Characterization of gestational brain remodeling in human mothers

by

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A dissertation submitted by in partial fulfillment of the requirements for the degree of Doctor of Philosophy in

Biomedical Science and Technology
Universidad Carlos III de Madrid

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October 2023

A mi madre Maida. Por darme la vida y las ganas de investigar.

To my mother Maida. To give me life and the will to research.

ACKNOWLEDGEMENTS

La finalización de esta tesis coincide con mi 29 cumpleaños. Alguien muy especial me dijo el otro día: "qué bonito que juntes el día de tu nacimiento con otro nacimiento". Empiezo una nueva etapa. Acabar esta tesis no ha sido duro para mí, pero si siento que llevo un tiempo preparándome para oficialmente ser investigadora posdoctoral. Para mucha gente la tesis es un camino lleno de amargura. Para mi han sido años muy felices. Y Susana es la responsable principal de haberme sentido tan cómoda en este recorrido. Ella ha hecho lo que toda buena líder debe de hacer, pero muy pocos acaban haciendo: me ha guiado, enseñado y acompañado, sin invadirme. Y me ha enseñado uno de los mayores placeres de mi vida: escribir. Susana, gracias a ti he aprendido a disfrutar de la escritura científica, y a verla como lo que realmente es: una *hard skill*. También quiero agradecerte que, en su día, hace ya seis años, depositaras tu confianza en mí y me incorporaras a este grupo de investigación. Neuromaternal es y seguirá siendo la investigación más importante de mi vida. Gracias a este grupo, he cumplido un sueño: trabajar rodeada de mujeres, y para mujeres. Como feminista que soy, estaré eternamente agradecida. Por último, gracias por introducirme al deporte más maravilloso del mundo y por compartir esta pasión conmigo. En cada clase, vuelvo a ser niña. Eres un ejemplo de vitalidad, curiosidad, motivación, y talento. Y tu maravillosa hija ha heredado tu mejor cualidad: tu sentido del humor.

De mi felicidad durante estos años también son responsables todas mis amigas y amigos del laboratorio. María Paternina, la compañera de laboratorio que todo el mundo querría tener y la persona que mejor me complementa investigando. Mano a mano en todas las investigaciones y artículos. María, gracias por enseñarme tanto de neuroimagen, por supervisar mis pasitos en R, y por ayudarme en todo lo que te he pedido, sin excepción. Tengo muchísimas ganas de conocer a tu hija, y de tocarle los piececitos. Marta Casquero, de las primeras personas que me acogieron en el laboratorio. No conozco a nadie tan fiel a sus amistades como ella. Marta, gracias por acompañarme en tantos momentos importantes durante estos años, y por darme tan buenos consejos. Nunca olvidaré las noches de Spritz en el italiano, hablando y riendo hasta las mil. Diego Romero, en mi top 3 de personas con mejor sentido del humor, y un magnífico escritor. Diego, estoy muy celosa de tu nueva casa, y espero que en esta nueva etapa de tu vida encuentres una profesión que te haga muy feliz. Y Nicolas Lamanna, de las personas más alegres que conozco. Un ejemplo de optimismo en la vida. Nico, gracias por todos los planes

tan divertidos que hemos compartido. Contigo he probado los mejores asados argentinos que existen.

Esta tesis también se la agradezco a mi mejor amiga Elisa Garcia. La única persona con la que soy capaz de estar noches enteras hablando sin dormir. Tú y yo sabemos que nuestros videos del piso de Barcelona bien podrían ser obras maestras. Gracias por tantas conversaciones sobre nuestra experiencia como mujeres. Por tantas risas, fiestas, amistades compartidas, viajes y festivales. Por nuestra sensibilidad y vulnerabilidad compartida. Soy muy feliz con nuestra relación.

También quiero agradecer estos últimos dos años a mi pareja, Adolfo Llopis. La persona con quien más he hablado de mi investigación fuera del laboratorio. Adolfo, gracias por hacerme de editor tantas veces. He aprendido muchísimo de escritura y comunicación contigo. Gracias por ser tan comprensivo y escuchar atentamente todo lo que te he contado. Como Gina le dice a Paul en *In treatment*: *You have a great sense of empathy. And a great ear.*

Por último, quiero agradecer todo el apoyo y ayuda de todo el resto de mi equipo de laboratorio: Daniel Martín (aka El Estrellitas), Alberto Fernández, Luis Marcos, Javier Navas, Inés Noguero, Laura Carretero, Yasser Alemán y David García. Gracias por ayudarme con el procesamiento y análisis estadístico de imagen, y con muchas figuras de los estudios de esta tesis. Y por revisarme muchos de mis trabajos. También agradezco a Manuel Desco haberme dado la oportunidad de trabajar todos estos años en el laboratorio, y su confianza en mí como investigadora independiente.

Aunque voy a seguir trabajando en el mismo grupo próximamente, siento que este trabajo es de algún modo una despedida. Me despido de mi etapa de supervisión, para empezar una etapa de autonomía y coordinación. De algún modo, siento que llevo desde 2020 con este proceso de duelo. Pero he necesitado un tiempo para acabar de asimilar el cambio. Entro en esta nueva etapa con muchas ganas de empezar a explorar otros horizontes y coordinar mis propios proyectos.

PUBLISHED AND SUBMITTED CONTENT

- Carmona, S., **Martínez-García, M.,** Paternina-Die, M., Barba-Müller, E., Wierenga, L. M., Alemán-Gómez, Y., ... & Hoekzema, E. (2019). **Pregnancy and adolescence entail similar neuroanatomical adaptations: a comparative analysis of cerebral morphometric changes.** Human brain mapping, 40(7), 2143-2152. https://doi.org/10.1002/hbm.24513. First co-authorship. This item is wholly included in Chapter 1 of this Thesis.

  (The material from this source included in this thesis is not singled out with typographic means and references.)

- **Martínez-García M**, Paternina-Die M, Cardenas S.I …, Saxbe D.E (2022). **First-time fathers show longitudinal gray matter cortical volume reductions: evidence from two international samples.** Cerebral Cortex. First authorship. This item is wholly included in Chapter 4 of this Thesis.

  (The material from this source included in this thesis is not singled out with typographic means and references.)

- **Martínez-García, M.,** Paternina-Die, M., Barba-Müller, E., Martín de Blas, D., Beumala, L., Cortizo, R., ... & Carmona, S. (2021). **Do pregnancy-induced brain changes reverse? The brain of a mother six years after parturition.** Brain sciences, 11(2), 168. https://doi.org/10.3390/brainsci11020168. First co-authorship. This item is wholly included in Chapter 2 of this Thesis.

  (The material from this source included in this thesis is not singled out with typographic means and references.)

