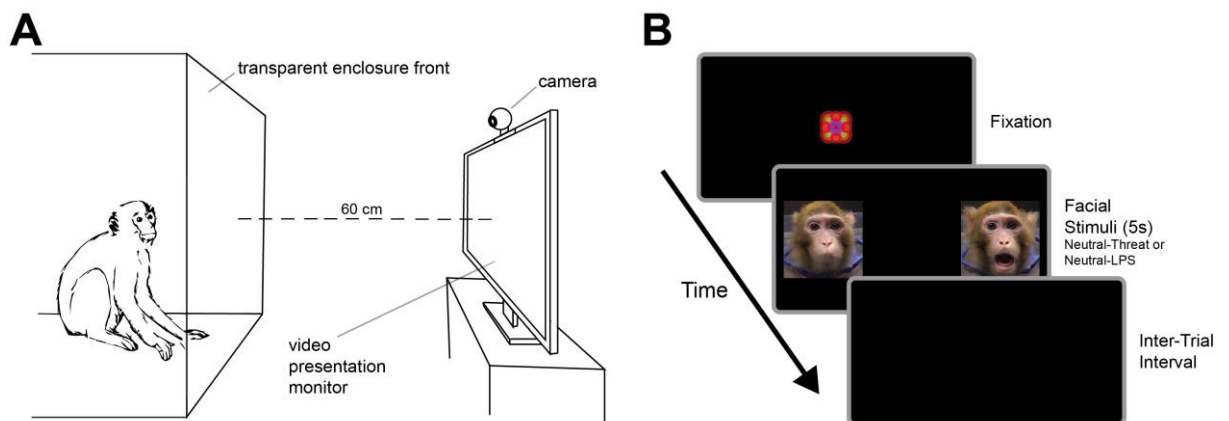


Figure 2: A) Schematic illustration of the gaze bias task set-up; B) Illustration of a trial in the gaze bias task. An experimenter triggered the appearance of fixation and facial stimuli screens when the subject looked towards the monitor.

3. Threat Sensitivity: At all three project time-points, subjects' responses to novel social and non-social stimuli differing in threat level were observed.

- a. **Human Intruder (social task).** A version of the human intruder paradigm (Kalin & Shelton, 1989) was used to assess subjects' affective reactivity to potential social threat; an unfamiliar human (i.e. the 'intruder'). Each subject was

temporarily separated from their social group and placed into the testing area in another section of the room; an 87 × 100 × 120cm enclosure with a clear panelled front. Animals were given five minutes to habituate to the enclosure once separated. Note, all animals had already been well familiarized with this process of separation into the testing enclosure before the day of assessment. Animals were recorded during the task using two cameras (50fps), which were then synchronized offline.

The task comprised four conditions differing in levels of threat to macaque monkeys: i) 'alone', where no intruder was present in the room; ii) 'profile', where the intruder entered the room and stood with their head turned away from the subject to avoid eye contact (low threat); iii) alone, where again, no intruder was present in the room; and iv) 'stare', where the intruder entered the room and stood with their head facing forward and making eye contact with the subject (high threat). In the profile and stare conditions, the intruder stood 1m from the front of the testing enclosure. Each condition lasted 2 minutes, and no other experimenter was in the room during the task. The task set-up is illustrated in Fig. 3.

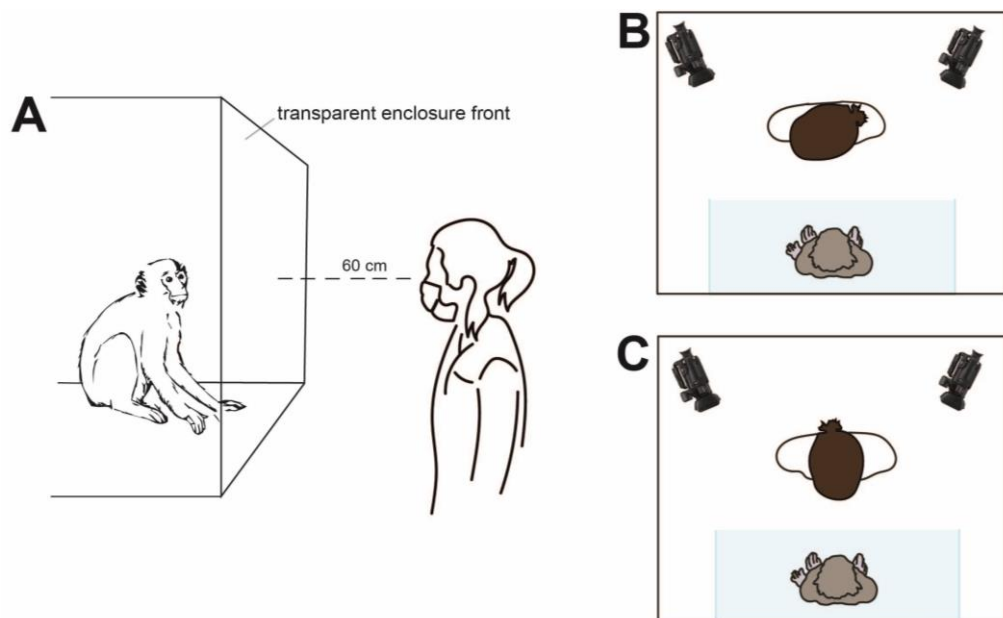


Figure 3: Schematic illustration of the human intruder task set-up; A) Task setting; B) Low threat "profile" condition; C) High threat "stare" condition.

Behaviours of interest such as freezing, aggressive gestures, affiliative gestures in the different conditions were scored second-by-second from the videos by trained coders. One experimenter coded all of the videos, with a second experimenter coding a random 15% at the first time-point to establish reliability (all $\kappa > 0.9$). More details and related results are presented in Appendix X.

b. Novel Object (non-social task)

A version of this paradigm (Howell, 2014) was used to assess subjects' affective reactivity to potential non-social threat; an unfamiliar neutral object (e.g. a cup) or an unfamiliar more threatening object (e.g. a plastic snake). Each subject was temporarily separated from their social group and placed into the testing area in another section of the room; an 87 × 100 × 120cm enclosure with a clear panelled front. Animals were given five minutes to habituate to the enclosure once separated. Note, all animals had already been well familiarized with this process of separation into the testing enclosure before the day of assessment. Animals were recorded during the task using two cameras (50fps), which were then synchronized offline.

There were two holes in the transparent front panel of the testing area that allowed subjects to access objects on a table outside the enclosure; though this were not large enough to enable animals to bring objects inside the testing area. One experimenter was present during the task. The task comprised three conditions differing in levels of threat to macaque monkeys: i) 'food alone', where a piece of food was placed on one side of the table (baseline); ii) 'neutral', where a neutral unfamiliar object was placed on the table on the opposite side to a piece of food; and iii) 'threat', where a threatening unfamiliar object was placed on the table on the opposite side to a piece of food. The position of both the food and the objects was counterbalanced for each subject. The first condition was always 'food alone', while the order of 'neutral' and 'threat' conditions was counterbalanced for each subject. Each condition

lasted 2 minutes. At the beginning of each of the three conditions, the experimenter moved forward to the table in front of the testing area, always looking down, and placed the food or the food and object (simultaneously) on the table. From that moment the 2 minutes for that condition began, and the experimenter stepped back and remained in a still position with their head down and arms crossed. The task set-up is illustrated in Fig. 4.

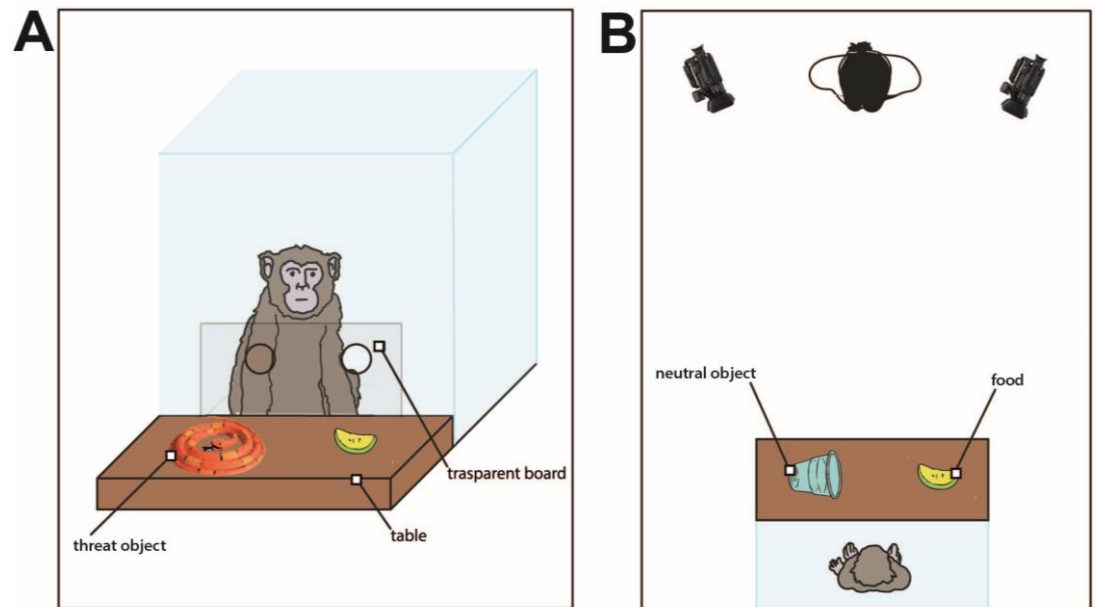


Figure 4: Schematic illustration of the novel object task set-up; A) Setting with example of threat condition; B) Setting with example of neutral object condition.

4. Executive Function: At time-points two and three (3.5 and 5 years), an 'A not B' task adapted from the human developmental literature (Holmboe, 2008; Diamond, 1989) was used to assess EF. Each subject was temporarily separated from their social group and placed into the testing area in another section of the room; an 87 × 100 × 120cm enclosure with a clear panelled front. Animals were given five minutes to habituate to the enclosure once separated. Note, all animals had already been well familiarized with this process of separation into the testing enclosure before the day of assessment. Behaviour was scored live, with animals also recorded during the task using one camera (50fps).

There were two holes in the transparent front panel of the testing area that allowed subjects to access objects on a table outside the enclosure. A box with two wells and sliding doors was placed on the table. Subjects were familiar with the process of being placed in the testing area and with the equipment used in the task. Two experimenters were present during the task. The task set-up can be seen in Fig. 5.

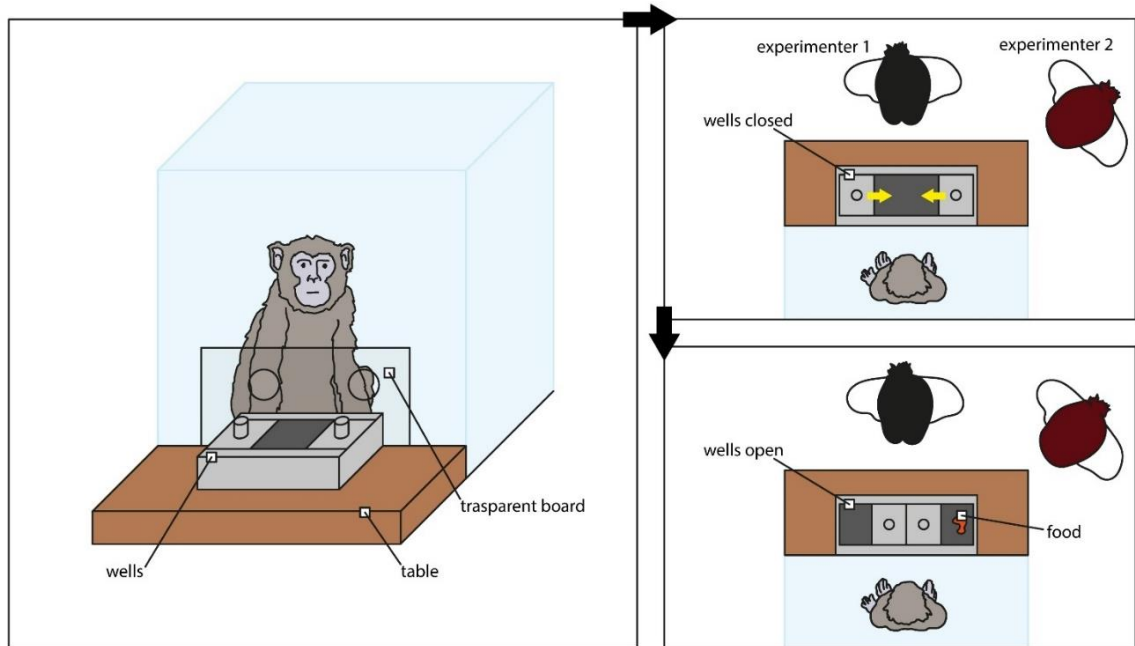


Figure 5: *Task set-up.* A frontal view of the test enclosure is illustrated on the left. Two views from above are illustrated on the right: upper right, the wells are closed with the yellow arrows indicating a sliding mechanism; upper left, the wells are open and a piece of food is placed in one of them.

In each trial, an experimenter presented a piece of food to the subject, and then placed it in one of the wells and covered both wells with the sliding doors. The trial only proceeded if the subject looked at the food when it was presented and when it was hidden. After a delay period, during which the subject's physical and visual access to the wells was blocked, the subject was allowed to reach for the wells. If the subject reached for the correct well, they were allowed to eat the food, otherwise, the correct well was opened to show them the food. If the subject touched both wells, didn't respond, or the response was unclear, the trial was repeated. The location of the food was changed after the subject reached for the correct well on two consecutive trials. The subjects completed two testing sessions at each assessment time point, with a maximum of 25 trials per session. The delay period was adjusted based on the subject's

performance. The trial responses were live-coded by one experimenter. More details and results are presented in Chapters 3 and 4.

Magnetic Resonance Imaging (MRI)

To investigate changes in brain anatomy and both structural and functional connectivity, magnetic resonance imaging (MRI) data were collected at each project time point (2.5, 3.5, and 5 years) using a 3T Siemens Prisma MR scanner. The subjects were separated from their social group for a total of approximately 5 hours to undergo the scanning process. This time period included 45 minutes for preparation, including anaesthesia induction, intubation, and positioning in the scanner, 3 hours of active scanning, and 1 hour of post-anaesthesia recovery. During the scan, the subjects were positioned in a supine position and anaesthetized with isoflurane (1.5%/2% isoflurane, intubation). A stereotaxic frame was employed to secure the head and prevent movement in order to mitigate motion artifacts. A Vitamin E capsule was placed on the right temple to serve as a marker for the right brain hemisphere. Alphaxalone was administered for anaesthesia induction, and NaCl was administered intravenously for hydration during the scan. We used local anesthetic cream (Anesederm) in the external ears conduct prior the installation of the macaque in the stereotaxic frame. The subjects were also placed under an MRI-compatible heating blanket to maintain body temperature. Physiological measures such as heart rate, temperature, and blood oxygenation were continuously monitored and maintained in accordance with veterinary protocols throughout the scan. Upon completion of the scan and full recovery from anaesthesia, the subjects were returned to their social group.

More information and results are presented in Chapter 4 and Appendix A and C.

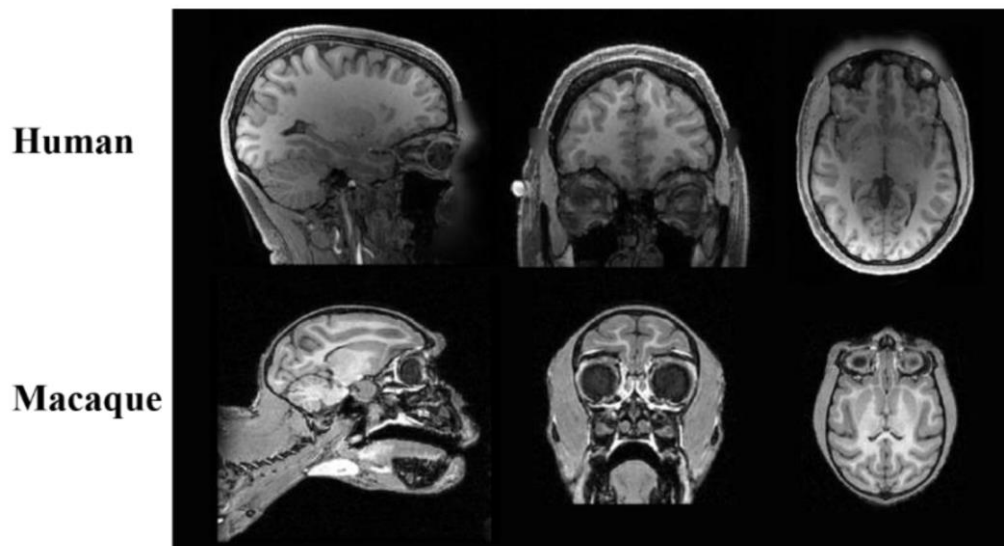


Figure 6: Example of an anatomical T1 scan in axial, coronal, and sagittal views in both humans and macaques (Wang, 2022).

Three types of scan were collected:

- **High resolution anatomical MRI (T1/T2) scans** were obtained in order to look at brain morphology: MPRAGE T1-weighted images, 0.5mm isotropic, a bandwidth of 250Hz, a TR of 3000ms, a TE of 3.62ms, a TI of 1100ms, a flip angle of 8° and 144 cross-sections, and SPACE T2-weighted images with a spatial resolution of 0.5mm isotropic, a bandwidth of 710Hz, a TR of 3000ms, a TE of 366ms, a flip angle of 120° and 144 cross-sections acquired.
- **High resolution diffusion MRI scans** were acquired to investigate brain microstructure: custom pulse sequence based on a segmented 3-dimensional EPI sampling of Fourier space with the following parameters; diffusion weighted images (b-value of 1000s/mm²), 46 directions diffusion gradient encoding with two B₀, a spatial resolution of 0.8mm isotropic, a bandwidth of 776Hz, a TR of 750 ms, a TE of 71ms, 4 segments, a FOV of 105x125x56mm³ and an acquisition time of 60 minutes 38 (Tounekti et al., 2018).
- **Resting-state functional MRI scans** were obtained to investigate functional brain connectivity: optimized gradient-echo T2*-weighted Echo Planar Imaging (EPI) sequence, in partially parallel acquisition mode with foot-head acceleration direction

and an acceleration factor of 2 (Grappa 2). The field of view (FOV) of read-out will be 105mm and that of phase 95mm. The repetition time (TR) were 2000ms and the voxel resolution 1.27x1.27x1.27mm. Molday ION, a contrast agent, were injected intravenously prior to the resting-state functional MRI scans at the quantity of 11mg/kg to enhance the signal-noise ratio and have a better spatial resolution for the analysis.

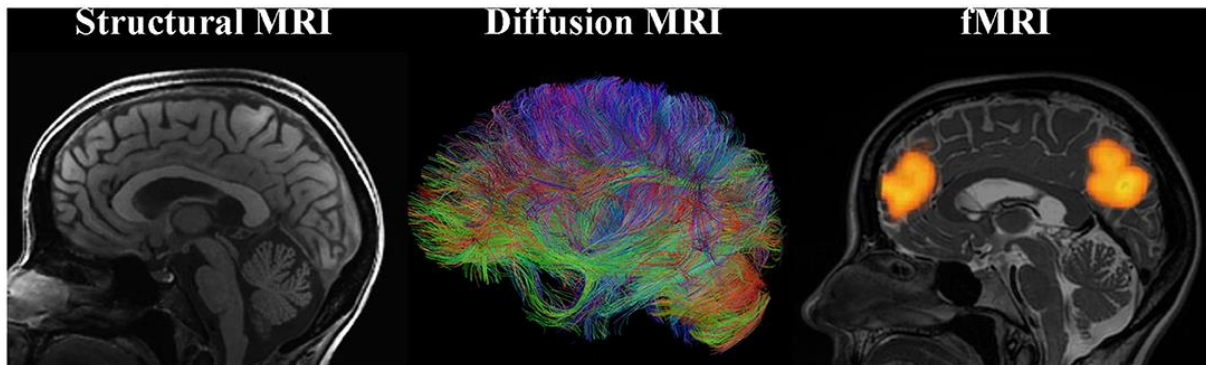


Figure 7. Example of structural, diffusion, and functional MRI images in humans. (Olga Tymofiyeva, 2021)

Endocrine and genetic assessments

At each project time-point, blood and hair samples were collected after anesthesia induction for the MRI acquisition. These were collected so that relevant endocrine and genetic measures could also be considered (e.g. genetic polymorphisms of the serotonin transporter gene and hair cortisol levels, both of have been linked to emotion dysregulation).

- 1. Blood sampling:** Blood samples (4ml/kg) were collected while the animals were sedated, and were then stored in a refrigerator at -80 degrees Celsius prior ready for processing.
- 2. Hair sampling:** Hair samples were obtained by shaving the subject's fur with an electric razor from the back of the neck (to the ears) and stored in a refrigerator at -20 degrees Celsius.

This thesis

The focus of the research presented in this thesis is on the development of executive function across adolescence in rhesus macaques exposed to psychosocial adversity, as well as the structural measures of brain development that may modulate executive function in this

context. Executive function is a key predictor of academic, socio-emotional, and occupational success, as well as risk for psychopathology, and is thought to rely on a sensitive period of brain development characterized by maturation of the prefrontal cortex (PFC) and related networks. Results are presented in two separate studies (Chapters 3 and 4).

Chapter 3 comprises a submitted paper (Massera et al., *under review*) in which the longitudinal effects of early psychosocial deprivation on executive function in rhesus macaques is investigated. The study utilized the 'A-not-B' task described above to assess general executive function at 3.5 and 5 years, with the fitting of a computational model of decision-making also used to functionally decompose the precise EF mechanisms (i.e. working memory and inhibitory control) that may underlie task performance. In Chapter 4, the longitudinal relationship between these executive function measures and structural measures of brain development in the PFC and in fronto-parietal and fronto-striatal white matter tracts (i.e. crucial circuitry implicated in components of executive function) using anatomical and diffusion MRI.

Some additional findings relating to emotion regulation and anxiety are also included in Appendix X, as well the modulating role of structural brain connectivity. This includes a published paper based on behavior at the first time-point (Rayson et al., 2021), and preliminary results involving diffusion MRI. In Appendix C, 4 posters are also presented involving preliminary resting-state fMRI results on amygdala whole brain connectivity in relation to anxiety, as well as pulvinar whole brain connectivity. Posters on behavioural results are also included in this appendix.

Note, the MRI portion of this worked was completed in collaboration with the research teams of Bassem Hiba and Suliann Ben Hamed. All work involving anatomical and diffusion MRI was led by Bassem Hiba; Hind Errame, with the help of Nathalie Richard and Yann Bihan-Poudec, preprocessed all the data and performed some of the analyses presented in Chapter 4 under the supervision of Dr Hiba. All work involving resting-state MRI was led by Dr Ben Hamed; Simon Clavengier and Mathilda Froesel preprocessed and analysed the data. Both teams were involved in MRI acquisitions, with Mathilda Froesel, Maeva Garcoin under the supervision of Dr Ben Hamed, and Yann Bihan-Poudec, under the supervision of Dr Hiba.

CHAPTER 3. Behavioural effect of early psychosocial deprivation: Executive function

LONGITUDINAL EFFECTS OF EARLY PSYCHOSOCIAL DEPRIVATION ON MACAQUE EXECUTIVE FUNCTION: EVIDENCE FROM COMPUTATIONAL MODELLING

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ABSTRACT

Executive function (EF) describes a group of cognitive processes underlying the organization and control of goal-directed behaviour. Environmental experience appears to play a crucial role in EF development, with early social adversity often linked to EF impairment. However, many questions remain concerning the developmental trajectories of EF after exposure to adversity, especially concerning specific mechanisms. Accordingly, using an 'A-not B' paradigm and a macaque model of early social adversity, we investigated how early psychosocial deprivation influences EF development longitudinally from adolescence into early adulthood. The contribution of working memory and inhibitory control mechanisms were examined specifically via the fitting of a computational model of decision making to the choice behaviour of each individual. As predicted, peer-reared animals (i.e. those exposed to early adversity) performed worse than mother-reared animals across time, with the fitted model parameters yielding novel insights into the functional decomposition of group-level EF differences underlying task performance. Results indicated differential trajectories of inhibitory control and working memory development in the two groups. Such findings not only extend our knowledge of how psychosocial deprivation influences EF longitudinally, but also provide support for the utility of computational modelling to elucidate specific mechanisms linking early social adversity to long-term poor outcomes.

Executive function (EF) refers to a group of cognitive processes that underlie the organization and control of goal-directed behaviour. One prominent theory defines EF as a construct comprising a number of interrelated, but distinct, components (1). These include working memory, inhibitory control, and cognitive flexibility, with some common underlying processes connecting them (2). EF develops rapidly between birth and two years of age, with steady development then continuing into early adulthood (3,4). Such protracted development suggests continued plasticity of EF, as does the extended developmental trajectory of neural networks supporting EF (e.g. fronto-parietal network) (3,5,6). Critically, individual differences in childhood EF are related to later social competence, academic performance, and occupational functioning (7–9), as well as risk for interpersonal problems and risky behaviour during adolescence and adulthood (10,11). EF deficits are also characteristic of a number of neurodevelopmental disorders, including attention deficit/hyperactivity disorder (ADHD) and autism spectrum disorders (ASD) (12–14). However, despite the clear importance of EF for healthy development (15), many questions remain concerning the factors underlying different developmental trajectories of EF, and thus the individuals most at risk for poor outcomes.

Evidence is growing for early environmental experience playing an important role in EF development. Indeed, early social adversity has been consistently linked to EF impairments in childhood (e.g. (16–18)), with early psychosocial deprivation, a specific form of early social adversity involving removal of an infant from their primary caregivers, having a particularly profound effect on EF components such as working memory and inhibitory control (19–22). Relevantly, increased risk for ADHD is also associated with early psychosocial deprivation (23,24), as is risk for symptoms such as disinhibited social engagement and repetitive and stereotyped behaviour (25). A lack of complexity and socio-cognitive stimulation in the early social environment, along with increased levels of stress, could explain why early psychosocial deprivation has such a significant impact on EF. For example, this can lead to excessive and less selective pruning of synapses in the cortex, including prefrontal regions implicated in higher cognitive abilities (54). In support of this, deprivation has regularly been associated with a generalized reduction of grey matter in prefrontal, parietal, and temporal cortical regions (55).

The few longitudinal studies conducted on this topic suggest that poor EF associated with early psychosocial deprivation in early life can persist into adolescence and adulthood (21,24,26,27), but as most studies only evaluated EF at one time-point (19,20,28,29), many questions about long-lasting effects on EF and the neural mechanisms underlying this relationship across time remain unanswered. Longitudinal research is now essential for bettering our understanding of how various aspects of cognition develop in terms of both typical and atypical trajectories. The tracking of EF across adolescence into adulthood will be a particularly key period of transition for future examination in studies of psychosocial deprivation. Adolescence is a period of heightened brain plasticity defined by

significant development of prefrontal cortex (38,39), as well as important refinements of EF (6). As such, adolescence may be a particularly useful target for interventions aimed at preventing or ameliorating adverse effects of early deprivation on cognitive development.

The use of a nonhuman primate (NHP) model such as rhesus macaques (*Macaca mulatta*) could be very helpful to address outstanding questions about the effects of early psychosocial deprivation on EF across development. Macaque monkeys are one of the closest species to humans in terms of genetics, physiology, and behaviour, and have an extended period of development comprising distinct infant, juvenile (pre-adolescent and adolescent), and adult stages. Like humans, their early social environment is also comprised predominantly of mother-infant interactions. Notably, the use of a macaque model can help address some limitations defining developmental studies with humans: e.g. lack of control over the early environment, very long developmental time-frames, and difficulty in tracking neural mechanisms underlying development. Although limited, research focused on EF development in macaques suggests that it parallels that in humans (30–33), and in adult macaques, EF relies on the same cortical networks (34,35). Limited research focused on early psychosocial deprivation effects on cognition in infant macaques have produced mixed findings (e.g. (36,37)), with research comparing deprived versus non-deprived animals especially rare. Discrepancies in findings concerning effects of early deprivation on cognitive ability may stem from differences in age at assessment and use of different measures at different time-points, and no studies thus far have focused on EF specifically.

A classic paradigm used to investigate EF in human infants and young children is the ‘A-not-B’ task (40), which is thought to measure two EF components: inhibitory control and working memory (41–43). Modified versions of the ‘A-not-B’ task have also been used to investigate EF in trained adult macaques. Macaque behaviour during the task is comparable to human performance (41,46). No previous research has used an ‘A-not-B’ task to look at macaque EF development or effects of early psychosocial deprivation, though a version such as that used with human infants could offer a valuable way to test EF without removal of the animals from their home enclosure and extensive training. A data collection approach that requires these two elements is starting to be adopted by other researchers (37,47), who argue that it enables a more direct comparison of cognitive development in macaques exposed to early psychosocial deprivation versus non- exposed macaques.

Although the ‘A-not-B’ task is assumed to involve both working memory and inhibitory control mechanisms, it has been recently argued that these components are difficult to disentangle using classic measures of response accuracy according to condition (48,49). Developing tasks that do not rely on instruction in order to assess specific EF components in infancy and early childhood (48,49) is very

challenging, but another option to help isolate mechanisms underlying behaviour in the 'A-not-B' task is to use computational modelling. Specifically, using a computational model of decision making to functionally decompose choice behaviour into working memory and inhibitory control mechanisms would enable both examination of these specific EF components, as well as a way to evaluate how well previously utilized 'A-not-B' performance measures actually assess these components.

Accordingly, the current study was designed to investigate the longitudinal effects of early psychosocial deprivation on EF in rhesus macaques using a computational modelling approach. We assessed EF in a rare sample of macaques comprised of two groups that differed in exposure to early psychosocial deprivation; one mother-reared and one peer-reared (i.e. exposed to early psychosocial deprivation; separated from their mothers and other adults at birth and raised in a nursery of peers by human caretakers); at two time-points corresponding to adolescence (3.5 years) and early adulthood (5 years). We used a version of the 'A-not-B' paradigm often used with human infants (43), with animals completing the task in a section of their home enclosure. The choice behaviour of each animal at each time-point was fit with a stochastic computational model of decision-making based on a weighted sum of exponentially decaying working memory and choice history influences. Choice history correspond to inhibitory control, the tendency to suppress previous responses or to repeat them. We then compared the fitted model parameters between groups and across time, as well as to previously utilized performance measures (43,50). We predicted that peer-reared animals would perform worse than mother-reared animals on the task at both time-points, and that both working memory and inhibitory control would be poorer in the peer-reared group.

METHODS

Subjects

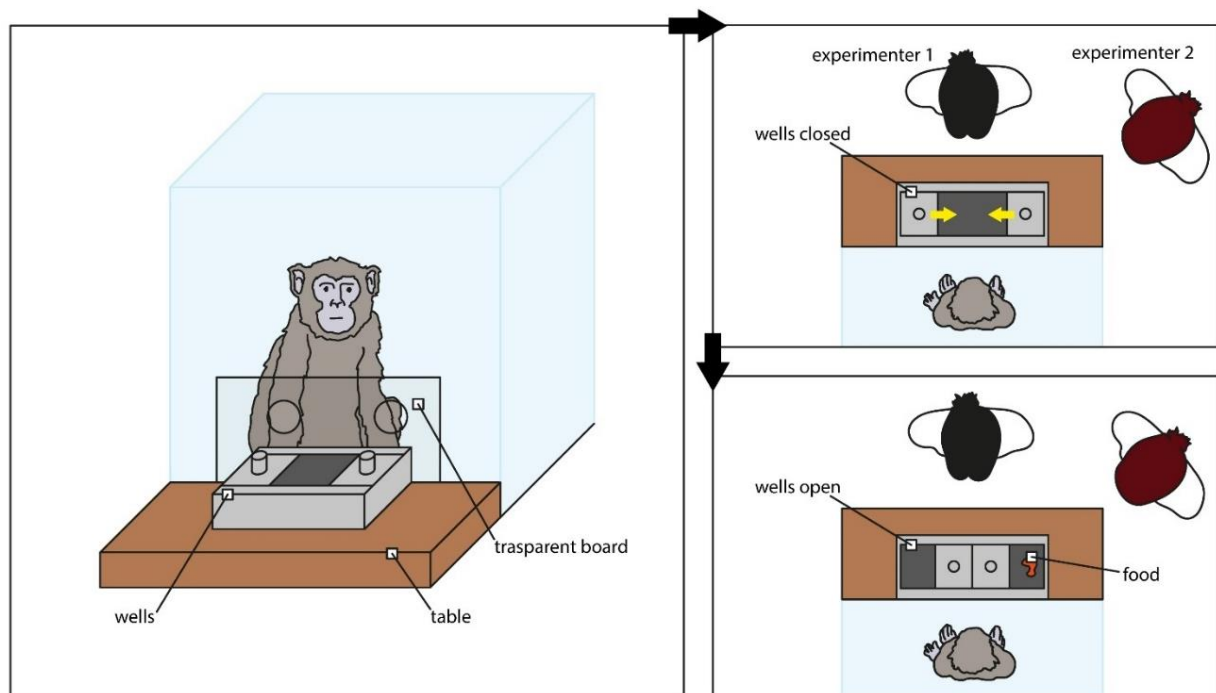
The sample consisted of 21 Rhesus macaque monkeys (*Macaca mulatta*), 11 mother-reared (five female) and 10 peer-reared (six female). Subjects were aged around 3.5 years at the first assessment time-point (mother-reared; M = 1368 days, SD = 104 days: peer-reared; M = 1363 days, SD = 101 days) and 5 years at the second assessment time-point (mother-reared; M = 1806 days, SD = 100 days: peer-reared; M = 1809 days, SD = 95 days). Subjects were housed at the *Institut des Sciences Cognitives Marc Jeannerod*, CNRS, during the assessment period in mixed mother- and peer-reared social groups of 5–6 animals (see SI for rearing protocol) Although new instances of maternal separation in monkeys' for research is largely and rightly now being prohibited, it was decided that further study of this particular sample of juvenile animals was the most ethical course of action. Due to the closure of the center in the US where animals were born and lived for the first two years of life, it was agreed that our team at

the ISC-MJ would receive these animals rather than allow them to be euthanized for medical research whereby effects of their early social experiences would not be considered; and consequently, would fail therefore to maximize the scientific benefit that could be derived from this existing sample. Every effort has been made to ensure that these animals now live in the most enriching environment possible, including their social environment, and the tasks included in the current study was designed to be as noninvasive as possible. All housing and procedures conformed to current guidelines concerning the care and use of laboratory animals (European Community Council Directive No. 86-609), and were approved by our local ethics board, Comité d’Ethique Lyonnais pour les Neurosciences Expérimentales (CELYNE) C2EA #42 (03.10.18), and the French Ministry of Research (10.10.18); project reference APAFIS#15091_2018071014483295_v2. All reporting here conforms to the recommendations in the ARRIVE Guidelines for Reporting Animal Research.

‘A-not-B’ task set-up and procedure

Each subject was temporarily separated into the testing area, which was a section of the home enclosure; an 87 × 100 × 120cm area with a clear-paneled front. All equipment required for the task was installed before separating the subject. Note, all animals had already been well familiarized with this process of separation into the testing area and with the task equipment.

The task set-up included two holes in the clear-panelled front of the testing area which enabled the subject to reach objects placed on a table outside the enclosure (Figure 1). A transparent board was used to block these holes at times when the subject was not allowed to reach (i.e. during the hiding and delay portions of the task; see Procedure section). A box with two wells and two sliding doors to



cover the wells was positioned centrally on the table in front of the two holes. The wells were 11.2 cm in diameter and were positioned 18 cm apart. Two experimenters were present for the entire task. Experimenter 1 stood facing the subject, while Experimenter 2 stood on one side of the table, with the side counterbalanced across subjects and sessions.

Figure 1: *Task set-up.* A frontal view of the test enclosure is illustrated on the left. Two views from above are illustrated on the right: upper right, the wells are closed with the yellow arrows indicating a sliding mechanism; upper left, the wells are open and a piece of food is placed in one of them.

To begin a trial, Experimenter 1 showed a piece of food to the subject in the centre of their visual field (Figure 2A). Once the subject looked at the food, Experimenter 1 placed the food in one of the wells and closed the two sliding doors to cover both wells. The trial only proceeded if the subject looked at the food when it was presented and when it was hidden. As soon as the wells were covered, a delay period started. During this delay, as well as physical access to the wells being blocked by the transparent board, Experimenter 2 blocked the subject's visual access to the wells with an opaque screen placed between the box and the enclosure front. At the end of the delay, the opaque screen and the transparent board were removed, allowing the subject to reach for the wells. If the subject reached for the correct well, the experimenter allowed the subject to eat the piece of food (Figure 2B). If the subject reached for the incorrect well, the experimenter opened the correct well to show the position of the food to the subject (Figure 2C). If the subject touched both wells, did not respond, or the response was unclear, the trial was repeated. The food was hidden in the same location (left or right well) until the subject reached for the correct well on two consecutive trials. After two consecutive correct trials, Experimenter 1 changed locations and hid the food in the other well (i.e. it was a 'change' trial). After the change trial, the experimenter then repeated this sequence but on the same side as the preceding change trial. For the first trial of each session, the delay period when visual and physical access to the wells was blocked lasted 2s, and then depending on the subject's performance, it was increased or decreased in subsequent trials. The delay period was decreased by 2s if two consecutive trials were incorrect, and was increased by 1s if two consecutive change trials were correct (43,50). Each subject completed two testing sessions at each assessment time point, with a maximum of 25 trials per session (i.e. 50 trials total per assessment time-point). During each session, Experimenter 1 live-coded correct and incorrect trials, and animals were also video recorded throughout the session with a camera placed to capture a view of the enclosure and Experimenter 1.

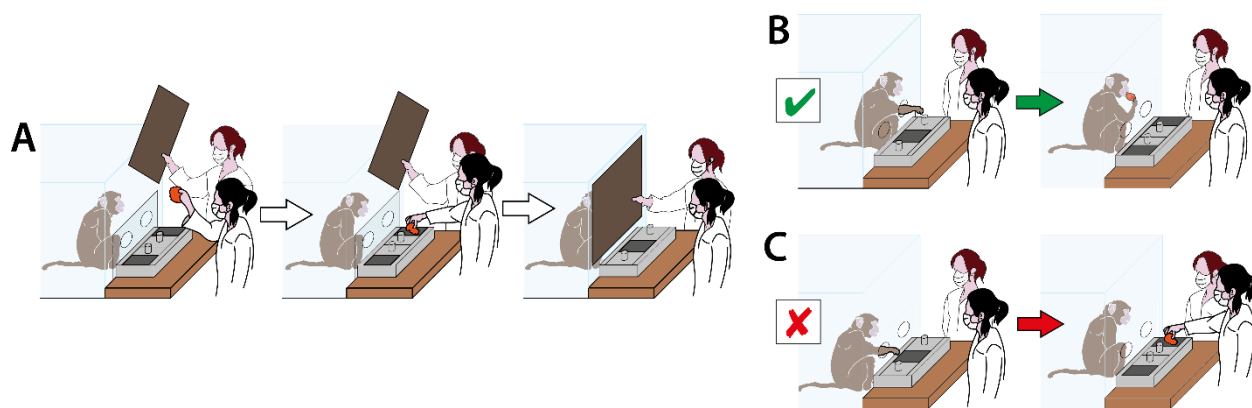


Figure 2: Task procedure. The series of images illustrates the sequence of a single trial. In the first three images (A) Experimenter 1 shows the food to the subject, places it in the well, and Experimenter 2 blocks the vision of the subject during the delay period. The top right images (B) illustrate a “correct” choice, with the subject reaching for the well where the food was hidden and then eating the food. The bottom right images (C) illustrate an “incorrect” choice, with the subject choosing the well containing no food and Experimenter 1 then highlighting where the food was actually hidden.

Control task set-up and procedure

At the second assessment time-point, all subjects also completed a control task to verify that results from the original version of our ‘A-not-B’ task were not a consequence of the structure of the task. In the original version, the length of the delay period depended on the performance of the subject, with poorly performing subjects not being tested at higher delays. Moreover, if the subjects were always correct, they could have possibly learned the pattern of the hiding locations (e.g. A, A, B, B, B, A, A, B). Therefore, while the task set-up was the same as in the original ‘A-not-B’ task, the procedure was different. In this new randomized control version of task, the position of the food hiding location (i.e. left or right well) and the length of the delay period were pseudo-randomized across trials. The hiding position could not be the same for more than five consecutive trials, and the delay period was maximum of five seconds. All subjects completed the same number of trials with a specific delay in a specific location (e.g. 3 seconds, right well). Each subject completed two sessions, with a maximum of 24 trials per session (i.e. 48 trials total). With this version of the task, then, all animals were tested equally on all the different possible delays (i.e. from a 0 second delay to a 5 second delay).

Video coding

A number of parameters were coded offline from the video recordings made of each subject during the testing sessions, including the actual length of delay periods (which may have varied slightly from the intended delay period) in seconds and the inter-trial-interval (ITI) lengths in seconds (see SI).