OTHER RESEARCH MERITS

ARTICLES

- **Martínez-García, M**., Cardenas, S. I., Pawluski, J., Carmona, S., & Saxbe, D. E. (2022**). Recent Neuroscience Advances in Human Parenting**. Patterns of Parental Behavior, 239-267. 10.1007/978-3-030-97762-7_8
- Cardenas, S. I., Morris, A. R., Marshall, N., Aviv, E. C., **Martínez García, M.,** Sellery, P., & Saxbe, D. E. (2021). **Fathers matter from the start: The role of expectant fathers in child development.** Child Development Perspectives, 00, 1–6. https://doi.org/10.1111/cdep.12436.
- **Martínez-García, M.,** Paternina-Die, M., Desco, M., Vilarroya, O., & Carmona, S. (2021). **Characterizing the Brain Structural Adaptations Across the Motherhood Transition.** Frontiers in Global Women's Health, 76. https://doi.org/10.3389/fgwh.2021.742775. First authorship. This item is wholly included in Chapter 5 of this Thesis.
- Hoekzema, E., Tamnes, C. K., Berns, P., Barba-Müller, E., Pozzobon, C., Picado, M., Lucco, F., **Martínez-García, M.,** (…) & Carmona, S. (2020). **Becoming a mother entails anatomical changes in the ventral striatum of the human brain that facilitate its responsiveness to offspring cues.** Psychoneuroendocrinology, 112, 104507. https://doi.org/10.1016/j.psyneuen.2019.104507. Co-authorship. This item is wholly included in Chapter 3 of this Thesis.
- Paternina-Die M, **Martínez-García M,** Pretus C, et al. **The paternal transition entails neuroanatomic adaptations that are associated with the father's brain response to his infant cues.** Cerebral Cortex Communications. 2020, tgaa082. https://doi.org/10.1093/texcom/tgaa082.
- Comitre-Mariano, B., **Martínez-García, M.,** et al (2021**). Feto-maternal microchimerism: memories from pregnancy.** iScience, 103664. https://doi.org/10.1016/j.isci.2021.103664.

INTERNATIONAL CONGRESSES

- May, 2022. Oral session in **Parental Brain 2022 7th International Meeting on the Neuroscience of Parenting.** Saint Malo, France. Title: First-time fathers show longitudinal grey matter cortical volume reductions: Evidence from two international samples. **Young Investigator award.**

- November, 2021. Coordinator, co-chair and speaker in **ISDP 2021 Hybrid: 54th Annual Meeting of the International Society for Developmental Psychobiology**. Chicago. The emerging parental brain: longitudinal research on parents' prenatal neurobiology and its implications for postpartum caregiving behavior.

- April, 2021. Poster in **Social & Affective Neuroscience Society Virtual Conference.**

Prospective, longitudinal subcortical grey matter volume change in first-time fathers across two samples.

- Nov, 2019. Poster in **2nd Brainstorming research assembly for young neuroscientists.** Milan, Italy. Neural Plasticity in First-Time Mothers: a neuroimaging perspective.

- July, 2019 Posters in **Organization for Human Brain Mapping.** Rome, Italy.
  - A prospective MRI study of the human father's brain changes within the default mode network.
  - Long-term brain changes associated to human pregnancy.
  - Pregnancy and adolescence entail similar neuroanatomical adaptations: A comparative analysis of cerebral morphometric changes.

- March, 2019. Oral session in **International Convention of Psychological Science.** Paris, France. Neural Plasticity in First-Time Fathers. Symposium of Interdisciplinary Perspectives on Fatherhood: Integrating Neurological, Hormonal, and Observational Data to Understand Men's Transition to Parenthood.

- March, 2019. Posters in **International Convention of Psychological Science.** Paris, France.

- Pregnancy and adolescence entail similar neuroanatomical adaptations: A comparative analysis of cerebral morphometric changes.
- Long-term brain changes associated to human pregnancy.

NATIONAL CONGRESSES

- October, 2021. Oral session in **Congreso Nacional de Psiquiatria.** Valencia. Spain. *Adaptaciones cerebrales en la transición a la maternidad.*

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OUTLINE OF THE DOCUMENT

In this PhD thesis, three research articles examine the brain morphometrics, intrinsic and extrinsic mediating factors, and the durability of the structural brain changes occurring during the transition to parenthood. These studies follow first-time mothers and fathers, scanning their brains with Magnetic Resonance Imaging (MRI) before pregnancy and then again at different moments of the postpartum period. Section 1 describes the motivation behind this thesis, as well as the implications each research article has in the health care system, including perinatal mental wellbeing, family policies, and brain aging. Section 2 introduces the terms neuroplasticity and maternal behavior, explains how pregnancy hormones and infant cues activate and maintain maternal behavior, and revises the current knowledge of the maternal brain adaptations both in rodents and humans. The section finishes by stating which gaps of knowledge of the parental brain field are addressed by the present thesis. Section 3 states the three objectives of the thesis. The following three sections correspond to the research articles, entitled: *"Pregnancy and adolescence entail similar neuroanatomical adaptations: A comparative analysis of cerebral morphometric changes", "First-time fathers show longitudinal gray matter cortical volume reductions: evidence from two international samples",* and *"Do Pregnancy-Induced Brain Changes Reverse? The Brain of a Mother Six Years after Parturition".* Then, Section 7 discusses the broader significance of these findings, the insight they have provided of the neuroplasticity of the parental brain, and future directions of the current work. Finally, Section 8 gathers the general conclusions of the work. Of note, this thesis will refer to *mothers* as persons who identify as women that undergo pregnancy through natural or assisted processes, and to *transition to motherhood* as the process including pregnancy and the postpartum period.

1. MOTIVATION

During the nine months of human pregnancy, a woman's body undergoes extreme changes to gestate, accommodate, deliver, and nourish her baby: her blood volume almost doubles, her mammary glands begin to produce milk and a new organ appears, the placenta, which will secrete enormous amounts of hormones that will coordinate all these adaptations. Another system in a mother's body undergoes major adaptations during pregnancy: her brain. The perinatal period, including pregnancy and the postpartum period, is a unique period of neuroplasticity in the adult female brain. In humans, such neuroplasticity manifests in pronounced volume changes in the mother's brain during pregnancy (Hoekzema et al., 2017; Oatridge et al., 2002) and early postpartum periods (Kim et al., 2010; Lisofsky et al., 2019; Luders et al., 2020; Oatridge et al., 2002). However, the nature of this brain remodeling is still in its early years of research. The present thesis constitutes the first approach in literature to characterize previously observed changes in brain volume in mothers, which may shed light on the underlying neuroplasticity. It covers three main objectives: 1) characterizing the brain morphometric features being remodeled, 2) identifying the pregnancy intrinsic and parenting extrinsic factors that mediate these changes, and 3) tracking the durability of the changes. This work examines in detail the neuroscience behind a remarkable lifetime transition that, like other women-specific processes, has been historically overlooked by the scientific community. This research will hopefully inspire others to gradually reduce the sex-bias in neuroscience.

Characterizing the maternal brain adaptations has important implications for the health care system. During periods of extreme brain plasticity, such as pregnancy, the brain is more sensitive to assaults -a plastic brain is a vulnerable brain. In fact, the perinatal period is one of the stages in a woman's life with the highest risk for various mental disorders. An estimated 17% of worldwide mothers suffer from postpartum depression (Wang et al., 2021), which is a "major depression event with perinatal onset" often characterized by high levels of anxiety, avoidance, and intrusive behaviors towards the baby (American Psychiatric Association, 2013). If left mistreated or misdiagnosed, postpartum depression can put the mother at risk for recurrent major depression and self-harm, as well as compromise the child's safety and cognitive and emotional development. Characterizing

the morphometric features sensitive to change in expectant mothers (Objective 1, Study 1) is a first step towards investigating which brain systems are disrupted in women who suffer perinatal mental disorders. Hopefully, this will ultimately contribute to the development of preventive strategies as well as safer and more effective treatments.

Non-human animal models indicate that the parental brain is shaped both by pregnancy- and parental experience-induced neuroplasticity (Stolzenberg & Champagne, 2016). Identifying the specific contributions of each of these two mechanisms to human brain plasticity across the parental transition (Objective 2) is currently a major milestone in the field. As of yet, most research into brain changes associated with parenthood has focused on biological mothers (Martínez-García, et al., 2021a), making it difficult to separate pregnancy-hormonal influences from other extrinsic factors associated with parenthood that may also lead to neural changes (e.g., caregiving experience, sleep deprivation, and other life-style changes that can lead to health disparities). Study 1 of this thesis compares maternal brain changes directly with those occurring during female adolescence, a well-described period of hormonally induced neuroplasticity. As an alternative approach to understand the mediating factors, the studies of this thesis follow the brains of both biological mothers (Studies 1 and 3) and their male partners (Study 2), who experience the cognitive, and emotional demands of caring for a newborn without going through pregnancy. Studying the paternal brain (Study 2) can reveal the extent to which brain changes in parents can be induced by experiential and environmental factors other than the reproductive experience. This information has a direct impact on public health policies aimed to support optimal pregnancy and parenting atmospheres.

Finally, this thesis has also important implications on the field of brain aging. In neuroscience, the influence of hormonal transitions on the aging brain remains largely understudied, and this gap disproportionately affects women (Barth & de Lange, 2020; Taylor et al., 2019). Women's endocrine lifespan, including reproductive experience, is often overlooked among all "lifespan" factors that are used to predict typical and atypical brain aging (e.g., years of education, lifetime physical activity, smoking history, or substance abuse). Large-scale neuroimaging studies in middle-aged and older women suggest that parity may influence the course of women's brain aging (Aleknaviciute et al., 2022; de Lange et al., 2019; de Lange, Barth, Kaufmann, Anatürk, et al., 2020; Orchard

et al., 2020) and their risk of neurodegenerative diseases such as Alzheimer (Beeri et al., 2009; Colucci et al., 2006; de Lange, Barth, Kaufmann, Maximov, et al., 2020; Jang et al., 2018). While these studies have demonstrated a link between reproductive history and the late-life brain status, their retrospective nature does not allow to establish causality. The present thesis includes the first study (Study 3) to track the long-term effects of pregnancy on the human brain with a longitudinal design (Objective 3). In doing so, this research unlocks a pathway in the study of female-specific brain aging trajectories.

In conclusion, through a series of original longitudinal studies, this thesis addresses the morphometric features, mediating factors, and durability of the brain adaptations to motherhood, which expands the knowledge of parental mental health in particular and women's health in general.

2. INTRODUCTION

2.1. Neuroplasticity of the human brain

An extraordinary feature of the brain is its capacity to change. Brain plasticity or neuroplasticity is the intrinsic ability of the nervous system to make adaptive changes in response to internal and external stimuli through processes that regulate cellular function, structure and connectivity (Mateos-Aparicio & Rodríguez-Moreno, 2019). Neuroplasticity involves changes on multiple scales, ranging from molecular changes to cellular and morphological changes, the two latter conforming structural plasticity (Brinton, 2009; García-Segura, 2009). At the molecular level, plasticity takes the form of alterations in intracellular cascades, neurotransmitters, and brain receptors. Cellular plasticity includes the generation of new functional neurons (i.e., neurogenesis) from neural stem cells or neural progenitor cells. Morphological changes include modifications of dendritic branching, post-synaptic spine density, and soma size. Brain plasticity also includes molecular, cellular, and morphological changes in glial cells (microglia, astrocytes, and oligodendrocytes), including the reversible extension of their cellular processes, myelination, and gliogenesis. By cross-talking with neurons, glial cells such as microglia (Crapser et al., 2021; Ikegami et al., 2019) and astrocytes (Perez-Catalan et al., 2021) also regulate neuronal plasticity, directing the formation and remodeling of synapses and neuronal circuits. Collectively, molecular, cellular, and morphological changes sculpt the brain's architecture by modulating synaptic strength, neuronal firing, neuronal connectivity, glial activity, neurogenesis, and gliogenesis. This phenomenon allows our brain to continuously respond to changes in our internal state (e.g., hormonal, immune, or growth factors) and the environment (e.g., stress, experience, and sensory stimulation). The present thesis will consider "brain plasticity" and "neuroplasticity" as synonymous.

Humans retain a basal level of neuroplasticity throughout life (Kinsley et al., 2008). The day-a-day learning and creation of new memories require different forms of molecular plasticity that selectively intensify or weaken synaptic transmission between neurons. In addition to basal levels of brain plasticity, the brain also exhibits pronounced, larger-scale structural changes during "sensitive periods" (Kinsley et al., 2008; Knudsen, 2004).

Researchers once believed that these “sensitive periods” were confined to early development and adolescence, when critical cognitive, social, and emotional milestones are reached. Brain plasticity during early development involves the creation of a functionally wired network from scratch, beginning with cell birth (neurogenesis and gliogenesis) and progressing to cell migration, differentiation, maturation, synapse formation, and myelin formation (Kolb & Gibb, 2011). During childhood, the brain overproduces both neurons and connections (Kolb & Gibb, 2011). From there and until late adolescence, the brain eliminates redundant cells and synapses while maintaining and strengthening the remaining ones. These plasticity trajectories translate into dynamic macroscopic cortical changes over time (Bethlehem et al., 2022; Blakemore, 2008; Giedd et al., 1999). The cortex of a child displays an initial strong increase in cortical grey matter volume from mid-gestation until six years, followed by a near linear decrease throughout childhood and adolescence (Bethlehem et al., 2022).

According to studies of the last decade, the windows of enhanced brain plasticity should be extended to a later stage of life marked by tremendous physical and cognitive demands and extreme hormonal fluctuations: pregnancy and the transition to motherhood.

2.2. Maternal behavior

Parental behavior can be defined as any behavioral response displayed by one member of a species towards infants, with the ultimate goal of increasing the likelihood of survival of the offspring (Numan, 2020, Chapter 1). Parental behavior occurs in a variety of vertebrates, including fish, amphibians, reptiles, birds, and mammals, being essential for the survival of the young in the two latter (Rosenblatt, 2003). Parenting comprises multiple social behaviors that can be influenced by several internal and external factors. Internal factors can include species-specific conditions such as the level of maturity of the young at birth, the litter size, and the mode of delivering nutrients to the offspring, among others. External factors can include environmental and social pressures such as food shortage, predation, and disease. Human parental behavior is additionally shaped by culture, socioeconomic status, and individual beliefs and motivations.

In mammals (class *Mammalia*), due to the necessity of infant nutrition by lactation, maternal behavior is the dominant form of parental care, and will be the main focus of this thesis. Yet, in a few mammalian species males exhibit paternal behavior, and many species display "alloparenting" or "cooperative breeding", in which relatives or conspecifics help to raise the offspring (Numan, 2020, Chapter 7). In humans, paternal investment is affected by socio-cultural and historical differences, as well as individual beliefs, producing behaviors that range from a father being fully absent to being the primary caregiver (Abraham & Feldman, 2022). Also, humans rely heavily on alloparents to provide childcare, including relatives like grandparents or siblings; adoptive and foster parents; and caregivers not related biologically, such as babysitters or teachers (Abraham & Feldman, 2018).

Rodents (order *Rodentia*), especially rats (*Rattus norvegicus*) and mice (*Mus Musculus*) models, are the best-studied mammals to understand the neural underpinnings of parental behavior (Pereira et al., 2022). This is due to a number of practical reasons: they are easy to maintain and breed in captivity, have short gestation times, and produce many offspring per litter. In rodents, maternal behaviors include nest building, pup grooming and licking, retrieving all pups to the nest, and nursing. Occasionally, maternal behavior also refers to abilities or behaviors that are indirectly related to caring for the pups, such as learning, foraging strategies, and enhancement of problem solving in a novel context. Through this thesis, the expression "maternal behavior" or "maternal care", when used to describe rodents, will refer to any of the above-mentioned pup-specific behaviors, unless otherwise noted.