Computational model

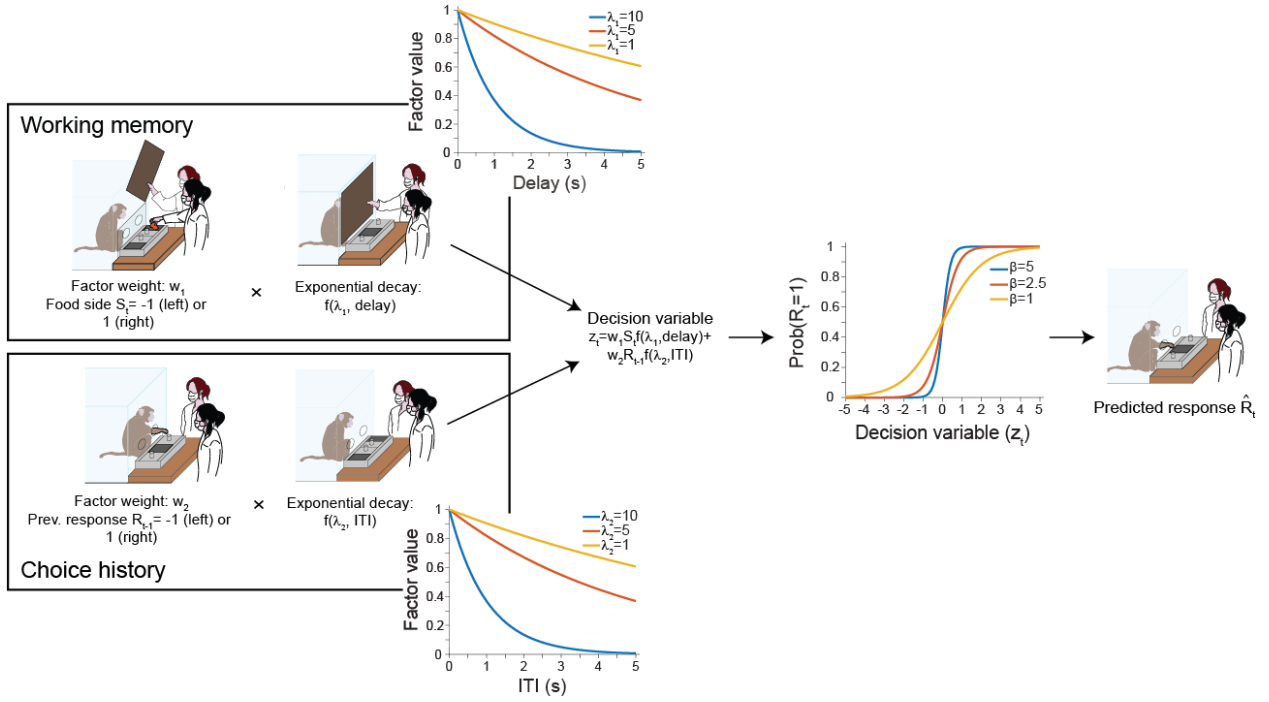


Figure 2: Model architecture. The model included a decaying working memory trace of the food location (top left, the plot shows the influence of decay rate: with higher decay rates, the influence of working memory diminishes faster), and a decaying trace of the previous choice (bottom left, the plot shows the influence of decay rate: with higher decay rates, the influence of the previous choice diminishes faster). The weighted sum of these influences is used to compute the probability of choosing one side or the other using a softmax function (the plot shows the influence of the inverse softmax temperature parameter: at higher values, the decision is more deterministic).

A computational model of decision-making was fitted to the decision behaviour of each subject (chosen out of eleven candidate models based on model comparison; see SI). The model included two influences on the decision to choose the left or right side (Figure 2). The first influence was a decaying working memory trace, m , of where the food was hidden, modeled as an exponential decay function according to the delay on trial t , d_t :

$$m_t = w_1 S_t e^{-\lambda_1 d_t}$$

where w_1 is the weight of this factor when the delay is 0, S_t is the side that food was hidden on in trial t (left = -1, right = 1), and λ_1 is the working memory decay rate. The second influence was that of the previous two choices, p , modeled as an exponential decay function according to the inter-trial interval before trial t , i_t :

$$p_t = w_2 (R_{t-1} e^{-\lambda_2 (i_t + d_t)} + R_{t-2} e^{-\lambda_2 (i_{t-1} + d_{t-1} + i_t + d_t)})$$

where, w_2 is the weight of this factor when the inter-trial interval and delay is 0, R_{t-1} is the response made on the previous trial (left = -1, right = 1), and λ_2 is the decay rate. Positive values of w_2 therefore

represent a tendency to repeat the previous choice, whereas negative values represent a tendency to suppress the previous choice, via different degrees of inhibitory control.

The decision variable, z , was computed as the sum of these two factors, m and p , with negative values of representing a tendency to choose the left side, and positive values a tendency to choose the right side. The decision variable was then transformed into the probability of choosing the right side on trial t :

$$P(R_t = 1) = \frac{1}{1 + e^{-\beta z_t}}$$

The inverse softmax temperature, β , determines the steepness of the softmax function, and thus how sensitive the choice probability is to z . We refer to this parameter as representing ‘choice stochasticity’.

The model was fit separately to each subject’s choice behaviour using maximum log-likelihood estimation in Matlab (v R2018a). To avoid local maxima, a grid search over all parameter values was used to initialize model parameters before fitting. The weight of the working memory factor, w_1 , was restricted to the range [0, 1], and the weight of the choice history factor, w_2 was restricted to [-1, 1] in order to detect both a tendency to inhibit or repeat the previous choice reflecting different degrees of inhibitory control. The decay rate parameters, λ_1 and λ_2 , and the inverse softmax temperature, β , were constrained to be in the range [0,10].

Data analysis

Before analysis, we checked if any coded behaviours that occurred during either a trial or the ITI were affecting the ability of the model to predict the responses of the subjects. We found that locomotion during trials was the only behaviour negatively impacting the model’s prediction accuracy, and therefore we excluded all the trials in which locomotion occurred (see SI). We also removed trials in which the ITI was more than 2.5 standard deviations above the mean, and we excluded subjects with less than 25 remaining trials (one mother-reared subject from both the original task at the first time-point and the randomized control version at the second time-point). We then checked if there was a difference in ITI duration between the two rearing groups, and we found no difference (see SI).

To compare performance on the original ‘A-not-B’ task between the two rearing groups over time, we computed three measures based on previous literature (e.g. (43,50)): i) percentage of correct responses (i.e. proportion of correct trials out of all trials completed); ii) cumulative score (the sum of successful change trial delays divided by the number of total trials completed); and iii) maximum delay reached. These measures were computed for each subject at each assessment time-point. The percentage of correct responses was also computed for the randomized control version of the task

completed at the second time-point. For percentage of correct responses, we conducted a generalized linear mixed model with group (mother-reared/peer-reared), time-point (3.5/5 years), and their interaction included as fixed effects, and subject, time-point, and session as nested random intercepts. For the cumulative score and maximum delay, we conducted a linear mixed model with group, time-point, and their interaction included as fixed effects, and subject-specific random intercepts. To compare the percentage of correct responses in the original task versus the randomized control version at the second time-point, we conducted a generalized linear mixed model with group, task (original/control), and their interactions included as fixed effects, and subject, task (original/control), and session as nested random intercepts.

The computational model was fitted separately for each subject at each assessment time-point, and for the two versions of the tasks. To compare the fitted model parameters between the two rearing groups across time, we used linear mixed models with group, time-point, and their interactions included as fixed effects, and subject-specific random intercepts. To compare the fitted parameters between the two rearing groups and tasks at the second time-point, we used linear mixed models with group (mother-reared/peer-reared), task (original/control), and their interactions included as fixed effects, and subject-specific random intercepts. To investigate whether the fitted model parameters were related to task performance, and thus to what extent the performance measures reflected involvement of working memory and inhibitory control, we used separate linear mixed models for each model parameter and performance measure in the original version across time. For percentage of correct responses, cumulative score, and maximum delay, we computed linear mixed models with model parameter (i.e. w_1 , λ_1 , w_2 , λ_2 , or β), group (mother-reared/peer-reared), time-point (3.5/5 years), and group by time-point interaction included as fixed effects, and subject-specific random intercepts. We then used separate linear mixed models for each model parameter and performance measure across tasks at the second time-point. For the percentage of correct responses, we fit linear mixed models with model parameter (i.e. w_1 , λ_1 , w_2 , λ_2 , or β), group (mother-reared or peer-reared), task (original/control) and group by task interaction included as fixed effects, and subject-specific random intercepts.

R v4.0.5 (52) was utilized to conduct all analyses presented here (see SI for package information). P-values for fixed effects and interactions were obtained using Type III Wald χ^2 tests for generalized linear models. To account for smaller sample sizes and normality violations, permutation tests (grouped by subjects) with 10,000 permutations were used to assess significance of linear model factors and to follow-up significant interactions.

RESULTS

Task performance

We first sought to determine if task performance varied between groups and time-points by comparing each of the performance measures (percentage of correct responses, cumulative score, and maximum delay) between groups and time-points for the original version of the 'A-not-B' task. A significant main effect of group was revealed for the percentage of correct responses [$\chi(1) = 9.462$; $p < 0.01$], with a higher performance for the mother-reared compared to peer-reared group (Figure 3A). No significant main effect of time-point or group by time-point interaction was found. A significant main effect of group was also revealed for a cumulative score [$F(1) = 12.643$, $p < 0.01$], with a higher score for mother-reared compared to peer-reared group (Figure 3B). No significant effect of time-point or group by time-point interaction was found. Finally, a significant main effect of group was found for the maximum delay [$F(1) = 17.721$, $p < 0.001$], with a higher delay for mother-reared compared to peer-reared group (Figure 3C). Again, no significant effect of time-point or group by time-point interaction was revealed.

Having established that the mother-reared group had higher performance on the 'A-not-B' task than the peer-reared group at each time-point, we then compared their performance between the original 'A-not-B' task and the random control task at the second time-point. A significant main effect of group was found [$\chi(1) = 6.323$; $p = 0.011$], with a higher percentage of correct responses for the mother-reared compared to peer-reared group (Figure 3D). No significant main effect of task or task by group interaction was revealed.

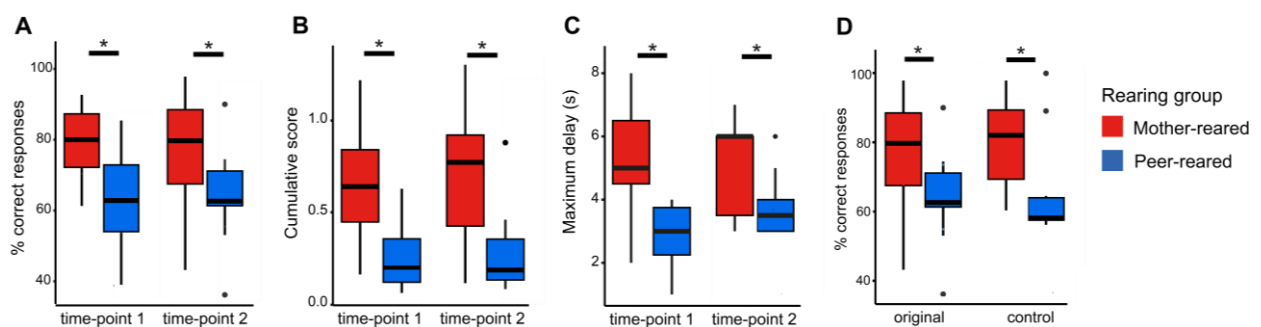


Figure 3. Task performance. The mother-reared group had a significantly higher percentage of correct responses (A), cumulative score (B), and maximum delay (C) in the original 'A-not-B' task than the peer-reared group at both time-points. Mother-reared animals also had a significantly higher percentage of correct responses in the randomized control task at the second time-point (D), and the performance of each group was not different between tasks.

Computational model fits

In order to determine the cognitive mechanisms underlying the difference in performance between the two groups, we then compared the fitted model parameters between groups and time-points for

the 'A-not-B' task. A significant main effect of group was revealed for working memory decay (λ_1) [$F(1) = 7.008$, $p = 0.014$], with the peer-reared group demonstrating a significantly faster rate of decay compared to the mother-reared group (Figure 4A; 4B). There was no significant effect of time-point or group by time-point interaction, and no significant differences in terms of the weight of the working memory factor (w_1). There were no main effects of group or time-point on the weight of the choice history factor (w_2), but a significant interaction between group and time-point was found [$F(1) = 7.159$, $p = 0.021$], with w_2 decreasing from time-point 1 to time-point 2 in mother-reared group and increasing in the peer-reared group. No significant main effects or interactions were found for choice history decay rate (λ_2 ; Figure 4C; 4D), or choice stochasticity (β).

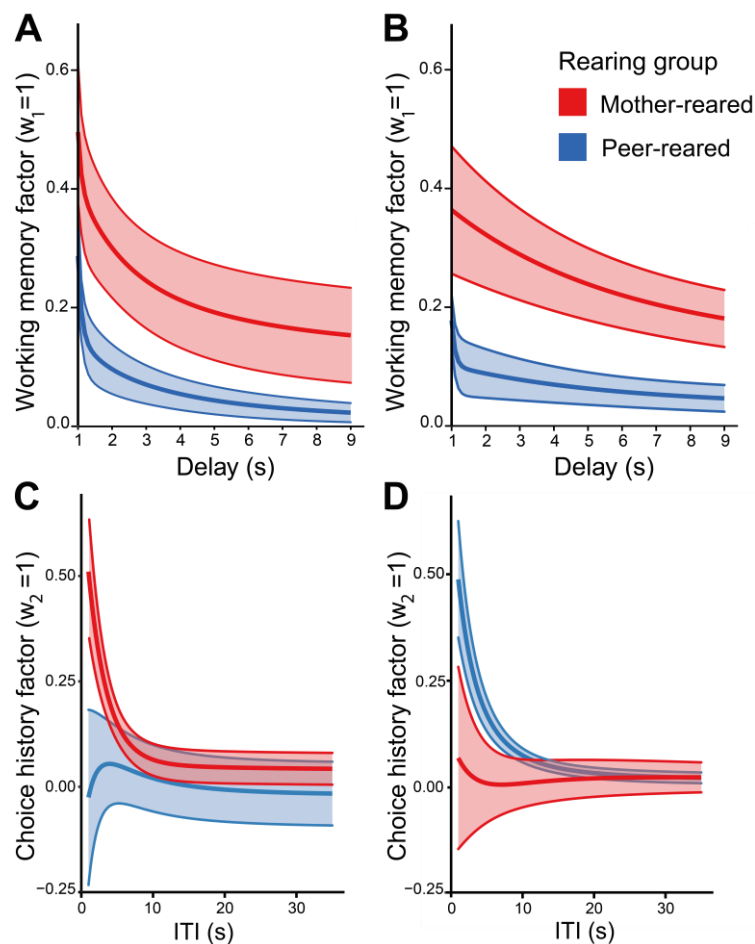


Figure 4. Fitted model parameters for the original 'A-not-B' task at both time-points. A) Decay in the value of the working memory factor at the first time-point, given λ_1 , and fixing $w_1=1$, for various delay durations. The solid lines represent the mean over subjects (red = mother-reared, blue = peer-reared), and the shaded areas represent the standard error. B) As in (A), for the second time-point. C) Decay in the value of the choice history factor rate at the first time-point, given λ_2 , and fixing $w_2=1$, for various inter-trial intervals. D) As in (C), for the second time-point.

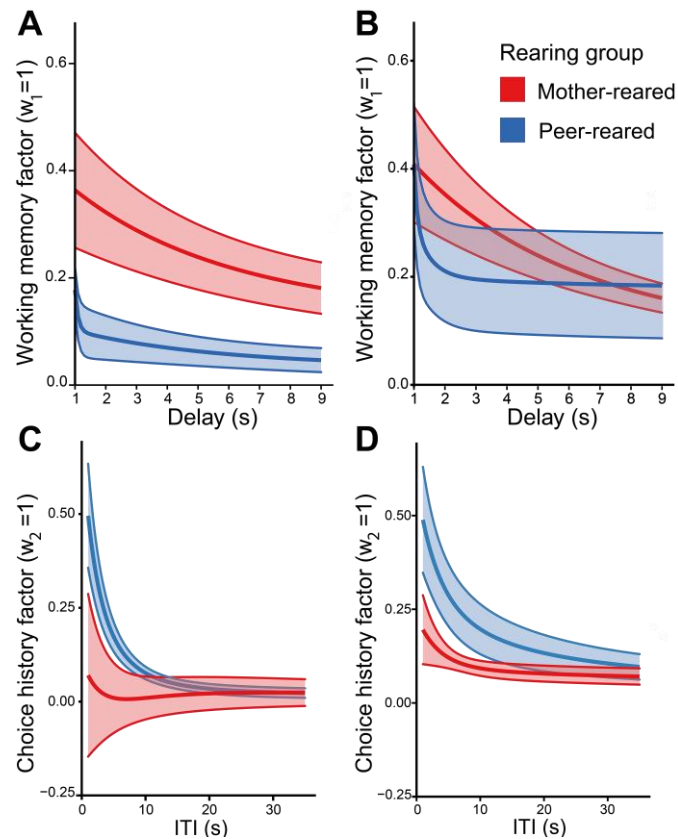


Figure 5. Fitted model parameters for the original and random control tasks at the second time-point. (A) Decay in the value of the working memory factor for the original ‘A-not-B’ task at the second time-point, given λ_1 , and fixing $w_1=1$, for various delay durations. The solid lines represent the mean over subjects (red = mother-reared, blue = peer-reared), and the shaded areas represent the standard error. B) As in (A), for the random control task at the second time-point. C) Decay in the value of the choice history factor rate for the original ‘A-not-B’ task at the second time-point, given λ_2 , and fixing $w_2=1$, for various inter-trial intervals. D) As in (C), for the random control task at second time-point.

We then aimed to establish if the mechanisms behind the difference in performance between groups were similar in both versions of the task by comparing their fitted model parameters between the original ‘A-not-B’ task and the random control task at the second time-point. For working memory decay rate (λ_1), a significant main effect of group was revealed [$F(1) = 5.314$, $p = 0.022$], with the peer-reared group demonstrating a significantly faster rate of decay compared to the mother-reared group (Figure 5A; 5B). There was no significant effect of task, or a group by task interaction. Again, there were no significant differences in terms of the weight of the working memory factor (w_1) (Figure 5A; 5B). For choice history, a significant main effect of group was revealed, with a greater choice history factor weight (w_2) found for the peer-reared compared to mother-reared group [$F(1) = 5.454$, $p = 0.031$] (Figure 5C; 5D). There was no significant effect of time-point or interaction effects. No significant main effects or interactions were found for decay rate of the choice history factor (λ_2) (Figure 5C; 5D). For choice stochasticity (β) there were no significant main effects or interactions.

Relationship between fitted model parameters and performance measures

Having shown that the groups vary similarly in terms of their performance in the original 'A-not-B' and random control tasks, and that the model predicts group differences in working memory decay and the influence of choice history, we then wanted to find out if these mechanisms were reflected by any of the performance measures calculated. We therefore related each fitted parameter to each performance measure for the original version of the task at both time-points. The weight of the working memory factor (w_1), and the rate of working memory decay (λ_1) predicted the percentage of correct responses [w_1 : $F(1) = 11.104$, $p < 0.01$; λ_1 : $F(1) = 10.564$, $p < 0.01$; $F(1) = 5.180$, $p = 0.048$], with higher working memory factor weight and longer working memory decay rate predicting a higher percentage of correct responses. There was no relationship between the weight of the choice history factor (w_2), the decay rate of the choice history factor (λ_2), or choice stochasticity (β) and the percentage of correct responses. The cumulative score was predicted by the weight of the working memory factor (w_1) [$F(1) = 4.576$, $p = 0.038$], the rate of working memory decay (λ_1) [$F(1) = 17.052$, $p < 0.01$], and choice stochasticity (β) [$F(1) = 8.880$, $p < 0.01$], with higher working memory factor weight, longer working memory decay rate, less choice stochasticity predicting higher cumulative scores. There was no relationship between the weight of the choice history factor (w_2) or the decay rate of the choice history factor (λ_2) with the cumulative score. Finally, the rate of working memory decay (λ_1) [$F(1) = 19.487$, $p < 0.001$] and choice stochasticity (β) [$F(1) = 9.941$, $p < 0.01$] both predicted the maximum delay, with longer working-memory decay rate and less choice stochasticity linked to higher maximum delay. Neither the weight of the working memory factor (w_1), the weight of the choice history factor (w_2), nor the decay rate of the choice history factor (λ_2) predicted maximum delay.

We then repeated this analysis for the original 'A-not-B' task and the random control task at the second time-point, relating each fitted model parameter to the percentage of correct responses (the cumulative score and maximum delay metrics only apply to the original task structure). The weight of the working memory factor (w_1) [$F(1) = 9.921$, $p < 0.01$] and working memory decay rate (λ_1) [$F(1) = 4.696$, $p = 0.046$] both predicted the percentage of correct responses, with greater w_1 and longer decay rate related to a higher percentage of correct responses. The weight of the choice history factor (w_2), the decay rate of the choice history factor (λ_2), and choice stochasticity (β) did not predict the percentage of correct responses.

DISCUSSION

This study aimed to assess the longitudinal effects of early psychosocial deprivation on executive function (EF) across adolescence and early maturity in rhesus macaques, using an 'A-not-B' task. We found that early psychosocial deprivation had a negative effect on task performance, with the peer-reared group performing worse than the mother-reared group at both assessment time-points. Furthermore, fitting a computational model of decision making enabled us to identify the mechanistic processes likely contributing to performance on the task, with results suggesting that psychosocial deprivation has long-term effects on both working memory and inhibitory control components of EF. In addition to offering important insights into the longitudinal effects of early deprivation on EF, findings also support a protracted developmental trajectory of inhibitory control in macaques that

extends into adulthood. These results indicate that the mechanisms implicated in development of specific EF components are similar in macaques and humans, with specific EF components having different developmental timelines. Our study therefore also provides support for the use of macaque models to investigate the psychological and neural mechanisms through which executive function develops more generally.

Our findings provide clear evidence for the potential long-term negative impact of early psychosocial deprivation on EF in macaques. Mother-reared animals performed better on the 'A-not-B' task than peer-reared animals across adolescence (time-point 1: 3.5 years) and early adulthood (time-point 2: 5 years), which is in line with previous findings suggesting that early psychosocial deprivation effects on childhood EF in humans can persist into adulthood (21). Analysis of the fitted model parameters revealed differences between the groups in terms of working memory, with peer-reared animals having a faster rate of working memory decay across time. A difference in terms of inhibitory control (i.e. the influence of choice history) was also found, with peer-reared subjects having a greater tendency to repeat, rather than inhibit, their previous choice in early adulthood. Notably, results from the modified random version of the task, used as a control at the second time-point (5 years), indicate that these group differences were not simply due to learning the structure of the original 'A-not-B' task. These effects on specific components of EF are in keeping with evidence that adversity in the form of early deprivation may have a particularly severe impact on working memory and inhibitory control in humans (e.g. (22)).

Notably, analysis of the fitted model parameters suggests that early psychosocial deprivation was linked to poor working memory in adolescence and early adulthood, but to weaker inhibitory control in early adulthood only. This could be due to several different factors. One possibility is that the performance directly impacted the ability of the task to correctly assess inhibitory control. According to Diamond et al. (41), the 'B' error (i.e. an incorrect response after switching the hiding position) occurs only when performance is accurate enough. This would mean that inhibitory control is only assessed correctly when accuracy is relatively high. Many studies use a criterion for subject inclusion where the participant has to be successful in at least one 'B' trial to be included in analysis (e.g. 43). We also used this criterion, and the model successfully predicted the same proportion of responses for both rearing groups despite their differences in performance (see SI). It is therefore unlikely that the differences in the fitted model parameters would be due to performance differences. Another possibility is that working memory and inhibitory control follow different developmental trajectories. For example, evidence shows that inhibitory control starts to decline later (around 35 years) than working memory in human adults (4), and in macaques, evidence suggests that working memory is mature even pre-puberty whereas inhibitory control is not (56). However, a lack of longitudinal studies

that focus on both working memory and inhibitory control does not allow any clear conclusion to be made. Based on our findings, we propose that while early psychosocial deprivation may impact both aspects of EF, their developmental trajectories after exposure likely differ. Peer-reared animals could have accelerated development of inhibitory control compared to mother-reared, then during adolescence, they are less flexible and do not improve. On the contrary, working memory may be impaired or accelerated at an earlier stage of development, with a protracted and stable negative effect of early psychosocial deprivation then seen across the transition from adolescence to adulthood. These differing trajectories could be explained by distinct underlying brain networks that are differentially impacted by early psychosocial deprivation. Confirming this hypothesis now requires more studies assessing neural measures development and actual link to behaviour across various stages of development.

Importantly, the inhibitory control parameters of the computational model (i.e. the influence of choice history), did not predict any of the performance measures, including the cumulative score and maximum delay, which are thought to account for both inhibitory control and working memory EF components. On the contrary, the working memory parameters predicted all performance measures. This suggests that the performance measures used in previous research mainly capture the influence of working memory on behaviour. However, using the computational model, it was possible to assess the contribution of both working memory and inhibitory control separately using an 'A-not-B' task. These findings are in line with the recent suggestion that proper assessment of inhibitory control in the first years of life is difficult using 'A-not-B' and similar tasks because of the high demands of working memory necessary to complete such tasks (43,48). Fitting of computational models could thus help us to understand if EF follows an unitary construct in infancy or if different components can already be distinguished at a very early stage of development still using this task (3), and how these factors can lead to individual differences related to both positive and negative outcomes. Our results suggest that the 'A-not-B' task does correctly assess inhibitory control, but this EF component is not reflected by the measures of performance used in the previous literature.

There are a few limitations to the present study that should be acknowledged. First, due to the sample size, it is difficult to account for inter-individual variability within the two groups and the results may thus not be generalizable. Second, we did not have any time-points pre-puberty, which would be necessary to fully examine developmental trajectories of EF development after exposure to early psychosocial deprivation. Third, we only had the control version of the task at the second assessment time-point (5 years). It will be important in future research to replicate these results with careful controls in larger samples, and at time-points covering multiple key transition periods in development.

This study also has several strengths. First, we assessed the effect of early psychosocial deprivation on EF development at two time-points covering a key transition period (i.e. adolescence into early adulthood). Second, our sample included two groups of macaques exposed to different early social and highly controlled environments. Third, all assessments were conducted with the monkeys remaining in their home enclosure without the addition of invasive and stressful procedures, which also makes results more comparable to those from human studies. Finally, the use of a computational model enabled the distinction between specific aspects of EF. This approach could be of great use in future longitudinal assessment of EF, especially when including a wide range of key developmental transition points.

To conclude, results from this study demonstrate that early psychosocial deprivation is associated with long-term effects on EF, which are apparent in adolescence and persist into adulthood. The use of a computational model of decision-making to disentangle mechanisms underlying performance on the 'A-not-B' task provides an alternative way to analyse the data from such tasks, and may be of specific interest for developmental research, which is often defined by a lack of precise measurement tools. In the future, computational approaches such as that used here can easily be adapted to other EF tasks, with the results then more easily linked to specific aspects of neural development and related cognitive mechanisms than classic behavioural measures alone. Such research involving assessment of brain development is essential for clarifying how early deprivation effects EF over the lifespan, and identifying the factors that confer risk for or resilience against poor developmental outcomes. This will be critical for design of more effective treatments and interventions that target individuals more at risk for developing impairments in specific aspects of EF and associated difficulties after exposure to early psychosocial deprivation.

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SUPPLEMENTARY INFORMATION

METHODS

Subjects

All subjects were born and raised at the *Laboratory of Comparative Ethology at the National Institutes of Health, US*. Peer-reared animals were raised in a nursery with access to same-aged peers. See (1) for more information on the peer-rearing protocol. At eight months postpartum, mother-reared and peer-reared subjects were placed into a single social group. Rearing procedures were approved by the NICHD and the University of Maryland Animal Care and Use Committee, and adhered to the NIH Guide for the Care and Use of Laboratory Animals. Animals were relocated to their current location at the *Rousset Primatological Station, CNRS, France* at two years of age, and are housed altogether. Animals live in semi free-ranging conditions, with access to both indoor and outdoor areas. As part of a wider longitudinal study, animals are temporarily relocated once a year to a nearby location in groups of 5-6 animals. All groups contain a balanced mix of mother-reared and peer-reared animals, with groups housed in an indoor enclosure. This enclosure is enriched to allow for meaningful activities and expression of the animals' full behavioural repertoire. Assessments for the current study were conducted a minimum of 10 days after this relocation. All animals were kept alive at the end of the study.

'A-not-B' task set-up

The task set-up is illustrated in Figure S1.

Video coding

We coded a number of parameters offline from the video recordings made of each subject during the testing sessions. These included the actual length of the delay periods (i.e. from the moment the experimenter showed the food to the subject, to the moment the clear panel was removed to enable the subject to reach for the wells), inter-trial-interval (ITI) lengths, position of the subject in the testing enclosure during each trial, and other behaviours during and between trials that may have affected performance, such as locomotion, pacing, anxiety (i.e. self-scratch, self-groom, yawn), vocalizations, and threatening, fearful, or affiliative gestures. These behaviours were coded as being present or not during each trial and ITI. A random 15% of videos were coded by two researchers at each time-point to establish reliability for the original version of the task, and for the control version of the task at the second-time point (5 years). All reliability scores obtained between coders were very strong (i.e. all $\kappa = 0.86-1.00$).

Computational model

Computational models of decision-making were fit to the decision behaviour of each subject, at each time-point, in each task. All models computed a weighted sum, z , of various factors such as working memory, choice history (the tendency to repeat the previous choice, or alternate), and reward prediction error. Negative values of z represent a tendency to choose the left side, and positive values a tendency to choose the right side. The weighted sum was then transformed into the probability of choosing the right side on trial t :

$$P(R_{R,t}) = \frac{1}{1 + e^{-\beta z_t}}$$

The inverse softmax temperature, β , determines the steepness of the softmax function, and thus how sensitive the choice probability is to z . We tested 11 different models:

- 1) Working memory : $z_t = w_1 S_t e^{-\lambda_1 d_t}$, where w_1 is the weight of this factor when the delay is 0, S_t is the side that food was hidden on in trial t (left = -1, right = 1), and d_t is the delay
- 2) Previous choice: $z_t = w_2 R_{t-1} e^{-\lambda_2 (i_t + d_t)}$, where w_2 is the weight of this factor when the inter-trial interval and delay is 0, R_{t-1} is the response made on the previous trial (left = -1, right = 1), and i_t is the inter-trial interval before this trial.
- 3) Previous two choices: $z_t = w_2 (R_{t-1} e^{-\lambda_2 (i_t + d_t)} + R_{t-2} e^{-\lambda_2 (i_{t-1} + d_{t-1} + i_t + d_t)})$
- 4) Working memory and previous choice: $z_t = w_1 S_t e^{-\lambda_1 d_t} + w_2 R_{t-1} e^{-\lambda_2 (i_t + d_t)}$
- 5) Working memory and previous two choices: $z_t = w_1 S_t e^{-\lambda_1 d_t} + w_2 (R_{t-1} e^{-\lambda_2 (i_t + d_t)} + R_{t-2} e^{-\lambda_2 (i_{t-1} + d_{t-1} + i_t + d_t)})$
- 6) Expected reward: $z_t = w_3 (v_{R,t} - v_{L,t})$, where w_3 is the weight of this factor, $v_{R,t}$ is the expected reward at time t for choosing right, and $v_{L,t}$ is the expected reward at time t for choosing left. Expected reward for each side was updated using the standard Rescorla-Wagner rule: $v_{c,t+1} = v_{c,t} + \alpha (\hat{v}_{c,t} - v_{c,t})$, where $v_{c,t}$ is the expected reward for side c at time t , α is the learning rate, and $\hat{v}_{c,t}$ is the actual reward received for choosing side c at time t .
- 7) Working memory and expected reward: $z_t = w_1 S_t e^{-\lambda_1 d_t} + w_3 (v_{R,t} - v_{L,t})$
- 8) Previous choice and expected reward: $z_t = w_2 R_{t-1} e^{-\lambda_2 (i_t + d_t)} + w_3 (v_{R,t} - v_{L,t})$
- 9) Previous two choices and expected reward: $z_t = w_2 (R_{t-1} e^{-\lambda_2 (i_t + d_t)} + R_{t-2} e^{-\lambda_2 (i_{t-1} + d_{t-1} + i_t + d_t)}) + w_3 (v_{R,t} - v_{L,t})$
- 10) Working memory, previous choice, and expected reward: $z_t = w_1 S_t e^{-\lambda_1 d_t} + w_2 R_{t-1} e^{-\lambda_2 (i_t + d_t)} + w_3 (v_{R,t} - v_{L,t})$

11) Working memory, previous two choices, and expected reward: $z_t = w_1 S_t e^{-\lambda_1 d_t} + w_2 (R_{t-1} e^{-\lambda_2 (i_t + d_t)} + R_{t-2} e^{-\lambda_2 (i_{t-1} + d_{t-1} + i_t + d_t)}) + w_3 (v_{R,t} - v_{L,t})$

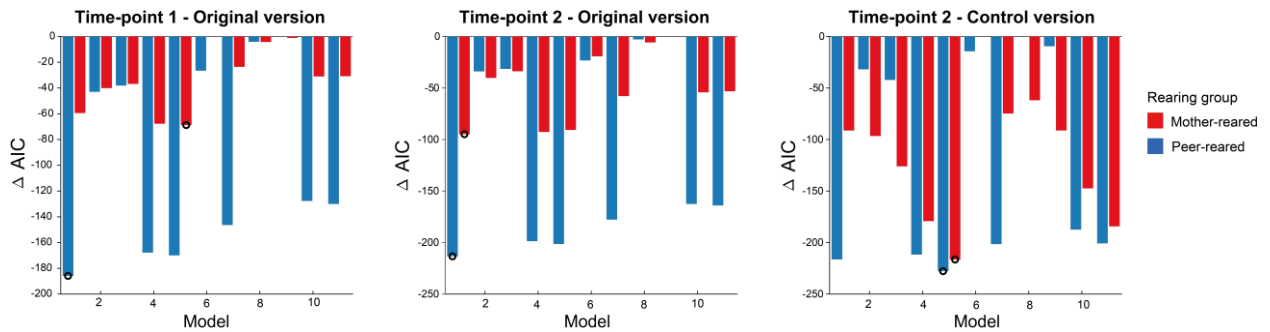


Figure S2: Model comparison. Akaike information criterion (AIC) for each model relative to the worst model for both groups at each assessment time-point and task version. Filled circles represent the models with the lowest AIC.

Model comparison was performed using AIC (computed as the sum of AIC for each subject) for each assessment time-point and task (Figure S2). Model 1 had the lowest AIC for the peer-reared group at time-points 1 and 2 and the mother-reared group at time point 2. Model 5 had the lowest AIC for the mother-reared group at time-point 1 and for both groups in the random version of the task. We therefore selected model 5 for the main analyses as it includes all the terms from model 1, and terms for the influence of the previous two choices.

DATA ANALYSIS

R v4.0.5 (2) and the lme4 v1.1-27.1 (3), lsmeans v2.30.0 (4), predictmeans v1.0.6 (5), and car v3.0.11 (6) packages were utilized to conduct the analyses.

Data pre-processing

To check whether any coded behaviours influenced the ability of any of the 11 tested computational model to predict the responses of the subjects, we used linear mixed models with the maximum of correctly predicted responses over all model versions as the outcome variable, and with each behaviour (e.g. locomotion during trials, locomotion during ITI, anxiety during trials, etc.), group (mother-reared/peer-reared), and their interaction included as fixed effects; each behaviour (e.g. locomotion during ITI) as random slope and subject, year (3.5/5 years), and session (1/2) as random intercepts were included as random effects. A permutation test was performed on the linear mixed models with 10,000 permutations (grouped by subject and block within session and task). The only behaviour found to impact the ability of the model to predict the choices of the subject was locomotion during the trials, with a significant main effect of locomotion [$F(1) = 4.671$, $p = 0.017$]. Therefore, we excluded all trials in which locomotion occurred. We also removed trials in which the ITI was more than 2.5 standard deviations above the mean. We set a threshold of 25 trials minimum per subject.

This resulted in one mother-reared subject being excluded from the analysis of the original task at the first time-point (3.5 years). The same subject was also excluded from analysis of the randomized control version of the task at the second time-point (5 years).

To investigate potential differences between rearing groups in terms of ITI duration in the original task at both time-points, we then used a linear mixed model with group (mother-reared/peer-reared), year (3.5/5 years), and their interaction included as fixed effects, and subject, year (3.5/5 years), and session (1/2) included as nested random intercepts. A permutation test was performed on the linear mixed model (grouped by subject and block within session and year). No significant main effects of group or year, or group by year interaction were found. To investigate potential differences ITI lengths at the second time-point (5 years) in the original and control versions of the task, we again used a linear mixed model with group (mother-reared/peer-reared), task (original/control), and their interaction included as fixed effects, and subject, task (original/control) and session (1/2) as nested random intercepts. A permutation test was performed on the linear mixed model (grouped by subject and block within session and task). No significant main effects of group, or task or group by task interaction were found.

Trial exclusion

Mean trials (out of the 50 trials for original task and 40 trials for the control task per subject) included for mother-reared animals were as follows: original task at the first time-point (3.5 years), $M = 44.63$, $SD = 6.36$; original task at the second time-point (5 years), $M = 47.1$, $SD = 2.96$; control task at the second time-point (5 years), $M = 45.2$, $SD = 5.69$. Mean trials included for peer-reared animals were as follows: original task at the first time-point (3.5 years), $M = 44.3$, $SD = 5.61$; original task at the second time-point (5 years), $M = 47.3$, $SD = 4.49$; control task at the second time-point (5 years), $M = 44.2$, $SD = 6.17$.

RESULTS

Descriptive statistics can be found in Table 1

Table 1: Behavioral measures of performance in original and control version of 'A-not-B' task. Percentage of correct responses (M and SD) is the proportion of correct trials out of all trials completed. Cumulative score (M and SD) is the sum of all successful change trial delays and then divided by the number of total trials completed. Maximum delay (M and SD) is the maximum observed delay period (seconds) coded from the videos.

Group	Mother-reared	Peer-reared
<i>Percentage of correct responses</i>		
3.5 years (original)	0.787 (0.105)	0.625 (0.146)
5 years (original)	0.77 (0.171)	0.642 (0.14)

5 years (control)	0.805 (0.132)	0.661 (0.154)
<i>Cumulative score</i>		
3.5 years (original)	0.643 (0.316)	0.265 (0.204)
5 years (original)	0.712 (0.4)	0.279 (0.244)
<i>Maximum delay</i>		
3.5 years (original)	5.181 (1.778)	2.8 (1.135)
5 years (original)	5.2 (1.619)	3.8 (1.032)

Proportion of responses predicted by the computational model

To check whether the computational model correctly predicted a different amount of responses between the two groups, we used linear mixed models with group (mother-reared/peer-reared) included as fixed effects, and subject-specific intercepts as a random effect. A permutation test was performed on the linear mixed models with 10,000 permutations (grouped by subject). No significant effect of group on the proportion of responses correctly predicted by the computational model was found.

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CHAPTER 4. Neurocognitive effects of early psychosocial deprivation

Structural brain development and executive function in macaques: Longitudinal effects of early psychosocial deprivation in adolescence and early adulthood

Early social adversity (ESA) involves violations in the early expectable caregiving environment (McLaughlin, 2016; Wade, 2022), and has been linked to poor development in numerous domains (Oeri, 2022; Luo, 2021; Zhang, 2017; Johnson, 2021). Psychosocial deprivation represents one model of ESA that has received particular attention in previous research, especially in the form of early institutionalization. Early institutional rearing involves the separation of an infant from their primary caregivers, and is often followed by the unpredictable presence of any regular caregiving figure and a related lack of sensitive socio-cognitive input. Early institutionalization is associated with negative long-term outcomes such as poor academic performance, occupational functioning, and social competence (Roy, 2006; Beckett, 2007; Caceres, 2021; Humphreys, 2019; Sonuga-Barke, 2017), as well as increased risk for psychiatric disorders such as mood and substance use disorders (van IJzendoorn, 2020; Bos, 2011; Zeneah, 2009; Humphreys, 2020). Increased prevalence of attention/deficit-hyperactivity disorder (ADHD) (Tibu, 2016; Golm, 2020; Boss, 2011) and quasi-autistic like behaviours (Levin, 2015; Rutter, 2007) have also been described in the context of early institutionalization. To design more effective early interventions and treatments for individuals who experienced early psychosocial deprivation, we now need to further knowledge on the factors conferring risk and resilience after such experience. This will require longitudinal studies investigating the specific neurocognitive mechanisms through which different poor outcomes arise after exposure to such adverse early experience, especially over important transitions later in development such as adolescence.

In terms of cognitive mechanisms, institutional rearing has been linked to abnormal threat and emotional processing (Bick, 2017; Tottenham, 2010), and notably, is a form of ESA most often associated with problems in executive function (EF) (Wade, 2019; Merz, 2016; Bos, 2009). EF refers to a group of cognitive processes that underlie the organization and control of goal-directed behaviour, and is often divided into three components: working memory, inhibitory control, and cognitive flexibility (Myiake, 2000, 2007). EF impairments play an important role in many of the poor outcomes linked to early institutionalization, including academic success and risk for internalizing and externalising psychopathology (Wade, 2020; Buzzell, 2020; Merz, 2016). EF deficits are also key features of neurodevelopmental disorders

associated with psychosocial deprivation (e.g. ADHD) (Friedman, 2022; Tibu, 2016). Previous institutionalization has been linked to EF deficits in childhood (Carlson, 2016) and adolescence (Wade, 2019), with children who spent more than two years in an institution demonstrating worse EF compared to those who spent less than two years in institutional care (Rutter, 2000). Institutional rearing may have particularly severe impacts on working memory and inhibitory control components (Johnson, 2021; McDermott, 2013; Wade, 2019; Massera, under review; Fox, 2011), however, as far less investigation into the effects of institutionalization on EF has been conducted so far compared to that on strictly emotional and social processes, much remains unknown about the trajectories of EF after early psychosocial deprivation. Critically, this includes the neurobiological mechanisms linking EF to the emergence of dysfunctional behaviour and psychopathology.

Evidence for poor outcomes related to early psychosocial deprivation being linked to specific aspects of brain development in humans is growing (McLaughlin, 2014; Holz, 2022; Tottenham, 2021). For example, it has been shown that the prefrontal cortex (PFC) plays a central role in EF (Diamond, 2002; Fiske, 2019; Friedman, 2022; Simmonds, 2014), with structural and functional differences in PFC found consistently in those exposed to early institutionalization (Hodel, 2015; Sheridan, 2022; Mackes, 2020). Although the PFC is functional from infancy, it is one of the last brain regions to reach full maturity (Fiske, 2019), undergoing significant development during adolescence (Spear, 2000; Caballero, 2016; Chini, 2021). Adolescence is proposed as a particularly important sensitive period for the refinement of EF, as notable changes in the PFC make this a time where EF is especially susceptible to environmental influences (Zeneah, 2013; Kolbe, 2012; Larsen, 2018; Schalbetter, 2022). Importantly, maturation of the frontal lobe does seem to parallel known improvements in EF across development, although relatively few studies have examined this relationship explicitly over time (Fiske, 2019; Werchan, 2017; Eng, 2022). Accordingly, adolescence may represent a prime period of opportunity to intervene and prevent long-term negative outcomes associated with EF after early psychosocial deprivation (Thompson, 2020), with adolescence also the time of greatest risk for the emergence of particular adverse outcomes linked to institutionalization such as mood disorders.