Importantly, some species can display maternal behavior through pup-stimulated sensitization (Stolzenberg & Champagne, 2016). For instance, although adult virgin female rats do not show maternal responsiveness when first exposed to conspecific young pups, they will eventually display maternal behavior if continuously housed with those young pups (Rosenblatt, 1967).

2.3. Hormones and maternal behavior

"Sensitive periods" for neuroplasticity coincide with life transitional stages accompanied by major hormonal surges. Hormones and the central nervous system maintain constant crosstalk during the life span (Been et al., 2021). Hormones send essential information to the brain and regulate its plasticity, allowing an adequate neural response to ongoing modifications of the inner and outer world (García-Segura, 2009, Chapter 1). In turn, brain changes affect hormone secretions from endocrine glands throughout the body, including the hypothalamus and pituitary glands in the brain (García-Segura, 2009, Chapter 2). Pregnancy is characterized by hormonal fluctuations that exceed any other neuroendocrine events of a female's lifetime (e.g., menstruation, adolescence, menopause) (Anderson et al., 2015; Deems & Leuner, 2020; Kuo et al., 2016; Voltolini & Petraglia, 2014). The circulating levels of estrogens and progesterone increase steadily across the three trimesters and fall off rapidly at parturition after placental detachment. Prolactin levels also rise steadily until parturition. During the postpartum period, steroid levels plummet and oxytocin and prolactin begin fluctuating in close coordination with breastfeeding, resulting in a markedly different hormonal environment than during pregnancy. As a result of its extensive hormonal exposure and distinctive endocrine trajectory, the maternal brain provides a unique window into hormonally induced neuroplasticity.

20th century neuroendocrinology studies with non-human mammals show that the hormones associated with pregnancy and parturition prime the mother's brain to display maternal behavior right after parturition (Numan, 2020, Chapter 3). Rat models have provided a great understanding of the hormonal regulation of maternal behavior. Early studies of Dr. Jay S. Rosenblatt show that blood transfused from a newly parturient female to a virgin female stimulated maternal behavior in terms of pup retrieving (Terkel & Rosenblatt, 1972), suggesting that the onset of maternal behavior was regulated by hormonal factors associated with parturition. These theories were later confirmed by Dr. Michael Numan, Dr. Robert R. Bridges, and colleagues, who created a new groundbreaking model in rats for the study of the hormonal regulation of maternal responsiveness (Bridges, 1984; Moltz et al., 1970). In rats, early and mid-pregnancy are characterized by progressive increases in estradiol and progesterone, while late pregnancy

is characterized by high levels of estradiol and pituitary prolactin, superimposed with an abrupt decline in progesterone (Pereira et al., 2022). Ovariectomized virgin females treated with an equivalent hormonal regimen of estradiol, progesterone, and prolactin reduced their latency to behave maternally from typical 6-7 days to 35-40 hours (Bridges, 1984; Moltz et al., 1970). These findings demonstrate that estradiol and prolactin of late pregnancy, along with the pre-delivery decline in progesterone, facilitate the onset of maternal behavior. A number of other studies suggest that oxytocin plays an additional role in arousing maternal behavior (Numan, 2020, Chapter 4). Specifically, a combination of oxytocin and estradiol stimulates maternal behavior in ovariectomized virgin female rats, while oxytocin alone takes longer to induce the maternal response (Fahrbach et al., 1985; Pedersen et al., 1982).

2.4. A neuroscience perspective of the maternal brain

2.4.1. Maternal brain adaptations in rodents

The neural mechanisms sustaining the link between hormones and maternal behavior have been identified in rodent models. Pregnancy hormones such as estradiol, prolactin, and oxytocin target the brain and remodel core neural circuits that regulate maternal behavior. As the activation of this circuit facilitates the onset of maternal behavior, researchers refer to it as the "maternal brain circuit" (Numan, 2020, Chapter 5). How do these peripheral hormones reach the brain? Estradiol, prolactin, and oxytocin receptors are densely expressed in the brain (Almey et al., 2015; Grattan et al., 2001; Quintana et al., 2019). Given its lipid-like nature, steroid hormones cross the blood-brain barrier by transmembrane diffusion, whereas peptide hormones such as prolactin are believed to cross using transporters (Banks, 2012). Oxytocin makes an interesting exception. This neuropeptide is synthesized in neurons of the hypothalamic paraventricular nucleus, from where it reaches diverse brain areas via long axonal projections, or via local diffusion through the extracellular fluid (Busnelli & Chini, 2018).

Once they reach the brain, peripartum hormones promote changes in the "maternal brain circuit" (Numan, 2020 Chapter 5). Following the decrease in progesterone before parturition, prolactin and estradiol bind to receptors in the medial preoptic area (mPOA)

of the hypothalamus and enhance their excitability via cytoplasmic kinase cascades and genomic signaling pathways. The hormonal priming of mPOA stimulates dopamine release from the mesolimbic reward system, in particular, from ventral tegmental area to the nucleus accumbens. The action of dopamine on the nucleus accumbens releases the steady inhibition of the ventral pallidum, increasing its responsiveness to pup-stimuli that arrive from the amygdala. Projections from the ventral pallidum to other basal ganglia, motor areas and medial prefrontal cortex, are key to eliciting maternal approach behaviors such as pup retrieval. Once the "maternal brain circuit" is properly primed, it becomes responsive to centrally released oxytocin, which up-regulates the neural firing of multiple regions including the mPOA, ventral tegmental area, nucleus accumbens, and amygdala. Functional activation of the "maternal brain circuit" is achieved through hormonally induced neuroplasticity changes in spines, dendritic branches, somas, axons, and the surrounding glial cells, which altogether result in enhanced synaptic transmission and conducting velocity (Hillerer et al., 2014; Pawluski et al., 2021). Importantly, while gestational hormones activate this circuit and initiate maternal care, once maternal behavior is established, it emancipates from hormonal stimulation and is maintained solely by infant stimuli (Stolzenberg & Champagne, 2016).

2.4.2. Maternal brain adaptations in humans

The brain networks that support maternal behavior in humans have been inferred predominantly from maternal neural responses to infant stimuli. Functional MRI (fMRI) based on Blood-Oxygen-Level-Dependent (BOLD) response has been the main neuroimaging technique used to study human brain function (Attwell & Iadecola, 2002). BOLD-fMRI uses regional changes in blood oxygenation to indirectly infer neuronal activity. With this technique, we can infer which regions become active when a person performs a specific task or is exposed to specific stimuli. In research on the human maternal brain, fMRI is commonly used to determine which brain regions are activated by baby-related stimuli, often comparing the mother's own child with a non-related child (Martínez-García et al., 2022a). Several brain regions show consistent activation upon visual, auditory, tactile, and olfactory infant cues (Bjertrup et al., 2019; Paul et al., 2019; Rigo et al., 2019; Shih et al., 2022), forming a putative "maternal caregiving brain network". This network shares core regions of the rodent "maternal brain", but also

includes later-evolved components that are unique to humankind (Feldman, 2015; Numan, 2020, Chapter 8). Similar to rodents, subcortical regions commonly activated in human mothers in response to their infant cues include the amygdala, and dopaminergic reward regions like the striatum, especially the nucleus accumbens, and the ventral tegmental area. This network may maintain aspects of parental behavior highly conserved among mammals such as vigilance for the child's safety and reward from the mother-infant bond. Beyond the subcortical network, cortical regions commonly activated in human mothers when presented with stimuli or their infant include the insula, inferior frontal gyrus and the prefrontal cortex. Other subcortical regions such as the hippocampus and the hypothalamus, and cortical regions such as the temporo-parietal junction and the posterior cingulate cortex, also appear activated upon infant stimuli, but less consistently. Altogether, these brain areas have been associated with higher-order cognitive processes related to parenting such as emotional empathy, mentalizing, and emotion regulation. Regional activation of the "maternal caregiving brain network" is linked to maternal sensitivity towards the infant, suggesting that this network may contribute to initiation and/or maintenance of maternal behavior.

The impact of motherhood on brain function has been explored, to a lesser extent, through resting-state fMRI. Resting-state fMRI allows to extract an index of the spontaneous intrinsic neural activity based on BOLD's low-frequency fluctuations (Murphy et al. 2013). During the acquisition, subjects are instructed to lie on the scanner and remain awake during the acquisition, allowing to reveal global changes in brain function beyond responses to infant stimuli. This technique is also suitable to measure functional connectivity, that is, the degree of co-activation between the functional time-series of anatomically separated brain regions at rest. Seed-based resting-state fMRI studies show differences between mothers and non-mothers in prefrontal cortex (Bak et al., 2020; Zheng et al., 2020), posterior cingulate cortex (Zheng et al., 2020), and temporo-parietal junction (Bak et al., 2020), which are critical nodes of the mentalizing or default mode network. Also, the stronger the functional connectivity between two core subcortical regions of the "maternal circuit", the amygdala and the nucleus accumbens, the better the maternal structuring measured during mother-child interactions (Dufford et al., 2019). At one year postpartum, mothers differ from non-mothers in effective functional connectivity between key regions of the "maternal caregiving brain network" such as the

prefrontal cortex, posterior cingulate cortex, parahippocampal gyrus, amygdala, and nucleus accumbens (Orchard et al., 2022).

While functional neuroimaging studies typically inform about brain activity at a certain time during the postpartum, structural MRI can capture more stable brain changes. The pituitary gland, a small neuroendocrine organ, was the first structure studied in the human maternal brain. The pituitary gland constantly produces prolactin during pregnancy, which stimulates milk production for the postpartum breastfeeding (Duthie & Reynolds, 2013). Such hyperprolactinemia leads to hypertrophy of prolactin cells and a pituitary enlargement (Dinç et al., 1998).

As new neuroimaging techniques developed, researchers began to analyze finer aspects of the brain's morphology, including changes to cortical volume, thickness, surface area, and folding. Structural MRI produces high-resolution images with well-defined gray and white matter interfaces, so it is the gold standard imaging method to capture changes in brain structure and morphometry (Backhausen et al., 2021; Mills & Tamnes, 2014). The majority of studies have analyzed changes in gray matter, while white matter modifications remains underexplored (Zhang et al., 2020). Most studies focused on changes during the postpartum period (Chechko et al., 2021; Kim et al., 2010, 2018; Lisofsky et al., 2019; Luders et al., 2020, 2021a, 2021b, 2021c), whereas a few examined brain changes throughout the gestational period (Hoekzema et al., 2017, 2020; Oatridge et al., 2002). Some of the cortical regions that consistently change in mothers include the precuneus, superior temporal gyrus, medial prefrontal gyrus extending to the anterior cingulate, medial temporal areas (including the hippocampus, parahippocampal gyrus, and insula), dorsolateral prefrontal cortex, and ventral striatum including the nucleus accumbens. Gray matter changes vary in direction depending on the timeframe captured. First-time mothers scanned before conceiving and again at two months after birth display prominent and widespread gray matter brain volume decreases compared to nulliparous women (Hoekzema et al., 2017). Immediately after childbirth, mothers display smaller gray matter volumes compared to non-mothers (Chechko et al., 2021; Lisofsky et al., 2019). Finally, studies scanning mothers at two time-points after parturition reveal brain volume increases during the first months of postpartum (Kim et al., 2010; Lisofsky et al., 2019; Luders et al., 2020). The unified trajectories resulting from these longitudinal

studies draw a dynamic evolution of gray matter volumetric decreases during pregnancy and gray matter volume increases during the postpartum (Martínez-García et al., 2021a).

Studies suggest a mechanistic link between structural and functional changes in the human maternal brain. Brain regions whose structure changes in mothers coincide with functional modifications reported during postpartum (Section 3.4.1). Furthermore, the degree of structural brain adaptations has been associated with different indirect measures of the mother-infant relationship. During pregnancy, the magnitude of gray matter volume changes predicts higher scores of the mother-to-infant attachment (Hoekzema et al., 2017) and greater brain activations with the infants' visual stimuli (Hoekzema et al., 2020). During the postpartum, gray matter volume increases in a midbrain cluster comprising the hypothalamus, amygdala, and globus pallidus, predicts a better mother's subjective perception of her baby (Kim et al., 2010). All in all, the structural brain changes observed during pregnancy and postpartum seem to reflect a neural adjustment that facilitates sensitive and timely behaviors towards the newborn.

2.5. Current research lines

Our understanding of the maternal brain has grown significantly over the past 20 years, but many questions remain unresolved. Neuroimaging studies have proved that motherhood transition is marked by pronounced changes in brain volume that overlap with brain regions that support different aspects of maternal behavior such as motivated behavior, empathy, and emotion regulation (Martínez-García et al., 2021a). Rodent models suggest that these brain changes may reflect an adaptive process of enhanced neuroplasticity in the "maternal circuits". However, parental behavior is species-specific, as may be the underlying brain mechanisms, making it difficult to translate rodent findings to humans. Currently, human parental brain researchers face the challenge of elucidating the neuroplasticity behind the macroscopic brain changes detected by MRI. The present thesis constitutes the first attempt in the neuroimaging field to unravel the initially observed brain volume changes in mothers, which may reveal different aspects of the underlying neuroplasticity. This thesis uses longitudinal structural MRI to examine the cortical morphometry (Study 1), mediating factors (Studies 1 and 2), and durability (Study 3) of the brain changes of first-time parents from preconception to the postpartum.

Study 1 decomposes the volumetric reductions associated with pregnancy into their specific morphometric details. In addition, this study compares this profile of brain changes with that occurring during female adolescence to determine if these two hormonally driven transitions leave similar traces in the brain. Study 2 examines the neuroanatomic adaptations of men transitioning into fatherhood to determine the contribution of other factors outside the reproductive experience. Finally, no study has explored beyond two years postpartum whether these brain changes are maintained or instead return to pre-pregnancy levels. Study 3 analyzes for the first time whether the brain volume reductions observed in primiparous women persist six years after delivery.

3. OBJECTIVES AND HYPOTHESES

The present thesis aims to characterize the cortical morphometry, mediating factors, and durability of the brain volume changes observed in first-time mothers from preconception to the postpartum. The specific objectives and hypotheses are as follows:

- **Objective 1:** To characterize the specific brain morphometric features being remodeled during the motherhood transition (**Study 1: Carmona et al., (2019)**). **Hypothesis 1:** Pregnancy will produce a profile of morphometric brain changes that will resemble the cortical flattening of female adolescence, the other major period of hormonal surges in a woman's life.
- **Objective 2:** To discern the contribution of pregnancy hormones (**Study 1: Carmona et al., (2019)**) and the postpartum parenting experience (**Study 2: Martínez-García et al., (2022b)). Hypothesis 2:** Gestational hormones will be the main drivers of the neuroanatomical adaptations, while childbearing experience will lead to subtler adaptations. Consequently, gray matter volume reductions will be more pronounced in first-time mothers than in first-time fathers.
- **Objective 3:** To determine whether these brain changes are confined to the postpartum period or endure beyond that period (**Study 3: Martínez-García et al., (2021b)). Hypothesis 3:** Gray matter volume reductions in first-time mothers will partially recover during the postpartum but will not reach preconception levels at six years postpartum.