As well as more immediate effects on neurocognitive development in infancy and early childhood, early social experience may have delayed developmental effects. These are often

referred to as "sleeper effects" or an "incubation period" (Maurer, 2007; Lupien, 2009; Maurer, 2007; Merz, 2010). For example, institutionalized children who do not exhibit externalizing psychopathology in childhood, i.e. symptoms of defiant or conduct disorders, have been found to demonstrate such symptoms in early adolescence (Zeanah et al, 2009; 2014; McGoron et al, 2013). This suggests that early institutional experiences may affect the development of certain skills and dispositions that become more important at later developmental stages, possibly under the influence of additional stressful and demanding environmental factors associated with adolescence (Zeanah, 2011). However, more research is now needed to fully understand the potential delayed impact of early experiences on development of processes such as EF during adolescence and its underlying neural bases.

Beyond the PFC in isolation, EF relies on connectivity of the PFC with other brain regions for the integration and control of cognitive representations (Menon, 2022; Smalker, 2015), and thus specific components of EF such as working memory and inhibitory control (Costantinidis, 2016; Morein-Zamir, 2015; Nowrangi, 2014; Fiske, 2019; Goddings, 2021). Distinct and overlapping neural networks involving PFC likely underly the emergence of general and specific components of EF, with the protracted trajectory of EF development following a different course for different components (Tervo-Clemmens, 2022). In particular, separately and in interaction, fronto-parietal and fronto-striatal circuits are thought to play an essential role in EF across development (Darki, 2014, 2019), with striatal circuits playing an increasingly important role across adolescence into adulthood (Simmonds, 2014; Ohja, 2022; Crone, 2016), having a more protracted developmental trajectory. Although cortico-cortical connectivity such as that of the fronto-parietal network, which is strongly implicated in EF (Yao, 2020; Nowrangi, 2014; Fiske, 2019), has traditionally been the main focus of EF research, findings increasingly demonstrate that subcortical regions are also vitally important. For example, Webb (2020) found that deficits in fronto-striatal white matter microstructure was negatively associated with EF, with this association becoming stronger with increasing age between early and mid-adulthood. Additionally, changes in ventrofronto-striatal white matter tracts are correlated with the development of inhibitory control throughout childhood and adolescence (Liston et al., 2006), with the caudate nucleus activated during working memory tasks in children (Klingberg et al., 2002; Ziermans et al., 2012) and adults (Postle et al., 2000). White matter microstructure in fronto-striatal tracts has also been related to future working memory

in adolescence and into early adulthood, with caudal activity predicting future working memory capacity (Darki, 2015). However, studies so far have been primarily been cross-sectional, and have not fully clarified the neural dynamics underlying changes in working-memory and inhibitory control across development (Vestergaard, 2011; Darki, 2015; Goddings, 2021).

There has been relatively little examination of the impact of early psychosocial deprivation on the neurophysiological substrates of EF specifically, or the actual relationship between brain development and performance on EF tasks. Using EEG, institutionalized children were found to have higher theta power and lower alpha and beta power at 22 months of age compared to a control group, with this pattern having previously being linked to ADHD and learning disorders (Marshall, 2004, McLaughlin, 2010). Institutionalized children continue to display lower alpha power and higher theta power at 16 years compared to controls (Vanderwert, 2010, 2016; Debnath, 2020). Such reduced alpha power and elevated theta power is proposed to reflect a deficit in cortical maturation in institutionalized children (Debnath, 2020), likely linked to cognitive function. At 12 years, EEG has also been used to show that institutionalized children demonstrate worse inhibition of planned actions, with larger P2 and smaller N2 event-related potential (ERP) amplitudes moderating the association between psychosocial deprivation and symptoms of externalizing behavior (Lamm, 2018). A couple of functional MRI studies have shown that worse cognitive control in preschool children exposed to early institutionalization is related to altered prefrontal resting-state connectivity (Demir, 2016), and that stronger coupling between the ventral striatum and the medial prefrontal cortex is associated with parent-reported social problems in previously institutionalized adolescents (Fareri, 2017). In terms of structural brain development, a few studies have also used MRI to investigate the relationship between institutional rearing, EF development, and ADHD symptoms (Mackes, 2020; Demir, 2016; Wade, 2019; Sheridan, 2022; Bick, 2015). In these studies, deprivation was associated with reduced grey and white matter volume, as well as cortical thickness in prefrontal regions (ACC, IFG, mPFC), which in turn, mediated the relationships between deprivation, lower IQ, and ADHD symptoms in childhood (McLaughlin, 2014). However, thicker cortex in prefrontal regions in adolescents and young adults exposed to early psychosocial deprivation has been related to worse behavioural outcomes and

increased risk for psychopathology (Mackes, 2020; Sheridan, 2022), with delayed thinning for exposed individual compared to non-exposed (Mackes, 2020; Sheridan, 2022).

Although relatively few studies have looked at *structural* brain development after psychosocial deprivation in humans, the use of techniques such as diffusion MRI and diffusion tensor imaging (DTI) for studying structural connectivity between EF-related brain regions in future research will be extremely important, with EF involving multiple functions supported by multiple brain regions that are connected structurally via different brain networks (Goddings, 2021). Additionally, myelination, which is critical for neural circuit formation (Malave, 2022), may be an aspect of brain development severely impacted by ESA (Teissier, 2020; Lutz, 2017; Islam, 2021).. One recent study that used DTI to examine the effects of institutionalization on structural brain connectivity during adolescence found reduced integrity in the superior longitudinal fasciculus (SLF), a white matter tract connecting the prefrontal and parietal cortices (Sheridan, 2022) and linked consistently to EF (Vestergaard, 2011; Koshiyama, 2020). Early institutionalization has also been found to impact development of fronto-striatal tracts from childhood until early adulthood, with reduced white-matter integrity in the corona radiata (CR), the internal capsule (IC), and the external capsule (EC) (Bick, 2016, 2017; Mackes, 2020; Sheridan, 2022).

Because longitudinal studies of early adversity assessing both neural and cognitive changes over time are rare, many questions remain about the trajectories of neurocognitive development linked to risk or resilience after early psychosocial deprivation (Goddings, 2021), especially in terms of EF. The use of a nonhuman primate (NHP) model such as rhesus macaques (*Macaca mulatta*) could be very helpful to address these outstanding questions. Macaque monkeys are one of the closest species to humans in terms of genetics, physiology, and neural organization, and have an extended period of development comprising distinct infant, juvenile (pre-adolescent and adolescent), and adult stages (Wang, 2020; Phillips, 2014). Like humans, their early social environment is also comprised predominantly of mother-infant interactions (Maestriperieri, 2009). The use of a macaque model can also help address some limitations defining developmental studies with humans, such as a lack of control over the early environment, very long developmental time-frames, and difficulty in tracking neural mechanisms underlying cognitive development.

Research focused on cognitive development in macaques suggests that it parallels that in humans (Hara, 2012; Gray, 2019), and in adult macaques, EF relies on the same brain networks (Lacreuse, 2020; Constantinidis, 2016; Levy, 1997; Oguchi, 2021). The few studies that have looked at effects of early psychosocial deprivation on cognitive function in macaques have produced mixed findings (e.g. Murphy, 2021; Sanchez, 1998), with research comparing ESA-exposed versus non-ESA-exposed animals especially rare. Additionally, these studies only looked in infancy, and no studies have examined EF specifically, or its different components explicitly. However, in our recent work (Massera et al., under review) looking at macaque EF in adolescence and early adulthood (Chapter 3 of this thesis), we showed that early psychosocial deprivation is associated with worse overall EF performance in macaques exposed to early psychosocial deprivation. Using a computational model of decision making, we were also able to suggest that poor performance in these animals reflected worse working memory across adolescence, as well as worse response inhibition as they reached maturity. Longitudinal studies tracking both EF and brain development across key developmental transitions such as adolescence are now required to elucidate the neural mechanisms through which such differences in EF arise.

Accordingly, the research presented here was designed to investigate how structural brain development in PFC and related networks may underlie EF deficits longitudinally in macaques exposed to early psychosocial deprivation. Our sample was made up of a rare group of macaques including two subgroups differing in exposure to early psychosocial deprivation; one mother-reared and one peer-reared (i.e. reared in a nursery of peers by human caregivers). We used anatomical MRI to measure cortical thickness in dorsolateral PFC (dlPFC) and ventrolateral PFC (vlPFC) at two time-points corresponding to adolescence (3.5 years) and early adulthood (5 years). Both dlPFC and vlPFC are subregions of PFC often linked to specific EF components, such as working memory and inhibitory control (e.g. Hertrich, 2021; Petrides, 2005; Nejati, 202; Nelson, 2011; Fiske, 2019). Additionally, with diffusion MRI/DTI at the same time-points (3.5 and 5 years), we assessed integrity of white-matter tracts involved in EF circuitry (Goddings, 2021), and known to be affected by early social adversity (Sheridan, 2022; Fareri, 2016; Ojha, 2022). Findings from the behavioural task used to assess EF were presented in our previous paper (Massera et al., under review; Chapter 3 of this thesis). Here, measures of EF from the behavioural tasks were related to gray and white matter ROIs across time. We

hypothesized that: i) cortical thickness in PFC regions (vIPFC and dIPFC) would decrease over time, but would be greater in the peer-reared versus mother-reared group; ii) integrity in fronto-parietal and fronto-striatal white matter tracts would increase over time, but would be lower in the peer-reared versus mother-reared group; iii) EF measures would be related to cortical thickness in dIPFC and vIPFC and white matter integrity in fronto-parietal and fronto-striatal tracts, with better EF linked to thinner cortex and higher integrity, respectively; iv) working memory and response inhibition would be related to both overlapping (e.g. superior longitudinal fasciculus) and distinct patterns of structural connectivity and cortical thickness in these different regions and circuitry; and v) cortical thickness and white matter integrity would modulate the relationship between psychosocial deprivation and EF across time, with fronto-striatal tracts having greater effects in adulthood compared to adolescence.

METHODS

Subjects

The sample consisted of 21 Rhesus macaque monkeys (*Macaca mulatta*), 11 mother-reared (five female) and 10 peer-reared (six female). Subjects were aged around 3.5 years at the first assessment time-point (mother-reared; M = 44.97 months, SD = 3.41 months: peer-reared; M = 44.81 months, SD = 3.32 months) and 5 years at the second assessment time-point (mother-reared; M = 59.37 months days, SD = 3.28 months: peer-reared; M = 59.47 months, SD = 3.12 months). Subjects were housed at the *Institut des Sciences Cognitives Marc Jeannerod*, CNRS, during the assessment period in mixed mother- and peer-reared social groups of 5–6 animals (see SI for more information about rearing protocol). Although new instances of maternal separation in monkeys for research is largely prohibited, it was decided that further study of this particular sample of juvenile animals was the most ethical course of action. Due to the closure of the center in the US where animals were born and lived for the first two years of life, it was agreed that our team at the ISC-MJ would receive these animals rather than allow them to be euthanized for medical research whereby effects of their early social experiences would not be considered; and consequently, would fail therefore to maximize the scientific benefit that could be derived from this existing sample. Every effort has been made to ensure that these animals now live in the most enriching environment possible, including their social environment, and the tasks included in the current study was

designed to be as noninvasive as possible. All housing and procedures conformed to current guidelines concerning the care and use of laboratory animals (European Community Council Directive No. 86-609), and were approved by our local ethics board, Comité d'Ethique Lyonnais pour les Neurosciences Expérimentales (CELYNE) C2EA #42 (03.10.18), and the French Ministry of Research (10.10.18); project reference APAFIS#15091_2018071014483295_v2. All reporting here conforms to the recommendations in the ARRIVE Guidelines for Reporting Animal Research.

'A-not-B' task set-up and procedure

To measure EF, subjects completed a 'A-not-B' task at both time-points. Subjects were placed in a designated testing area within their home enclosure for the experiment. The testing area was 87 cm x 100 cm x 120 cm with a transparent front panel. The subjects were familiar with the process of being placed in the testing area and with the equipment used in the task. There were two holes in the transparent front panel of the testing area, which allowed the subjects to access objects on a table outside the enclosure. A transparent board was used to block the holes at certain times during the task. A box with two wells and sliding doors was placed on the table in front of the holes. The wells were 11.2 cm in diameter and were spaced 18 cm apart. Two experimenters were present during the task, with one facing the subject and the other standing on one side of the table. The side that the second experimenter stood on was counterbalanced across subjects and sessions (see Fig.1 for set-up).

During a trial (Fig. 1), one experimenter presented a piece of food to the subject and placed it in one of the wells, then covered both wells with sliding doors. The trial only proceeded if the subject looked at the food when it was presented and when it was hidden. After a delay period during which the subject's physical and visual access to the wells was blocked, the opaque screen and transparent board were removed and the subject was allowed to reach for the wells. If the subject reached for the correct well, they were allowed to eat the food. If they reached for the wrong well, the correct well was opened to show them the food. If the subject touched both wells, did not respond, or the response was unclear, the trial was repeated. The location of the food was changed after the subject reached for the correct well on two consecutive trials. Each subject completed two testing sessions at each assessment time point, with a maximum of 25 trials per session. The delay period was adjusted based on the subject's performance. The trial was live-coded by one experimenter and recorded by a camera.

To assess differences between general EF performance between the two rearing groups, we computed a 'cumulative score' based on previous developmental studies using a comparable 'A not B' paradigm; i.e. the sum of successful change trial delays divided by the number of total trials completed (Holmboe, 2008), as a measure of performance.

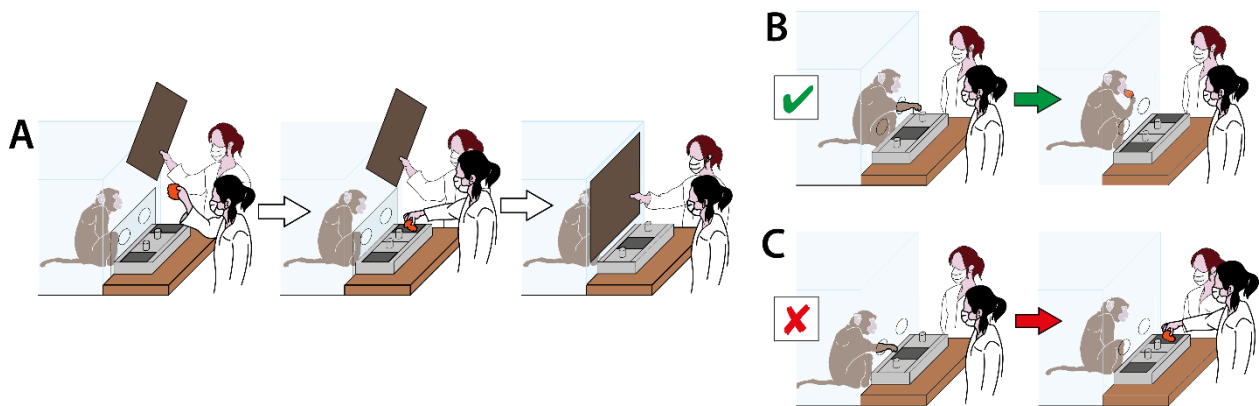


Figure 1. Task procedure. The series of images show the sequence of a single trial. In the first three images (A) Experimenter 1 presents the food to the subject and places it in the well. Experimenter 2 then blocks the subject's vision while a delay period occurs. The top right images (B) show a "correct" choice, where the subject reaches for the well with the food and consumes it. The bottom right images (C) show an "incorrect" choice, in which the subject selects the empty well and Experimenter 1 points out where the food was actually located."

Video coding

During the testing sessions, video recordings were made of each subject and a number of parameters were coded from these recordings offline. These parameters included the actual length of the delay periods (which may have differed slightly from the intended duration) in seconds, and the length of the inter-trial intervals (ITIs) in seconds (see SI).

Computational model

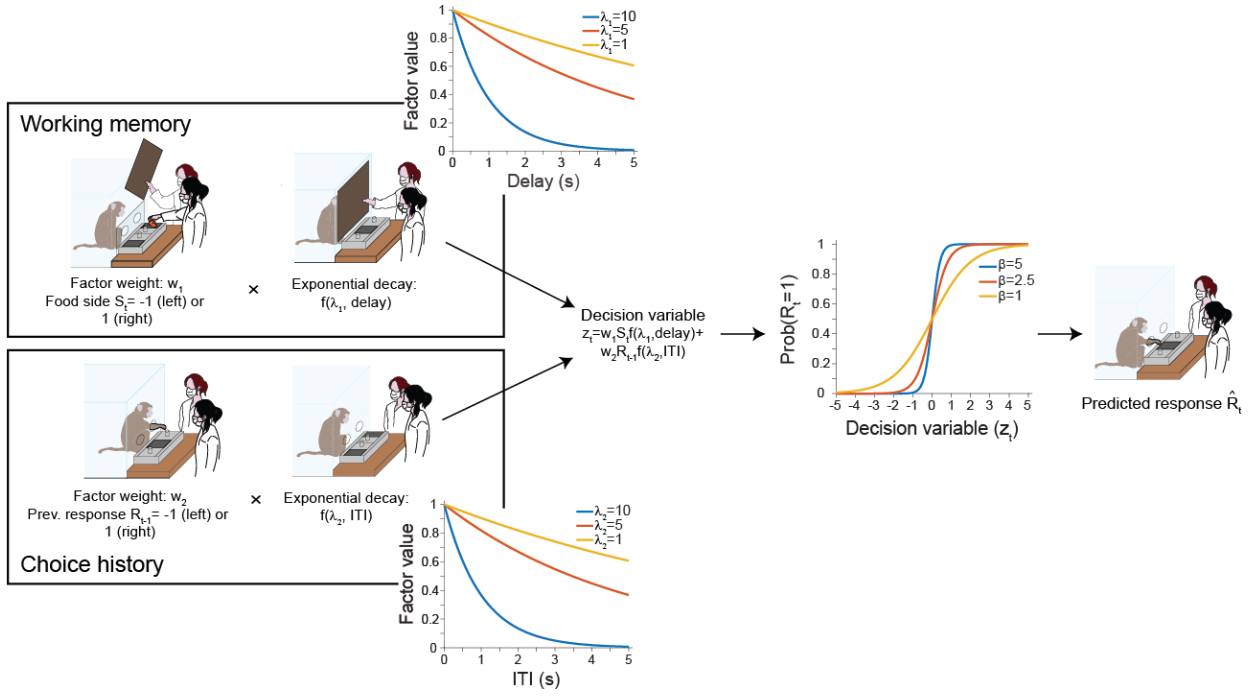


Figure 2. Model architecture. The model included two decaying memory traces: one for the location of food (shown in the top left plot, where higher decay rates correspond to a faster diminishment of working memory influence) and one for the previous choice made (shown in the bottom left plot, where higher decay rates correspond to a faster diminishment of previous choice influence). The probability of choosing one side or the other was calculated using a softmax function, with the weighted sum of these influences as input (the right plot shows the influence of the inverse softmax temperature parameter: at higher values, the decision is more deterministic).

A computational model of decision-making was applied to the decision behaviour of each subject, with the specific model being chosen from among eleven candidate models based on model comparison (see SI). The model consisted of two influences on the decision to choose the left or right side (Figure 2). The first influence was a decaying working memory trace, m , of where the food was hidden, modelled as an exponential decay function according to the delay on trial t , d_t :

$$m_t = w_1 S_t e^{-\lambda_1 d_t}$$

where w_1 is the weight of this factor when the delay is 0, S_t is the side that food was hidden on in trial t (left = -1, right = 1), and λ_1 is the working memory decay rate. The second influence was that of the previous two choices, p , modelled as an exponential decay function according to the inter-trial interval before trial t , i_t :

$$p_t = w_2 (R_{t-1} e^{-\lambda_2 (i_t + d_t)} + R_{t-2} e^{-\lambda_2 (i_{t-1} + d_{t-1} + i_t + d_t)})$$

where, w_2 is the weight of this factor when the inter-trial interval and delay is 0, R_{t-1} is the response made on the previous trial (left = -1, right = 1), and λ_2 is the decay rate. Positive values of w_2 therefore represent a tendency to repeat the previous choice, whereas negative values represent a tendency to suppress the previous choice, via different degrees of inhibitory control.

The decision variable, z , was computed as the sum of these two factors, m and p , with negative values representing a tendency to choose the left side, and positive values representing a tendency to choose the right side. The decision variable was then transformed into the probability of choosing the right side on trial t :

$$P(R_t = 1) = \frac{1}{1 + e^{-\beta z_t}}$$

The inverse softmax temperature, β , controls the slope of the softmax function, thereby determining the sensitivity of the choice probability to the decision variable, z . This parameter is referred to as the "choice stochasticity." The model was fit separately to each subject's choice behaviour using maximum log-likelihood estimation in Matlab (v R2018a). To avoid local maxima, a grid search of all parameter values was utilized to initialize the model parameters prior to fitting. The weight of the working memory factor, w_1 , was restricted to the range of [0, 1], while the weight of the choice history factor, w_2 , was restricted to the range of [-1, 1] in order to detect both a tendency to inhibit or repeat the previous choice, thus reflecting different degrees of inhibitory control. The decay rate parameters, λ_1 and λ_2 , and the inverse softmax temperature, β , were constrained to the range of [0,10].

Therefore, five model parameters were available for subsequent analysis: w_1 (the weight of the working memory factor when the delay is 0), λ_1 (decaying working memory trace of the food location), w_2 (the weight of the choice factor when the inter-trial interval and delay is 0), λ_2 (decaying trace of the previous choice), and β (choice stochasticity).

MRI acquisition

To investigate changes in brain anatomy and both structural and functional connectivity, magnetic resonance imaging (MRI) data were collected at each project time point (2.5, 3.5, and 5 years) using a 3T Siemens Prisma MR scanner. The subjects were separated from their social group for a total of approximately 5 hours to undergo the scanning process. This time

period included 45 minutes for preparation, including anaesthesia induction, intubation, and positioning in the scanner, 3 hours of active scanning, and 1 hour of post-anaesthesia recovery. During the scan, the subjects were positioned in a supine position and anaesthetized with isoflurane (1.5%/2% isoflurane, intubation). A stereotaxic frame was employed to secure the head and prevent movement in order to mitigate motion artifacts. A Vitamin E capsule was placed on the right temple to serve as a marker for the right brain hemisphere. Alphaxalone was administered for anaesthesia induction, and NaCl was administered intravenously for hydration during the scan. We used local anesthetic cream (Anesederm) in the external ears conduct prior the installation of the macaque in the stereotaxic frame. The subjects were also placed under an MRI-compatible heating blanket to maintain body temperature. Physiological measures such as heart rate, temperature, and blood oxygenation were continuously monitored and maintained in accordance with veterinary protocols throughout the scan. Upon completion of the scan and full recovery from anaesthesia, the subjects were returned to their social group.

High resolution anatomical MRI (T1/T2) scans were first obtained: MPRAGE T1-weighted images, 0.5mm isotropic, a bandwidth of 250Hz, a TR of 3000ms, a TE of 3.62ms, a TI of 1100ms, a flip angle of 8° and 144 cross-sections, and SPACE T2-weighted images with a spatial resolution of 0.5mm isotropic, a bandwidth of 710Hz, a TR of 3000ms, a TE of 366ms, a flip angle of 120° and 144 cross-sections (Figure 3). High resolution diffusion MRI scans were then also acquired to investigate differences in brain microstructure: custom pulse sequence based on a segmented 3-dimensional EPI sampling of Fourier space with the following parameters; diffusion weighted images (b-value of 1000s/mm²), 46 directions diffusion gradient encoding with two B0, a spatial resolution of 0.8mm isotropic, a bandwidth of 776Hz, a TR of 750 ms, a TE of 71ms, 4 segments, a FOV of 105x125x56mm³ and an acquisition time of 60 minutes (Tounekti et al., 2018) (Figure 3). Scans were acquired successfully from all subjects at both time-points.

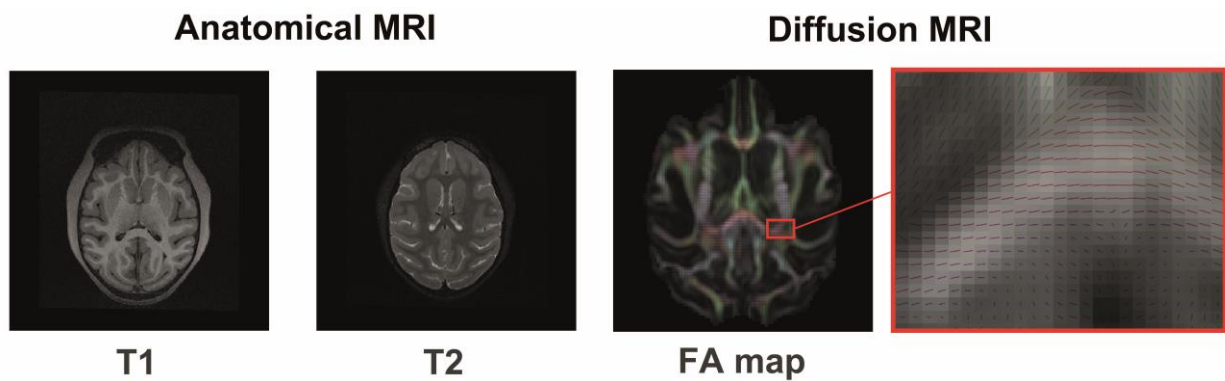


Figure 3. *Examples of raw MRI images from a macaque monkey.* The first two images are examples of different structural scans (T1, T2); the third is based on a diffusion MRI scan and shows a fractional anisotropy (FA) color map, displaying the diffusivity vector V_1 (principal direction vector) for each voxel (red represents left-right oriented fibers; blue represents dorsal-ventral oriented fibers; green represents anterior-posterior oriented fibers); the fourth image depicts a close-up view of the FA map.

MRI pre-processing

Anatomical:

The aim of the structural data analysis in this study was to extract and analyze morphometric features such as cortical thickness and volumes. The steps involved in this analysis are similar to the Human Connectome Project pipeline. First, brain extraction was performed using BET (Jenkinson, Pechaud, and Smith 2005). The T2w and T1w images were then registered and aligned to MNI space to create a native volume space. The resolution was lowered from 0.5 to 1 mm to run the freesurfer pipeline. Freesurfer was then used to generate a brain segmentation of the three tissues (white matter, grey matter, and subcortical matter) and brain surfaces (white matter, pial matter, and mid matter). Finally, the volumes and surfaces were rescaled back to the initial native resolution. Using FreeSurfer and Workbench anatomical white and pial 32k vertex surfaces were created for all the subjects both in their native space and in MNI nonlinear space. These surfaces were useful for brain cortical parcellation and tissue segmentation and the calculation of cortical thickness. The atlas Markov132 labels were projected onto the brain surfaces (Markov, 2014), which were aligned to the MNI nonlinear space. The cortical thickness values in each region of interest (ROI) were then extracted by calculating the mean of the cortical thickness at every vertex within the ROI. This method enabled analysis of morphometric features, specifically here, cortical thickness, within specific regions of the brain. We focused on specific areas corresponding to broader functional regions of the prefrontal cortex. The areas 9/46d, 9/46v, 46d, 46v corresponds to

dorsolateral PFC (dlPFC); the areas 44, 45a, 45b, correspond to ventrolateral PFC (vlPFC) (see REF: Petridis 1994; 2000; 2002a; 2005; 2012).

DTI TBSS:

The Diffusion Weighted Images (DWIs) underwent correction for movement and inhomogeneity distortions, as well as eddy current effects, utilizing FSL's topup and eddy algorithms (Woolrich, 2009; Anderson, 2003; Anderson, 2016a). The DTI maps were computed at the original resolution, and a study-specific template was generated at a higher resolution of 0.4 isotropic, through the use of the ANTs Optimal Template Construction algorithm (Avants, 2009). Subsequently, an affine registration of the fractional anisotropy (FA) maps of all subjects was performed onto the study template, resulting in transformation matrices that were employed to upscale the native DTI volumes to the resolution of the template. The decision to upscale was based on a comparison of the Tract-Based Spatial Statistics (TBSS) FA skeletons at both resolutions, with the 0.4 resolution resulting in more continuous FA skeletons and a maximum representation of white matter fibers. TBSS, a tool within FSL, enables a voxel-wise statistical approach while mitigating registration bias and multiple comparison issues by identifying the center of white matter bundles. For increased precision, TBSS non-linear scalar registration was replaced with a tensor registration using DTI-TK to the study-specific template (Zhang, 2007). Using the TBSS analysis, the impact of early psychosocial adversity on brain microstructure was studied by investigating the effects of rearing group on axial diffusivity (AD), radial diffusivity (RD), and FA.

DTI ROIs:

Diffusion parameters were computed for major brain fiber tracts. In order to obtain more accurate measurements of the DTI parameters in the white matter regions of interest, the extraction of these values was performed on the white matter skeletons generated by TBSS. In fact, the values of the skeletons correspond to the DTI values projected at the center of the bundles and are less impacted by the registration bias. To accomplish this, the ONPRC18 multimodal macaque atlas (REF: https://www.nitrc.org/projects/onprc18_atlas) was intersected with the skeleton masks. Then, for each region of the skeleton conventional DTI methods were used to calculate the mean values of FA, which represents the degree of directionality of random water diffusion, RD, which represents the diffusion of water perpendicular to axonal

fibers, and AD, which represents the diffusion of water parallel to axonal fibers. Higher FA is often interpreted to reflect greater white matter integrity, with the other metrics reflecting other measures and being used to calculate FA. FA is a widely used imaging metric that quantifies the degree of restriction in the direction of water molecule diffusion, and is elevated in white matter tracts that are tightly bundled and organized (Basser, 1995; Beaulieu, 2002; Sen and Basser, 2005). Although FA is highly sensitive to microstructural changes in white matter, it is less sensitive to the type of change. For example, lower FA could indicate either more diffusely organized white matter, lower axonal density, or reductions in myelination. RD and AD, reflect different properties of white matter microstructure (Schmithorst and Yuan, 2010). RD represents the amount of diffusivity that occurs perpendicular to the length of axons and is negatively correlated with myelination levels, reflecting developmental changes in myelination. AD represents the amount of diffusivity that occurs parallel to the length of axons and increases with maturational changes in development, potentially reflecting increased axonal organization and projection. In the current study, the primary analyses were based on FA values. All significant results involving FA in DTI ROIs were also followed up by examining differences in AD and RD in order to probe what may be driving differences in FA (reported in the SI).

Data analysis

Descriptive statistics for each of the brain measures are shown in Table S1 of the SI.

Executive function:

To compare performance on the original 'A-not-B' task between the two rearing groups over time, we computed a cumulative score (the sum of successful change trial delays divided by the number of total trials completed) for each subject at each assessment time-point. For the cumulative score, we conducted a linear mixed model with group, time-point, and their interaction included as fixed effects, and subject-specific random intercepts. The computational model was fitted separately for each subject at each assessment time-point. To compare the fitted model parameters between the two rearing groups across time, we used linear mixed models with group, time-point, and their interactions included as fixed effects, and subject-specific random intercepts. See Chapter 3 of this thesis for more details on the behavioural analyses.

Cortical thickness:

We then examined differences in cortical thickness in our *a priori* ROIs: the dorsolateral PFC (dlPFC), including areas 9/46d, 9/46v, 46d, 46v, and the ventrolateral PFC (vlPFC), including areas 44, 45A, 45B (see Fig. X). We used a mixed-model approach for analysis, with group (mother-reared/peer-reared), time-point (3.5/5 years), and their interactions included as fixed effects, and subject-specific random intercepts. For each area, right and left hemisphere analyses were run separately. Sex was controlled for in all analyses.

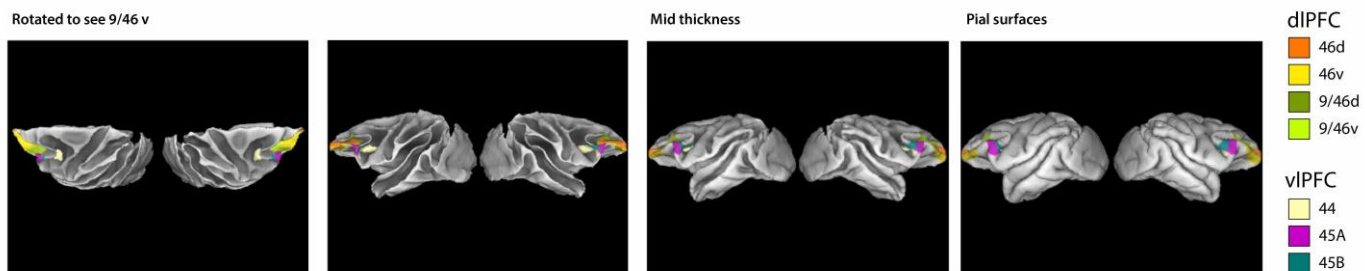


Figure x. ROIs for analyses of PFC cortical thickness. The different colours represent the different ROIs in dlPFC and vlPFC. These images show the ROIs in different layers of cortex.

White matter microstructure (TBSS):

To investigate differences between groups in white matter microstructure over the whole brain without specifying *a priori* regions of interest, we used a tract-based spatial statistics (TBSS) approach. Using the Randomise tool of FSL, a non-parametric t-test with 10000 permutations and threshold-free cluster enhancement was performed to compare white matter microstructure between the two rearing groups. This was done for each of the three assessment time points and with all time-points combined. The results were corrected using family-wise error correction to avoid false positives generated due to a multiple comparison problem. The results were considered significant at a $p < 0.05$. Sex was controlled for in all analyses.

White matter microstructure (ROIs):

We then examined differences in white matter integrity in our *a priori* ROIs: the superior longitudinal fasciculus (SLF), i.e. fronto-parietal circuitry linked to EF; and the anterior corona radiata (ACR), anterior limb of internal capsule (ALIC), and external capsule (EC), i.e. fronto-striatal circuitry linked to EF (see Figure 4). We used a mixed-model approach for analysis, with

group (mother-reared/peer-reared), time-point (3.5/5 years), and their interactions included as fixed effects, and subject-specific random intercepts. Separate analyses were run for each tract in each hemisphere. Sex was controlled for in all analyses.

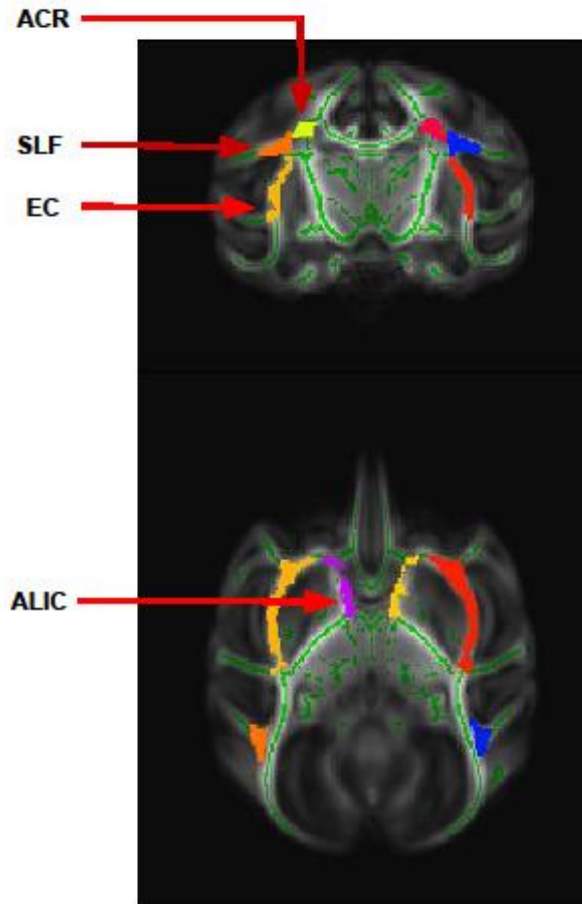


Figure 4. Coronal and axial views of the study template with FA skeleton (green) and symmetrical regions of the ONPCR18 atlas overlaid. ROIs are highlighted as follows: anterior corona radiata (ACR: left hemisphere – yellow; right hemisphere – red); superior longitudinal fasciculus (SLF: left hemisphere – orange; right hemisphere – blue); external capsule (EC: left hemisphere – light orange; right hemisphere – light red); and anterior limb of the interior capsule (ALIC: left hemisphere – purple; right hemisphere – dark yellow).

Relationships between cortical thickness in PFC regions and EF:

We also investigated the relationship between general EF performance (cumulative score), as well as the working memory (w_1, λ_1), inhibitory control (w_2, λ_2), and choice stochasticity (β) parameters of the computational model, and cortical thickness of dlPFC, and vlPFC both concurrently and prospectively. Separate models were run for each ROI and hemisphere. As a number of analyses were run predicting the same outcome (see below), our Bonferroni-

adjusted alpha level for significance was set to $p = 0.0036$ (i.e. $p = 0.05/14$; left and right hemisphere, 7 regions). Descriptive statistics for each variable are shown in Table S1 in the SI.

Concurrent statistical analyses

To examine concurrent relationships between cortical thickness and EF, a mixed modelling framework was adopted. For cumulative score, w_1 , w_2 , λ_2 , and β , we used linear mixed models with cortical thickness, group (mother-reared/peer-reared), time-point (3.5/5 years), and their interactions included as fixed effects, and subject-specific random intercepts. For λ_1 (which was binarized), we used a generalized linear mixed model with cortical thickness, group, time-point, and their interactions included as fixed effects, and subject-specific random intercepts.

Prospective statistical analyses

To examine how cortical thickness measures at 3.5 years may prospectively predict EF at 5 years, we also ran a number of regression analyses. For cumulative score, w_1 , w_2 , λ_2 , and β at 5 years, we used linear regressions with cortical thickness at 3.5 years, group (mother-reared/peer-reared), and their interactions as fixed effects. For λ_1 at 5 years, we used a generalized linear regression with cortical thickness at 3.5 years, group, and their interactions included as fixed effects.

Relationships between structural connectivity in fronto-parietal and fronto-striatal circuits and EF:

Finally, we investigated the relationships between general EF performance (cumulative score), as well as the working memory (w_1 , λ_1), inhibitory control (w_2 , λ_2), and choice stochasticity (β) parameters of the computational model, and white matter integrity of the SLF, ACR, ALIC, and EC, both concurrently and prospectively. Separate models were run for each ROI and hemisphere. As a number of analyses were run predicting the same outcome (see below), our Bonferroni- adjusted alpha level for significance was set to $p = 0.006$ (i.e. $p = 0.05/8$; left and right hemisphere, four tracts). Descriptive statistics for each variable are shown in Table S1 in the SI.

Concurrent statistical analyses

To examine concurrent relationships between DTI measures and EF, a mixed modelling framework was adopted. For cumulative score, w_1 , w_2 , λ_2 , and β , we used linear mixed models with DTI, group (mother-reared/peer-reared), time-point (3.5/5 years), and their interactions included as fixed effects, and subject-specific random intercepts. For λ_1 (which was binarized), we used a generalized linear mixed model with DTI, group, time-point, and their interactions included as fixed effects, and subject-specific random intercepts.

Prospective statistical analyses

To examine how DTI measures at 3.5 years may predict EF prospectively at 5 years, we also ran a number of regression analyses. For cumulative score, w_1 , w_2 , λ_2 , and β at 5 years, we used linear regressions with DTI at 3.5 years, group (mother-reared/peer-reared), and their interactions as fixed effects. For λ_1 at 5 years, we used a generalized linear regression with DTI at 3.5 years, group, and their interactions included as fixed effects.

R v4.2.1 was utilized to conduct all analyses presented here involving cortical and white matter ROIs (see SI for package information). *P*-values for fixed effects and interactions were obtained using Type III F tests for linear models, and Type III Wald χ^2 tests for generalized linear models. Sex was controlled for in all analyses, and presence of influential cases was checked for and outliers higher than 1 Cook's distance removed.

RESULTS

Summary of results from Massera et al. (under review): General EF, working memory, and inhibitory control

We first aimed to investigate potential variations in task performance between the two rearing groups and time-points by comparing the cumulative score from the 'A-not-B' task. The results show that the mother-reared group performed better than the peer-reared group, with a significant main effect of group revealed [$F(1) = 12.643$, $p < 0.01$]. There were no significant effects of time-point or the interaction between group and time-point. We then sought to investigate the cognitive mechanisms that underlie the observed performance differences between the two groups via comparison of the fitted computational model parameters. Results revealed a significant main effect of group on working memory decay (λ_1)

[$F(1) = 7.008, p = 0.014$], with the peer-reared group exhibiting a faster rate of decay compared to the mother-reared group. No significant effects of time-point or group by time-point interaction were found, nor were there any significant differences in the weight of working memory parameter (w_1). Additionally, no main effects of group or time-point were observed on the weight of the choice history parameter (w_2). However, a significant interaction between group and time-point was detected [$F(1) = 7.159, p = 0.021$], with w_2 decreasing from time-point 1 to time-point 2 in the mother-reared group, and increasing in the peer-reared group. However, we did not find a significant difference between the two groups and between time-points in follow-up analyses. No main effects or interactions were found for choice history decay rate (λ_2), or choice stochasticity (β). See Chapter 3, Figures 4- 6.

Therefore, peer-reared animals were found to demonstrate worse general EF, performing worse on the 'A-not-B' task at both time-points compared to mother-reared. This poor performance appears related to both problems in working memory at 3.5 and 5 years, and inhibitory control at 5 years. See SI for descriptive measures (Table S1).

Differences between groups in cortical thickness in dlPFC and vlPFC associated with EF

We then wanted to investigate the effects of early psychosocial deprivation on the development of two specific prefrontal regions important for EF (dlPFC, and vlPFC) across adolescence into adulthood. All main effects or interactions are also reported in the SI in Table S2.