4. Study 1: Pregnancy and adolescence entail similar neuroanatomical adaptations: A comparative analysis of cerebral morphometric changes

Carmona, S., **Martínez-García, M.**, Paternina-Die, M., Barba-Müller, E., Wierenga, L.M., Alemán-Gómez, Y., ... & Hoekzema, E. (2019). **Pregnancy and adolescence entail similar neuroanatomical adaptations: a comparative analysis of cerebral morphometric changes**. *Human brain mapping*, 40(7), 2143-2152.

Doi: https://doi.org/10.1002/hbm.24513

5. Study 2: First-time fathers show longitudinal gray matter cortical volume reductions: evidence from two international samples

Magdalena Martínez-García, María Paternina-Die, Sofia I Cardenas, Oscar Vilarroya, Manuel Desco, Susanna Carmona, Darby E Saxbe, (2022). **First-time fathers show longitudinal gray matter cortical volume reductions: evidence from two international samples**, *Cerebral Cortex*, ; bhac333.

Doi: https://doi.org/10.1093/cercor/bhac333

6. Study 3: Do Pregnancy-Induced Brain Changes Reverse? The Brain of a Mother Six Years after Parturition

brain sciences

MDPI

Article

Do Pregnancy-Induced Brain Changes Reverse? The Brain of a Mother Six Years after Parturition

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Citation: Martínez-García, M.; Paternina-Die, M.; Barba-Müller, E.; Martín de Blas, D.; Beumala, L.; Cortizo, R.; Pozzobon, C.; Marcos-Vidal, L.; Fernández-Pena, A.; Picado, M.; et al. Do Pregnancy-Induced Brain Changes Reverse? The Brain of a Mother Six Years after Parturition. *Brain Sci.* **2021**, *11*, 168. https://doi.org/10.3390/brainsci11020168

Academic Editors: María José Rodrigo and Livio Provenzi
Received: 16 December 2020
Accepted: 22 January 2021
Published: 28 January 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Neuroimaging researchers commonly assume that the brain of a mother is comparable to that of a nulliparous woman. However, pregnancy leads to pronounced gray matter volume reductions in the mother's brain, which have been associated with maternal attachment towards the baby. Beyond two years postpartum, no study has explored whether these brain changes are maintained or instead return to pre-pregnancy levels. The present study tested whether gray matter volume reductions detected in primiparous women are still present six years after parturition. Using data from a unique, prospective neuroimaging study, we compared the gray matter volume of 25 primiparous and 22 nulliparous women across three sessions: before conception (n = 25/22), during the first months of postpartum (n = 25/21), and at six years after parturition (n = 7/5). We found that most of the pregnancy-induced gray matter volume reductions persist six years after parturition (classifying women as having been pregnant or not with 91.67% of total accuracy). We also found that brain changes at six years postpartum are associated with measures of mother-to-infant attachment. These findings open the possibility that pregnancy-induced brain changes are permanent and encourage neuroimaging studies to routinely include pregnancy-related information as a relevant demographic variable.

Keywords: pregnancy; maternal brain; magnetic resonance imaging; neuroplasticity; postpartum

1. Introduction

Motherhood is a life-changing event that affects the social, psychological, and biological spheres. Biologically, pregnancy entails dramatic adaptations in the function and structure of all physiological systems, including the brain. Studies in non-human animal models indicate that pregnancy and motherhood modify the so-called maternal circuit: a set of brain regions that includes reward and social processing areas [1–3]. Such modifications, triggered by pregnancy and peripartum hormonal fluctuations and then by mother–pup interactions, play a critical role in the onset, maintenance, and adjustment of maternal care [4].

Results from neuroimaging studies in humans closely align with those obtained in non-human animal models. For instance, functional magnetic resonance imaging (MRI) studies show that the mother's brain responds to her child's stimuli by activating regions involved in reward and social processing [5]. According to this literature, activations in reward regions, especially in the nucleus accumbens, reflect the hedonic state that the mothers experience when presented with stimuli of their child [6]. Activations in areas involved in social processing, such as medial prefrontal cortex and precuneus, reflect mentalizing or Theory of Mind (ToM) processes, that is, the disposition of a person to interpret others' minds, in this case, the mother's disposition to interpret her infant's signs [7].

As compared to functional studies, very few have investigated whether pregnancy and motherhood modify brain anatomy. Existing longitudinal data indicate that the transition to motherhood also produces anatomical adaptations in reward and social cognition brain circuits [8]. These adaptations seem to be dynamic: They differ in direction and magnitude depending on the time frame studied (pregnancy vs. postpartum) [9–12]. Specifically, whereas studies comparing preconception to postpartum brains found gray matter (GM) volume decreases [13–15], those analyzing the brain from the early to late postpartum period found GM volume increases [13,16,17]. This suggests a U-shaped trajectory of volume decreases during pregnancy, followed by increases after parturition. However, it remains to be determined if brain volume ever fully returns to pre-pregnancy levels. The longest longitudinal study to date that tested the persistence of brain changes after the early postpartum compared the neuroanatomical MRI data of primiparous women across three sessions: a few months before their first pregnancy, during the early postpartum, and at two years after parturition [14]. In this study, we found pronounced GM volume reductions within ToM networks following the first pregnancy, and these reductions were still detectable two years after parturition.

The first two years postpartum—which together with the intrauterine period are known as "the first thousand days of life"—are considered a critical period to promote optimal child development [17]. As infants are highly dependent, this critical period of nurturance and care requires a tremendous maternal investment [18]. Since early maternal care has been associated with neuroanatomical changes during pregnancy [14] and postpartum [16,19], it is possible that mother's brain structure returns to pre-pregnancy levels after these first two critical years. If changes remit, they might indicate that they are confined to the period of maximal maternal investment and that, after that, the mother's brain is equivalent to that of nulliparous women. On the contrary, if changes are still detectable after this critical period, they might indicate that pregnancy has a long-lasting—perhaps irreversible—impact on women's brains. Still, it is unknown how the anatomy of a woman's brain evolves beyond the first two years postpartum.

Here, we assessed if a single pregnancy leaves a trace in the anatomy of a woman's brain that persists beyond the first two years of maximal maternal investment. For that, we followed the subjects of the prospective cohort study of Hoekzema et al., 2017 [14], and scanned them again at six years after parturition. We compared the brain of primiparous and nulliparous women across three sessions: before conception (PRE), during the early postpartum (POST), and six years after parturition (POST6y). We aimed to determine: (1) if the GM volume reductions detected in the mother's brain during their first and

only pregnancy were still present six years after parturition and (2) if, based on GM changes, we could classify women as never having been pregnant or as having been pregnant more than six years ago. Finally, to corroborate that brain changes detected were related to motherhood, we examined whether brain changes between the PRE and POST6y sessions predicted the measures of mother-to-infant attachment collected during the early postpartum.

2. Materials and Methods

2.1. Participants

In the previous study, we followed a prospective cohort study of 25 first-time mothers and 20 nulliparous women that was set up to examine the effects of pregnancy on the human brain [14]. For first-time mothers, the data set included one MRI session before conception (PRE) and another during the early postpartum (POST). Mean time between the PRE and POST sessions was 1.27 (±0.30) years. For nulliparous women, two MRI sessions were acquired at comparable time intervals (Table 1).

Table 1. Demographic characteristics of the sample.