For dlPFC, a significant main effect of group was found in several areas (Figure 5). Peer-reared group had thicker cortex in the left 46d area [$\chi(1) = 7.6423, p = 0.006$], in the right 46v area [$\chi(1) = 3.9604, p = 0.047$], in the right 9/46d area [$\chi(1) = 4.2627, p = 0.039$], and in both the right and left 9/46v areas [right, $\chi(1) = 4.8845, p = 0.027$; left, $\chi(1) = 5.5396, p = 0.019$]. In addition, we also found an effect of year in the right 46v area [$\chi(1) = 8.3011, p = 0.004$], with thinner cortex at 5 years in both groups. No other significant main effects or interactions were found in dlPFC.

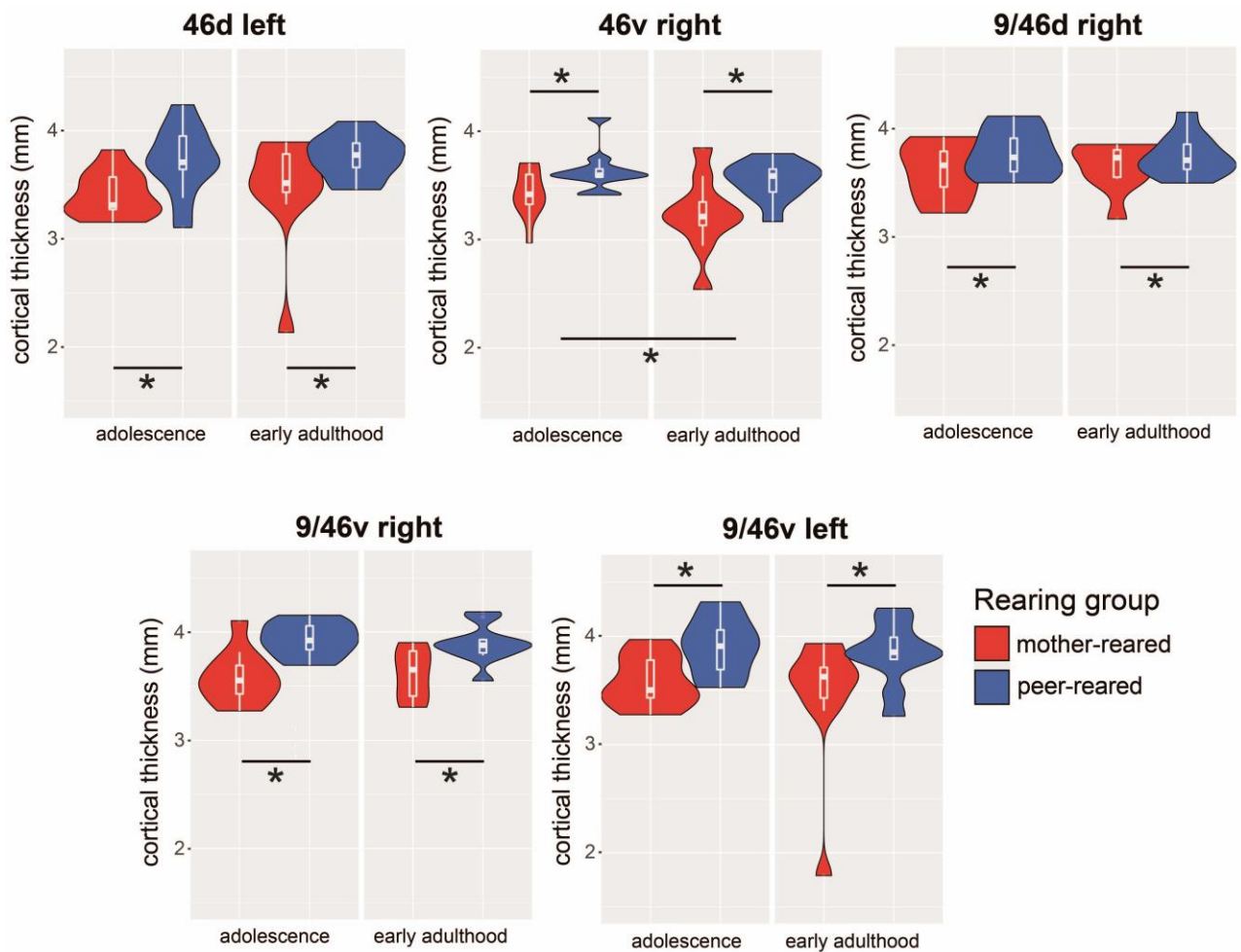


Figure 5. Group differences in dlPFC cortical thickness in the different ROIs. Across time, greater cortical thickness was found in the peer-reared (blue) versus mother-reared (red) group in the left 46d, the right 46v, the right 9/46d, and the right and left 9/46v areas. Only in the right 46v we found thinner cortex in early adulthood compared to adolescence in both groups. Significant effects are denoted by *. Violin plots represent the density of the variable, while box-plot represent summary statistics, with error bars reflecting +/- SE.

For vlPFC, a significant main effect of group was found in both the right and the left 45B areas [right, $\chi(1) = 10.8470$, $p < 0.001$; left, $\chi(1) = 22.1000$, $p < 0.001$], with thicker cortex in peer-reared group compared to the mother-reared group (Figure 6). No other significant main effects or interactions were found in vlPFC.

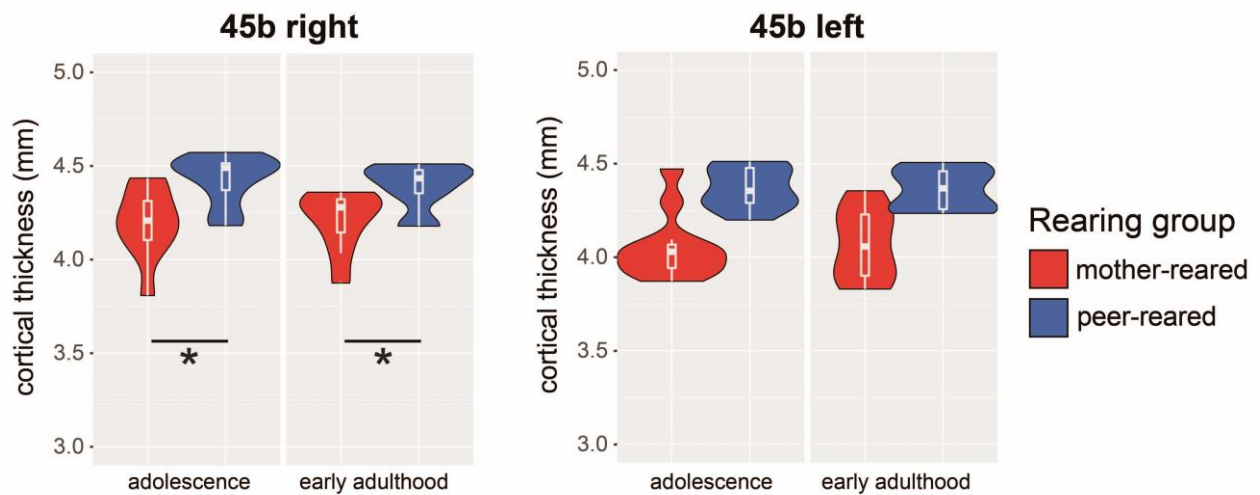


Figure 6. Group differences in vIPFC cortical thickness in the different ROIs. Across time, greater cortical thickness was found in the peer-reared (blue) versus mother-reared (red) group in the right and left 45B areas. Significant effects are denoted by *. Violin plots represent the density of the variable, while box-plot represent summary statistics, with error bars reflecting +/- SE.

Differences in whole-brain white matter between groups

We then examined the effects of early social deprivation on white-matter integrity overall. We compared whole brain white-matter integrity between the two groups using TBSS at three time points separately (2.5, 3.5, and 5 years) and with the three years pooled together. We did not find any significant results between the two groups in the analyses at the three time points separately. In addition, the results show no significant difference in FA between the two groups with the three years pooled together. However, we found a significant difference between the two groups in RD (Figure 7) in the right and SLF, right and left EC, left superior corona radiate, and right posterior limb of internal capsule. RD was greater in the mother-reared versus peer-reared group (all clusters $p < 0.05$).

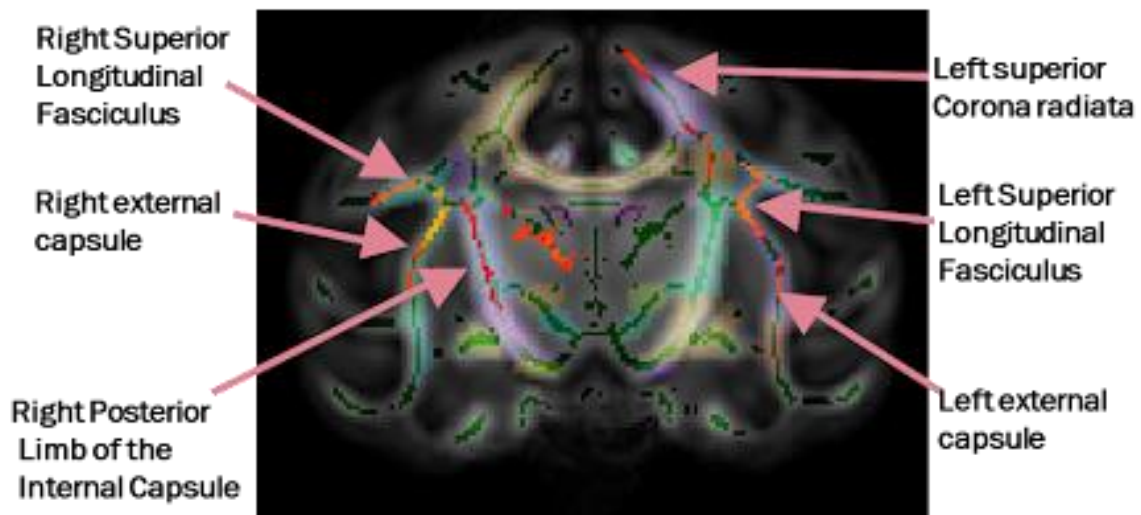


Figure 7: TBSS analysis results (RD). Main effects of group. Green voxels indicate the FA skeleton produced as part of the TBSS analysis. Red/yellow voxels represent the statistically significant clusters (thresholded at $p < 0.05$) where mother-reared animals had significantly higher RD than the nursery-reared ones. The ONPRC18 atlas is overlaid on the image to locate the regions where the clusters belong.

We also found significant differences between the two groups in AD (Figure 8), with mother-reared having higher AD in the left superior corona radiata, body of corpus callosum, and left dorsal posterior corona radiata (all $p < 0.05$).

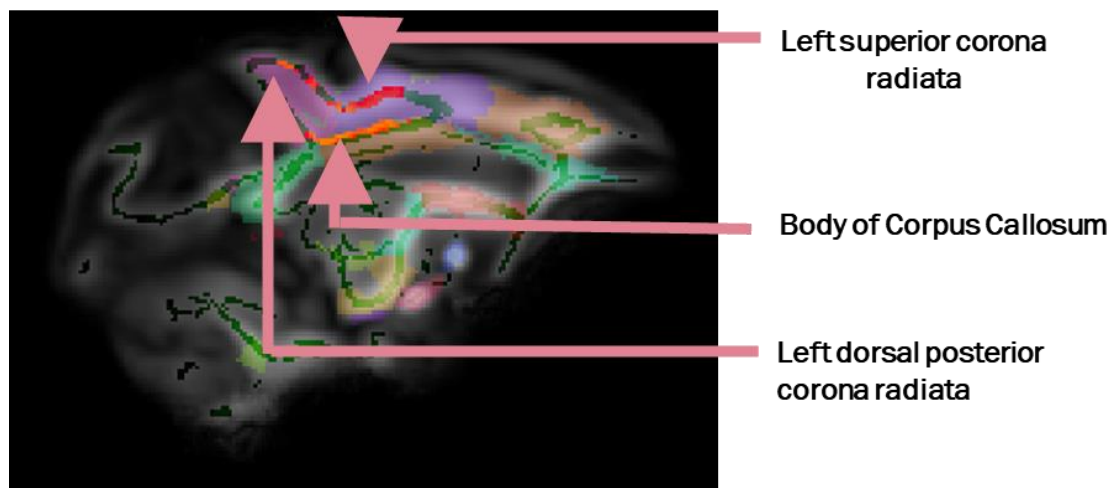


Figure 8: TBSS analysis results (AD). Main effects of group. Green voxels indicate the FA skeleton produced as part of the TBSS analysis. Red/yellow voxels represent the statistically significant clusters (thresholded at $p < 0.05$) where mother-reared animals had significantly higher AD than the nursery-reared ones. The ONPRC18 atlas is overlaid on the image to locate the regions where the clusters belong.

Differences between groups in specific white matter tracts associated with EF and early adversity

We then investigated the effects of early psychosocial deprivation on the development of four specific white-matter tracts important for EF (SLF, ALIC, ACR, EC) across adolescence into adulthood. All main effects or interactions are also reported in the SI in Table S2.

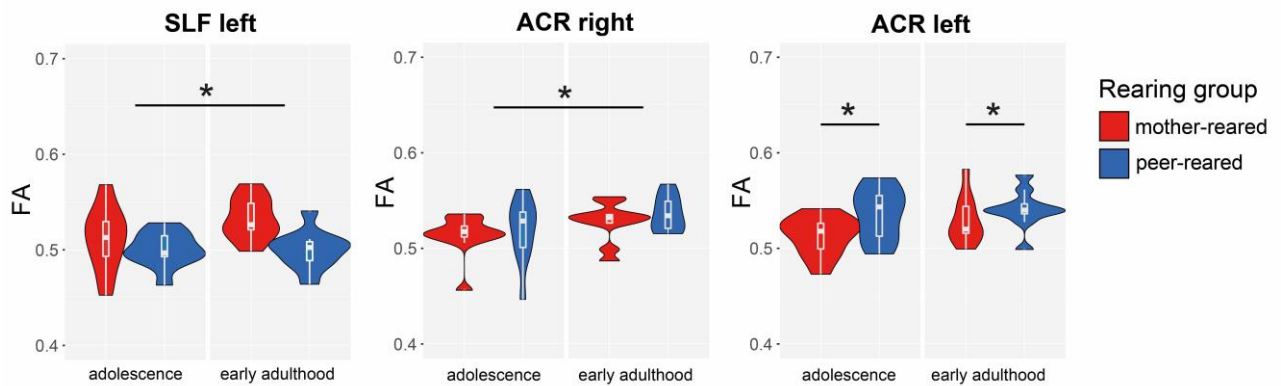


Figure 9. Group differences in FA in the SLF and ACR white matter tracts. Across time, greater white-matter integrity was found in the peer-reared (blue) versus mother-reared (red) group in the left anterior corona radiata (ACR). In both groups, greater white-matter integrity was found in early adulthood compared to adolescence in the left superior longitudinal fasciculus (SLF), and in the right ACR. Significant effects are denoted by *. Violin plots represent the density of the variable, while boxplot represent summary statistics, with error bars reflecting \pm SE.

For SLF, a significant main effect of time point was observed in the left hemisphere [$\chi(1) = 5.712, p = 0.017$]. Specifically, there was higher FA at 5 years compared to 3.5 years (see Figure 9). However, no significant effect of group or group by time point interaction was found in the left SLF, and no significant results were observed in the right FA SLF.

For ACR, we found a significant main effect of year in the right hemisphere [$\chi(1) = 4.647, p = 0.031$], with higher FA at 5 years compared to 3.5 years. We did not find a significant effect of group or group by time point interaction in right ACR. In contrast, in the left ACR, we identified a significant main effect of group [$\chi(1) = 4.632, p = 0.031$], with higher FA in the peer-reared group compared to the mother-reared group. In the left ACR we also detected a trend for time point [$\chi(1) = 3.6385, p = 0.056$], with higher FA at 5 years compared to 3.5 years. No significant group by time point interaction for FA was detected in the left ACR. These results are illustrated in Figure 9.

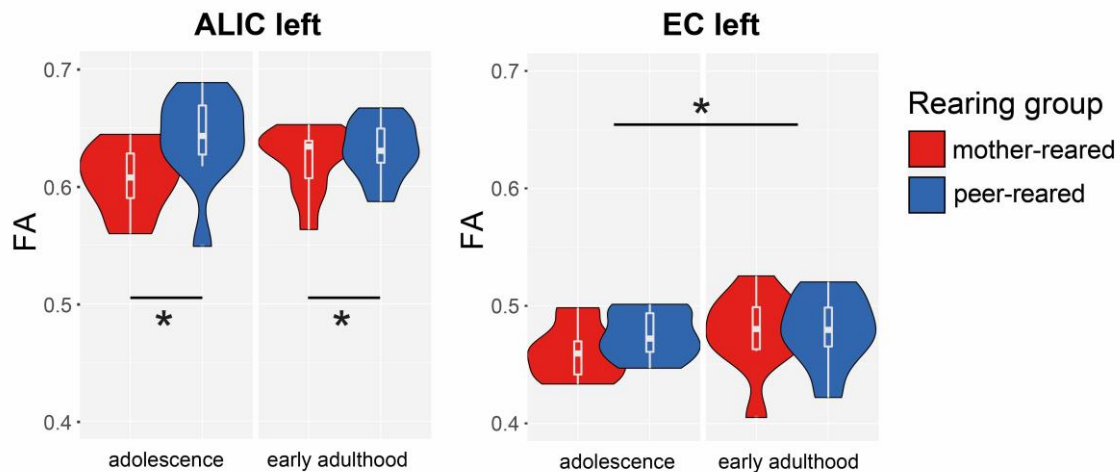


Figure 10. Group differences in FA in the ALIC and EC white matter tracts. Across time, greater white-matter integrity was found in the peer-reared (blue) versus mother-reared (red) group in the left anterior limb of internal capsule (ALIC). In both groups, greater white-matter integrity was found in early adulthood compared to adolescence in the left external capsule (EC). Significant effects are denoted by *. Violin plots represent the density of the variable, while box-plot represent summary statistics, with error bars reflecting +/- SE.

For ALIC, we found a significant main effect of group in the left hemisphere [$\chi(1) = 8.199, p = 0.004$], with higher values of FA in peer-reared animals compared to mother-reared animals. There was no effect of time point or group by time point interaction in the left hemisphere for FA. Furthermore, no significant results were obtained in the right ALIC. See Figure 10 for FA illustration of the FA results.

For EC, we identified a significant main effect of time point on FA in the left hemisphere [$\chi(1) = 4.970, p = 0.026$], with higher values at 5 years compared to 3.5 years (see Figure 10). No other significant relationships were found in the right and left EC for FA.

Concurrent cortical thickness ROI-EF relationships

In order to determine whether differences in EF between the two rearing groups were associated with concurrent differences in the cortical thickness of relevant neural circuitry over time, we examined the relationship between cortical thickness in two prefrontal areas (dlPFC, vlPFC) and EF as measured via the cumulative score and fitted model parameters. In the text the significant cortical thickness main effects or interactions are reported, while all the other effects and interactions reported in Table S3 in the SI.

For dlPFC, a number of significant interactions with cortical thickness were revealed. For the analysis predicting w_1 , in area 9/46d we found a significant interaction between cortical thickness, and time point in the left hemisphere [$\chi(1) = 10.9092, p = <0.001$]. Specifically, at 5 years, thicker cortex was related to lower w_1 (i.e. worse working memory), compared to 3.5 years [$t(1) = 2.771, p = 0.0143$]. For w_1 , we also found a significant interaction in area 9/46v between cortical thickness and time point in the right hemisphere [$\chi(1) = 11.2848, p < 0.001$]. However, the follow-up analyses were not significant. For β , we found a significant interaction in area 46d between cortical thickness, and group in the right hemisphere [$\chi(1) = 8.6568, p = 0.003$]. Specifically, the mother-reared group differed from the peer-reared group [$t(1) = 2.352, p = 0.0257$], but no other significant follow-ups were found.

For vlPFC, a number of significant interactions with cortical thickness were revealed. For cumulative score, we found a significant interaction in area 44 between cortical thickness, and time point in the right hemisphere [$\chi(1) = 8.6102, p = 0.003$]. However, the follow-up analyses were not significant. For cumulative score we also found a significant interaction in area 45B between cortical thickness and time point in the right hemisphere [$\chi(1) = 18.7350, p < 0.001$], with the cortical thickness – cumulative score relationship changing from 3.5 years to 5 years [$t(1) = 2.896, p = 0.01$], but no other significant follow-ups were found. For the analysis predicting w_1 , we found a significant interaction in area 45B between cortical thickness and group [$\chi(1) = 9.9779, p = 0.001$], and between cortical thickness and year in the left hemisphere [$\chi(1) = 9.0307, p = 0.002$] (see Figure 11). The follow-up tests for the group by time point interaction were not significant, but for the mother-reared animals, thicker cortex in area 45B was related to higher w_1 (i.e. better working memory) [$t(1) = 2.043, p = 0.05$], compared to the peer-reared group [$t(1) = 2.445, p = 0.0208$]. For the analysis predicting λ_1 , we found a significant interaction in area 45A between cortical thickness, time point, and group in the right hemisphere [$\chi(1) = 9.2507, p < 0.001$]. At 3.5 years for the mother-reared group, thicker cortex was related to lower λ_1 (i.e. slower working memory decay / better working memory) [$t(1) = -2.765, p = 0.006$], compared to the peer-reared group [$t(1) = -2.778, p = 0.005$], and to 5 years [$t(1) = -2.983, p = 0.003$]. For the analysis predicting λ_2 , we found a significant interaction in area 45B between cortical thickness, time point, and group in the right hemisphere [$\chi(1) = 8.5167, p = 0.003$] (see Figure 11). Specifically, at 3.5 years for the peer-reared group, thicker cortex was related to lower λ_2 (i.e. slower decay/ worse inhibitory

control) [$t(1) = -4.544, p < 0.001$], compared to the mother-reared group [$t(1) = 4.841, p < 0.001$], and to 5 years [$t(1) = -2.866, p = 0.01$]. For the analyses of β , we found a significant interaction between cortical thickness, group, and time point in area 45A in the left hemisphere [$\chi(1) = 9.1172, p = 0.002$]. That is, in the peer-reared group at 3.5 years, thicker cortex was related to a lower β (i.e. more random choices) [$t(1) = -2.193, p = 0.0364$], compared to 5 years [$t(1) = -2.131, p = 0.0471$], and differing from the mother-reared group at 3.5 years [$t(1) = 2.609, p = 0.014$]. Additionally, the relationship between cortical thickness and β in the mother-reared group changed from 3.5 years to 5 years [$t(1) = 2.169, p = 0.0469$], but no other significant follow-ups were found. For β , an interaction between cortical thickness and group was found in area 45B in the right hemisphere [$\chi(1) = 8.5956, p = 0.003$], with thicker cortex related to lower β (i.e. more random choices) in the peer-reared group [$t(1) = -2.360, p = 0.025$].

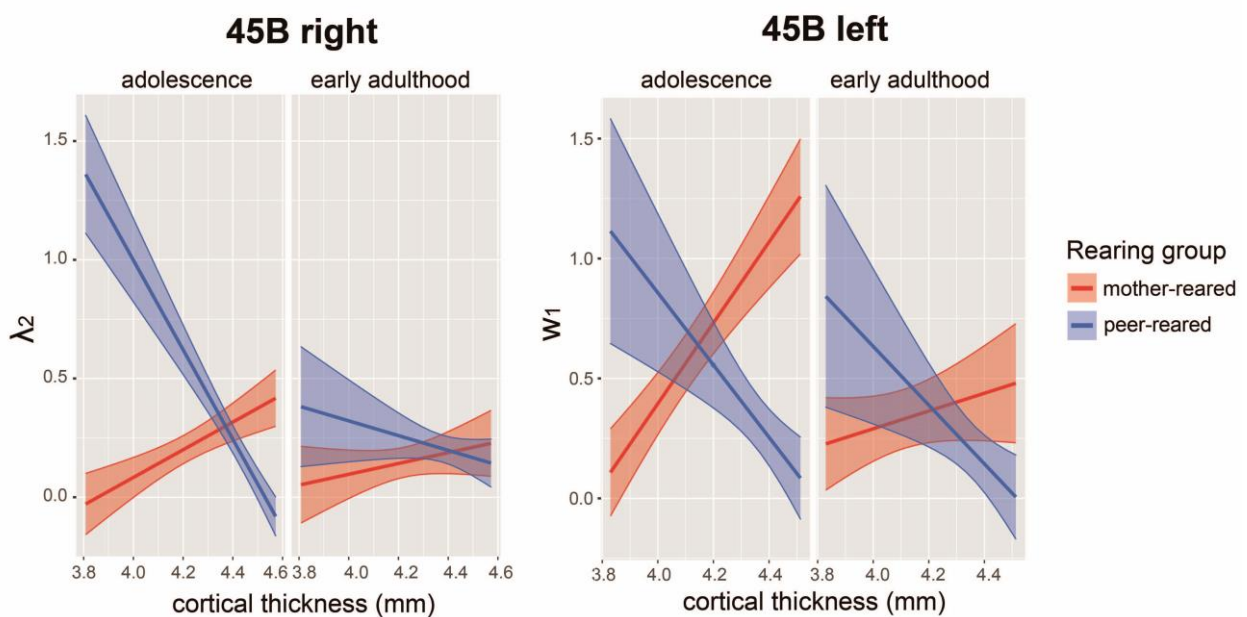


Figure 11. *vIPFC thickness and parameters interaction.* Greater cortical thickness predicted lower λ_2 (i.e. slower decay/ worse inhibitory control) in the peer-reared (blue) versus mother-reared (red) group in right area 45B, during adolescence but not in early adulthood. Thicker cortex in left area 45B predicted higher w_1 (i.e. better working memory) in mother-reared group compared to peer-reared group across adolescence into early adulthood. Error bars represent +/- SE.

Prospective cortical thickness ROI-EF relationships

We then sought to determine whether cortical thickness in the same prefrontal areas (dlPFC, vlPFC) during adolescence predicted prospective EF in early adulthood. No significant results were revealed.

Concurrent DTI ROI-EF relationships

In order to determine whether differences in EF between the two rearing groups were associated with concurrent differences in the structural connectivity of relevant neural circuitry over time, we examined the relationship between white matter integrity in four WM tracts (SLF, ACR, ALIC, EC) and EF as measured via the cumulative score and fitted model parameters. In the text the significant DTI main effects or interactions are reported, while all the other effects and interactions reported in Table S3 in SI.

For the SLF, we found a significant interaction between FA and time point related to cumulative score in the right hemisphere [$\chi(1) = 7.5402, p = 0.006$]. Specifically, at 3.5 years, higher FA was related to greater cumulative score (i.e. better performance) [$t(1) = 2.677, p = 0.011$], being different from 5 years [$t(1) = 2.280, p = 0.032$]. Additionally, higher FA was related to a greater cumulative score (i.e. better EF performance) in the left hemisphere, with a main effect of FA revealed [$\chi(1) = 16.106, p < 0.001$]. We did not find any other significant effects of time point, group, or interactions for SLF for other outcome variables.

For the ACR, for analyses predicting λ_1 , a significant interaction between FA, group, and time-point was revealed in the left hemisphere [$\chi(1) = 2043.7960, p < 0.001$]. For the mother-reared group at 5 years, higher FA was related to lower λ_1 (i.e. slower working memory decay/better working memory) [$z(1) = -35.237, p < 0.001$], compared to the peer-reared group at 5 years [$z(1) = -35.696, p < 0.001$], and to 3.5 years [$z(1) = 48.370, p < 0.001$]. We did not find any other significant effects of time point, group, or interactions for ACR for other outcome variables.

For the ALIC, a number of significant interactions with FA were revealed. For the analysis predicting w_1 , we found a significant interaction between FA, group, and time point in the right hemisphere [$\chi(1) = 15.234, p < 0.001$] (see Figure 12). That is, in the mother-reared group, higher FA was related to higher w_1 (i.e. better working memory) at 3.5 years [$t(1) = 2.688, p = 0.011$] significantly different from the peer-reared group [$t(1) = 2.701, p = 0.012$], but to a lower w_1 (i.e. worse working memory) at 5 years [$t(1) = -2.290, p = 0.03$] significantly different

from the peer-reared group [$t(1) = -2.444, p = 0.021$], and differing between the two time points [$t(1) = 3.446, p = 0.002$]. Additionally, for the analysis predicting the decay rate of working memory (λ_1), we also found a significant interaction between FA, group, and time point in the right hemisphere [$\chi(1) = 2.6082e+07, p < 0.001$]. In the mother-reared group, higher FA was related to higher λ_1 (i.e. faster working memory decay /worse working memory) at both time points [3.5 years, $z(1) = 9850.001, p < 0.001$; 5 years, $z(1) = 2398.029, p < 0.001$], with a stronger effect seen at 3.5 years [$z(1) = 6459.734, p < 0.001$]. In the peer-reared group, higher FA was related to lower λ_1 (i.e. slower working memory decay / better working memory) at both time points [3.5 years, $z(1) = -2835.353, p < 0.001$; 5 years, $z(1) = -2691.382, p < 0.001$], with a stronger effect seen at 5 years [$z(1) = 970.664, p < 0.001$]. The relationship between FA and λ_1 also differed between the mother-reared and peer-reared groups at both time points [3.5 years, $z(1) = 13862.528, p < 0.001$; 5 years, $z(1) = 6205.178, p < 0.001$]. Finally, for the analysis of the weight of the choice history factor (w_2), a significant interaction between white matter integrity (FA), group, and time point in the right hemisphere was found [$\chi(1) = 8.134, p = 0.004$] (see Figure 12). Specifically, higher FA in the mother-reared group was related to higher w_2 (i.e. repeating previous choices more frequently/worse response inhibition) at 5 years [$t(1) = 2.250, p = 0.031$], differing from 3.5 years [$t(1) = -2.242, p = 0.032$], and from the peer-reared group [$t(1) = 2.332, p = 0.026$]. No significant effects of FA were found on the decay rate of choice history (λ_2), choice stochasticity (β), or cumulative score.

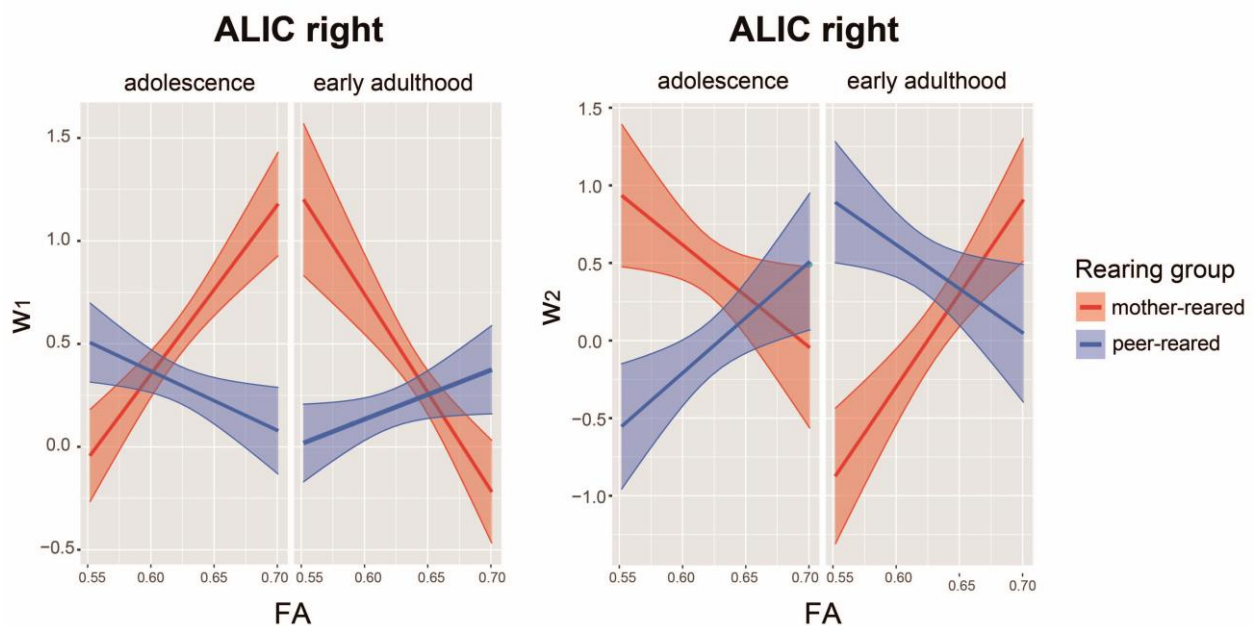


Figure 12. *Right anterior limb of internal capsule (ALIC) FA and parameters interactions.* Higher FA in the right ALIC was related to higher w_1 (i.e. better working memory) during adolescence, but to a lower w_1 (i.e. worse working memory) in early adulthood in the mother-reared group (red) compared to the peer-reared group (blue). In the right ALIC, higher FA was also related to higher w_2 (i.e. repeating previous choices more frequently/worse response inhibition) in early adulthood in the mother-reared group (red) compared to the peer-reared group (blue). Error bars represent +/- SE.

For the EC, we found a significant interaction between FA, group, and time point for the analysis predicting the decay rate of working memory (λ_1) in both hemispheres [right, $\chi(1) = 120.5388, p < 0.001$]; left, $\chi(1) = 2.5229e+10, p < 0.001$]. Specifically, in the right EC, higher FA was related to lower λ_1 (i.e. slower working memory decay / better working memory) at 5 years in the mother-reared group [$z(1) = -14.588, p < 0.001$], compared to 3.5 years [$z(1) = 12.970, p < 0.001$], and compared to the peer-reared group [$z(1) = -12.187, p < 0.001$]. In the left EC, at 3.5 years, higher FA was also related to higher λ_1 (i.e. faster working memory decay/ worse working memory) in both groups [mother-reared, $z(1) = 1261.430, p < 0.001$; peer-reared, $z(1) = 6639.270, p < 0.001$], with a stronger effect in the peer-reared group [$z(1) = -7873.180, p < 0.001$]. At 5 years, higher FA was related to lower λ_1 (i.e. slower working memory decay / better working memory) in both groups [mother-reared, $z(1) = -161383.850, p < 0.001$; peer-reared, $z(1) = -24695.320, p < 0.001$], with a stronger effect in the mother-reared group [$z(1) = -129133.570, p < 0.001$]. For both rearing groups the FA - λ_1 relationship differed between the two time points [mother-reared, $z(1) = 207545.560, p < 0.001$; peer-reared, $z(1) = 37728.890, p < 0.001$]. We also did not find any interaction between FA and the weight of working memory (w_1), weight of choice history (w_2), decay rate of choice history (λ_2), choice stochasticity (β), or cumulative score.

Prospective DTI-EF relationships

We then sought to determine whether white matter integrity (FA) in the same four WM tracts (SLF, ALIC, ACR, EC) during adolescence predicted prospective EF in early adulthood.

For the SLF at 3.5 years, in the models predicating w_2 at 5 years, we found a main effect of FA, with higher FA related to lower w_2 (i.e. repeating fewer previous choices/worse response inhibition) [$F(1) = 13.296, p = 0.003$] in the right hemisphere. Finally, no other main effects of FA or FA, group, time point interactions were found in any other models.

DISCUSSION

The purpose of this study was to examine how effects of early psychosocial deprivation on macaque executive function (EF) may be associated with differences in structural brain development across adolescence. Using both anatomical and diffusion MRI, we sought to clarify this relationship between EF and specific brain regions and circuitry critical for EF using different metrics of assessment, including gray matter thickness in prefrontal cortex (PFC) and white matter microstructure in fronto-parietal and fronto-striatal fiber tracts. Results indicate a functional division in the lateral PFC, with the dorsolateral PFC (dlPFC) associated with working memory and stochasticity of choice behavior (or ‘randomness’ in decision making), and the ventrolateral PFC (vlPFC) associated with all measures of EF. Differences between rearing groups were found for vlPFC thickness, with these differences also predicting differences in EF between groups. Early psychosocial deprivation was also linked to differing developmental trajectories of white matter microstructure in fronto-parietal and fronto-striatal circuits. Both fronto-parietal and most fronto-striatal tracts underwent protracted development across adolescence into adulthood, with increased white matter integrity predicting EF in opposite directions in the peer-reared versus mother-reared group in particular fronto-striatal tracts. These findings provide new insights into the longitudinal and long-term effects of early psychosocial deprivation on neurocognitive development, highlighting the involvement of the vlPFC and fronto-striatal circuits in the poor EF seen in affected individuals, during the transition between adolescence and adulthood. Additionally, these results offer novel information about the neural bases of general and specific components of executive function across normative macaque development.

Cortical thickness in lateral prefrontal cortex

Findings regarding cortical thickness in the lateral prefrontal cortex (PFC) support our hypothesis, with thicker cortex found in the peer-reared compared to mother-reared group in both the dlPFC and vlPFC. An effect of age was found only in dlPFC, with thinner cortex in both groups in early adulthood compared to adolescence. These findings are in line with studies on institutionalization in humans, which have found greater cortical thickness (including in PFC) in institutionalized adolescents and young adults compared to non-institutionalized individuals (Sheridan, 2022; Mackes, 2020). This is also in keeping with studies on normative development in terms of cortical thickness in humans and macaques, with thinning seen

across childhood and adolescence (e.g. Ramirez, 2021). Our results also support the idea that, like in humans, the lateral PFC plays an important role in the refinement of EF in macaques across adolescence. More specifically, the dlPFC was linked to working memory in both groups, with thicker cortex predicting worse sensory-spatial working memory (i.e. w_1) in early adulthood in all animals. The vlPFC was linked to different and overlapping measures of EF, with changes in some of these relationships seen over time, and more differences between rearing groups found. Notably, vlPFC thickness in the right hemisphere predicted general EF differently across time, and better information retrieval/longer-term working memory maintenance (i.e. λ_1) in adolescence in mother-reared group. Conversely, thicker vlPFC in the right hemisphere predicted worse inhibitory control maintenance (i.e. λ_2) in adolescence and more random decisions across time in the peer-reared group. Additionally, thicker cortex in the left vlPFC in the mother-reared group predicted better sensory-spatial working memory across time and predicted decision-making (i.e. θ) across time differently, while in the peer-reared group, thicker cortex in this region predicted more random choices (i.e. θ) during adolescence. We did not find prospective relationships between dlPFC and vlPFC and EF. This may suggest that other brain measures better predict future capacity, such as functional activity, but this lack of findings also may be attributed to limited statistical power (e.g. mixed models could not be used).

These results concerning cortical thickness partially align with previous research indicating significant contributions of both the dlPFC and vlPFC to working memory and inhibitory control in humans. For example, dlPFC has been associated with domain general executive control functions, cognitive control, and aspects of working memory (Hertrich, 2021; Petrides, 2005; Nejati, 2021; Haber, 2022), while vlPFC has been linked to active valuation, inhibition, rule acquisition, and for the delay phase of working memory tasks (Nelson, 2011; Haber, 2022). Evidence also suggests that both dlPFC and vlPFC play an important role in working memory and inhibitory control across development (Fiske, 2019). Previous research with macaques suggests that dlPFC is more clearly linked to working memory monitoring and abstract rules, while vlPFC is to first-order executive processes such as active selection, comparison, and judgment of stimuli held in shorter-term and longer-term memory (Petrides, 1998, 2002). Our findings do support the existence of a functional division between dlPFC and

vIPFC, with dIPFC being more involved in working memory specifically and randomness of choices, and vIPFC related to multiple measures including general EF performance.

To summarise findings in terms of PFC cortical thickness presented, the peer-reared group had greater thickness in both dIPFC and vIPFC. In dIPFC, thicker cortex predicted better EF in both groups in early adulthood. In vIPFC, thicker cortex predicted worse EF in the peer-reared group, while it predicted better EF in mother-reared group across time. Notably, while the peer-reared group had thicker cortex in both dIPFC and vIPFC, it also appeared that both groups have greater thickness in vIPFC compared to dIPFC (though we did not test the latter statistically; see SI for descriptive statistics). This could suggest that for vIPFC in mother-reared animals, thicker cortex at the ages examined in the current research reflect normative and beneficial development, enabling the continuation of refinements in EF, and thus flexibility and plasticity in terms of subsequent development. In the peer-reared animals, however, greater vIPFC thickness (which was also thicker overall in peer versus mother groups) may have reflected supra-optimal development, with a lack of expected thinning taking place due to accelerated development prior to adolescence, and hence being associated with impaired EF. If dIPFC undergoes less protracted development than vIPFC, thicker cortex in dIPFC here could reflect mean delayed or less flexible development in all animals, thus being associated with worse behavioural outcomes but not differently between groups. In fact, evidence suggest that working memory develops largely prior to puberty, as well as associated cortical development (e.g. dIPFC) (Zhou, 2016), while inhibitory control and vIPFC undergo a more protracted period of development and plasticity, in both human and macaques (Sydnor, 2021). Also in support of this, previous studies on the effects of early psychosocial deprivation in humans found thicker cortex in institutionalized individuals only during adolescence and early adulthood, while at earlier ages, they found thinner cortex in the context of institutional rearing (Sheridan, 2022, Hodel, 2015). The more advanced thinning of PFC in the mother-reared group could thus be explained by both typical levels of synaptic pruning and progressive myelination in these cortical areas during adolescence, which is linked to better cognitive performance when it occurs during the expected sensitive period of development (Marsh, 2009). However, a more extreme and perhaps early closing of the expected developmental window of PFC development is linked to worse EF in the peer-reared group; i.e. early psychosocial deprivation accelerates the development of certain brain networks that

leads to the premature closing of an adolescent plasticity window in PFC, which related to greater cortical thickness in the long-term (Tooley, 2021).