Group	**MOTHERS**			**NULLIPAROUS**		
Session	**PRE**	**POST**	**POST6y**	**PRE**	**POST**	**POST6y**
n of participants	25	25	7	22	21	5
Age (mean ± s.d.) (years)	33.87 ± 3.89	35.14 ± 3.87	40.55 ± 3.14	31.17 ± 5.77	32.12 ± 6.03	38.83± 6.37
Education (*n* of subjects)	2	2	0	2	2	1
-School	4	4	2	4	3	1
-College	19	19	5	16	16	3
-University						
Means of conception (*n* of subjects)	9	9	3			
-Natural	16	16	4	-	-	-
-Fertility assisted						
Time since the PRE session (mean ± s.d.) (years)	-	1.27 ± 0.30	7.22 ± 0.50	-	1.11 ± 0.31	7.57 ± 0.56
Time since parturition date (mean ± s.d.) (months)	-	2.45 ± 1.59	75.80 ± 7.07	-	-	-

The variables "age", "education", and "time since the PRE session" did not differ significantly between the groups of mothers and nulliparous women at POST6y (age: *p*-value = 0.545, education: *p*-value = 0.462, time since the PRE session: *p*-value = 0.285). The means of conception at the PRE and POST sessions were skewed toward fertility treatment. However, as stated in Hoekzema et al., 2017 [14], "there were no gray matter volume differences between the groups." Abbreviations are as follows: PRE = pre-pregnancy session, POST = early postpartum session, POST6y = six years after parturition session, s.d. = standard deviation.

To assess the long-term effects of pregnancy, we re-contacted the participants for a new MRI scanning session six years after parturition (POST6y) [14]. Among the 25 mothers included in the original sample, five already had another child, one was pregnant, one had a traumatic brain injury, three had moved out of the country, and eight either did not reply or were no longer interested in participating in the study. This resulted in a final sample of seven mothers (mean age 40.55 ± (3.14) years). For these seven mothers, we also recovered their scores on the Maternal Postnatal Attachment Scale (MPAS), administered during the first months of postpartum. Mean values for the three MPAS subscales were as follows: "Quality of Attachment" = 36.87 (±4.75), "Absence of Hostility" = 16.19 (±2.94), and "Pleasure in Interaction" = 22.00 (±1.83). To control for the effects of other unknown confounding variables, we also contacted the nulliparous women of the original sample of Hoekzema et al., 2017 [14]. Among the 20 nulliparous women, 14 had become mothers, and three were no longer interested in participating in the study. To increase the sample, for the present study, we also scanned two nulliparous women who were not included in the previous study, but whose anatomical scans were obtained at comparable dates, in the same scanner and using identical sequence parameters. For one of these women, a session

matching the POST session time interval was also available, resulting in a sample of five nulliparous women (mean age 38.83 ± (6.37) years).

Informed consent for the six-year follow-up session was obtained from all participants. Table 1 provides a general description of the participants at each session. For further details about the original sample and the analyses investigating the impact of demographic or clinical factors on the results, see Hoekzema et al., 2017 [14] and Supplementary Tables S1 and S2.

2.2. MRI Data Acquisition

High-resolution anatomical MRI brain scans were acquired in a Philips Achieva 3 Tesla scanner located at Hospital del Mar in Barcelona. The protocol was identical to that of the previous sessions [15], consisting of a T1-weighted gradient echo pulse sequence (repetition time = 8.2 milliseconds, echo time = 3.7 milliseconds, voxel size = 0.9375 mm × 0.9375 mm × 1 mm, field of view = 240 mm × 240 mm × 180 mm, and flip angle = 8°). During the POST6y session acquisition, there was a software update on the Philips scanner (from version 5.1.7 to version 5.3.1), and six participants out of 12 were scanned with a different software version. The effects caused by the software update were isolated and corrected before the image processing (see Supplementary Methods and Results 1 for the correction details).

2.3. Image Processing

Images were processed with SPM12 (https://www.fil.ion.ucl.ac.uk/spm/), implemented in Matlab 2017b (https://es.mathworks.com/). We used the longitudinal symmetric diffeomorphic modeling pipeline [20], which produces for each subject a map of volume change between sessions. We calculated the maps of volume change between PRE and POST, and between PRE and POST6y sessions.

As previously mentioned, this study was a follow-up analysis, built upon the results obtained in a previously published paper [20]. Thus, to avoid any potential bias due to image processing steps, we applied a pipeline identical to that used in the previous study (see Figure S2) [20]. Specifically, the anatomical images of each participant were longitudinally registered. This step included rigid-body registration, intensity inhomogeneity correction, and nonlinear diffeomorphic registration. The subjects' images were then registered to the within-subject average image to avoid biases associated with asymmetry in pairwise registration, giving as a result the Jacobian determinants of the longitudinal pairwise registration. The midpoint average images were segmented into specific tissues using the unified segmentation algorithm [21]. Jacobian determinants were subsequently multiplied by each subject's GM segmentation, creating maps of GM volume change. These maps of GM volume change were normalized into Montreal Neurological Institute (MNI) space using Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) tools and smoothed with a 12-mm, full-width, half-maximum smoothing kernel [22].

2.4. Statistical Analyses

2.4.1. Region of Interest Analyses

We performed a region of interest (ROI) analysis to focus on the specific areas known to be affected during pregnancy. These ROIs were obtained from the results reported in Hoekzema et al., 2017 [14], and correspond to regions where GM volume decreases more in the mothers than in nulliparous women (GM volume changes ((Nulliparous POST-PRE) − (Mothers POST-PRE))). Those ROIs, named based on their location within the MNI space, are: Left Fusiform, Left Hippocampus, Left Inferior Frontal, Left Inferior Orbitofrontal, Left Middle Frontal, Left Superior Temporal, Medial Frontal, Precuneus, Right Fusiform, Right Inferior Frontal, and Right Superior Temporal (Figure S1). We created an additional ROI including all the above mentioned regions named "All ROIs."

For each ROI, we tested whether the mean GM volume changes (POST6y-PRE) and the slopes (PRE to POST, and POST to POST6y) differed between groups after correcting for the potential age effects. We used the non-parametric Wilcoxon–Mann–Whitney test to determine the significance of the group comparisons. The test was limited to one side

as we specifically expected to find more decreases in the mothers than in the nulliparous group. The threshold was set to False Discovery Rate-Adjusted p-value (q-value) of 0.05 to correct for multiple comparisons [23].

2.4.2. Whole-Brain Analyses

As exploratory analyses, we examined if groups showed GM volume increases or decreases (between the PRE and POST6y) in other regions not included within the ROIs. We did a whole-brain, voxel-based analysis through a General Linear Model, including age as a covariate. The significance threshold was set to p-value < 0.05 family-wise error-corrected, and a cluster of 25 contiguous voxels.

2.4.3. Pattern Recognition Analyses

For classification and regression tests, we performed the multivariate pattern recognition analyses in PRoNTo (version 2.10; http://www.mlnl.cs.ucl.ac.uk/pronto/), implemented in Matlab. Briefly, the pipeline searches for regularities in the maps of GM volume changes and trains a decision function. This function is used to predict the label of the images based on the signal regional contribution within the image. When the labels are discrete (e.g., mothers vs. nulliparous women), the learned function is called the classifier model; when they are continuous (e.g., MPAS scores), it is called regression model. Here, we performed both approaches. First, we used a support vector machine model to test the power of the GM changes (POST6y-PRE) to discriminate between the groups while including age at POST6y as a potential confounder. Then, with a multiple kernel ridge regression algorithm, we tested whether mothers' brain changes could predict MPAS scores. To evaluate the performance of the pattern recognition analyses, we examined the accuracy of the classification and the goodness of fit measures for the regression models using a leave-one-out, cross-validation strategy. The models' statistical significances were calculated non-parametrically with 10,000 permutations at a threshold of p-value < 0.05.