Finally, accelerated or altered development of gray matter in PFC, reflected by less synaptic proliferation, early pruning, and/ or a slower myelination in the peer-reared, could initially represent a compensatory mechanism after exposure to early psychosocial deprivation. Intriguingly, some research indicates that early social adversity can result in the prioritization of working memory development over inhibitory control (Ellis, 2017). In an unstable, threatening and/or deprived environment, it may be adaptive to prioritize the quick identification of salient stimuli and their location, and to not inhibit responses to these stimuli in order to act on them promptly; e.g. in the presence of a potentially threatening conspecific or in the context of low food resources, especially in the absence of social support, it may be adaptive to identify related stimuli quickly and accurately and respond to them in a reactive manner. Over time, however, this could end up being detrimental for various aspects of development. For instance, although this may be adaptive in macaque pre-puberty, this could then lead to less social success and more aggression and threats from other monkeys during adolescence, a time when peer relationships are becoming increasingly important and the establishment of hierarchy takes place. There is also evidence that inhibitory control and information retention aspects of working memory are more heavily impacted by early psychosocial deprivation in children and adolescents compared to cognitive flexibility and monitoring aspects of working memory (McDermott, 2013; Mittal, 2015; Johnson, 2021; Young, 2016). Our behavioural results provide some support for this difference in effects on certain aspects of working memory and inhibitory control. Only working memory maintenance (i.e. λ_1) was impacted by early deprivation, while the more sensory-spatial aspect of working memory (i.e. w_1) was not different between rearing groups. In addition, inhibitory control (i.e. w_2) was significantly worse for peer-reared compared to mother-reared animals in adulthood only. If the PFC of peer-reared animals already underwent extensive synaptic pruning and re-organization earlier on in development to adapt to their environment, they would likely be less flexible in adulthood. This could result in long-lasting impairment of inhibitory control and general EF, including randomness in decision making; i.e. this may reflect a sleeper effect. This idea is also supported by the results on cortical thickness, which show that dlPFC, an area implicated more in monitoring aspects of working memory, predicted this specific aspect of

working memory in the same way in both groups, with thicker cortex predicting a worse outcome. In vIPFC, thickness predicted better working memory (both sensory and long-term aspects) in mother-reared group, but more randomness in decision-making and impaired inhibitory control maintenance in peer-reared group. Therefore, compensatory mechanisms promoting particular aspects of working memory in the peer-reared group may also involve slightly different brain networks than those supporting these aspects in the mother-reared group; i.e. this represents an alternative developmental mechanism underlying working memory performance.

White matter microstructure in fronto-parietal and fronto-striatal fiber tracts

The results of the whole-brain white matter microstructure analysis using tract-based spatial statistics (TBBS) revealed an effect of early psychosocial deprivation on white-matter development in the superior longitudinal fasciculus (SLF), dorsal posterior (dpCR) and superior corona radiata (sCR), external capsule (EC), and corpus callosum (CC), but only when our three assessment time-points were combined. This somewhat aligns with previous research documenting effects of early psychosocial deprivation on fronto-parietal and fronto-striatal tracts in humans, as well as the corpus callosum (Sheridan, 2012; Sheridan, 2022). Higher radial diffusivity (RD; see SI), likely signifying less myelination, in SLF, SCR, and EC in the mother-reared group could suggest an ongoing sensitive period of development, but lower reflecting accelerated development in the peer-reared group which led to the premature closure of the sensitive period (Larsen, 2022). Additionally, higher axial diffusivity (AD), perhaps signifying greater axonal organization and projection, in CC, dpCR and sCR, could suggest more advanced development in these areas in mother-reared group, though understanding of typical development in terms of AD is currently very limited. Unfortunately, however, it is difficult to derive clear conclusions based on these findings due to the lack of change over time, with most other studies also having found differences in fractional anisotropy (FA) which was not the case here; FA can be driven by changes in RD and AD, and is commonly interpreted to reflect white matter integrity. A lack of differences in FA here could be related to sample size and a lack of power to detect significant differences using TBBS; this involves a large amount of multiple comparisons correction and it is not possible to look at changes over time (separate analyses have to be run for each time-point).

The examination of white-matter integrity in specific regions of interest (ROIs) did reveal group and age effects on FA, partially supporting our hypotheses. Early psychosocial deprivation was related to differences in FA in two fronto-striatal tracts in the left hemisphere (ACR and ALIC). However, the peer-reared group had higher rather than lower FA compared to the mother-reared group in these tracts. This is in opposition to FA findings in previous studies on institutionalization in children and adolescents (Bick, 2015; Sheridan, 2022). This could be in part due to age of assessment, and/or it could again reflect a compensatory mechanism in the peer-reared group. This idea will be explored more later in the chapter, also considering links between effects on grey and white matter measures. Meanwhile, in the right ACR, the left SLF, and the left EC, we found higher FA in early adulthood compared to adolescence in both groups, which is in accordance with typical development in humans and macaques (Dean, 2015; Imperati, 2011; Pierre, 2008), and supported our hypothesis.

Specific white matter tracts were differentially associated with measures of EF, with these relationships also impacted differently by psychosocial deprivation. The integrity of the SLF in both hemispheres was found to predict general measure EF concurrently at both assessment time-points in all animals. SLF integrity in adolescence also prospectively predicted inhibitory control (i.e. w_2) in early adulthood in both groups. No other prospective relationships were found between white-matter integrity and EF. This lack of prospective findings could reflect real effects, but may also result from a lack of statistical power. However, differences in RD may have driven differences in FA (see SI) in SLF, with higher RD in this tract also being linked to worse general EF in both groups. This would be in line with the hypothesis that myelination is one of the mechanisms responsible for the closure of sensitive period (Larsen, 2022); as mentioned before regarding the TBSS analysis, RD in SLF was higher in the mother-reared group, which could suggest ongoing plasticity in this tract. Rather concurrently supporting EF, this could support future EF, in line with the typical protracted trajectory of EF development. For peer-reared group, accelerated development would be positive related to EF concurrently, but could lead to less flexible and impaired EF later on.

FA in fronto-striatal tracts was found to predict EF more widely in the mother-reared compared to the peer-reared group. In mother-reared animals, in early adulthood, higher concurrent integrity in the left ACR and right and left EC was related to better information retrieval/longer-term working memory maintenance (i.e. λ_1), while higher integrity in the right

ALIC was associated with worse sensory-spatial (i.e. w_1) and maintenance (i.e. λ_1) aspects of working memory, as well as inhibitory control (i.e. w_2). In addition, in adolescence, higher concurrent integrity in the left EC and in the right ALIC predicted worse working memory maintenance (i.e. λ_1), but higher integrity in the right ALIC predicted better sensory-spatial working memory (i.e. w_1). Interestingly, RD (see SI) seemed to drive FA changes in the ACR, with higher RD linked to better working memory in the mother-reared group. This may indicate that protracted development of these tracts is linked to better cognitive outcomes as part of normative development, reflecting a reorganization of these brain circuits during adolescence.

Some relationships between fronto-striatal integrity and EF were also found for the peer-reared group. In this group, higher integrity (FA) in the right ALIC was associated with better working memory in adolescence and early adulthood, with a stronger effect seen in adulthood. Higher integrity in the EC was related to worse working memory maintenance (i.e. λ_1) in adolescence, but to better working memory in adulthood in peer-reared animals. Notably, the integrity of EC predicted working memory in the same direction in the mother-reared and peer-reared groups, but during adolescence, this relationship was stronger in the peer-reared group, and in early adulthood, this relationship was stronger in the mother-reared group. This suggests that early psychosocial deprivation can impact development of certain fronto-striatal tracts related to aspects of EF during adolescence, supporting recent findings that highlight the importance of the striatum and its connectivity in poor EF outcomes (Monchi, 2006; Tian, 2019).

To summarise the results involving white-matter integrity in fronto-striatal and fronto-parietal tracts, a number of group and age effects were revealed in specific ROIs. Early deprivation was linked to differences in FA in fronto-striatal tracts, with the peer-reared group having higher white matter integrity compared to the mother-reared group. In early adulthood, FA in the right ACR, left SLF, and left EC was higher in both groups compared to adolescence. No effect of early deprivation was found on the SLF or EC. This contradicts earlier findings on the effects of early institutionalization on FA in these tract (Sheridan, 2022), however, this discrepancy may be associated with the ages of assessment; here, SLF could have reached a high level of maturity in both groups, with peer-reared animals 'catching-up' by early adulthood. However, general EF and future inhibitory control were related to SLF integrity, and long-term working

memory was related to EC integrity, highlighting the importance of looking at individual differences when looking at relations between brain and behaviour (there may have been no group effect on SLF and EC, but aspects of EF known to be worse in the peer-reared group were associated with FA). The results showing higher FA in the ACR and ALIC in peer-reared animals may reflect accelerated development in this circuitry. Finally, higher white matter integrity in the right ALIC was associated with worse working memory and inhibitory control in the mother-reared group, but to better working memory in the peer-reared group.

We did find fewer relationships between white matter integrity and EF in the peer-reared compared to mother-reared group, and an opposite relationship between groups in ALIC. This could result from impaired development in these tracts and/or greater variability in terms of development in the peer-reared group, or a lack of power to detect relationships. However, a potentially explanation could also be that accelerated development in fronto-striatal circuits, as shown for cortical thickness in vPFC, acts as a compensatory mechanism prioritizing monitoring working memory aspects, with less inhibition helping to adapt faster, but to the detriment of other aspects of EF in the long-term. Interestingly, one study has shown FA decreases in frontoparietal white matter following larger PFC lesions, but increases in some thalamocortical tracts during behavioral recovery (Adam, 2021). This implies that fronto-striatal tracts rearrange with fronto-parietal tracts to compensate for impairments in PFC, an idea that is supported by the findings presented in this chapter.

Relationships between grey matter thickness and white matter integrity results

As noted above, the uncovering of significant age interactions for some brain measures but not others, with opposite directions sometimes found in adolescence and adulthood in the two rearing groups, may indicate that alterations in development of particular frontal networks serves as a compensatory mechanism in relation to PFC in the context of early psychosocial deprivation (Darki, 2019; Adam, 2021). Recent research has highlighted the involvement of fronto-striatal (or similarly, fronto-thalamic) circuits in EF (Roy, 2022; Darki, 2015; Friedman, 2022). The dlPFC is more closely connected to the SLF, and interestingly we found that thinner cortex in dlPFC and higher SLF white matter integrity was associated with better overall EF in both groups; note, white matter integrity in SLF during adolescence also predicted future inhibitory control in both groups. However, in the left SLF, we also found an effect of group on AD, with higher AD in mother-reared group compared to peer-reared group.

In addition, higher RD was found in TBSS analyses in mother-reared group, and higher RD during adolescence and adulthood predicted worse EF in both groups. This could suggest an accelerated development in fronto-parietal tract in peer-reared group, with an overall increased myelination in this tract, reflected by RD, suggesting an earlier closure of plastic of sensitive period during adolescence. This is also in line with peer-reared group having thicker dlPFC, that in both groups predicted worse EF. Furthermore, the fact that higher white matter integrity in ALIC predicting better working memory in the peer-reared group could be related to thicker vlPFC predicting worse inhibitory control and decision-making in terms of randomness. In fact, ALIC connects the vlPFC with the striatum and the thalamus (Petrides, 2005a, Haber, 2022), and in line with the discussion in the previous two sections, we propose that higher integrity in the ALIC in the peer-reared animals reflects a compensatory mechanism prioritizing working memory over inhibitory control in early development. This mechanism may impact the development of the vlPFC and related EF refinement. Another option could be that earlier impairment in PFC results in reorganization of fronto-striatal and fronto-parietal circuits. Further research is now needed clarify the causal relationships in terms of how different neural networks interact and influence each other to predict different aspects of EF.

How do these results fit with current models on the effects of early social adversity?

Both stress and plasticity models of early social adversity propose that experiences such as early psychosocial deprivation can have profound impacts on development of the prefrontal cortex (PFC) via effects on the HPA-axis and processes such as synaptic pruning (Tottenham, 2010; McLaughlin, 2014). Such impacts on the PFC, as well as related development of PFC cortico-cortical and cortico-subcortical networks, would consequently have huge effects on the development of executive function (EF); with existing literature strongly supporting that psychosocial deprivation negatively impacts both executive function and PFC development (Sheridan, 2022; Wade, 2019). Dimensional models of early adversity also posit that working memory and inhibitory control play particularly key roles in the emergence of various poor outcomes associated with early psychosocial deprivation (e.g. ADHD) (Johnson, 2021; Ho, 2021), and that these processes are particularly affected by this specific type of early adversity. More discussion about how results presented in this thesis may support or contradict current

models of development after exposure to early psychosocial adversity will be presented in Chapter 5 of this thesis.

Normative development of EF and its neural bases in macaques

As well as important information on the effects of early psychosocial adversity, our findings also add to the literature about normative development of EF and its underlying neural correlates in macaques. Our results suggest a thinning in PFC across adolescence into adulthood in the dlPFC, with higher thickness in vlPFC compared to dlPFC perhaps reflecting later maturation. In addition, our results suggest a functional differentiation between dlPFC and vlPFC in all animals, and differential trajectories in their development in relation to EF. Thicker dlPFC predicted worse working memory in adulthood, while thicker vlPFC predicted better working memory, and differences in general EF and choice randomness (i.e. decision-making) across adolescence into adulthood. Overall, this is in line with previous research suggesting that cortical thinning in PFC occurs in macaques during adolescence (Brito, 2014; Ramirez, 2021), with earlier development and less changes in dlPFC across this period also related to working memory (Zhou, 2016). This is in keeping with the proposal of adolescence being a sensitive period for EF refinement and its neural correlates in macaques, as in humans, when major changes in PFC occur (Thompson, 2020; Sheridan, 2022; Ferguson, 2021; Verrico, 2011; Larsen, 2022). Additionally, white-matter integrity in fronto-striatal and fronto-parietal tracts predicted EF across time, revealing different developmental trajectories of various tracts as well. Interestingly, more typical developmental patterns in fronto-striatal tracts matched those in PFC; higher integrity in SLF was associated with better overall performance, with integrity in SLF in adolescence also predicting future inhibitory control. In early adulthood, higher integrity in EC and ACR predicted better working memory and inhibitory control, and thicker dlPFC predicted worse working memory. This in line with evidence demonstrating cortical thinning and increasing integrity in white matter tracts across development, as well as relation between this and healthy cognitive outcomes. However, in early adulthood, higher integrity in ALIC predicted worse working memory, and thicker vlPFC predicted better working memory and inhibitory control. This could be because vlPFC and related connectivity with other regions undergoes more protracted development, for example, and or non-concurrent changes (i.e. one predicts the other). It is important to note that not all brain networks and regions development in parallel, with some changes occurring

at different times than others (Oldham, 2019; Tau, 2009). The results here are in keeping with this, and suggest a complex relationship between development in cortical regions, structural connectivity, and their interaction across development.

Although both dlPFC and vlPFC are connected with fronto-striatal tracts, we know that dlPFC has more structural connections with association cortices, including the parietal cortex via the SLF, and vlPFC has more connections with subcortical regions such as the striatum and thalamus (Haber, 2022). We also know that the fronto-parietal network and dlPFC may develop earlier compared to fronto-striatal circuits and vlPFC (ref). Results in this chapter could therefore support a more protracted development for vlPFC and fronto-striatal circuits, that could thus be more susceptible to effects of early psychosocial deprivation across development. Finally, randomness in decision-making was only predicted by cortical thickness and not structural connectivity, which could be in accordance with previous research in humans and macaques showing that PFC has a central role in decision-making (Oguchi, 2022), but is in contrast with other research findings in human relating white-matter development to this also (ref). Longitudinal studies across more developmental time points looking at developmental changes in various PFC regions are now needed to clarify these differences.

Strengths and limitations

The current research has several strengths. Firstly, it is a longitudinal study focusing on a developmental transition that represents a critical time for refinement of executive function, and one that may reveal delayed effects and/or long-term effects of early psychosocial adversity on neurocognitive development. Secondly, brain and behavioral measures were made at both time points, which is a rarity in both human and macaque research, and adds to our understanding of developmental trajectories after exposure to early adversity. Thirdly, as well as providing key insights into how early psychosocial deprivation impacts executive function and its underlying neural correlates in macaques, by including the mother-reared group, it also provides new information about typical versus atypical development in macaques. Finally, the use of computational modelling to analyze behavior, utilization of advanced methods including that resulting in acquisition of very high-quality MRI data, and an integrative neuroscientific, ethological, psychological, and computational approach are also strengths of this research.

Nevertheless, this research also possesses some limitations. Firstly, the sample size is limited, which may have influenced findings such as those for the TBSS and prospective analyses in particular. Furthermore, we have no executive function measures prior to adolescence. In general, the literature demonstrates that other brain circuits, including limbic areas, also play an essential role in working memory and inhibitory control. As such, future studies should focus on wider and more complex brain networks in relation to EF development and early psychosocial deprivation, in both human and macaques, to more fully comprehend how structural brain differences may contribute to differences in EF. For instance, future research could expand its focus beyond fronto-parietal and fronto-striatal tracts to include other limbic areas believed to be involved in executive function in emotional contexts, look more closely at other DTI parameters (e.g. RD, AD, MD), and adopt a network-analysis approach to better investigate the complex and interacting development of neural circuitry implicated in executive function. It would also be useful to incorporate other measures of brain development such as changes in functional activity (e.g. fMRI, EEG) in order to understand how functional and structural changes interact to predict EF refinement in macaques. Another future direction of inquiry could be examination of the dopaminergic system, given its crucial role in both executive function and activity in fronto-striatal circuitry (Sajed, 2022; Smith, 2020; Nour, 2019). Finally, it will be interesting in future studies to see how a relationship between brain development and EF predict poor outcomes associated with EF, such as internalizing and externalizing symptoms, and symptoms of ADHD. In general, replication of the findings presented in this chapter with larger samples and inclusion of more time-points across development will be important in future research.

Conclusion

To conclude, this study aimed to examine the impact of early psychosocial deprivation in macaques on structural brain development associated with executive function across adolescence and into adulthood. Our results indicate that early deprivation has a negative influence on executive function during this period via effects on vIPFC and connectivity in fronto-parietal and fronto-striatal networks. These findings also contribute significantly to our understanding of normative executive function and underlying brain development in macaques in addition to the effects of early psychosocial deprivation on this.

SUPPLEMENTARY INFORMATION

METHODS

Subjects

All subjects were born and raised at the *Laboratory of Comparative Ethology at the National Institutes of Health, US*. Peer-reared animals were raised in a nursery with access to same-aged peers. See (1) for more information on the peer-rearing protocol. Rearing procedures were approved by the NICHD and the University of Maryland Animal Care and Use Committee, and adhered to the NIH Guide for the Care and Use of Laboratory Animals. Animals were relocated to their current location at the *Rousset Primatological Station, CNRS, France* at two years of age, and are housed altogether. As part of a wider longitudinal study, animals are temporarily relocated once a year to a nearby location in groups of 5-6 mixed mother- and peer-reared animals. All animals were kept alive at the end of the study.

'A-not-B' task set-up

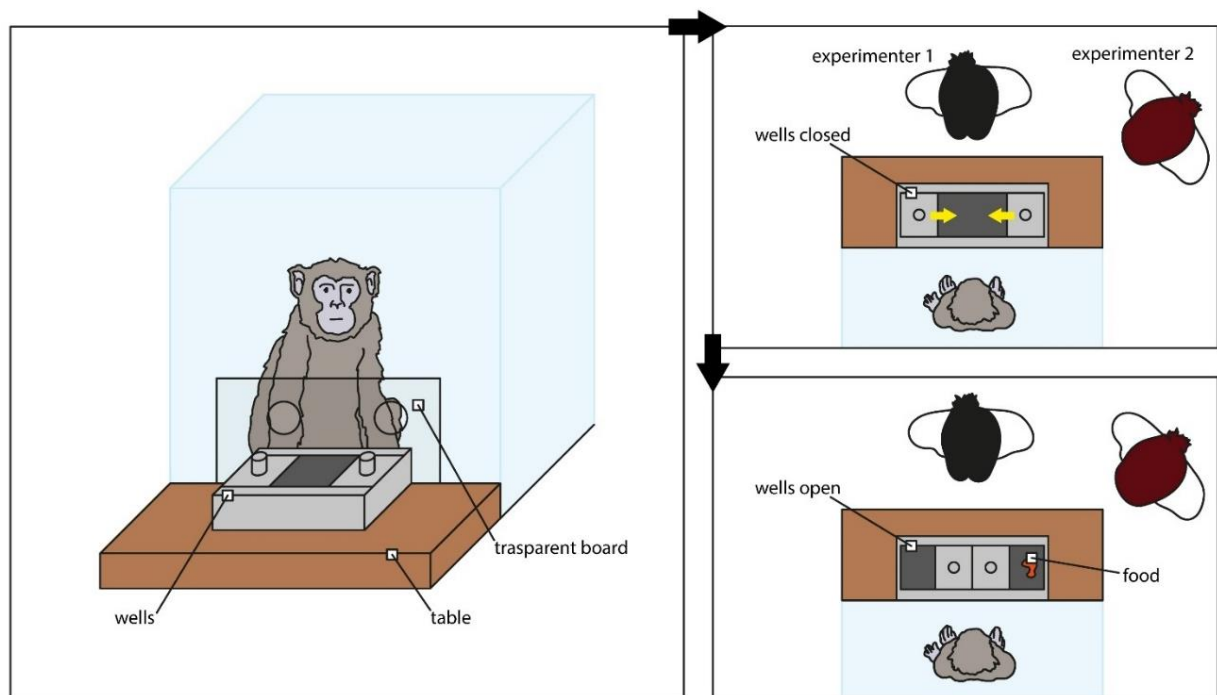


Figure S1: *Task set-up.* A frontal view of the test enclosure is illustrated on the left. Two views from above are illustrated on the right: upper right, the wells are closed with the yellow arrows indicating a sliding mechanism; upper left, the wells are open and a piece of food is placed in one of them.

'A-not-B' task procedure

For the first trial of each session, the delay period when visual and physical access to the wells was blocked lasted 2s, and then depending on the subject's performance, it was increased or decreased in s

ubsequent trials. The delay period was decreased by 2s if two consecutive trials were incorrect, and was increased by 1s if two consecutive change trials were correct (REF). Each subject completed two testing sessions at each assessment time point, with a maximum of 25 trials per session (i.e. 50 trials total per assessment time-point).

Video coding

We coded a number of parameters offline from the video recordings made of each subject during the testing sessions. These included the actual length of the delay periods (i.e. from the moment the experimenter showed the food to the subject, to the moment the clear panel was removed to enable the subject to reach for the wells), inter-trial-interval (ITI) lengths, position of the subject in the testing enclosure during each trial, and other behaviours during and between trials that may have affected performance, such as locomotion, pacing, anxiety (i.e. self-scratch, self-groom, yawn), vocalizations, and threatening, fearful, or affiliative gestures. These behaviours were coded as being present or not during each trial and ITI. A random 15% of videos were coded by two researchers at each time-point to establish reliability for the original version of the task, and for the control version of the task at the second-time point (5 years). All reliability scores obtained between coders were very strong (i.e. all $\kappa = 0.86-1.00$).

Computational model

We tested 11 different computational models that differed in terms of the factors that contributed to the decision variable, z :

- 1) Working memory : $z_t = w_1 S_t e^{-\lambda_1 d_t}$, where w_1 is the weight of this factor when the delay is 0, S_t is the side that food was hidden on in trial t (left = -1, right = 1), and d_t is the delay
- 2) Previous choice: $z_t = w_2 R_{t-1} e^{-\lambda_2 (i_t + d_t)}$, where w_2 is the weight of this factor when the inter-trial interval and delay is 0, R_{t-1} is the response made on the previous trial (left = -1, right = 1), and i_t is the inter-trial interval before this trial.
- 3) Previous two choices: $z_t = w_2 (R_{t-1} e^{-\lambda_2 (i_t + d_t)} + R_{t-2} e^{-\lambda_2 (i_{t-1} + d_{t-1} + i_t + d_t)})$
- 4) Working memory and previous choice: $z_t = w_1 S_t e^{-\lambda_1 d_t} + w_2 R_{t-1} e^{-\lambda_2 (i_t + d_t)}$
- 5) Working memory and previous two choices: $z_t = w_1 S_t e^{-\lambda_1 d_t} + w_2 (R_{t-1} e^{-\lambda_2 (i_t + d_t)} + R_{t-2} e^{-\lambda_2 (i_{t-1} + d_{t-1} + i_t + d_t)})$
- 6) Expected reward: $z_t = w_3 (v_{R,t} - v_{L,t})$, where w_3 is the weight of this factor, $v_{R,t}$ is the expected reward at time t for choosing right, and $v_{L,t}$ is the expected reward at time t for choosing left. Expected reward for each side was updated using the standard Rescorla-Wagner

rule: $v_{c,t+1} = v_{c,t} + \alpha(\hat{v}_{c,t} - v_{c,t})$, where $v_{c,t}$ is the expected reward for side c at time t , α is the learning rate, and $\hat{v}_{c,t}$ is the actual reward received for choosing side c at time t .

- 7) Working memory and expected reward: $z_t = w_1 S_t e^{-\lambda_1 d_t} + w_3 (v_{R,t} - v_{L,t})$
- 8) Previous choice and expected reward: $z_t = w_2 R_{t-1} e^{-\lambda_2 (i_t + d_t)} + w_3 (v_{R,t} - v_{L,t})$
- 9) Previous two choices and expected reward: $z_t = w_2 (R_{t-1} e^{-\lambda_2 (i_t + d_t)} + R_{t-2} e^{-\lambda_2 (i_{t-1} + d_{t-1} + i_t + d_t)}) + w_3 (v_{R,t} - v_{L,t})$
- 10) Working memory, previous choice, and expected reward: $z_t = w_1 S_t e^{-\lambda_1 d_t} + w_2 R_{t-1} e^{-\lambda_2 (i_t + d_t)} + w_3 (v_{R,t} - v_{L,t})$
- 11) Working memory, previous two choices, and expected reward: $z_t = w_1 S_t e^{-\lambda_1 d_t} + w_2 (R_{t-1} e^{-\lambda_2 (i_t + d_t)} + R_{t-2} e^{-\lambda_2 (i_{t-1} + d_{t-1} + i_t + d_t)}) + w_3 (v_{R,t} - v_{L,t})$

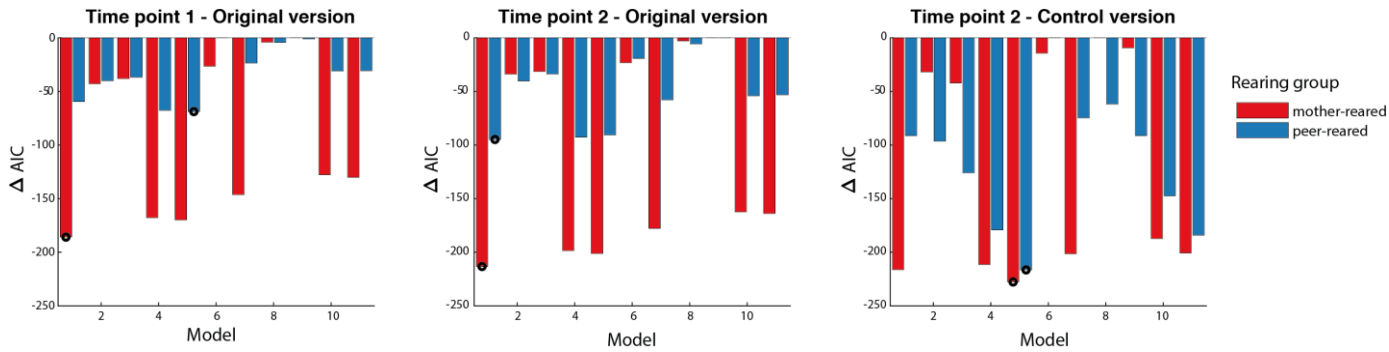


Figure S1: Model comparison. Akaike information criterion (AIC) for each model relative to the worst model for both groups at each assessment time-point and task version. Filled circles represent the models with the lowest AIC.

Model comparison was performed using the Akaike information criterion (AIC; Akaike, 1973), a metric that captures the trade-off between model goodness of fit and complexity. Lower AIC (relative to the worst model), indicates that the model better fits the data without being so complex that it overfits it. The sum of AIC for each subject was compared for each assessment time-point and task (Figure S1). Model 1 had the lowest AIC for the mother-reared group at time-points 1 and 2 and the peer-reared group at time point 2. Model 5 had the lowest AIC for the peer-reared group at time-point 1 and for both groups in the random version of the task. We therefore selected model 5 for the main analyses as it includes all the terms from model 1, and terms for the influence of the previous two choices.

DATA ANALYSIS

R v4.0.5 (REF) and the lme4 v1.1-27.1 (3), lsmeans v2.30.0 (4), predictmeans v1.0.6 (5), and car v3.0.11 (6) packages were utilized to conduct the analyses.

Data pre-processing

Before analysis, we checked if any behaviours that occurred during the trial or the inter-trial interval (ITI) were affecting the model's ability to predict the subjects' responses. We found that locomotion during trials negatively impacted the model's prediction accuracy, so we excluded those trials from the analysis. We also removed trials with ITIs that were more than 2.5 standard deviations above the mean and excluded subjects with less than 25 remaining trials. We then checked for differences in ITI duration between the two rearing groups and we found no difference (see Massera et al, under review).

Trial exclusion

Mean trials (out of the 50 trials for original task and 40 trials for the control task per subject) included for mother-reared animals were as follows: original task at the first time-point (3.5 years), $M = 44.63$, $SD = 6.36$; original task at the second time-point (5 years), $M = 47.1$, $SD = 2.96$; control task at the second time-point (5 years), $M = 45.2$, $SD = 5.69$. Mean trials included for peer-reared animals were as follows: original task at the first time-point (3.5 years), $M = 44.3$, $SD = 5.61$; original task at the second time-point (5 years), $M = 47.3$, $SD = 4.49$; control task at the second time-point (5 years), $M = 44.2$, $SD = 6.17$.

RESULTS

Table S1: Descriptive statistics. Behavioral measure of performance (i.e. cumulative score) in 'A-not-B' task at 3.5 and 5 years. Cumulative score M (SD) is the sum of all successful change trial delays and then divided by the number of total trials completed. Fitted parameters in 'A-not-B' task at 3.5 and 5 years M (SD): w_1 (the weight of the working memory factor when the delay is 0), λ_1 (decaying working memory trace of the food location), w_2 (the weight of the choice factor when the inter-trial interval and delay is 0), λ_2 (decaying trace of the previous choice), and θ (choice stochasticity). Grey matter cortical ROIs M (SD) at 3.5 and 5 years: dlPFC – areas 46d, 46v, 9/46d, 9/46v; vlPFC – areas 44, 45A, 45B. White matter ROIs M (SD) at 3.5 and 5 years: superior longitudinal fasciculus (SLF), anterior corona radiata (ACR), anterior limb of internal capsule (ALIC), external capsule (EC).

	Group	
	Mother-reared	Peer-reared
<u>Cumulative score</u>		
3.5 years	0.643 (0.316)	0.265 (0.204)
5 years	0.712 (0.4)	0.279 (0.244)

<i>Fitted parameters</i>		
<i><u>W₁</u></i>		
3.5 years	0.494 (0.387)	0.286 (0.255)
5 years	0.363 (0.339)	0.177 (0.134)
<i><u>λ₁</u></i>		
3.5 years	0.09 (0.301)	0.3 (0.483)
5 years	0.1 (0.316)	0.5 (0.527)
<i><u>W₂</u></i>		
3.5 years	0.51 (0.486)	minus 0.025 (0.657)
5 years	0.07 (0.686)	0.495 (0.438)
<i><u>λ₂</u></i>		
3.5 years	0.203 (0.182)	0.182 (0.254)
5 years	0.134 (0.139)	0.194 (0.199)
<i><u>β</u></i>		
3.5 years	9.029 (1.568)	6.577 (3.196)
5 years	7.664 (2.606)	8.157 (2.669)
<i>Grey matter ROIs</i>	Mother-reared	Peer-reared
<i><u>dIPFC</u></i>	cortical thickness (mm)	
<i><u>right 46d</u></i>		
3.5 years	3.49 (0.28)	3.627 (0.213)
5 years	3.505 (0.55)	3.681 (0.246)
<i><u>left 46d</u></i>		
3.5 years	3.412 (0.205)	3.725 (0.324)
5 years	3.435 (0.507)	3.762 (0.198)
<i><u>right 46v</u></i>		
3.5 years	3.434 (0.218)	3.638 (0.198)
5 years	3.232 (0.35)	3.548 (0.18)
<i><u>left 46v</u></i>		
3.5 years	3.528 (0.155)	3.668 (0.201)

5 years	3.414 (0.268)	3.59 (0.242)
<i>right 9/46d</i>		
3.5 years	3.624 (0.244)	3.77 (0.224)
5 years	3.418 (0.751)	3.745 (0.194)
<i>left 9/46d</i>		
3.5 years	3.665 (0.21)	3.76 (0.31)
5 years	3.508 (0.792)	3.85 (0.251)
<i>right 9/46v</i>		
3.5 years	3.58 (0.24)	3.935 (0.154)
5 years	3.387 (0.731)	3.892 (0.18)
<i>left 9/46v</i>		
3.5 years	3.58 (0.232)	3.908 (0.273)
5 years	3.453 (0.618)	3.837 (0.295)
<i>vIPFC</i>	cortical thickness (mm)	
<i>right 44</i>		
3.5 years	4.013 (0.186)	4.083 (0.197)
5 years	3.745 (0.961)	4.071 (0.228)
<i>left 44</i>		
3.5 years	3.908 (0.145)	4.08 (0.134)
5 years	3.584 (0.902)	4.08 (0.138)
<i>right 45A</i>		
3.5 years	4.63 (0.147)	4.744 (0.097)
5 years	4.244 (1.177)	4.755 (0.094)
<i>left 45A</i>		
3.5 years	4.588 (0.19)	4.68 (0.145)
5 years	4.251 (1.006)	4.677 (0.157)
<i>right 45B</i>		
3.5 years	4.2 (0.177)	4.429 (0.132)
5 years	3.879 (1.042)	4.39 (0.122)

<i>left 45B</i>						
3.5 years	4.056 (0.179)			4.371 (0.11)		
5 years	3.769 (0.968)			4.363 (0.109)		
	Mother-reared			Peer-reared		
<i>White matter ROIs</i>	FA	RD	AD	FA	RD	AD
<i>right SLF</i>						
3.5 years	0.514 (0.028)	0.839 (0.054)	1.208 (0.043)	0.503 (0.023)	0.831 (0.104)	1.156 (0.051)
5 years	0.527 (0.017)	0.785 (0.06)	1.193 (0.062)	0.504 (0.018)	0.81 (0.095)	1.168 (0.04)
<i>left SLF</i>						
3.5 years	0.512 (0.033)	0.688 (0.03)	1.2 (0.054)	0.5 (0.018)	0.653 (0.064)	1.15 (0.043)
5 years	0.533 (0.022)	0.67 (0.084)	1.193 (0.057)	0.5 (0.02)	0.621 (0.021)	1.14 (0.02)
<i>right ACR</i>						
3.5 years	0.513 (0.021)	0.681 (0.022)	1.192 (0.055)	0.519 (0.033)	0.67 (0.046)	1.182 (0.052)
5 years	0.527 (0.02)	0.668 (0.027)	1.17 (0.033)	0.536 (0.018)	0.653 (0.024)	1.193 (0.05)
<i>left ACR</i>						
3.5 years	0.513 (0.02)	0.714 (0.03)	1.209 (0.078)	0.536 (0.027)	0.709 (0.024)	1.216 (0.052)
5 years	0.529 (0.025)	0.695 (0.024)	1.186 (0.036)	0.54 (0.02)	0.72 (0.044)	1.208 (0.03)
<i>right ALIC</i>						
3.5 years	0.617 (0.028)	0.705 (0.032)	1.419 (0.089)	0.627 (0.037)	0.695 (0.044)	1.443 (0.139)
5 years	0.63 (0.035)	0.712 (0.052)	1.433 (0.13)	0.619 (0.035)	0.658 (0.041)	1.341 (0.09)
<i>left ALIC</i>						
3.5 years	0.606 (0.027)	0.713 (0.041)	1.396 (0.074)	0.64 (0.04)	0.709 (0.032)	1.466 (0.159)
5 years	0.623 (0.028)	0.709 (0.04)	1.417 (0.1)	0.631 (0.025)	0.694 (0.04)	1.364 (0.068)
<i>right EC</i>						
3.5 years	0.468 (0.031)	0.762 (0.033)	1.212 (0.051)	0.473 (0.022)	0.746 (0.051)	1.204 (0.066)
5 years	0.483 (0.025)	0.758 (0.067)	1.231 (0.057)	0.484 (0.021)	0.729 (0.035)	1.209 (0.028)
<i>left EC</i>						

3.5 years	0.461 (0.022)	0.739 (0.033)	1.222 (0.039)	0.475 (0.02)	0.717 (0.042)	1.216 (0.078)
5 years	0.478 (0.033)	0.739 (0.051)	1.227 (0.044)	0.479 (0.03)	0.706 (0.035)	1.202 (0.032)

Differences between groups in specific white matter tracts associated with EF and early adversity (AD and RD)

All the model results are shown in Table S2.

A significant main effect of group was also found in the left SLF in AD [$\chi(1) = 5.153, p = 0.023$], with the mother-reared group having higher AD compared to peer-reared group. No other significant effects were observed in RD and AD in the left SLF.

No effects were found for RD or AD in right and left ACR.

Additionally, we identified a significant interaction between group and time point on AD in the left ALIC [$\chi(1) = 4.203, p = 0.04$], however follow-up analyses were not significant. There was no effect of group, time point or group by time point interaction in the left hemisphere for RD. No significant results in the right ALIC for RD and AD.

No significant effects were observed for RD and AD in the right and left EC.

Table S2: *Significant results summary.* Main effects of group and time point and their interaction in all models with ROIs.

ROIs - Group and time point effects			
Cortical Thickness (CT)	Main effects and interactions	$\chi(1)$	p value
<i>dIPFC</i>			
Left - 46d	Group	7.6423	0.005702
Right - 46v	Group	3.9604	0.046582
	Year	8.3011	0.003962

Right - 9/46d	Group	4.2627	0.03896
Right - 9/46v	Group	4.8845	0.0271
Left - 9/46v	Group	5.5396	0.01859
<i>vIPFC</i>			
Right - 45B	Group	10.8470	0.0009895
Left - 45B	Group	22.1000	2.588e-06
Tract and diffusivity (DTI) measures	Main effects and interactions	$\chi(1)$	<i>p</i> value
Left - SLF FA	Year	5.7116	0.01685
Left - SLF AD	Group	5.1539	0.02319
Right - ACR FA	Year	4.6473	0.0311
Left - ACR FA	Group	4.6316	0.03139
	Year	3.6385	0.05646
Left - ALIC FA	Group	8.1988	0.004192
Left - ALIC AD	Group:Year	4.2033	0.04035
Left - EC FA	Year	4.9705	0.02578

Concurrent DTI ROI-EF relationships (AD and RD)

All the model results are shown in Table S3.

For SLF, higher RD was also found to be related to lower performance (cumulative score) in the right hemisphere [$\chi(1) = 8.519, p = 0.003$]. No significant relationships between AD and

cumulative core were found. We did not find any other significant effects of time point, group, or interactions for SLF for other outcome variables.

For ACR, in the analyses predicting λ_1 , significant interactions were also found between RD, group, and time-point in the left hemisphere [$\chi(1) = 1.9568e+10, p < 0.001$]. At 3.5 years, for the mother-reared group, higher RD was related to higher λ_1 (i.e. faster working memory decay/worse working memory) [$z(1) = 56181.330, p < 0.001$], while for the peer-reared group, higher RD was related to lower λ_1 (i.e. slower working memory decay/better working memory) [$z(1) = -166468.380, p < 0.001$]. At 5 years, the opposite was true, with the mother-reared group having higher RD related to lower λ_1 (i.e. slower working memory decay/better working memory) [$z(1) = -91022.770, p < 0.001$], and the peer-reared group having higher RD related to higher λ_1 (i.e. faster working memory decay/worse working memory) [$z(1) = -91022.770, p < 0.001$]. Therefore, the relationship between RD and λ_1 was significantly different between the two rearing groups at 3.5 years [$z(1) = 114274.020, p < 0.001$], and at 5 years [$z(1) = -21006.150, p < 0.001$]. The relationship between RD and λ_1 was also significantly different in the mother-reared group [$z(1) = 44924.730, p < 0.001$], and in the peer-reared group [$z(1) = -69245.630, p < 0.001$] between 3.5 years and 5 years. In the analyses predicting λ_1 , a significant interaction was also revealed between AD, group, and time-point in the left hemisphere [$\chi(1) = 2.1800e+08, p < 0.001$]. At 3.5 years, for both groups, higher AD was related to lower λ_1 (i.e. slower working memory decay/better working memory), [mother-reared, $z(1) = -48474.280, p < 0.001$; peer-reared, $z(1) = -68847.190, p < 0.001$] but with the peer-reared group having a stronger negative relationship compared to the mother-reared group [$z(1) = 40446.830, p < 0.001$]. At 5 years, the opposite was true: for both groups, higher AD was related to higher λ_1 (i.e. faster working memory decay/worse working memory) [mother-reared, $z(1) = 31139.790, p < 0.001$; peer-reared, $z(1) = 26321.170, p < 0.001$], but with the peer-reared group having again a stronger negative relationship [$z(1) = -2061.840, p < 0.001$]. The relationship between AD and λ_1 was also significantly different between 3.5 years and 5 years in the mother-reared group [$z(1) = -94945.480, p < 0.001$], and in the peer-reared group [$z(1) = -113943.170, p < 0.001$]. No other significant relationships were found in analyses predicting the other model parameters.

For ALIC, for the analysis of w_1 , we found a significant interaction between AD, group, and time point in the right hemisphere [$\chi(1) = 6.4916, p = 0.0108$]. For the mother-reared group at

5 years, higher AD was related to lower w_1 (i.e. worse working memory) compared to 3.5 years [$t(1) = 2.266, p = 0.034$] [$t(1) = -2.207, p = 0.035$]. No significant results were found for radial diffusivity (RD). Additionally, for the analysis of λ_1 , in the right hemisphere, we found a significant interaction between AD, group, and time point [$\chi(1) = 4.0404e+09, p < 0.001$]. At both time-points for the mother-reared group, higher AD was related to higher λ_1 (i.e. faster working memory decay /worse working memory) [3.5 years, $z(1) = 7367.100, p < 0.001$; 5 years, $z(1) = 15898.120, p < 0.001$], with a stronger relationship at 5 years [$z(1) = -12743.040, p < 0.001$]. On the contrary, for the peer-reared group, higher AD was related to lower λ_1 (i.e. slower working memory decay /better working memory) [3.5 years, $z(1) = -22653.570, p < 0.001$; 5 years, $z(1) = -48411.860, p < 0.001$], with a stronger relationship at 5 years [$z(1) = 38208.170, p < 0.001$]. The relationship between AD and λ_1 also differed between the two rearing groups at 3.5 years [$z(1) = 40680.970, p < 0.001$], and at 5 years [$z(1) = 72303.460, p < 0.001$]. No relationship with RD was found. Finally, for the analysis of w_2 , a significant interaction between AD, group, and time point in the right hemisphere was found [$\chi(1) = 6.926, p = 0.008$]. At 3.5 years, for the peer-reared group, higher AD was related to higher w_2 (i.e. repeating previous choices more frequently/worse response inhibition) [$t(1) = 2.050, p = 0.049$], differing from the mother-reared group at the same time point [$t(1) = -2.118, p = 0.042$]. No relationship with RD was found.

For EC, no significant relationships were found between RD and AD, and λ_1 .

Table S3: Significant results summary. Main effects and interaction effects in all models with ROIs and EF.

ROIs - behaviour models				
Cortical Thickness (CT) measures	Behavioural measures	Main effects and interactions	$\chi(1)$	p value
<i>dIPFC</i>				
Left - 9/46d	w_1	Year	10.3168	0.0013182
		CT:Year	10.9092	0.0009569
Right - 9/46v	w_1	CT	11.0015	0.0009104
		Year	10.4446	0.0012301

		CT:Year	11.2848	0.0007814
Right - 46d	β	CT:Group	8.6568	0.003258
<i>VIPFC</i>				
Right - 44	Score	Year	8.6763	0.003224
		CT:Year	8.6102	0.003343
Right - 45B	Score	Year	18.7506	1.490e-05
		CT:Year	18.7350	1.502e-05
Left - 45B	w_1	CT	11.0148	0.0009039
		Group	9.2890	0.0023053
		CT:Group	9.9779	0.0015843
		CT:Year	9.0307	0.0026548
Right - 45A	λ_1	Group	83.5506	<2e-16
		Year	98.5762	<2e-16
		CT:Year	8.9006	0.002851
		Group:Year	89.6153	<2e-16
		CT:Group:Year	9.2507	0.002354
Right - 45B	λ_2	Group	25.8237	3.741e-07
		CT:Group	25.6612	4.069e-07
		Group:Year	8.5919	0.003377
		CT:Group:Year	8.5167	0.003519
Left - 45A	β	Group:Year	8.7055	0.003172
		CT:Group:Year	9.1172	0.002532
Right - 45B	β	CT:Group	8.5956	0.003370
Tract and diffusivity (DTI) measures	Behavioural measures	Main effects and interactions	$\chi(1)$	<i>p</i> value
Right - SLF FA	Score	DTI	10.1368	0.001453
		Year	7.5889	0.005873
		DTI:Year	7.5402	0.006034

Left - SLF FA	Score	DTI	16.1057	5.99e-05
Right - SLF RD	Score	DTI	8.5194	0.0035137
		Group	8.5723	0.0034132
Left - ACR FA	λ_1	Year	7229.847	<2e-16
		DTI:Year	1742.9785	<2e-16
		Group:Year	684.0890	<2e-16
		DTI:Group:Year	2043.7960	<2e-16
Left - ACR RD	λ_1	DTI	9.9258e+08	<2e-16
		Group	6.8112e+09	<2e-16
		Year	1.0249e+09	<2e-16
		Sex	2.6456e+05	<2e-16
		DTI:Group	1.3059e+10	<2e-16
		DTI:Year	2.0182e+09	<2e-16
		Group:Year	9.6751e+09	<2e-16
		DTI:Group:Year	1.9568e+10	<2e-16
Left - ACR AD	λ_1	DTI	2.3696e+09	<2e-16
		Group	1.1163e+09	<2e-16
		Year	1.4404e+10	<2e-16
		Sex	7.2964e+05	<2e-16
		DTI:Group	6.1820e+08	<2e-16
		DTI:Year	1.0331e+10	<2e-16
		Group:Year	3.1055e+08	<2e-16
		DTI:Group:Year	2.1800e+08	<2e-16
Right - ALIC FA	w_1	DTI	7.8644	0.0050417
		Group	7.4105	0.0064844
		Year	12.9403	0.0003216
		DTI:Group	7.9663	0.0047657
		DTI:Year	13.3854	0.0002536
		Group:Year	15.1225	0.0001008

		DTI:Group:Year	15.2336	9.5e-05
Right - ALIC AD	w_1	Year	5.386	0.02030
		DTI:Year	5.587	0.01809
		Group:Year	6.5724	0.01036
		DTI:Group:Year	6.4916	0.01084
Right - ALIC FA	λ_1	DTI	9.7835e+07	<2e-16
		Group	7.9330e+07	<2e-16
		Year	1.6785e+07	<2e-16
		Sex	2.7203e+03	<2e-16
		DTI:Group	1.9397e+08	<2e-16
		DTI:Year	4.2036e+07	<2e-16
		Group:Year	1.0101e+07	<2e-16
		DTI:Group:Year	2.6082e+07	<2e-16
Right - ALIC AD	λ_1	DTI	4.1621e+07	<2e-16
		Group	2.2523e+09	<2e-16
		Year	1.0689e+09	<2e-16
		Sex	2.8287e+01	1.046e-07
		DTI:Group	1.1024e+09	<2e-16
		DTI:Year	4.1351e+08	<2e-16
		Group:Year	7.7152e+09	<2e-16
		DTI:Group:Year	4.0404e+09	<2e-16
Right - ALIC FA	w_2	Group:Year	8.9801	0.002729
		DTI:Group:Year	8.1345	0.004343
Right - ALIC AD	w_2	Group	5.5487	0.018495
		Year	4.6944	0.030261
		DTI:Group	4.8938	0.026954
		DTI:Year	4.3189	0.037690
		Group:Year	7.9294	0.004864
		DTI:Group:Year	6.9257	0.008497

Right - EC FA	λ_1	Year	113.2647	<2e-16
		DTI:Year	168.2154	<2e-16
		Group:Year	106.9007	<2e-16
		DTI:Group:Year	120.5388	<2e-16
Left - EC FA	λ_1	DTI	7.4265e+03	<2e-16
		Group	9.1017e+06	<2e-16
		Year	8.9928e+09	<2e-16
		DTI:Group	6.1987e+07	<2e-16
		DTI:Year	4.3075e+10	<2e-16
		Group:Year	5.0013e+09	<2e-16
		DTI:Group:Year	2.5229e+10	<2e-16

Prospective DTI-EF relationships

No significant relationships were found between RD and AD, and w_2 .

CHAPTER 5. GENERAL DISCUSSION

The main purpose of this thesis was to investigate the longitudinal effects of early psychosocial deprivation on macaque executive function (EF), as well as the neural mechanisms through which such adverse early experience may lead to deficits in EF. Findings demonstrate that early deprivation can result in long-lasting EF impairments, with structural alterations in the prefrontal cortex (PFC) and both cortico-cortical and cortico-subcortical connectivity of PFC modulating the relationship between early rearing conditions and different measures of EF. This is the first research to investigate explicitly how early psychosocial adversity impacts executive function in macaques, and is some of the first to look at EF and associated brain development longitudinally across adolescence into adulthood after exposure to adversity. Altogether, these findings provide important insights into the neurocognitive mechanisms that may underlie poor long-term developmental trajectories linked to cognitive aspects of self-regulation in those exposed to early psychosocial deprivation, and furthers our understanding of the factors that may increase risk for poor outcomes such as psychopathology risk and ADHD-like symptoms in this population.

Results from Chapter 3 supported the hypothesis that peer-rearing would be related to worse executive function across adolescence into adulthood, in keeping with human research on the effects of early institutionalization (Wade, 2019; Wade, 2022). This was found in terms of general EF, and via the novel computational modeling approach used to functionally decompose EF mechanisms contributing to 'A not B' task performance, in terms of working memory across time and inhibitory control in early adulthood. Concerning related brain development, structural differences presented in Chapter 4 in ventrolateral and dorsolateral PFC (i.e. cortical thickness) and in white matter tracts (i.e. fractional anisotropy) connecting the PFC with the parietal cortex and striatum were found between the mother-reared and peer-reared groups. As predicted, cortical thickness in these PFC regions was greater in the peer-reared group. This is in keeping with human studies of early institutional rearing effects in adolescence (Mackes, 2020; Sheridan, 2022). Some interesting but unexpected findings were revealed in terms of white-matter integrity, with higher FA in some tracks found in peer-reared animals, rather than the predicted lower integrity (Sheridan, 2022; Bick, 2015). Notably, thickness in vlPFC and white matter integrity in certain frontostriatal tracts predicted EF measures in the peer-reared group, with thicker vlPFC predicting worse inhibitory control, but greater ALIC integrity predicting better working memory.

Small lesions in PFC have been linked to an increase in fractional anisotropy (FA) (i.e. greater white matter integrity) in the superior longitudinal fasciculus (SLF), as well as in thalamocortical tracts. Increased FA in these tracts connect remote areas of the frontoparietal network, and thus this could be interpreted as a compensatory mechanism promoting behavioural recovery. However, *larger* lesions in the PFC lead to decreases in FA in the SLF, but still an increase in thalamocortical tracts. This indicates that more extensive damage can activate somewhat different pathways of compensation to support behavioral recovery, via thalamocortical connectivity (Adam, 2021). It is possible that the results presented in Chapter 4 reflect a compensatory mechanism in the peer-reared group, whereby profound deficits in PFC development lead to increased integrity of frontostriatal tracts, which supports EF in the peer-reared group when FA is high in fronto-striatal tracts such as ALIC. However, this appears to promote working memory specifically. Other research has found that in the context of early psychosocial deprivation, inhibitory control and information retrieval is impacted negatively, while cognitive flexibility and monitoring are not (Ellis, 2017; Young, 2018; Sheehy-Skeffington, 2020). More flexible behaviour and less inhibition, could be an adaptive mechanism that is beneficial in the context of early deprivation initially, but could result in a premature closing of certain plasticity windows, and therefore poor EF outcomes in adolescence and into adulthood as accelerated development occur. Network analyses could help to test this idea more explicitly in future studies with more time-points of assessment to see how changes in different PFC networks may interact together to promote certain components of EF versus others after early psychosocial deprivation.

How do these results fit with current theories of how early psychosocial adversity affects executive function?

The findings in this thesis could provide some support for theories emphasizing the effects of early psychosocial deprivation on neural plasticity and different effects of specific types of adversity (i.e. dimensional models) (Ho, 2021; Humphreys, 2015; McLaughlin, 2021). These models suggest that early psychosocial deprivation specifically will have profound impacts on cognitive functioning and underlying cortical development, compared to other forms of adversity characterized by threat versus deprivation (e.g. in the context of abuse; Johnson, 2021; Machlin, 2019), which may have more influence on emotion processing. While proliferation and pruning of synaptic connections in the PFC occurs in infancy, pruning of these connections also occurs extensively in adolescence (Huttenlocher, 1997; Spear, 2013). Neural

plasticity models propose that absence of expected social and cognitive inputs in the early environment in the context of psychosocial deprivation result in decreased synaptic proliferation or early pruning as sensitive periods in the PFC open, such as in adolescence. This may then lead to the premature closing of plasticity windows before key transition point in development, when cortical regions affected in the context of early deprivation are most likely to be influenced by experience (i.e. adolescence in the case of PFC). This has severe consequences on subsequent brain development, resulting in a lack of refinement and reorganization in PFC going into adulthood. This may explain why in previous research, reduced gray matter volume and cortical thinning in those exposed to early institutional rearing has been found earlier in development (Hodel, 2015; McLaughlin, 2014), but increased thickness is seen later in adolescence (Sheridan, 2022). Our results suggest this may also be the case in peer-reared macaques.

Additionally, results presented in the Appendix A of this thesis, where analyses were performed including all three assessment time-points in the wider project (late childhood, adolescence, and early adulthood); rather than the only the two time-points included in this chapter (adolescence and early adulthood), which are also the ages at which EF was assessed. In these analyses, we see that in the majority of PFC regions in more dorsal dIPFC (i.e. left 46d, right and left 46v), cortical thickness decreases across time in both rearing groups. Conversely, in more ventral regions of dIPFC (right and left 9/46v), and in the majority of vIPFC regions (left 44, right and left 45b), peer-reared animals have higher thickness across time, with a decrease across time seen in both groups only in left area 45A. The lack of group effects in the dIPFC, but seen in the main that are found in the analyses presented in this chapter investigating adolescence and adulthood only, could suggest that while in late childhood the groups don't differ, earlier in childhood, peer-reared would have shown thinner cortex compared to the mother-reared animals. In vIPFC, in area 45a where greater cortical thickness is and perhaps one of the last areas to reach maturity, no group differences are found in any analyses. However, to confirm this, research including more assessment time points covering a longer developmental time-period is necessary, as well as larger sample sizes and the use of other statistical approaches, such as growth curve analyses, that may enable something more specific to be concluded about trajectories of development that may differ between groups.

And SLF

Although plasticity models have focused mostly on cortical development, effects of reduced plasticity and differences in cortico-subcortical connectivity has also been hypothesized, which is supported by previous findings of altered structural connectivity in fronto-limbic and fronto-striatal tracts (Sheridan, Howell, 2019), and the finding presented in Chapter 4. Notably here, fewer changes with age were found in the peer-reared group compared to the mother-reared group in terms of structural brain development. This finding may also support that early psychosocial deprivation, via plasticity mechanisms, accelerates certain aspects of neural development as a form of adaptation to the early social environment; but that then results in the peer-reared individuals undergoing fewer refinements during adolescence, a period typically characterized by significant reorganization of brain circuits and the pruning of synapses.

While the results presented here provide some support for plasticity and dimensional models, this does not necessarily mean that other mechanisms are not affecting brain development linked to EF in early life. In support of stress models of early adversity, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has also been linked to early psychosocial deprivation (Tottenham, 2011; Silvers, 2017; Callaghan, 2019). This dysregulation can lead to alterations in the brain's stress response system, potentially causing long-term negative impacts on various aspects of self-regulation, including executive function, due to the close interplay between the HPA-axis and development of brain regions including PFC. Specifically, evidence suggest that early life stress can negatively affect GABAergic release in the PFC, and other subcortical areas (e.g. amygdala), with reduced inhibitory regulation in PFC but increased in amygdala. This would affect the excitatory/inhibitory balance, and the protracted absence of GABA would prevent the opening of sensitive period later on in development, including adolescence. Notably, few recent studies posited the key role of GABAergic increase, and subsequent decrease of the excitatory/ inhibitory ratio, for the opening of sensitive period in associative cortex, in addition to the well-established sensory and visual cortex. Additionally, dysregulation in this mechanism is seen in several psychopathologies that include EF impairments, but also emotional dysregulation (e.g. mood disorders). For instance, one study in rats found that maternal separation would results in reduced GABA and increased anxiety in adolescents.

Therefore, both plasticity and stress mechanisms (and both domain-specific domain general) could contribute to the negative outcomes observed in the context of early psychosocial deprivation.

More recently, both of these models have been extended to incorporate the likely importance of striatal development, with a few studies having already demonstrated effects of early institutionalization on both functional (Fareri, 2017) and structural striatal connectivity (Bick et al., 2017). Fronto-striatal circuitry has an important role in the emergence of poor emotional regulation and executive function (Eluvathingal, 2006; Fareri, 2017), and therefore may play a critical role in the emergence of poor self-regulation in the context of early psychosocial adversity. The results presented in Chapter 4 support this proposal, with alterations in fronto-striatal connectivity linked to specifically to differences in EF between rearing groups.

Importantly, while early psychosocial deprivation is characterized by lack of socio-cognitive stimulation in the early caregiving environment, it also can involve increased levels of threat, as well as elements of unpredictability. Therefore, more complex models are likely necessary to explain how different types of early social adversity may lead to both overlapping and distinct outcomes. While dimensional models emphasize a stronger link with cognitive outcomes and underlying brain development that threat and emotion processing deficits (with results concerning effects on subcortical structures such as the amygdala also less consistent; Mackes, 2020; Hodel, 2015; VanTieghem, 2021), previous research has found associations between early institutionalization and both executive function and emotion regulation deficits (Nelson, 2019; Humphreys, 2020; Sonuga-Barke, 2017), which are linked to an increased risk of psychopathology (Bos, 2011; Wade, 2022; Golm, 2020). Indeed, results from the wider project suggest that both executive function and emotion regulation were impacted negatively in our peer-reared group of macaques, with preliminary results concerning emotion regulation discussed more in the next section of this chapter.

Future directions

Next steps for the work described here include consideration of additional measures of brain development which will enable a more comprehensive understanding of the neural mechanisms underlying poor EF linked to early psychosocial deprivation. First, analysis of

other diffusivity metrics will be carried out, such as mean diffusivity (MD) in addition to fractional anisotropy (FA), and myelination will be examined. This will provide more information about what may be driving changes in FA, and about what specific mechanisms could be leading to a premature closure of sensitive period/ plasticity during adolescence in peer-reared group. Second, analyses of cortical surfaces will be conducted. Evidence, suggests that cortical thickness and surface area follow different developmental trajectories and are inversely related to cognitive outcomes. For instance, during childhood higher IQ has been linked to thinner cortex but greater surface area, and in adulthood, thicker cortex and earlier expansion/quicker decrease in surface area are associated with higher IQ (Schnack et al., 2014). Third, analysis of the resting-state fMRI data, which reflects functional brain activity whilst not actively engaging in a task, collected as part of the wider project will also offer insights into potential differences in the functional organization of brain networks, both in terms of typical and atypical development.

Another promising direction will be to conduct genetic analyses to examine dopaminergic dysregulation, using the blood samples collected before MRI acquisition. Dopamine (DA) dysregulation in fronto-striatal circuits is proposed to play a crucial role in the emergence of cognitive difficulties after early psychosocial deprivation (Smith, 2020; Brett, 2015). Notably, imbalance of DA levels in the PFC can result in perseveration, while too much DA in the striatum can lead to distractibility and too much flexibility (Sajed, 2022). Previous investigations have explored the correlation between dopamine function, cortical activity, and task performance. The ideal balance between stability and flexibility of neural representation, which is critical for optimal performance, depends on the interaction of cortical D1 receptors and striatal D2 receptors (Nour, 2019). Different subtypes of dopamine receptors have divergent effects on working memory and learning, suggesting that while the impaired working memory performance seen in psychiatric conditions such as schizophrenia may result from supra-optimal D2R signaling, non-pathological increases in D2R availability in healthy populations may be linked to improved performance (Martel, 2020; Gallo, 2019; Nour, 2019). Results presented in Chapter 4 concerning an opposite pattern of associations between fronto-striatal connectivity and EF in the two rearing groups and with greater cortical thickness in vIPFC in peer-reared animals could be in keeping with these findings.

Interestingly, different patterns in fronto-thalamic circuitry and dopamine signaling is associated with different pathologies involving EF impairments, including schizophrenia, bipolar disorder, mood disorders, and ADHD (Ashok, 2017; Mamah, 2019, Shaw, 2006). Cortical thickness in PFC seems related to both the amount of dopamine release and to cognitive performance (Hettwer, 2022; Jaworska, 2017); in healthy individuals, thicker cortex is related to a smaller amount of dopamine release and better working memory (Casey, 2013; Cherkasova, 2017). This is line with findings here in the mother-reared group, with thicker vIPFC predicting better working memory in this group. On the contrary, in individuals diagnosed with ADHD, a thicker cortex has been associated with more dopamine release and poor working memory in terms of more perseveration (Cherkasova, 2017). This is also in line with our results, with thicker vIPFC predicting worse inhibitory control and more random choices in the peer-reared group.

Therefore, based on such research looking at the dopaminergic system, and accordance with previously discussed models of stress and plasticity, effects of early psychosocial deprivation on EF could be related to epigenetic dysregulation in dopamine signaling, with such early experience leading to higher release in the PFC but not in the striatum.

In fact, the GABA regulation is also mutually connected with dopaminergic regulation. DA can directly modulate the physiology of nonpyramidal, presumably GABAergic neurons in the rat PFC. Evidence suggest that D1 agonists caused larger evoked IPSCs by increasing the intrinsic excitability of interneurons and their axons. In contrast, D2 agonists had no effects on sIPSCs but did produce a significant reduction in mIPSCs, suggestive of a decrease in GABA release probability. In addition, D2 agonists reduced the postsynaptic response to a GABAA agonist. At the same time, evidence also suggest that decrease of GABA in PFC, would affect dopamine signaling, with an inverted U mode pattern.

This could be associated with slower thinning of vIPFC and increases in FA in fronto-striatal tracts across adolescence, which acts as a compensatory mechanism in early development. It is important to note, however, that other cortical and subcortical regions are likely involved (e.g. parietal, limbic). Further research is necessary to fully comprehend the relationship between various aspects of dopamine function and its role in poor executive function in the context of early psychosocial deprivation in human and nonhuman primates.

Examination of how both cognitive and emotional aspects of self-regulation predict specific poor outcomes, including increased risk for anxiety and ADHD-like symptoms, will also be a critical next stage of the wider research project presented here. In Appendix A of this thesis, preliminary results concerning emotion regulation and anxiety are presented. In a published paper (Rayson et al., 2021), we found that the peer-reared group demonstrated an attention-bias toward threat in pre-adolescence, which may indicate poor emotion regulation. This was associated with greater anxiety in peer-reared animals. Indeed, the peer-reared group demonstrated higher levels of anxiety-like behaviour at all three project time-points (i.e. late childhood, adolescence, early adulthood), as well as greater emotional reactivity to social threat which predicted anxiety in adulthood. In this Appendix, we also show that early psychosocial deprivation has an effect on development in fronto-limbic white matter tracts; higher FA was found in the superior cingulum (SC) during adolescence, and in the fornix during late childhood in the peer-reared compared to the mother-reared group. Rearing group also modulated the relationship between white matter integrity in fronto-limbic/fronto striatal tracts and anxiety. In the mother-reared group, higher integrity in the right anterior corona radiata (ACR) predicted less anxiety in late childhood and early adulthood. However, increased right ACR integrity was linked to more anxiety in adulthood in the peer-reared group. This result is in line with higher integrity predicting positive outcomes in terms of EF presented in Chapter 4 in the mother-reared group, but perhaps suggests that compensatory mechanisms in frontostriatal circuits can also lead to increased risk of anxiety in the peer-reared group. Interestingly, integrity in the left uncinate fasciculus (UF), with the UF constituting the main white matter tract connecting the amygdala with PFC, a negative relationship FA and anxiety was stronger in the peer-reared group across time, even though this relationship became weaker over time in general. This is in line with previous literature showing that in typical development, a relationship between UF integrity and anxiety weakens (Hein et al., 2018). It is possible that the in our peer-reared group, maintenance of this relationship serves as a protective mechanism, but it could also suggest atypical development with normative changes not taking place or being delayed; this may lead to negative outcomes later on in life, and may impact development of other networks, including that involving fronto-striatal circuitry. This will idea will be explored more as part of the wider project presented here.

Furthermore, next steps will involve looking at how emotional and cognitive processes interact to predict poor outcomes in the context of early psychosocial adversity. For example, attention shifting and working memory may aid in the detection of threats in the environment and exploitation of rare opportunities in chaotic environments. This is supported by the attention biases results presented in Appendix A. However, these benefits may come at the expense of inhibitory control and performance in tasks requiring long-term storage and retention despite distractions (Mittal et al., 2015; Young et al., 2016). In addition, these mechanisms could lead to an increase of anxiety like behaviours, also supported by results in Appendix A. A compensatory mechanism involving fronto-striatal circuits may enhance working memory, as evidenced by higher integrity in the anterior limb of the internal capsule in the peer-reared group (Chapter 4), but may also impair inhibitory control, working-memory decay, and related emotion regulation. Notably, it is known that inhibitory control and general EF are intimately linked with emotional processes, with implicated brain networks in these two aspects of self-regulation overlapping (e.g...REFS). As such, investigating the neural substrates that could mediate the relationship between executive function and emotional reactivity will help us to gain a deeper insight into the mechanism underpinning risk for outcomes such as anxiety disorders and ADHD after early deprivation.

Finally, future research on the impact of early social adversity on development in general should aim to elucidate the specific learning mechanisms through which psychosocial deprivation leads to poor outcomes. Associative learning and reinforcement learning mechanisms have proposed to affect socio-cognitive and behavioural development in this context, including how reward processing could interact and/or affect self-regulation. According to associative learning theory (Gallistel, 2013), the brain adjusts itself to the experienced world without fully comprehending the underlying causes of improved performance. Reinforcement learning theory, derived from Hebb's work, posits that long-lasting learning derives from incrementally reinforced and rewarding actions through accumulated experience. We know that social experiences in the early years play a crucial role in shaping an individual's ability to process information from their environment, including social information (Wade, 2022; Pirazzoli, 2022). Social information processing theory (Dodge & Crick, 1990) explains that children with disruptive behavior problems often perceive, interpret, and make decisions about social information in ways that increase the likelihood of

aggressive behavior. These difficulties could stem from issues with attachment or the presence of coercive cycles in the family environment (Martel, 2019). Social information processing models have been established for several mental disorders, including depression, aggression, anxiety disorders, oppositional defiant disorder, and schizophrenia (Lau, 2017; Dillon, 2020; Martel, 2019). In the context of early social adversity, attention biases towards threat have been found, which increases the risk for anxiety (Troller-Renfree, 2017; Sheridan, 2018; Dodge, 1995; Rayson et al., 2021). These information processing biases, which result in an over-identification of situations as threatening, may serve a protective function in hazardous environments but have also been linked to internalizing and externalizing problems later on (Reid, 2006). Research comparing anxious and non-anxious parents suggests that disruptions in associative learning could be a critical mechanism by which social adversity increases the risk of mental impairment (Sheridan, 2018). In view of these findings, it has been proposed that difficulties in social information processing and changes in emotional processing are two core mechanisms linking threat exposure and negative mental health outcomes in children and youth (McLaughlin, 2020; Wade, 2022).

Reinforcement learning is linked to reward processing. Maternal separation along with food insecurity has been associated with poor reward performance, as well as lower fronto-striatal white matter integrity that mediates an association with depressive symptoms (Dennison, 2019). Multiple human studies focused on effects of early psychosocial deprivation in human and non-human samples suggest decreased responding or an inability to use feedback and reward-related cues (REFS). Changes in these motivation-related behaviors appear to be founded on a host of neurobiological changes, including lower functional activity in the ventral striatum, altered glutamatergic signaling, changes in dopaminergic modulation, and altered molecular signatures of brain plasticity that may be regulated by epigenetic mechanisms (Hanson, 2021). In Chapter 3, we did test some versions of the computational model with reinforcement learning included as a factor, but none of these were the best model to explain the performance in both groups. However, we cannot exclude that this process could have an important role in the negative outcomes seen in context of early social adversity. In addition, a few studies recent studies suggest that as well as reinforcement learning, 'punishment learning' should be taken into account (Liebenow, 2022). Further research is now needed to investigate these different proposed learning mechanisms are impacted in the case of early

social adversity, with multiple mechanism at distinct and overlapping stages of development likely playing an important role in native outcomes.

Strengths and limitations of this research

The research presented in this thesis possesses several strengths. First was the use of a longitudinal design to track development across adolescence. This type of design enables examination of the dynamic and evolving nature of the relationship between brain and behavior across a time of increased vulnerability for poor outcomes that are often associated with early psychosocial deprivation. Second, the use of more objective measures to assess cognitive and emotional development is an important advantage compared to a lot of human research, where questionnaires completed by caregivers, teachers, or participants themselves are often used in isolation. The observational approach adopted here minimizes the potential for the bias inherent to such measures. Third, the multidisciplinary approach used here is a key strength. The combination of various techniques including magnetic resonance imaging (MRI), behavioural assessment, and computational modelling allowed for thorough exploration of the neurocognitive mechanisms underlying the association between early psychosocial adversity and self-regulation. Finally, meticulous control of early rearing conditions was achieved by using a nonhuman primate model, resulting in reduced variability in both the timing and duration of early psychosocial experiences. These factors play a significant role in determining later behavioral and neurological outcomes (refs), and therefore are important to account for in research such as that presented here.

However, there are also some limitations to the current project that need to be acknowledged. One of these is the small sample size, which restricts the generalizability of findings to the larger population and may have reduced power to identify some meaningful effects. Another limitation is the lack of assessments at ages prior to late childhood and after early adulthood, which limits understanding of the trajectories of neurocognitive development from the time of initial caregiver deprivation and across later life. Finally, although results further our knowledge on the neurocognitive mechanisms that likely underlie poor outcomes in the context of early psychosocial deprivation, they do not reveal anything about the specific learning mechanisms through which alterations in development occur, or how trajectories may differ after different types of early social adversity. All these limitations

will have to be addressed in future research to more fully elucidate how early social adversity impacts neurocognitive development and risk for long-term poor outcomes.

Conclusion

To conclude, findings presented in this thesis provide novel and important insights into the effects of early psychosocial deprivation on self-regulation and its neural bases across adolescence. The main results suggest that early deprivation in macaques leads to impairments in executive function that persist into adulthood. These are related to structural development of the prefrontal cortex and connectivity within frontoparietal and frontostriatal brain circuitry. Findings also provide some support for a compensatory mechanism after early psychosocial deprivation in terms of structural brain development. Additionally, results in Appendix X indicate that increased emotion regulation problems persist into adulthood after experience of psychosocial deprivation in macaques, which is associated with an increased risk for anxiety; this relationship is modulated by structural brain connectivity in networks linked to emotion processing and regulation. Further research, including longitudinal studies across more stages of development (e.g. infancy, childhood, adolescence, and adulthood), is now necessary to more fully understand how different developmental trajectories of self-regulation emerge after different types of social adversity, as well as the learning mechanisms that may contribute to healthy versus pathological psychological and behavioural outcomes. This will be essential for the future design of treatments and interventions to ameliorate and prevent poor outcomes in the context early social adversity, and thus improve the quality of life in affected individuals.

APPENDIX A. Early Psychosocial Deprivation, Emotion Processing, and Anxiety Risk

1. Published manuscript (accepted version): Affect-biased attention and anxiety in the context of early psychosocial deprivation

Rayson, H., Massera, A., Belluardo, M., Ben Hamed, S., & Ferrari, P. F. (2021). Early social adversity modulates the relation between attention biases and socioemotional behaviour in juvenile macaques. Scientific reports, 11(1), 1-11.

In this paper we looked at how early psychosocial deprivation influences affect-biased attention and relates to anxiety at the first project time-point (2.5 years). Affect-biased attention is thought to play a fundamental role in early socioemotional development (e.g. prosocial behaviour and risk for anxiety disorders), but factors influencing its emergence are unclear, as are associations with typical versus pathological outcomes (Morales et al., 2016). Atypical affect-biased attention is demonstrated by children exposed to institutional rearing (Troller-Renfree et al., 2015; 2017), and may reflect an important aspect of emotion dysregulation increasing the risk for mood disorders and antisocial behaviour in individuals exposed to early psychosocial deprivation. Like early institutionalization in humans, peer-rearing in macaques has been associated with increased anxiety-like behaviour (e.g. Dettmer et al., 2012), though whether this was linked to affect-biased attention was previously unexplored.

In this study, we aimed to: a) establish whether pre-adolescent macaques demonstrate attention biases to both threatening and reward-related dynamic facial gestures; b) examine the effects of early deprivation on such biases; and c) investigate how this relation may be linked to socioemotional functioning (anxiety and social engagement). We presented our two groups of macaques (mother-reared/peer-reared) with pairs of dynamic facial gestures comprising two conditions: neutral-threat and neutral-lipsmacking. Note, lip-smacking is an affiliative facial gesture in macaques. Attention biases to threat and lipsmacking were calculated as the proportion of gaze to the affective versus neutral gesture. Measures of anxiety and social engagement (i.e. social grooming) were also acquired from videos of the subjects in their everyday social environment over a number of weeks.

Results revealed that while both groups demonstrated an attention bias towards threatening facial gestures, a greater bias linked to anxiety was demonstrated by the peer-reared group only. Only the mother-reared group demonstrated a significant attention bias towards lipsmacking, and the degree of this positive bias was related to duration and frequency of social engagement. Such results suggest that early psychosocial deprivation does indeed impact affect-biased attention in macaques, and that threat biased attention is linked to increased anxiety in this early rearing context. These findings offer important insights into the effects of early social experience on affect-biased attention and related socioemotional behaviour in nonhuman primates, and demonstrate the utility of this model for future

investigations into the neurocognitive mechanisms underlying these relationships across development. The full paper (Rayson et al., 2021) is included in pages X-X of this Appendix.

2. Preliminary results: Longitudinal relationships between deprivation, structural brain connectivity, and anxiety

We are currently running analyses to look at whether structural brain connectivity in frontolimbic and frontostriatal tracts modulate a relationship between early psychosocial deprivation and anxiety over time, as well as how emotional reactivity modulates these relationships. This brain circuitry is implicated in anxiety disorders () and is affected by early institutionalization ().

Detailed information and results are presented on pages X-X of this Appendix, but briefly, preliminary analyses reveal that: i) anxiety-like behaviour is more frequent in the peer-reared versus mother-reared group across time; ii) emotional reactivity is greater in the peer-reared versus mother-reared group over time and is related to anxiety at the 5 year time-point; iii) year/group ROI diffs...; and iv) there are differing relationships between white matter integrity in specific tracts and rearing group/reactivity that predict anxiety over time.

The MRI/DTI work presented here was led by Dr. Bassem Hiba and his team; Hind Errame, who is supervised by Dr. Hiba, with the help of Nathalie Richard and Yann Bihan-Poudec, completed all preprocessing of the MRI data. Thanks also to Suliann Ben Hamed, Mathilda Froesal and Maeva Garcoin for their help with the acquisition of the MRI data.

1) Published manuscript in Scientific Reports (2021; accepted version): Affect-biased attention and anxiety in the context of early psychosocial deprivation

EARLY SOCIAL ADVERSITY MODULATES THE RELATION BETWEEN ATTENTION BIASES AND SOCIOEMOTIONAL BEHAVIOUR IN JUVENILE MACAQUES

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Abstract

Affect-biased attention may play a fundamental role in early socioemotional development, but factors influencing its emergence and associations with typical versus pathological outcomes remain unclear. Here, we adopted a nonhuman primate model of early social adversity (ESA) to: i) establish whether juvenile, pre-adolescent macaques demonstrate attention biases to both threatening and reward-related dynamic facial gestures; ii) examine the effects of early social experience on such biases; and iii) investigate how this relation may be linked to socioemotional behaviour. Two groups of juvenile macaques (ESA exposed and non-ESA exposed) were presented with pairs of dynamic facial gestures comprising two conditions: neutral-threat and neutral-lipsmacking. Attention biases to threat and lip-smacking were calculated as the proportion of gaze to the affective versus neutral gesture. Measures of anxiety and social engagement were also acquired from videos of the subjects in their everyday social environment. Results revealed that while both groups demonstrated an attention bias towards threatening facial gestures, a greater bias linked to anxiety was demonstrated by the ESA group only. Only the non-ESA group demonstrated a significant attention bias towards lipsmacking, and the degree of this positive bias was related to duration and frequency of social engagement in this group. These findings offer important insights into the effects of early social experience on affect-biased attention and related

socioemotional behaviour in nonhuman primates, and demonstrate the utility of this model for future investigations into the neural and learning mechanisms underlying this relationship across development.

Introduction

In the face of limited perceptual and cognitive resources, attention mechanisms enable the brain to manage competing demands in the everyday environment by prioritizing a subset of stimuli for dedicated processing. Such mechanisms guide behaviour from the earliest months postpartum, serving as a fundamental base for learning, self-regulation, and memory (Buss et al., 2018; Rueda et al., 2005). *Affect-biased* attention specifically is posited to play a broad and pervasive role in early socioemotional development (Morales et al., 2016; Todd et al., 2012), with emerging affect biases shaping an infant's experience of their environment via preferential processing of threat- and reward-related information. This, in turn, is thought to support the emergence of adaptive approach and avoidance behaviour (Peltola et al., 2013, 2015). However, specific affect-biases have also been linked to poor socioemotional functioning later on in development (e.g. Pérez-Edgar et al., 2010; Roy et al., 2008), and many questions remain concerning the mechanisms through which affect-biased attention arises and may relate to both typical and pathological outcomes.

Biased attention towards threat-relevant information serves an essential survival function (Pourtois et al., 2013). It is unsurprising, therefore, that most affect-bias studies have focused on threatening stimuli such as angry or fearful versus neutral faces. An attention bias to threat (ABT) emerges during the first year postpartum (Morales et al., 2017; Peltola et al., 2013), and has been linked to positive socioemotional outcomes in the form of secure infant attachment (Peltola et al., 2015). Conversely, ABT has also been associated with emotion regulation difficulties, social withdrawal, and anxiety in both adults and younger populations (Bar-Haim et al., 2007; Dudeney et al., 2015), with cognitive models of anxiety attributing a causal relation to ABT in the development and or maintenance of anxiety (Beck & Clark, 1997; MacLeod & Mathews, 2012; Mathews & MacLeod, 2002). This apparent contradiction suggests that any increased vulnerability conferred by threat-biased attention may arise from an early-emerging, normative threat bias (Field and Lester 2010; Dodd et al., 2015), with

excessive ABT or a failure to inhibit ABT exacerbating the risk for psychopathology (Kindt & Van Hout, 2001). Inconsistent findings concerning a link between ABT and anxiety in childhood, with no relation often found despite ABT presence (e.g. Dodd et al., 2015), is in keeping with this idea, but so far, very little is known about how ABT and socioemotional function interact across development.

An attention bias to positive stimuli (ABP) such as happy faces may also play an important role in early socioemotional functioning. Such positive biases are often conceptualized as a bias towards rewarding stimuli (Morales et al., 2016). Relatively little is known about the emergence of ABP, but evidence suggests this also arises at an early stage in development (Burris et al., 2017; Morales et al., 2017). ABP has been associated with several aspects of positive socioemotional functioning in children and adults, including social engagement and prosocial behaviour (Troller-Renfree et al., 2015), adaptive emotion regulation skills (Joormann & Gotlib, 2007), and positive affect (Grafton et al., 2012). Notably, ABP has also been linked to lower levels of anxiety (e.g. Taylor et al., 2011), and may act as a protective factor in developmental populations at increased risk for poor socioemotional outcomes. This includes risk for anxiety and internalizing problems in behaviourally inhibited and previously institutionalized children (Dodd et al., 2020; Troller-Renfree et al., 2015, 2017; VanTieghem et al., 2017). Nevertheless, findings linking ABP to anxiety in younger populations are again mixed, with some studies failing to find any relation between ABP and anxiety in childhood (Roy et al., 2008; Waters et al., 2010).

Nonhuman primate (NHP) models could add significantly to our understanding of affect-biased attention and its role in early socioemotional functioning. Adopting a comparative developmental approach can provide unique insights into the origins of human cognition, highlighting similarities and divergences in our evolutionary history (Rosati et al., 2014, 2018). However, it is currently unclear whether affect biases comparable to those in humans are present in early NHP development, and how these may relate to other specific developmental outcomes. Rhesus macaque (*Macaca mulatta*) monkeys are very similar to humans in terms of cognition, socio-affective characteristics, and brain organization, and are thus commonly utilized to investigate the aetiology of various psychiatric and neurodevelopmental disorders, including anxiety (see Coleman & Pierre, 2014; Kalin & Shelton, 2003). Macaques also live in large social groups, have an extended period of development comprising distinct infant,

juvenile (pre-adolescent and adolescent), and adult periods, and the early macaque mother-infant relationship shares many commonalities with humans (Ferrari et al., 2009). The macaque model is, therefore, especially well-suited to developmental studies, and could provide particularly valuable information concerning the mechanisms underlying ABT and ABP emergence. Accordingly, we adopted a macaque model in the current study with the goal of furthering our understanding of affect-biased attention in NHPs, and investigating the suitability of this as a translational model of its development and related socioemotional outcomes.

Early-emerging ABT also appears normative in macaques, assessed via presentation of threatening versus neutral faces (Mandalaywala et al., 2014; Morin et al., 2019; Rosati et al., 2018). To our knowledge, only one macaque study has investigated ABP specifically, with no bias towards a positive, affiliative facial gesture ('lip-smacking') versus a neutral face revealed at any stage in development (Rosati et al., 2018). Nevertheless, this lack of bias may have resulted from difficulty in discriminating between the two static images, as lip-smacking, a highly rhythmic and dynamic facial gesture, is very difficult to portray in a static stimulus. To date, the majority of both human and NHP studies that have utilized paired affective vs. neutral facial stimuli to investigate affect-biased attention have used static images. The use of dynamic facial stimuli, however, improves various aspects of perception and enhances attention biases in human adults (Ambadar et al., 2005; Cudek et al., 2017; Kamachi et al., 2013), and recruits dissociable neural pathways from those involved in the perception of static faces (Kilts et al., 2003; Pitcher et al., 2014). This issue of static versus moving faces is especially pertinent for developmental research. Dynamic stimuli can enhance neural and behavioural discrimination of emotional versus neutral faces from early infancy (Ichikawa et al., 2014; Quadrelli et al., 2019), and attention to emotional expressions is modulated by stimulus motion across childhood, adolescence, and into adulthood (Nelson & Mondloch, 2018). Altogether, this highlights the importance of adopting more ecologically valid, dynamic facial stimuli in studies of affect-biased attention, and indeed, there is a growing movement within the wider research community towards the use of more naturalistic stimuli in studies of social attention with humans and NHPs (see Fan et al., 2021).

To address outstanding questions concerning the mechanisms through which affect-biased attention emerges and relates to both typical and atypical functioning, it is critical to consider

which factors may contribute to individual differences in ABT and ABP, and how such differences may confer vulnerability or resilience. It is well established that early social adversity can increase risk for a number of adverse socioemotional outcomes, including anxiety and reduced social engagement in humans and macaques (e.g. Almas et al., 2015; Dettmer et al., 2012; McGoron et al., 2012; Winslow et al., 2003). A small number of studies have linked elevated ABT specifically to early social deprivation (Troller-Renfree et al., 2015, 2017) and maternal anxiety (Morales et al., 2017) in children and human infants, with maternal abuse and over-protectiveness associated with ABT magnitude in infant and adolescent macaques (Mandalaywala et al., 2014; Morin et al., 2019). Early social deprivation may also impact ABP, with 'care-as-usual' versus foster home placement related to a reduced or absent bias towards happy faces (Troller-Renfree et al., 2015, 2017), and greater ABP to more social engagement and fewer internalizing problems (Troller-Renfree et al., 2015, 2017) in the context of early institutionalization. It remains unknown whether early social adversity has similar effects on the relation between affect-biased attention and comparable socioemotional outcomes in NHPs.

The current study was designed to investigate both ABT and ABP in pre-adolescent macaques using dynamic facial stimuli, and to examine whether such biases are linked to socioemotional functioning; specifically, anxiety-like behaviour and social engagement. To consider the effects of early social adversity, we assessed two groups of juvenile macaques (aged 2.5 years), one mother-reared and one peer-reared. Peer-rearing is often adopted in macaque models of early social adversity, and has been associated with both increased anxiety and decreased social behaviour (Dettmer et al., 2012; Winslow et al., 2003). Our hypotheses were: i) although all animals will demonstrate attention biases, peer-reared animals will demonstrate greater ABT, and mother-reared animals will demonstrate greater ABP; ii) greater ABT will be related to more anxiety-like behaviour, but greater ABP will be related to less; and iii) greater ABP will be related to more social engagement.

Methods

Subjects and housing conditions

The sample consisted of 21 juvenile rhesus macaques (*Macaca mulatta*), 11 mother-reared (six female) and 10 peer-reared (five female). Subjects were aged around 2.5 years at the time of this study (mother-reared; M = 943.18 days, SD = 17.98; peer-reared; M = 956.2 days, SD = 20.24). Subjects were housed at the *Institut des Sciences Cognitives Marc Jeannerod* (ISC-MJ), CNRS, in mixed mother- and peer-reared social groups of 5-6 animals. All housing and procedures conformed to current guidelines concerning the care and use of laboratory animals (European Community Council Directive No. 86–609), and were approved by our local ethics board, ‘Comité d’Ethique Lyonnais pour les Neurosciences Expérimentales’ (CELYNE) C2EA #42 (03.10.18), and the French Ministry of Research (10.10.18); project reference APAFIS#15091_2018071014483295_v2. All reporting here conforms to the recommendations in the ARRIVE Guidelines for Reporting Animal Research.

All subjects were born and raised at the Laboratory of Comparative Ethology at the National Institutes of Health, US. Peer-reared animals were raised in a nursery with access to same-aged peers; see Shannon et al. (1998) for more rearing protocol details. Rearing procedures were approved by the NICHD and the University of Maryland Animal Care and Use Committee, and adhered to the NIH Guide for the Care and Use of Laboratory Animals. Animals were relocated to the Rousset Primatological Station, CNRS, France at two years of age. More information about the rearing protocol and housing can be found in the SI.

Facial gesture stimuli

Stimuli for the gaze bias task consisted of short, dynamic video clips (5s) of an unfamiliar adult female macaque performing three types of facial movements: i) neutral facial movements (i.e. no gesture but with small movements of, for example, the nose and mouth); ii) lip-smacking (LPS), comprised of the rapid, rhythmic opening and closing of the mouth, and pursing of the lips; and iii) open-mouth threat, comprised of the wide opening of the mouth and lowering of the jaw, with lips held in a tense position covering the teeth. The onset and duration of movement, size, brightness, contrast, spatial frequency, and overall motion levels were controlled for (see SI) to ensure that neutral, lip-smacking, and threat video stimuli did not differ in terms of low-level visual features. All videos started with a 500ms static period showing the first frame of the video (with a neutral facial expression), followed by 4500ms of movement (i.e. two consecutive instances of each gesture or neutral movement sequence).

Gaze bias task

Each subject was temporarily separated from their social group and placed into the testing area in another section of the room; 87 × 100 × 120cm enclosure with a clear panelled front. Before commencing the task, a widescreen computer monitor (35 × 61cm; 2560 × 1440 resolution) was placed 60cm from the front of the enclosure, and animals were given five minutes to habituate to the enclosure once separated. Note, all animals had already been well familiarized with this process of separation into the testing enclosure and presentation of non-social video stimuli before the day of assessment. Animals were recorded during the task using a webcam (30fps) placed on the top-centre of the monitor.

Animals were presented with pairs of neutral-affective gesture stimuli comprising two conditions: i) Neutral-Threat (five trials per subject); and ii) Neutral-LPS (five trials per subject), i.e. the positive or reward condition. Video pairs were presented for 5s per trial, with condition order and position (left or right) of neutral-affective gesture videos counterbalanced across subjects. Before the stimuli appeared, a moving geometric pattern accompanied by a non-social sound was presented in the centre of the screen to attract the subject's attention, with stimuli presentation then triggered by an experimenter watching the animal live on a separate monitor (not in view of the subject). Additionally, a calibration procedure was conducted before presentation of experimental stimuli, whereby images of objects (e.g. ball, toy car) were presented on the right, centre, and left of the screen. Each image was jittered up and down slightly to attract attention and was accompanied by a non-social sound. Psychopy v1.90.2 (Peirce et al., 2019) was used for stimulus (calibration and experimental) presentation, with video recording onset and offset automatically triggered at the start and end of each presentation. This sequence and the experimental set-up is illustrated in Fig. 1.

Subjects' gaze (left, right, other, offscreen) was manually coded offline, frame-by-frame, by a researcher blind to the condition being presented and the position of the neutral-gesture stimuli. A random 15% of videos were coded by a second researcher to establish reliability, with very good reliability scores obtained ($\kappa = 0.84$).

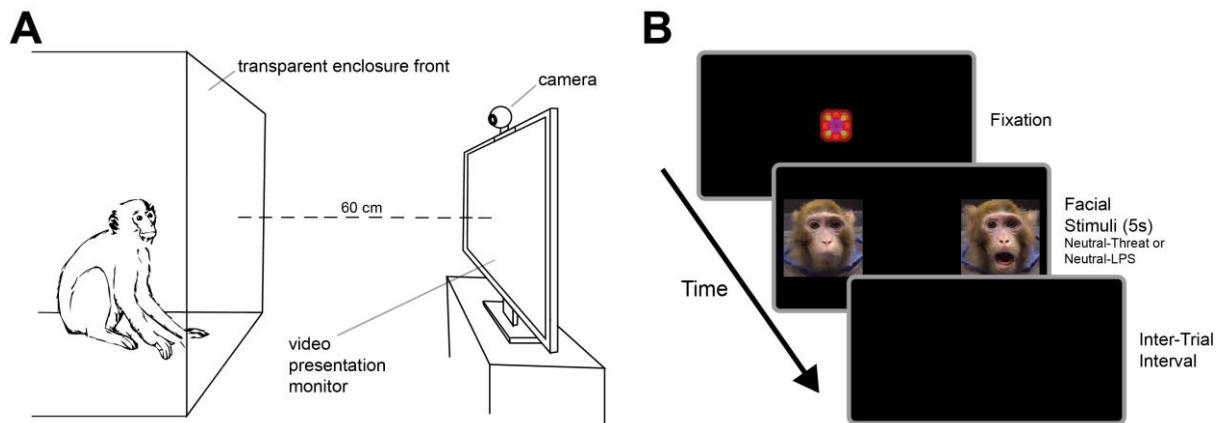


Figure 1: A) Schematic illustration of the gaze bias task set-up; B) Illustration of a trial in the gaze bias task. An experimenter triggered the appearance of fixation and facial stimuli screens when the subject looked towards the monitor.

Behavioural observation

Video recordings of the social group (50fps) were made two times per week, once in the morning and once in the afternoon, for three weeks. Two cameras were used to capture the whole home enclosure and were synchronized offline for the manual coding of behaviour. Each animal was coded second-by-second for five minutes per recording session, totalling 30 minutes per animal. One experimenter coded all of the videos, with a second experimenter coding a random 15% to establish reliability ($\kappa = 0.93$). The following behaviours were coded using the focal sampling method: a) self-scratching, self-grooming, yawns, and body shakes (i.e. behaviours reflecting anxiety in macaques; see Coleman & Pierre (2014)); and ii) social grooming (both give and receive), which is a primary means by which macaques maintain and strengthen social relationships (Dunbar, 1998).

Data analysis

To calculate attention bias to threat (ABT), the proportion of time looking at the neutral and threat stimuli (out of total time looking onscreen) was calculated separately for each neutral-threat trial, with the neutral proportion then subtracted from the threat proportion. The equivalent approach was used to calculate attention bias to LPS stimuli, i.e. positive stimuli (ABP). A linear mixed model was then utilized to investigate potential differences between rearing groups and attention bias type at the trial level, with group (mother-reared or peer-

reared), condition (threat or LPS), and their interaction included as fixed effects, and subject-specific intercepts as a random effect. Social rank (randomized Elo-ratings) was also included as a covariate, and z-scored for analysis. Model residuals were checked for normality and homogeneity. Before analysis, trials where animals failed to look at the screen during facial gesture presentation or were 2.5 SDs above or below the mean were excluded. More details about rank calculation and trial exclusion can be found in the SI.

The following behavioural indices were computed based on the group observation coding: i) *anxiety frequency*, obtained by summing occurrences of self-scratching, self-grooming, yawns, and body shakes; and ii) both *duration and frequency of social engagement*, obtained by calculating total time spent in social grooming interactions and the frequency of social grooming interactions, respectively. To explore the relation between attention biases and anxiety or social engagement at the observation session level, generalized linear mixed models were run separately for average ABT and ABP per participant, with negative binomial error distribution and a log link function. This was done with either *frequency* or *duration* of behaviour as the outcome variable. Group (mother-reared or peer-reared), ABT or ABP, and their interaction were included as fixed effects, rank (randomized Elo-ratings) as a covariate, and subject-specific intercepts as a random effect. Elo-ratings and attention biases were z-scored for analysis. R v3.6.3 (R Core Team, 2020) was utilized to conduct these analyses (see SI for package information). *P*-values for fixed effects and interactions were obtained using Type III *F* tests for linear models, and Type III Wald χ^2 tests for generalized linear models. Significant interactions between factors were followed up by planned pairwise comparisons of estimated marginal means which were Tukey-corrected for multiple comparisons. Significant interactions between factors and continuous variables (i.e. ABT or ABP) were followed up by planned comparison of the estimated marginal means of the linear trends of the continuous variable to 0 at each level of the factor. Effect sizes are reported as unstandardized model parameter estimates (in the scale of the model response variable). All animals were included in these analyses ($n = 21$; 11 mother-reared, 10 peer-reared). Descriptive statistics can be found in Table 1.

Table 1: *Gaze measures and socioemotional behaviours. Proportion onscreen is the proportion (M and SD) of trial time spent attending to the screen. Proportion threat, neutral, and LPS are the proportions (M and SD) of time attending to the screen spent attending to the threat,*

neutral, and LPS face, respectively. ABT and ABP (i.e. affective versus neutral stimuli) is the difference between proportion threat (or LPS) and neutral in the neutral-threat, and neutral-LPS trials, respectively. Anxiety and social groom frequency are the frequencies (M and SD) of anxiety-like behaviours and social grooming during the observation period. Social groom duration (M and SD) is the total amount of time (seconds) spent in grooming interactions during the observation period.

Group	Mother-reared	Peer-reared
Gaze measures		
Proportion onscreen	0.60 (0.15)	0.44 (0.19)
Proportion threat (neutral-threat trials)	0.455 (0.1)	0.536 (0.17)
Proportion neutral (neutral-threat trials)	0.264 (0.103)	0.145 (0.065)
Proportion LPS (neutral-LPS trials)	0.456 (0.094)	0.324 (0.1)
Proportion neutral (neutral-LPS trials)	0.225 (0.069)	0.292 (0.133)
Attention bias to threat (ABT)	0.19 (0.164)	0.386 (0.151)
Attention bias to LPS (ABP)	0.231 (0.086)	0.032 (0.14)
Socioemotional Behaviour		
Anxiety frequency	1.561 (0.743)	2.383 (1.114)
Social groom frequency	1.106 (0.987)	0.983 (0.713)
Social groom duration (s)	50.606 (48.466)	37.117 (31.096)

Results

Attention bias to threat and LPS

First we compared the attention biases to affective stimuli in the neutral-LPS and neutral-Threat conditions between the mother- and peer-reared groups. This revealed a significant main effect of group [$F(1) = 8.288, p = 0.007$, effect size (mother - peer) = 0.036], as well as a significant group \times condition interaction [$F(1) = 22.101, p < 0.0001$, mother-reared effect size (LPS – threat) = 0.036, peer-reared effect size (LPS – threat) = -0.364] (Fig. 2). Attention bias to threat (ABT) was greater in the peer-reared compared to mother-reared group [$t(33.1) = -2.079, p = 0.045$], and attention bias to LPS (i.e. positive bias; ABP) was greater in the mother-reared compared peer-reared group [$t(34.6) = 2.879, p = 0.007$]. In the mother-reared group, ABT and ABP were not significantly different from each other [$t(176) = 0.628, p = 0.531$] but in the peer-reared group, ABT was significantly greater than ABP [$t(178) = -5.827, p < 0.0001$].

One sample t -tests also confirmed that in the mother-reared group, ABT [$t(10) = 3.841, p = 0.003$] and ABP [$t(10) = 8.905, p < 0.0001$] were significantly different from zero, whereas in the peer-reared group, only ABT [$t(9) = 8.093, p < 0.0001$] was significantly different from zero.

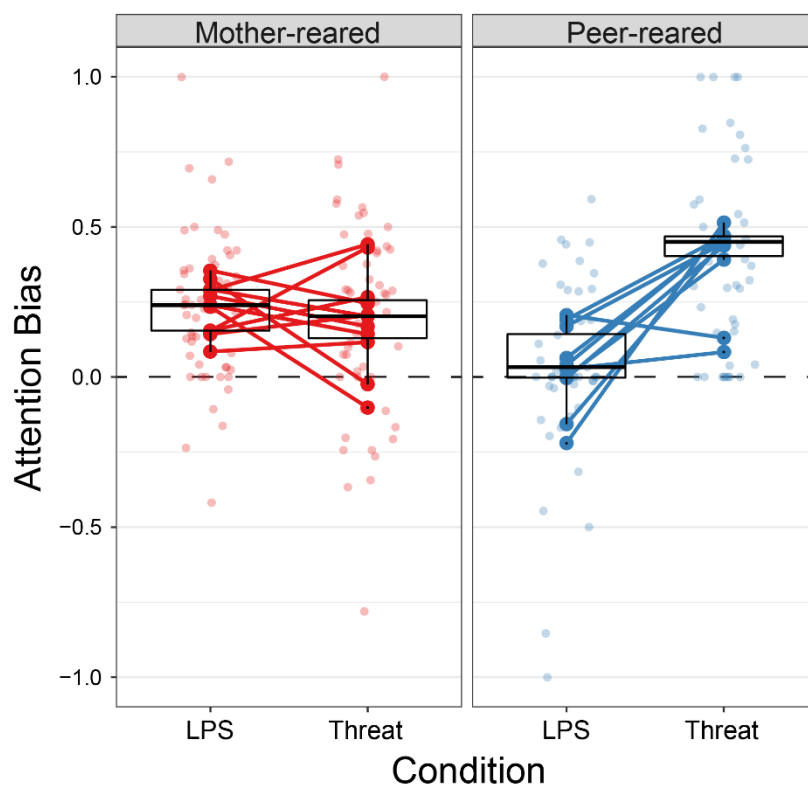


Figure 2: Attention biases in the neutral-LPS and neutral-threat conditions, for the mother-reared group (red; left) and peer-reared group (blue; right). Zero indicates no bias, positive values a bias towards LPS or threat versus neutral, and negative values a bias towards neutral versus LPS or threat. Light-coloured dots represent the bias in each trial, large dark-coloured

dots indicate the average bias for each subject, and lines connect the average bias in the two conditions per subject. The median bias per group, first and third quartiles, and + or - 1.5 times the inter-quartile range from the first and third quartiles are also shown in the box plots.

Relation between attention biases and socioemotional behaviour

Having established a difference in attention biases between groups and conditions, we then sought to determine if attention biases were related to the frequency of anxious behaviour in the two rearing groups. We found a group \times ABT interaction [$\chi^2(1) = 6.256, p = 0.012$, mother-reared effect size = -0.193, peer-reared effect sized = 0.506] (Fig. 3), with greater ABT related to more frequent anxiety-like behavior in the peer-reared group [$z = 2.261, p = 0.024$]. There was no significant main effect of ABP or the relation between ABP and frequency of anxious behaviour in either rearing group (both $p > 0.458$).

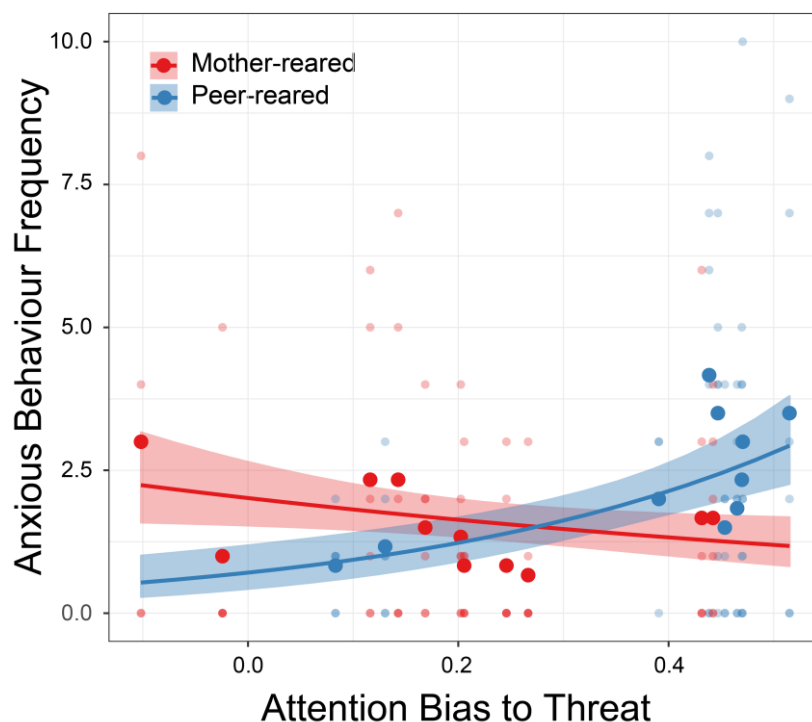


Figure 3: The relation between attention bias to threat (ABT) and frequency of anxiety-like behaviour in the mother-reared (red) and peer-reared (blue) group. Each light-coloured dot represents an individual behavioural observation session, large dark-coloured dots represent the subject mean, dark lines indicate the model fit, and shaded regions around the lines denote + or - SE.

We then went on to investigate relations between attention biases and the frequency and duration of social engagement. There were significant main effects of ABP [$\chi^2(1) = 10.064$, $p = 0.002$, effect size = 1.070], group [$\chi^2(1) = 7.445$, $p = 0.006$, effect size (mother – peer) = -1.199], and an ABP \times group interaction [$\chi^2(1) = 6.194$, $p = 0.013$, mother-reared effect size = 1.931, peer-reared effect size = 0.211] for social engagement frequency (Fig. 4a), with greater ABP related to more frequent social engagement in the mother-reared group [$z = 3.172$, $p = 0.002$]. For social engagement duration, we found significant main effects of both ABP [$\chi^2(1) = 18.397$, $p \leq 0.0001$, effect size = 2.56] and group [$\chi^2(1) = 12.746$, $p \leq 0.001$, effect size (mother – peer) = -60.590], and an ABP \times group interaction [$\chi^2(1) = 9.793$, $p = 0.002$, mother-reared effect size = 4.456, peer-reared effect size = 0.665]; greater ABP was related to a longer duration of social engagement [$z = 4.289$, $p < 0.0001$ in the mother-reared group (Fig. 4b)]. There were no significant main effects of ABT or relations between ABT and frequency or duration of social engagement (all $p > 0.236$).

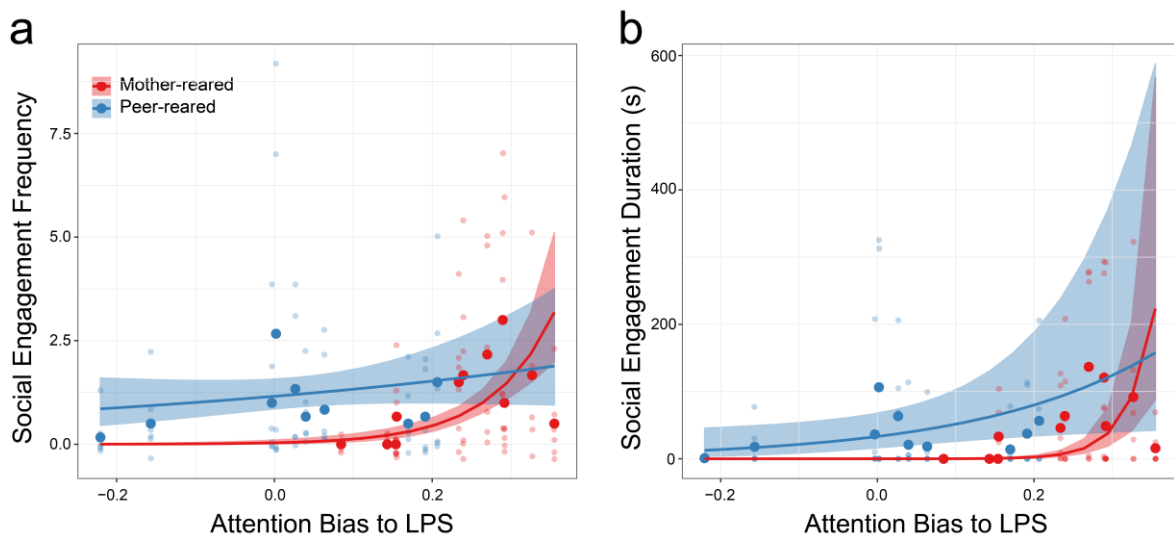


Figure 4: The relations between attention bias to LPS (i.e. to positive stimuli; ABP) and social engagement in the mother-reared (red) and peer-reared (blue) group. Each light-coloured dot represents an individual behavioural observation session, large dark-coloured dots represent the subject mean, dark lines indicate the model fit, and shaded regions around the lines denote \pm or - SE.

Discussion

In this study, juvenile macaques demonstrated biased attention to both threatening and affiliative dynamic facial gestures. This is in keeping with a previously demonstrated bias towards static images of open-mouth threat (Mandalaywala et al., 2014; Morin et al., 2019; Rosati et al., 2018), and provides evidence for a bias towards positive or reward-related facial gestures also. Early social deprivation was associated with the degree of bias towards both types of affective stimuli, with a greater attention bias to threat (ABT) found in peer-reared animals, and a greater attention bias to positive stimuli (ABP) found in animals reared by their mothers. Notably, ABT was also linked to anxiety-like behaviour in peer-reared animals, and ABP to levels of social engagement in mother-reared animals. These findings offer novel insights into the effects of early social adversity on affect-biased attention, and suggest that such biases could play an important role in early macaque socioemotional functioning.

The presence of a bias towards dynamic open-mouth threat in both our rearing groups provides some support for ABT emergence being part of typical macaque development. This bias was, however, greater in the peer-reared compared to mother-reared group. Therefore, although ABT emergence is likely normative in macaques (Mandalaywala et al., 2014; Rosati et al., 2018), it is possible that very early social deprivation can exacerbate this bias, even into the preadolescent juvenile period. Interestingly, such effects of early social experience on ABT are similar to those seen in human children in the context of early institutionalization (Troller-Renfree et al., 2015, 2017). Mother-reared animals also showed a bias towards dynamic lip-smacking (LPS) stimuli. No previous macaque study has investigated potential effects of early adversity on an attention-bias to dynamic LPS specifically, but in line with our results, there is some evidence that early rearing status can affect infants' social responses to LPS performed by a human experimenter (Vanderwert et al., 2015). As a whole, the peer-reared group did not show significant ABP, again paralleling results from the available human literature concerning the effects of early social deprivation on ABP in pre-adolescence (Troller-Renfree et al., 2015, 2017).

Here, a greater magnitude of ABT was related to more frequent anxiety-like behaviour in the peer-reared group, suggesting that early social adversity can confer greater risk for anxiety via exaggerated or uninhibited ABT. This finding aligns with evidence suggesting that in humans, the ABT-anxiety link is not found consistently during childhood, and may be found more

reliably in the context of early adverse experience. A relation between ABP and social engagement (i.e. social grooming) was also revealed in the mother-reared group, with more frequent and a longer time spent in grooming interactions associated with greater ABP. Therefore, it is possible that a bias towards reward-related stimuli also serves a positive function in macaque development. In contrast to some research with human children, we did not find a relation between greater ABP and reduced anxiety. There are a number of possible explanations for this result. For instance, some human research suggests that ABP is present in institutionalized children and linked to fewer internalizing problems only after stable fostering placement (Troller-Renfree et al., 2017). Additionally, in community samples, it may be that ABP only serves as a protective factor against anxiety in behaviourally inhibited children (White et al., 2017; Dodd et al., 2020).

In the case of atypical early parenting input, it is possible that uninhibited, exacerbated, or diminished affective attention biases are adaptive in the short-term, increasing offspring survival rates. Longer term, however, this may tie individuals to maladaptive trajectories of development (Morales et al., 2016; Pérez-Edgar et al., 2014), increasing the risk for psychopathology and social difficulties. Reduced attention to threat has already been associated with insecure and disorganized attachment in infants (Peltola et al., 2015, 2020). Parental sensitivity impacts the formation of infant-caregiver attachment (e.g. McElwain & Booth-Laforce, 2006), with insecure and disorganized attachment implicated in the development of numerous adverse outcomes (see Ranson & Urichuk, 2008). In the case of early social deprivation, an attachment figure is completely absent. Therefore, while it may be adaptive to avoid threat in the presence of, for example, an abusive caregiver, it may be adaptive for an infant with no social buffering to be hypervigilant towards threat. This highlights an important outstanding issue, with general versus specific mechanisms linking early adversity to negative socioemotional functioning being poorly understood (McLaughlin, 2016). Clarifying these mechanisms requires consideration of how specific types of adversity may increase risk for specific adverse outcomes. In terms of a positive bias, it may be that a lack of learning opportunities to associate positive facial gestures with reward in the absence of an attachment figure influences the emergence of a positive affect bias and related social outcomes. This idea needs to be explored more explicitly in subsequent studies, but it is in

keeping with evidence for atypical reward processing in the context of early social deprivation (e.g. Goff et al., 2013).

Although we found a link between ABT and anxious behaviour in juvenile macaques, it remains unclear whether ABT actually played a causal or maintaining role, or simply reflected current levels of anxiety. This remains a key unanswered question in the literature. Very little research thus far has investigated how ABT relates to or predicts anxiety longitudinally, or even the developmental trajectory of ABT itself in typical or at-risk populations. There is some evidence that ABT is unstable across childhood (Troller-Renfree et al., 2017; White et al., 2017), with ABT links to anxiety variable across this period (Nozadi et al., 2016; White et al., 2017; Dodd et al., 2020). ABP may also vary across childhood (Troller-Renfree et al., 2017; White et al., 2017). Limited research focused on how early ABP predicts subsequent anxiety and social behaviour (White et al., 2017; Dodd et al., 2020) suggests that greater ABP is linked to more positive outcomes and may serve as a protective factor against anxiety from very early childhood, but again, a lack of studies measuring both ABP and socioemotional functioning at more than one time-point limits understanding of the exact role ABP serves.

Clearly, longitudinal studies assessing both affect-biased attention and socioemotional functioning at multiple time points are now needed to better understand the role of attention biases in healthy and pathological development. Such studies will be vital to determine how increased vulnerability associated with atypical ABT might arise from a normative threat bias, and how the presence or stability of affect-biased attention across development relates to positive and negative outcomes in different populations. As childhood and adolescence represent the core period of developmental risk for anxiety disorders (Beesdo et al., 2009), longitudinal studies across this period are of particular importance. Examining the neural learning mechanisms through which attention biases and related outcomes arise will also be critical to address these outstanding questions, and to clarify the processes underlying these relations across different points in development. Our results suggest that the macaque model is ideal for such longitudinal research, and could add considerably to our understanding on whether universal mechanisms of development underlie affect-biased attention, and whether these evolved in primates to help offspring adapt to differences in their early social environment.

In terms of neural bases, the emergence of affect-biased attention is thought to involve attentional networks supporting alerting, orienting, and executive functions, mediated by emotion processing circuitry. This includes brain structures such as the amygdala, orbital frontal cortex (OFC), regions of prefrontal cortex, and anterior cingulate cortex (Morales et al., 2016; Todd & Anderson, 2013.), but evidence from developmental populations is severely lacking. The amygdala and related circuitry may play a particularly crucial role in the processes underlying a relation between early adversity and affect-biases, with a wealth of evidence from the animal and human literature linking adversity to atypical amygdala function and development (Callaghan & Tottenham, 2016). Although the amygdala is classically described as a central node of the fear network, this region probably supports both ABT and ABP (Morales et al., 2016), with more recent studies demonstrating a large overlap of fear and reward networks (e.g. Fernando et al., 2013; Morrison & Salzman, 2010). Investigating links between development in these networks and a relation between affect-biased attention and socioemotional functioning over time will be key in future studies.

Strengths of the current study include the use of ecological dynamic stimuli and investigation of both ABT and ABP links to socioemotional behaviour. In addition, the use of a macaque model allowed for very well-controlled consideration of early environmental effects. In human research, it is often difficult to distinguish effects of the early social environment from other factors such as physical neglect, but results here provide support for the effects of very early social deprivation specifically on affect-biased attention in the pre-adolescent period. However, there are some limitations to this study that should be noted. First, the modest sample size could represent a limitation, and it is important that the results are confirmed in larger samples. Second, these results do not speak to the issue of trait versus state anxiety, both of which have been linked to affect-biased attention (see Bar-Haim et al., 2007), which will require the measurement of anxiety-like behaviour in different contexts across an extended time scale. Third, atypical ABP may also play a role in adverse behavioural outcomes in human children (e.g. Morales et al., 2016), therefore prospective macaque research should consider this also.

There are three additional points concerning the group differences revealed here that need to be considered. Firstly, although animals in the mother-reared group remained in their natal group for the first eight months of life, they were also removed from their natal group post-

weaning. Therefore, it is possible that our results were affected by ‘earlier vs later’ maternal separation. Future studies would ideally include another group of animals that were not removed from their natal social group at any point. However, our results do demonstrate clear differences between the two rearing groups, and are in keeping with previous findings concerning development of affect biased attention in individuals who have been exposed to early psychosocial adversity versus not (e.g. Troller-Renfree et al., 2015, 2017). Behaviour in our peer-reared group (e.g. increased anxiety-like behaviour) does suggest that their risk for long-term poor outcomes is greater than in the mother-reared group, perhaps highlighting the first months of life as a sensitive period for the impact of social adversity on certain aspects of socioemotional development. Secondly, peer-reared animals often obtain a lower social rank than mother-reared animals (e.g. Dettmer et al., 2016), and hypothetically, exposure to more threat in the environment as a result of low rank may have a direct effect on anxiety, or indirectly via effects on attention biases. In our sample, peer-reared animals did tend to be lower-ranking (see SI), though the inclusion of rank as a covariate in our models did not change relations between early adversity, gaze biases, and socioemotional behaviour. This suggests that early social adversity affects rank and gaze bias/behaviour independently, but is still possible that rank moderates the mediating effects of attention biases. Indeed, some previous work with adult macaques has found that social dominance can influence attention towards social stimuli (e.g. Schülke et al., 2020). This possibility is an interesting avenue for future longitudinal work with larger samples, especially in the case of natural variation in early social experience. And thirdly, while social grooming is often used as a measure of prosocial engagement in macaques, grooming is a complex behaviour with context-dependent effects of hormonal and neural responses in NHPs (e.g. Shutt et al., 2007; Crockford et al., 2013). Elucidating these context-dependent effects such as the relative rank of the actor and receiver, and their relationship to affect-biased attention is an important avenue for future research.

To conclude, this study demonstrates that juvenile, pre-adolescent macaques are biased towards looking at both threatening and affiliative dynamic facial gestures, but that the degree of attention bias is influenced by early social deprivation. Furthermore, links between ABT and anxiety in the context of early adversity, and an absence of ABP links with social engagement, suggests that affect-biased attention could play an important role in rhesus

macaque development. Longitudinal research concerning the mechanisms underlying these relations is now required to determine the factors conferring greatest risk for anxiety, as well as those implicated in positive social outcomes and resilience.

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Author Contributions

HR and PFF designed the study; HR, AM, and MB collected the data; HR analysed the data; HR and PFF wrote the manuscript; HR, AM, MB, SBH, and PFF all reviewed the manuscript.

Conflict of Interest Statement:

The authors have no conflict of interest to declare.

Supplementary Information

Methods

Subjects

Peer-reared animals were raised from birth in a nursery with access to same-aged peers; this peer-rearing protocol was based on that of Shannon et al. (1998). At eight months postpartum, mother-reared and peer-reared subjects were placed into a single social group. Animals were relocated to their current location at two years of age and are housed altogether. Animals live in semi free-ranging conditions, with access to both indoor and outdoor areas. As part of a wider longitudinal study, animals are temporarily relocated once a year to a nearby location in groups of 5-6 animals. All groups contain a balanced mix of mother-reared and peer-reared animals, with groups housed in an indoor enclosure. This enclosure is enriched to allow for meaningful activities and expression of the animals' full behavioural repertoire. Assessments for the current study were conducted a minimum of 10 days after this relocation.

Facial gesture stimuli

To calculate brightness, contrast, spatial frequency, and overall movement, video frames were first converted to greyscale by averaging over the RGB colour dimension. Brightness was computed as the normalized average of all pixel values, and contrast as the standard deviation. Spatial frequency was computed using a two-dimensional Fourier transform, which was then averaged over the azimuth to create a one-dimensional vector of power at each spatial frequency. Motion was computed using the Farneback method (Farneback, 2003) for optical flow, using a classical pyramid image scale with 3 levels, an averaging window size of 15 pixels, 3 iterations per pyramid, a pixel neighborhood size of 5, and a Gaussian with SD equal to 1.2 for derivative smoothing; movement was then converted to pixels per second.

The values averaged over all frames from each video for each facial gesture type were as follows: a) Lip-smacking (LPS), brightness = 0.337, contrast = 0.168, motion = 52.13 pixels per second; b) Neutral, brightness = 0.335, contrast = 0.2, motion = 41.86 pixels per second; and c) Threat, brightness = 0.34, contrast = 0.2, motion = 42.81 pixels per second. Brightness, contrast, spatial frequency, and motion are illustrated in Figures S1-S4.

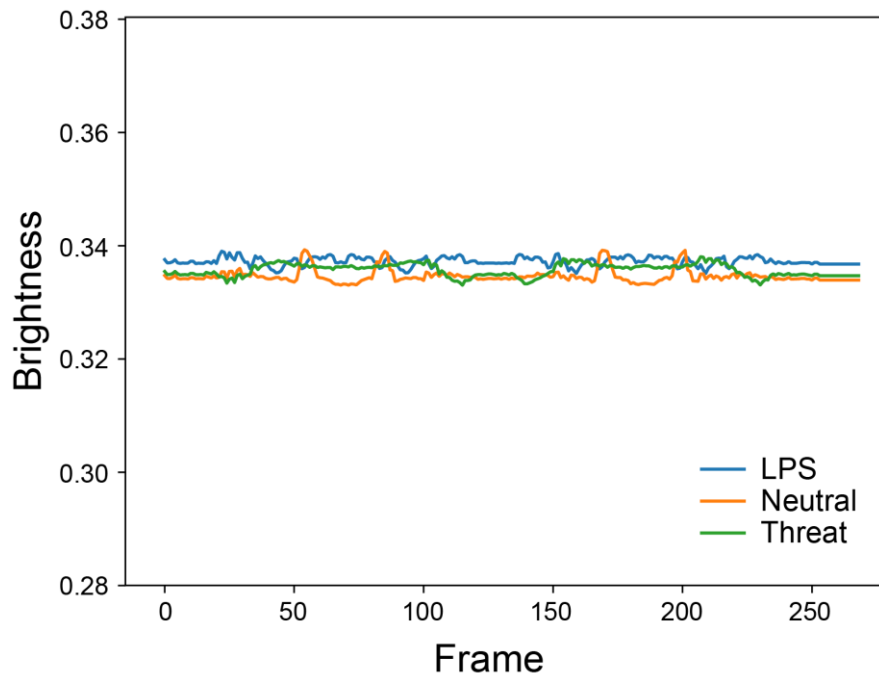


Figure S1: Brightness for LPS (blue), neutral (orange), and threat (green) videos computed as the normalized average of all pixel values.

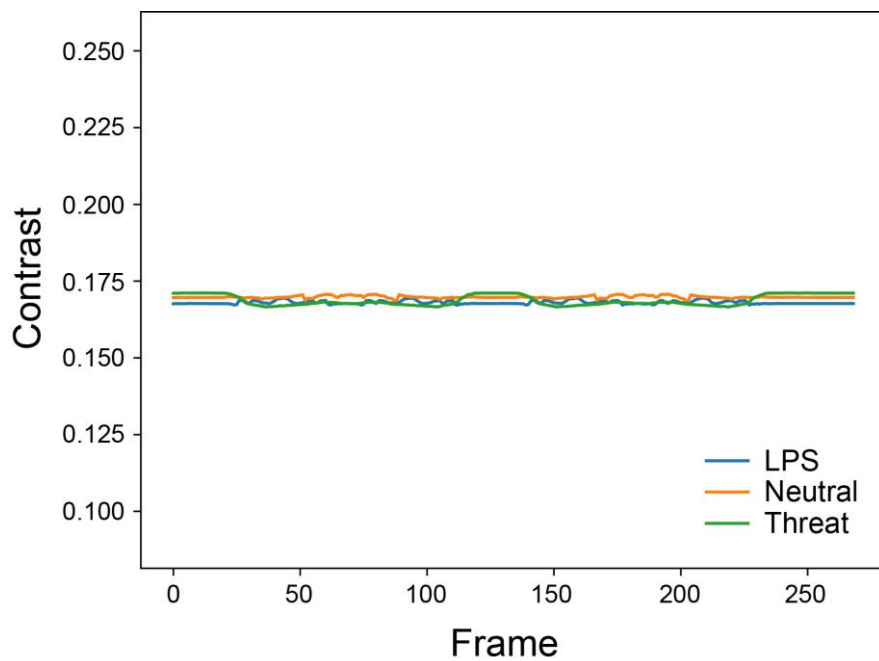


Figure S2: Contrast for LPS (blue), neutral (orange), and threat (green) videos computed as the standard deviation of all pixel values.

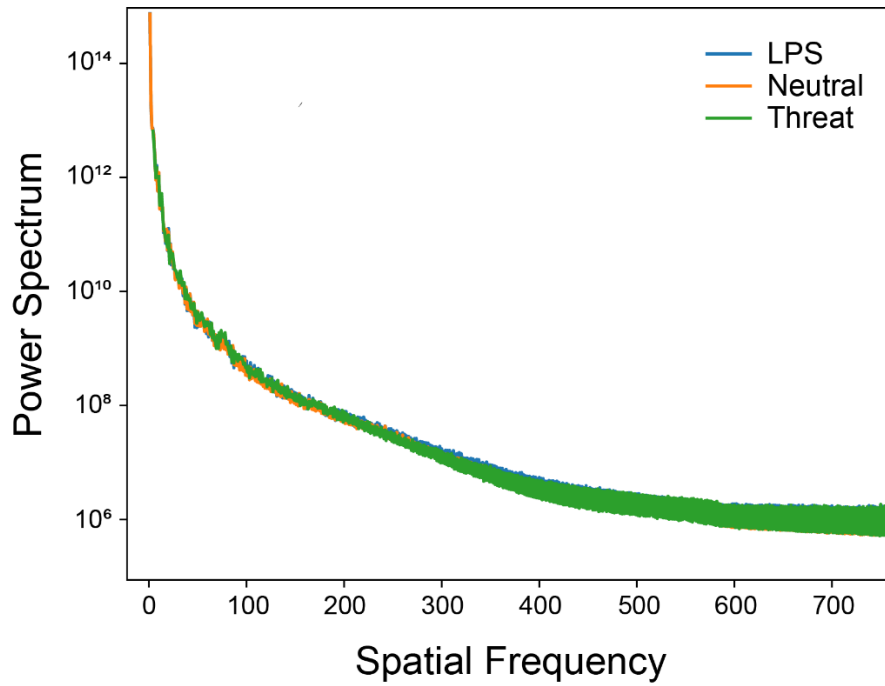


Figure S3: Spatial power spectrum for LPS (blue), neutral (orange), and threat (green) videos computed using a two-dimensional Fourier transform, averaged over the azimuth to create a one-dimensional vector of power at each spatial frequency.

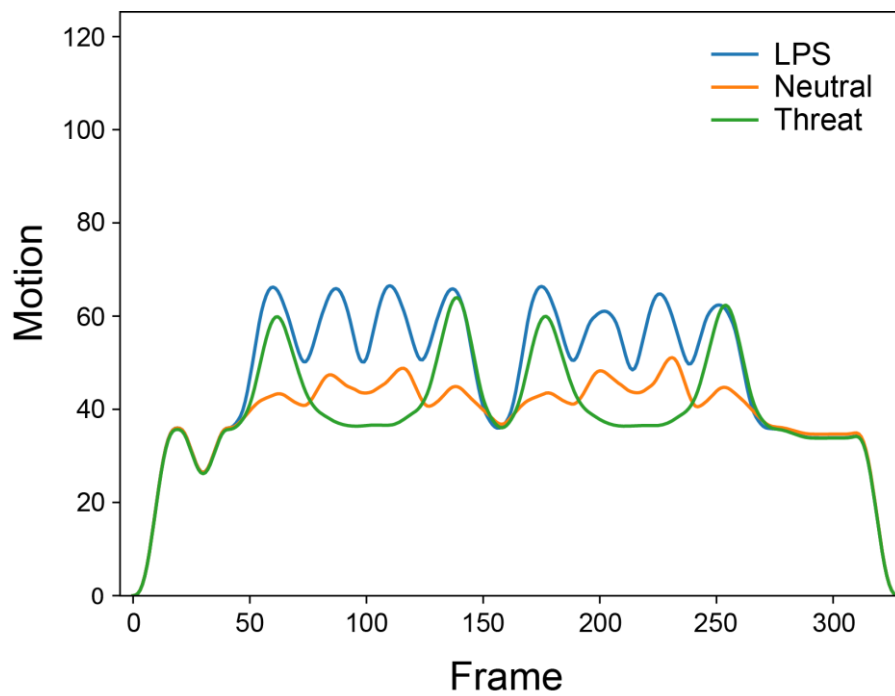


Figure S4: Motion (pixels per second) for LPS (blue), neutral (orange), and threat (green) videos computed using the Farneback method for optical flow.

Data analysis

R v3.6.3 (R Core Team, 2020) and the lme4 v1.1.21 (Bates et al., 2015), nlme v3.1.144 (Pinheiro et al., 2020), lsmeans v2.30.0 (Lenth, 2016), the glmmADMB v0.8.3.3 (Fournier et al., 2012; Skaug et al., 2016), and car v3.0.6 (Fox & Weisberg, 2019) packages were utilized to conduct these analyses.

Results

a) Gaze bias task: Trial exclusion in the different conditions

A generalized linear model with a Poisson distribution and logit link function was run to confirm that the number of gaze bias trials did not differ between groups or conditions after trial rejection. This revealed no effect of group, condition, or their interaction on the number of trials. Mean trials (out of the 5 ABT trials and 5 ABP trials per subject) included for mother-reared animals were as follows: ABT, $M = 4.818$, $SD = 0.405$, range 4-5; ABP, $M = 4.909$, $SD = 0.302$, range 4-5: Mean trials included for peer-reared animals were as follows: ABT, $M = 4.7$, $SD = 0.483$, range 4-5; ABP, $M = 4.6$, $SD = 0.699$, range 3-5.

b) Social rank calculation

Rank of individual animals was established via Elo-ratings (Sanchez-Tojar et al., 2018; R packages 'EloRating' v 0.46.11; Neumann & Kulik, 2020; 'aniDom' v0.1.5; Farine & Sanchez-Tojar, 2021), with winners and losers of agonistic interactions, classified as aggressive or submissive, identified from 60 minutes of behavioural observation per animal (four observations per week (two mornings, two afternoons) for three weeks). Coded behaviours included displacement, threat gestures, aggression, fear grimace, and present genitals (Dettmer et al., 2016; Wooddall et al., 2017). There were a total of 198 interactions, $M=9.41$ and $SD=1.81$ per group. As the hierarchies were highly stable over the course of the observation period (S index: 0.95 – 0.99; $M=0.97$; $SD=0.02$), we used randomized Elo-ratings (over 100 temporally shuffled randomizations). Animals in the peer-reared ($M=-201.25$, $SD=180.21$) group attained a lower rank on average than the mother-reared ($M=182.96$, $SD=141.87$) group [$t(17.11)=5.39$, $p < 0.001$].

c) Influence of sex

To control for a potential influence of sex on our results, we also ran all analyses reported in the main text with sex included as a covariate. All animals were included in these analyses; 11 mother-reared (six female) and 10 peer-reared (five female). The pattern of results revealed were identical to those reported in the main manuscript, suggesting that differences between groups remain even when accounting for sex.

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2) Preliminary results: Longitudinal relationships between deprivation, structural brain connectivity, and anxiety

Psychosocial deprivation in the form of institutional rearing is associated with poor outcomes spanning multiple developmental domains, including increased risk for various psychological and social difficulties. These include psychiatric disorders associated with emotion dysregulation, such as anxiety, depression, and externalizing problems (e.g. Bos et al., 2011; Humphreys et al., 2015). Previous research suggests that alterations in brain development linked to early social experience play a crucial role the increased emotional dysregulation often seen in institutionalized children (e.g. Tottenham et al., 2010; Troller-Renfree et al. 2015). However, many questions remain about the specific neurocognitive mechanisms that contribute to this dysregulation and subsequent risk for poor outcomes, especially across key developmental transition periods when these disorders are most likely to emerge, such as adolescence (Kessler et al., 2005).

Accordingly, we made longitudinal observations of our peer-reared and mother-reared animals to track anxiety-like behavior from pre-adolescence into early adulthood (i.e. 2.5, 3.5, and 5 years). At the same time-points, we acquired anatomical and diffusion MRI, enabling us to use DTI to investigate how development in structural neurocircuitry linked to emotion regulation and anxiety may be impacted by early psychosocial deprivation in macaques, and how this relates to actual anxiety-like behaviour. Note, early psychosocial has previously been linked to problems with emotion processing, regulation, and anxiety in macaques (e.g. Dettmer et al. 2012; Rayson et al., 2021). More specifically, we hypothesized that peer-reared animals would demonstrate greater anxiety across time, and that this would be linked to increased emotional reactivity. We also predicted that white matter microstructure in fronto-limbic and fronto-striatal tracts would modulate these relationships. Frontolimbic and frontostriatal structural connectivity has been associated with early institutionalization in a few studies (e.g. Bick et al., 2015), and limbic and striatal connectivity with prefrontal cortical regions is associated with anxiety disorders (Kenwood et al., 2022).

The particular white matter ROIs/tracts of interest for our analyses included the uncinate fasciculus (UF), the superior cingulum (SC), the hippocampal cingulum (HC), the fornix (FX), the anterior limb of the internal capsule (ALIC), the anterior corona radiata (ACR), and the external capsule (EC). These fronto-limbic and fronto-striatal tracts have all been linked to early psychosocial deprivation and to anxiety in humans, though little such research in the context of psychosocial deprivation has been conducted, particularly in terms of longitudinal development.

Note for all analyses presented here, R v3.6.3 (R Core Team, 2020) was utilized. *P*-values for fixed effects were obtained using Type III Wald χ^2 tests. Significant interactions between factors were followed up by planned pairwise comparisons of estimated marginal means which were Tukey-corrected for multiple comparisons. Significant interactions between factors and continuous variables were followed up by planned comparison of the estimated marginal means of the linear trends of the continuous variable to 0 at each level of the factor. Residuals for each model were inspected for outliers using Cook's distance, but none required removal.

Presented below is a summary of our preliminary findings thus far.

a. ***Peer-rearing is associated with increased risk for anxiety across time compared to mother-rearing.***

Methods: At each time-point (2.5, 3.5, and 5 years), video recordings of the social group (50fps) were made two times per week, once in the morning and once in the afternoon, for three weeks. Two cameras were used to capture the whole home enclosure and were synchronized offline for the manual coding of behaviour. Each animal was coded second-by-second for ten minutes per recording session, totalling 60 minutes per animal, per time-point. One experimenter coded all the videos, with a second experimenter coding a random 10% at each time-point to establish reliability (all $\kappa > 0.85$). The following behaviours were coded using the focal sampling method for the anxiety measure: a) self-scratching, self-grooming, yawns, and body shakes (i.e. behaviours reflecting anxiety in macaques; see Coleman & Pierre (2014)). For analysis, a negative binomial generalized mixed model was run with group (mother-reared/peer-reared) and time-point (2.5/3.5/5 years) as fixed effects, and subject-specific intercepts as a random effect. Frequency of anxiety-like behavior was used as the outcome measure, and sex was also controlled for in the model.

Results: Results revealed a main effect of group [$\chi^2(1) = 4.46, p = 0.035$], with greater anxiety in peer-reared animals across time. A main effect of time-point was also found [$\chi^2(1) = 17.7, p = 0.0001$], with anxiety increasing in all animals over time. These findings are illustrated in Fig. 1.

Conclusion: In accordance with our hypothesis, early psychosocial deprivation is linked to increased risk for anxiety across adolescence into early adulthood in rhesus macaques.

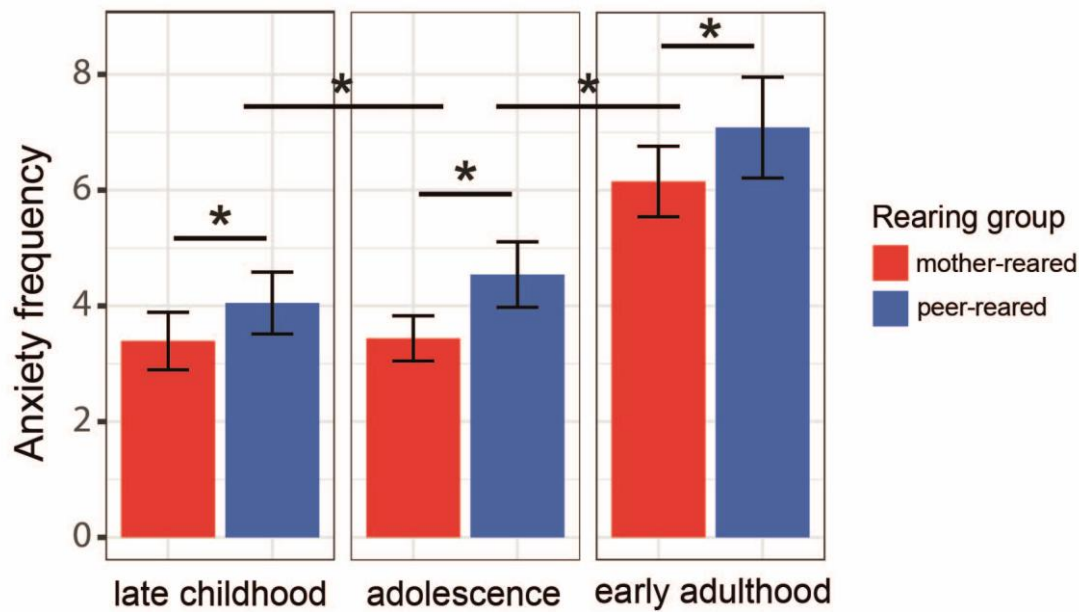


Figure 1: Frequency of anxiety-like behaviour at each assessment time-point. Across time, greater anxiety was found in the peer-reared (blue) versus mother-reared (red) group, with anxiety increasing in both groups with age. Significant effects are denoted by *, and error bars represent +/- SE.

b. Peer-rearing is associated with greater emotional reactivity to threat compared to mother-rearing, with higher emotional reactivity also related more anxiety.

Methods: At all time-points, a version of the HI paradigm (Kalin & Shelton, 1989) was used to assess subjects' reactivity to potential social threat; an unfamiliar human intruder. Each subject was temporarily separated from their social group and placed into the testing area in another section of the room; an 87 × 100 × 120cm enclosure with a clear panelled front. Animals were given five minutes to habituate to the enclosure once separated. Note, all animals had already been well familiarized with this process of separation into the testing enclosure before the day of assessment. Animals were recorded during the task using two cameras (50fps), which were then synchronized offline. The task comprised four conditions: i) 'alone 1', no intruder present; ii) 'profile', intruder stood with their head turned away from the subject to avoid eye contact (low threat); iii) 'alone 2', no intruder present; and iv) 'stare', intruder stood with their head facing forward and making eye contact with the subject (high threat). In the profile and stare conditions, the intruder stood 1m from the front of the testing enclosure. Each condition lasted 2 minutes. As some coded behaviours had a duration and some did not, a combined z-score measure of reactivity was

calculated based on all responses directed towards the intruder (i.e. threatening, fearful, and affiliative gestures) in the high threat condition (stare), which was used as the outcome variable for analysis. A linear mixed model was run with group (mother-reared/peer-reared) and time-point (2.5/3.5/5 years) as fixed effects, and subject-specific intercepts as a random effect. Emotional reactivity was the outcome variable, and sex was controlled for in the model. A negative binomial generalized mixed model was then run to examine the relationship between reactivity and anxiety-like behaviour in the everyday social environment (see section a) above). Reactivity and time-point (2.5/3.5/5 years) were included as fixed effects, and subject-specific intercepts as a random effect. Frequency of anxiety-like behavior was used as the outcome measure, and sex was controlled for.

Results: Results for the analysis predicting reactivity revealed a main effect of group [$\chi_2(1) = 4.52, p = 0.033$], with greater reactivity in the nursery-reared group compared to the mother-reared across time. A main effect of time was also revealed [$\chi_2(1) = 10.39, p = 0.006$], with reactivity decreasing across time overall. Note, no differences between groups were found in other types of defensive responses or behaviours during this task (e.g. freezing). These findings are illustrated in Fig. 2. The analysis predicting anxiety revealed a main effect of reactivity [$\chi_2(1) = 6.77, p = 0.009$], with greater reactivity associated with more anxiety. A main effect of time was also found, with anxiety increasing with age [$\chi_2(1) = 30.56, p < 0.0001$].

Conclusion: Although reactivity decreases between pre-adolescence and early adulthood overall, reactivity remains greater in the peer group compared to the mother group over time. At five years of age, higher reactivity also predicted more frequent anxiety.

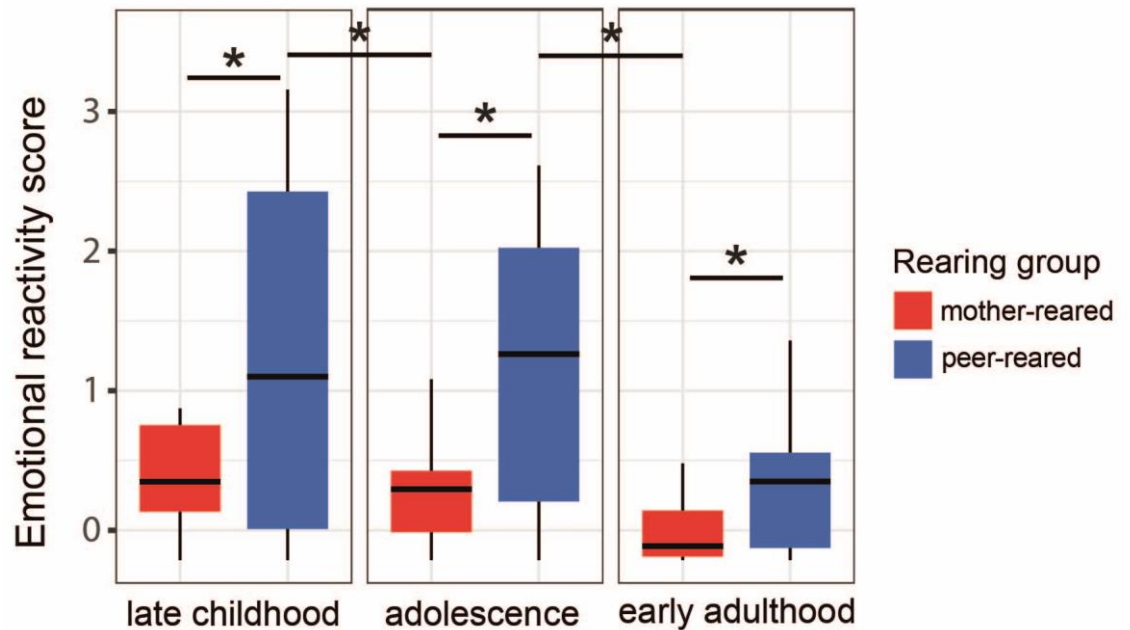


Figure 2: Emotional reactivity at each assessment time-point. Across time, greater reactivity in the stare (high threat) condition was found in the peer-reared (blue) versus mother-reared (red) group, though reactivity decreased with age overall. Significant effects are denoted by *, and error bars represent +/- SE.

c. Rearing condition was associated with differences in FA in the cingulum tracts and the fornix. White matter integrity tended to increase over time.

Methods: For each ROI, conventional DTI methods were used to calculate the mean values of fractional anisotropy (FA), which represents the degree of directionality of random water diffusion. Higher FA is often interpreted to reflect greater white matter integrity. Linear mixed models were run for each DTI ROI and hemisphere, with group (mother-reared/peer-reared) and time-point (2.5/3.5/5 years) as fixed effects, and subject-specific intercepts as a random effect. FA was the outcome variable, and sex was controlled for in all models.

Results: For the right SC, a group by year interaction was revealed [$\chi^2(2) = 8.95, p = 0.01$]; at the second time-point, FA in the peer-reared group was higher ($p = 0.03$), and FA was greater at time-points two and three compared to one in the peer group (both $p < 0.05$). Comparable results were found for the left SC. For HC, a group by year interaction was revealed [$\chi^2(2) = 9.62, p = 0.008$]; FA was greater at time-points two and three compared to one in the mother-reared group (both $p < 0.0002$). For the left HC, only a main effect of age was revealed [$\chi^2(2) = 9.51, p = 0.009$]. For the fornix, a group by year interaction was revealed [$\chi^2(2) = 9.67, p = 0.008$]; FA was greater in the mother-reared group at the first time-point ($p = 0.004$). A main effect of year was

found for left ALIC [$\chi^2(2) = 9.7, p = 0.008$], with FA increasing over time. For right ACR, there was a main effect of year [$\chi^2(2) = 14.56, p < 0.001$], with FA increasing over time. Finally, for right EC, a main effect of year was also found [$\chi^2(2) = 6.4, p = 0.04$], with FA increasing over time. No effects were found for the UF.

Conclusion: While white matter integrity tended to increase with age, changes in the cingulum and fornix were influenced by rearing condition. The superior cingulum integrates many cortical structures such as the anterior cingulate, ventromedial prefrontal, orbitofrontal and parietal cortices, as well as cortico-subcortical regions (e.g. amygdala/thalamus-PFC), and is associated with emotion, reward, and motor processes (Bubb et al., 2018). The fornix connects the hippocampus to subcortical areas including hypothalamus and to cortical areas such as prefrontal and cingulate regions, and is related to memory and spatial association (Catani et al., 2013). Both tracts are important parts of the limbic system and play an important role in emotion regulation.

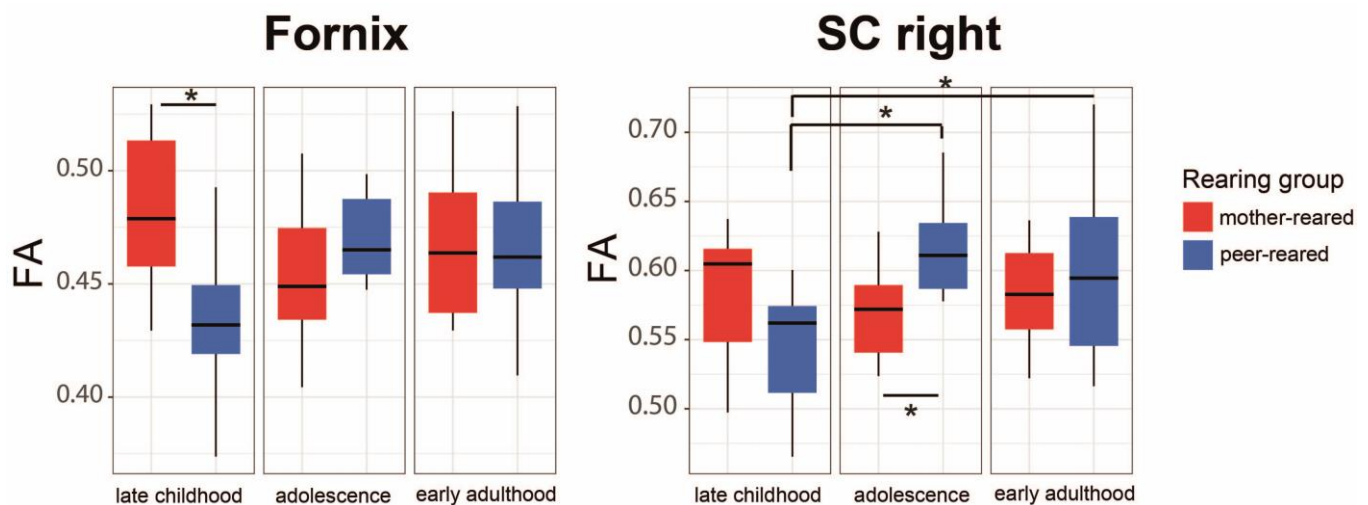


Figure 3: FA at each assessment time-point. A) FA in the right superior cingulum in the two rearing-groups. B) FA in the fornix in the two rearing-groups. A main effect of group was found in both these tracts. Significant effects are denoted by *, and error bars represent +/- SE.

- d. **Anxiety is related to structural connectivity differentially in fronto-limbic and fronto-striatal brain circuitry, with differences found for peer-reared versus mother-reared animals.**

Methods: At each time-point (2.5, 3.5, and 5 years) high resolution anatomical (T1/T2) and diffusion MRI scans were acquired. For analysis, separate negative binomial generalized mixed models were run for each ROI (UF, SC, HC, FX, ALIC, ACR, and EC) and hemisphere (right and left) with group (mother-reared/peer-reared), time-point (2.5/3.5/5 years), and FA included as fixed effects, and subject-specific intercepts as a random effect. Frequency of anxiety-like behavior was used as the outcome measure for analysis, and sex was also controlled for in all models. If significant effects of FA were found, this was followed-up with analyses of AD and RD. As a number of analyses were run predicting the same outcome (13 in total), our *p*-value for significance was set at 0.004 after Bonferroni correction.

Results: Results for the UF and ACR tracts are presented here. For the right UC, a significant main effect of FA was revealed [$\chi^2(1) = 30.83, p < 0.0001$], with greater FA predicting less anxiety across time. For the left UC, a significant interaction was found between group and FA was found [$\chi^2(1) = 16.60, p < 0.0001$], with a negative FA anxiety relationship stronger in the peer-reared compared to mother-reared group ($p < 0.0001$), and a significant interaction between year and FA was revealed [$\chi^2(2) = 21.77, p < 0.0001$], with a negative FA-anxiety relations disappearing by the third time-point (both $p < 0.006$). For the right ACR, a significant group by year by DTI interaction was revealed [$\chi^2(2) = 204.63, p < 0.0001$], with a negative FA-anxiety relationship at time points one and three but a positive FA-anxiety relationship at time-point two for the mother-reared group (all $p < 0.0001$), and a negative relationship only at the first two time-points for the peer-reared group (both $p = 0.003$). The two rearing groups were different from one another at the last two time-points (both $p < 0.001$). For the left ACR, a significant year by FA interaction was found [$\chi^2(2) = 280.8, p < 0.0001$] with higher FA linked to less anxiety at time-points one and two ($p < 0.0001$).

Conclusion: Early psychosocial deprivation has long-term effects on emotion regulation and anxiety in macaques. Development of structural connectivity in fronto- limbic and fronto-striatal brain circuitry appears to modulate this, with group differences found in the relationship between white matter integrity and anxiety in the uncinate fasciculus (UF; a tract that connects that amygdala and prefrontal cortex) and the anterior corona radiata (ACR; a tract containing thalamic and striatal projections from the internal capsule to the prefrontal cortex). Results for the left UF could suggest that while a relationship between higher FA remains for the peer-reared group across time, in general, this relationship weakens with age. This weakening relationship across adolescence is in keeping with findings in the human literature

(Hein et al., 2018), with the continuance of this relationship in the peer-reared group perhaps indicating a form of resilience or protection in the context of early psychosocial adversity. Alternatively, this could reflect atypical development in the peer-reared group, with normative changes not taking place or being delayed. The opposite relationship found between groups for the right ACR that emerged by the last time-point (i.e. early adulthood) is particularly interesting. Results in the mother-reared group suggest some reorganization takes place in fronto-striatal circuitry that relates to anxiety in adolescence. This does not happen in the peer-reared group, which could suggest a protracted effect of early psychosocial deprivation on fronto-amygdala connections, maybe also sustained by less involvement of fronto-striatal circuits across time because prioritizing other aspects such as EF (see Chapter 4 and 5 of this thesis).

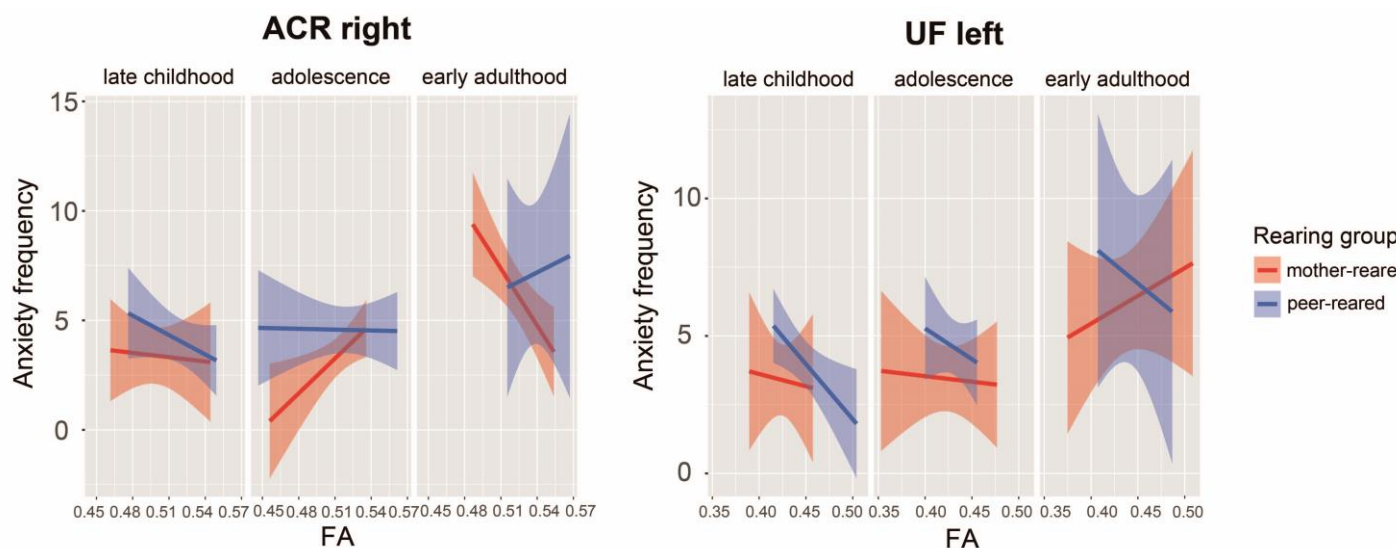


Figure 4. Anterior corona radiata (ACR) and uncinate fasciculus (UF) integrity interactions with anxiety frequency. Greater integrity in left UF predicted less anxiety across group, with a stronger relationship in peer-reared animals. In addition, this negative FA-anxiety relation decreased over time and disappeared by early adulthood. Greater integrity in the right ACR in mother-reared group predicted less anxiety in childhood and early adulthood, but more anxiety in adolescence; while in peer-reared group greater integrity in the right ACR predicted less anxiety in childhood and adolescence. Error bars represent +/- SE.

Next steps in the analysis of this data include the following: i) finish analyses concerning how DTI ROIs and rearing group predict anxiety; ii) consideration of how emotional reactivity rather than group interacts with white matter integrity to predict anxiety; iii) consider other structural measures of brain development, such as regional volumes and cortical thickness; iv) use of analyses to better examine trajectories of change (e.g. prospective analyses, hierarchical growth curve models).

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APPENDIX C. Poster presentations

1. Poster and short talk presented at NeuroFrance (2021) and at the Budapest CEU Virtual Conference on Cognitive Development (2021).

a. The work on which this poster is based has now been published. The manuscript is presented in Chapter 3 of this thesis.

Early social adversity influences executive function in juvenile macaques

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1. Introduction

Executive function (EF) is an umbrella term for a group of domain general, interrelated cognitive skills that underlie the organization and control of goal-directed behaviour.

EF deficits associated with neurodevelopmental disorders (e.g. ADHD, ASD, internalizing and externalizing disorders) [1,2]

Early social deprivation has been linked to EF deficits [3,4]

But, what are the effects of early social deprivation on EF across different periods of development?

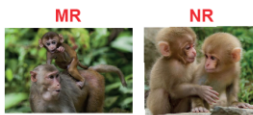
2. Project objectives and subjects

Objectives

Investigate the effect of early social deprivation on the development of EF in adolescent rhesus macaques

Subjects

- 2 groups of rhesus macaques
- mother-reared MR (11)
- nursery-reared NR (10)



3. Behavioural assessment

A not B task

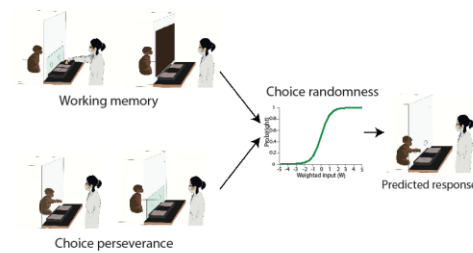
One experimenter stood in front of the monkey and hid a piece of food in one position (A trials), and then changed the hiding position (B trials) after the subject had the opportunity to retrieve the food from the first hiding place a number of times [5,6].



4. Analysis

$$A. \text{ CUMULATIVE MEASURE} = \frac{\text{addition of correct change trials delays}}{\text{number of total trials}}$$

B. COMPUTATIONAL MODEL

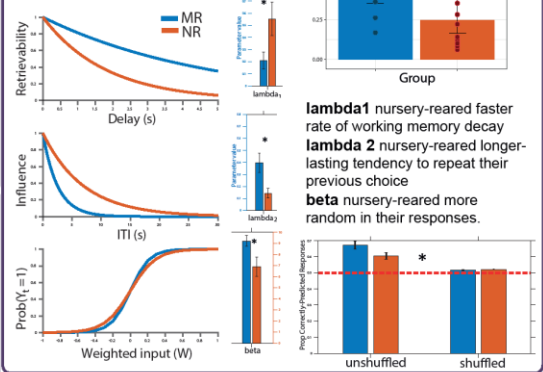


4. Results

Cumulative measure

Greater cumulative measure score in the mother-reared compared to nursery-reared group

Computational model



5. Conclusion

These are the first results to demonstrate that, like in humans, early social adversity is associated with poor executive function in young rhesus macaques, with the computational model providing novel insights into the neural mechanisms through which this may occur.

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2. Poster presented at the International Society for Developmental Psychobiology Virtual Conference (2021).

- a. The work on which this poster is based has now been published. The manuscript is presented in Appendix A of this thesis.

Early social adversity influences the relationships between attention biases and socioemotional behaviour in juvenile macaques

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1. Introduction

Affect-biased attention thought to play a fundamental role in early socioemotional development¹

- But **mechanisms and links to typical vs. adverse³ outcomes** unclear

Important question:

- What factors lead to **individual differences** in affect-biased attention and risk/resilience?

Macaque model may be particularly useful to address these questions
- But do similar attention biases to humans exist in macaques and do they relate to socioemotional function?

2. Subjects and Tasks

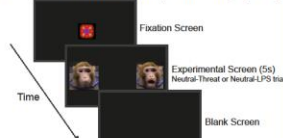
Subjects

21 juvenile rhesus macaques
11 mother-reared (MR)
10 peer-reared (PR)
~2.5 years of age/pre-adolescent

Attention Bias Task

2 conditions (dynamic stimuli):

Neutral-Threat/Neutral-Lipsmacking (LPS)



Behavioural Observation

Videos made of subjects in home enclosure to assess socioemotional function

3. Data Acquisition and Analysis

Procedure and Video Coding

Attention bias task:

Subjects separated into testing area
- 87 × 100 × 120cm with clear-panelled front
- Screen placed outside 60cm from front

Calibration

- Object+sound presented left/centre/right of screen

Stimuli presentation

- 5 Neutral-Threat & 5 Neutral-LPS trials
- Subjects recorded via webcam

Gaze coding

- Gaze during stimuli presentation coded offline (left/centre/right/other/offscreen)



Behavioural observation:

Subjects housed in mixed MR/PR social groups

Total 30 minutes observation per subject over three weeks

Socioemotional behaviour coding^{4,5}

- Anxiety-like behaviour identified (frequency)
- Involvement in grooming interactions identified (frequency and duration)

Statistics

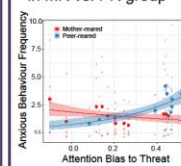
Mixed model framework: linear model (gaze bias), generalized linear model - negative binomial (behaviour)

4. Results

Attention Biases

Significant threat bias in both groups; greater threat bias in PR vs. MR group

Significant LPS bias in MR group; greater LPS bias in MR vs. PR group

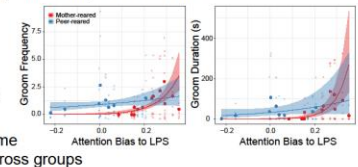


Bias-Socioemotional Behaviour Relationship

In PR group, greater threat bias linked to more frequent anxiety-like behaviour

Greater LPS bias linked to more frequent grooming interactions in MR group

Greater LPS bias linked to more time spent in grooming interactions across groups



5. Summary and Conclusions

Differences in attention biases between groups

- Threat bias greater in PR group
- LPS bias greater in MR group

Greater **threat bias** linked to **more frequent anxiety-like behaviour** in PR group

Greater **LPS bias** linked to more **affiliative behaviour** in both groups

Results similar to those with **human children** in context of **early institutionalization^{6,7}**

Results indicate **utility of macaque model** for future developmental affect-biased attention research

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Email: holly_rayson1@gmail.com @holly_rayson This work was supported by the EU's Horizon 2020 Marie Skłodowska-Curie grant agreement and the National Institute of Health



3. Poster presented at Federation of European Neuroscience Societies (2022).



a. A manuscript based on the work presented in this poster is currently in preparation.



9-13 July 2022 | Paris, France

Early social adversity in non-human primates interferes with the developmental trajectory of amygdalo-cortical functional connectivity

Simon Clavagner*, Holly Rayson*, Mathilda Froessel*, Maëva Gacoin, Alice Massera, Pier Francesco Ferrari, Sullian Ben Hamed

Introduction

Neuroimaging of the non-human social primate brain has been instrumental to forge our current understanding of primate social cognition. However, little is known to date on how this cognitive function develops from childhood into adolescence, and how early social adversity (ESA) interferes with typical brain functional developmental trajectories. Here, we used a longitudinal neuroimaging protocol and collected anesthetized resting-state fMRI data in 21 juvenile monkeys. We focus on the amygdala, a subcortical structure which plays a key role in socioemotional and attentional processing.

Material and Methods

3T Resting state MRI acquisitions under anesthesia + MION injection

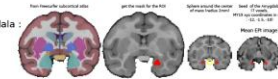
Control group : 11 Mother-reared (MR) macaques
ESA group : 9 Peer-reared (PR) macaques (Rayson et al, 2021)

3 Time points : - Childhood (age 1: 2.63y +/- 0.06)
 - Adolescence (age 2: 3.75y +/- 0.2)
 - Early adulthood (age 3: 4.9y +/- 0.23)

MRI anatomical procedure
 2 T1w + 1 T2w whole brain images
 Processing method using Matlab, AFNI, FSL, Freesurfer, ANTs and Connectome workbench (Adapted from Autio et al, 2020 and Seitz et al, 2017)

Resting state MRI procedure
 4 runs of 526 EPI images (28 slices, 1.5x1.5x1.6mm, TR = 1.7s, TE = 17ms)
 Processing method (using Matlab, AFNI and FSL)
 - Despiking - Slice time correction - Motion correction - Bias field correction
 - Non linear transformation to the individual reference T1w image and skull-stripping
 - Non linear transformation to MY19 atlas
 - Detrend - bandpass filter (0.01-0.1Hz), censoring -
 Regressing out head motion, mean white matter signal and mean ventricles signal
 Blurring at 3 mm - Signal normalisation
 - Hand classification and removal of ICA noise component with MELODIC

FC Analysis
 Left and Right Amygdala:

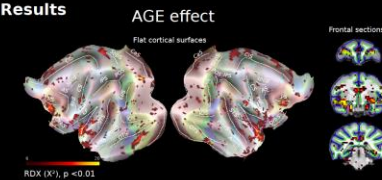


Seed-based correlation analysis per each run + Fisher's-to-Z score transformation
 T Test.
 "Right-to-left" transformation of Right Amygdala results.

Linear Mixed-Effects Modelling Approach analysis
 Random intercept and random slope : Age x Group + (1/Monkey)
 Random effects (RDX) : AGE, GROUP and AGExGROUP

Results

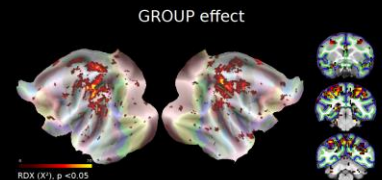
AGE effect



RDX (X²), p < 0.01

macaque N=21 atlas, Markov et al, 2013 parcellation

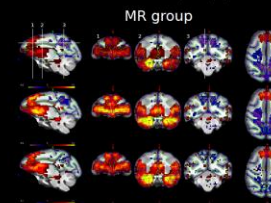
GROUP effect



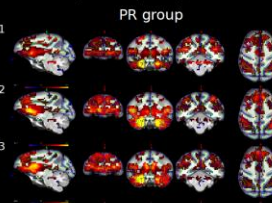
RDX (X²), p < 0.05

mean Zscore per age (inclusive mask from *FX all ages* FDR q<0.001)

MR group



PR group

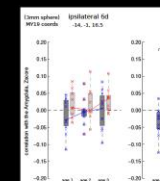


Fixed effect (FDR q<0.001)

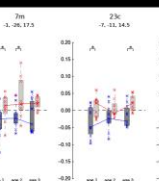
Age 1
Age 2
Age 3

The PR group, as compared to the MR group, retains part of its functional connectivity with the medial posterior parietal cortex (precuneus), the mid-cingulate and specific premotor and prefrontal regions.

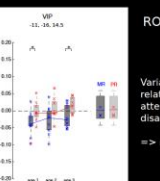
Left spheral seed Z score



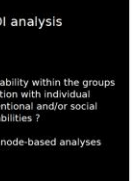
Right spheral seed Z score




3m



23c



VP



ROI analysis

Variability within the groups relation with individual attentional and/or social disabilities ?
 => node-based analyses

refinement of the amygdalo-cortical connectivity through adolescence, from a broad distributed pattern to a more adult-like restrained pattern.

No interaction between the two main effects

Conclusion

We propose that altered amygdalo-cortical functional connectivity in the peer-reared group accounts for the specific differences in affect-biased attention and high emotional reactivity observed in this group.

Rayson et al. "Early social adversity modulates the relation between attention biases and socioemotional behaviour in juvenile macaques." Sci Rep. 2021
 Autio et al. "Towards HCP-Style macaque connectomes: 24-Channel 3T multi-array coil, MRI sequences and preprocessing." Neuroimage, 2020
 Seitz et al. "A population MRI brain template and analysis tools for the macaque." Neuroimage 2018

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4. Poster presented at Federation of European Neuroscience Societies (2022).

a. A manuscript based on the work presented in this poster is currently in preparation.

DEVELOPMENTAL CHANGES OF PULVINO-CORTICAL FUNCTIONAL CONNECTIVITY

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Introduction

The pulvinar (PU) is a complex thalamic nucleus involved in high cognitive functions (Froesel et al. 2021). PU subregions are associated with different developmental trajectories; e.g. the inferior PU (PuI) is adult-like in function and cortical connectivity rapidly stabilizes after birth, whereas medial PU (PuM) maturation is more protracted and subject to individual variability. Adult anatomo-functional PU properties are associated with attention deficit & hyperactivity disorder (ADHD), autism spectrum disorders (ASD) & schizophrenia.

Pu functional connectivity with cortex:
1. Changes from childhood to adulthood?
2. Impact of early social adversity on development?

Materials and method

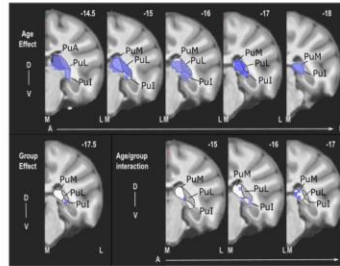
Two groups: Peer-reared (n=9) and mother reared (n=11; control) rhesus macaques.
Methods: 3T resting-state fMRI under controlled gas anesthesia.

Assessment time points: Childhood (2,63y+/-0,06). Adolescence (3,75y +/-0,2). Early adulthood (4,9y +/- 0,23).

Analysis: Linear Mixed-Effects Modelling (LME) on functional connectivity (FC) metrics.

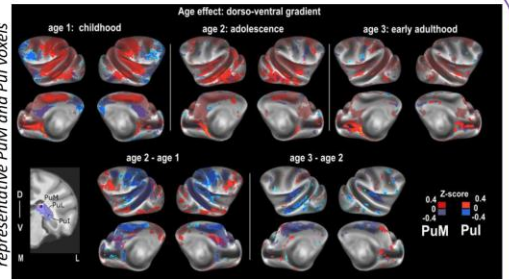
Results

1. LME main effects and interactions per individual PU voxels



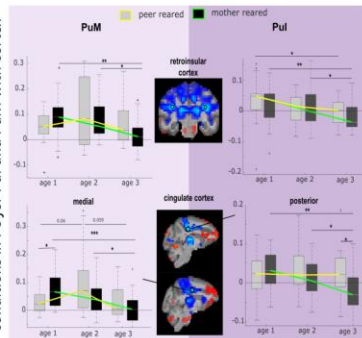
- All PU voxels show significant changes in cortical FC with age
- Specific PU voxels show a significant age x group interaction.

2. LME main effects of age for representative PuM and PuI voxels



- At all ages, FC of PuM is more extended than PuI
- Across time, FC of Pu becomes more spatially refined
- Changes in FC with posterior parietal & premotor prefrontal cortex culminate at adolescence
- Changes in FC with temporal cortex take place later.

3. Interaction between age and early life conditions in FC for PuI and PuM with cortex



- Changes in FC of PuM and PuI with age depends on group (brain maps); mostly characterized by delayed FC changes (blue)
- Differences in PuM FC are particularly marked in retroinsular and medial cingulate cortex
- Differences in PuI FC are particularly marked in retroinsular and posterior cingulate cortex
- Three developmental trajectories identified: stable, linear and inverted U-shaped.

Discussion & Conclusion

PuM is associated with multiple high level cognitive functions including action, decision-making and emotion regulation, while PuI is mostly involved in sensory perception. Here, developmental trajectories of PU FC with cortex varied between PuM and PuI. We found distinct developmental trajectories of PU with parietal, premotor, prefrontal and temporal cortex, initiated either before or after adolescence. Early adversity perturbed these trajectories, targeting specific functional networks. While developmental trajectories were mostly linear in mother-reared individuals, peer-reared individuals showed both stable (no change) or inverted U-shaped trajectories, indicating puberty related-brain changes in this group.