2.4.4. Positive Predictive Value (PPV)

Given our limited sample size at POST6Y, as a post hoc analysis, we calculated the positive predictive value (PPV) of our results [24,25]. The PPV is the probability that a "positive" or significant finding reflects a true effect. This probability was calculated for the between-group differences in GM volume change (POST6y-PRE) in the "All ROIs" mask (Supplementary Methods and Results 2).

2.4.5. Supplementary Analyses

Due to an unexpected technical problem, the radiofrequency head coil (RFHC) had to be replaced for three (two nulliparous and one primiparous woman) participants out of 12. To ensure that our findings did not depend on this variable, we repeated the main analysis excluding these participants. Supplementary Methods and Results 3 show that the main findings persisted after excluding the subjects with a different RFHC.

3. Results

3.1. Region of Interest Analyses

When comparing the GM volume changes between the PRE and POST6y in the "All ROIs" mask, we found larger decreases in the mothers' group than in the nulliparous' group (p-value = 0.015). Figure 1 and Table S3 show the estimated GM volume trajectories (means and slopes) in both groups, for every ROI and every session (PRE, POST, and POST6y). Results of the early postpartum session belonged to the previous study of Hoekzema et al., 2017 [14], and are included in Figure 1 as reference. As can be observed, most of the ROIs followed the same pattern of change and remained reduced six years after parturition (POST6y-PRE). When studying the group differences at POST6y, all the ROIs survived multiple comparisons, except for the Left Hippocampus (p-value = 0.053), Left Inferior

Orbitofrontal (p-value = 0.074), and Left Superior Temporal (p-value = 0.053) that did not reach the threshold established for statistical significance.

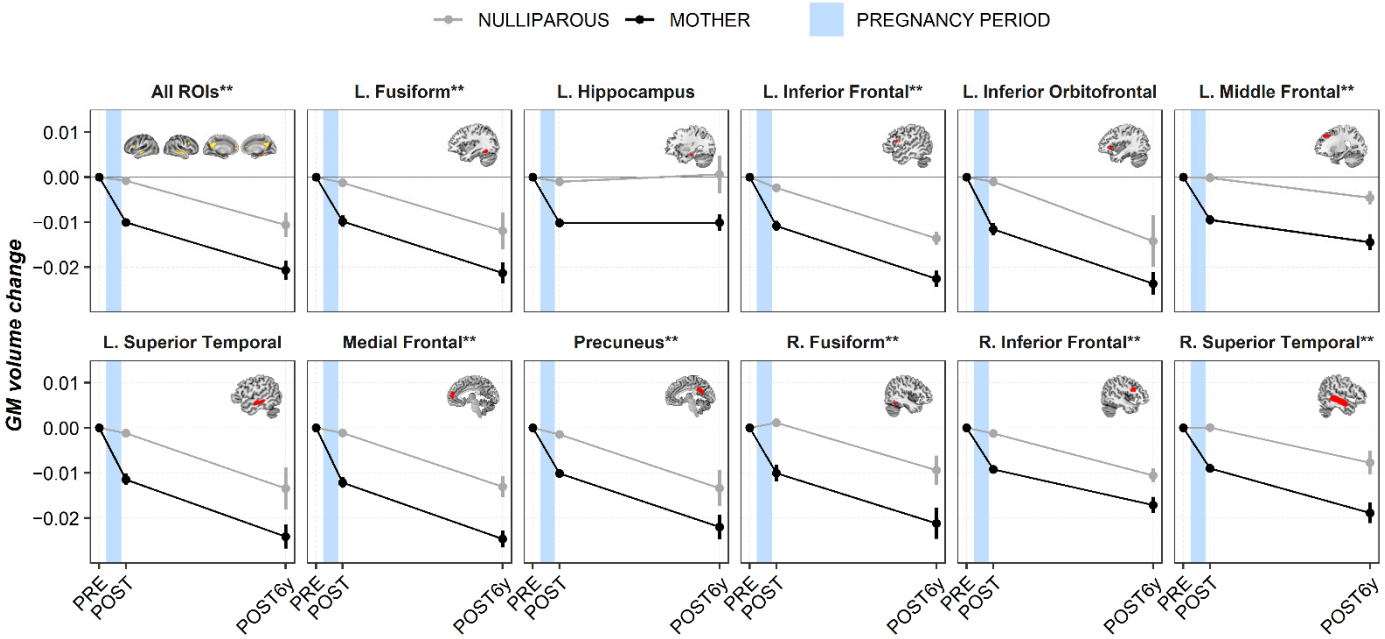

Figure 1. Gray matter volume changes for every region of interest at every session. Results of the early postpartum session were displayed as reference values [14]. Mean values (circle) with their respective standard error of the mean (vertical lines) and slopes (lines joining the circles) are represented. Black and gray lines represent mothers and nulliparous women, respectively. The blue shadow indicates the approximated period of pregnancy. Abbreviations are as follows: GM = gray matter, L. = left hemisphere, R. = right hemisphere, PRE = pre-pregnancy session, POST = early postpartum session, POST6y = six years after parturition session, and FDR = false discovery rate. ** Asterisks indicate group differences at $q < 0.05$ FDR-corrected for multiple comparisons.

3.2. Whole-Brain Analyses

Whole-brain, voxel-wise comparisons also indicated that prominent GM volume decreased in the mothers' group. As shown in Figure 2, these volume decreases affected regions within the explored ROIs, especially those of the medial wall such as the Medial Prefrontal cortex, the Precuneus, and other lateral areas outside the ROIs. We did not find any significant increase in the mothers' group or changes—either increases or decreases—within the nulliparous women.

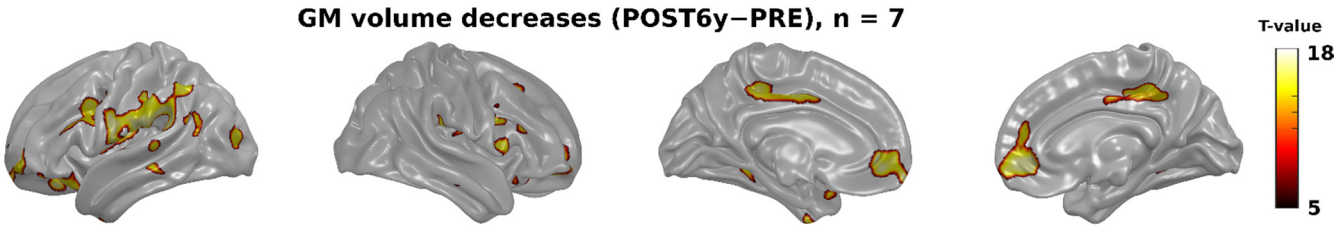

Figure 2. Gray matter volume decreases between the PRE and the POST6y sessions in mothers (n = 7), controlling for the effect of age (p-value < 0.05, FWE-corrected, and clusters bigger than 25 contiguous voxels). The vertical color bar shows the T statistical values. Abbreviations are as follows: GM = gray matter, PRE = pre-pregnancy session, POST6y = six years after parturition session, FWE = family-wise error.

3.3. Pattern Recognition Analyses

3.3.1. Classification

Figure 3 shows the results of the classification analysis. At six years after parturition, it is still possible to accurately discriminate between mothers and nulliparous women. Based on the GM volume changes between the PRE and the POST6y session, all mothers were correctly identified (class accuracy = 100%, p-value = 0.023), and only one nulliparous woman was misclassified as a mother (class accuracy = 80%, p-value = 0.059). This resulted in a total accuracy of 91.67% of women correctly classified and a group balanced accuracy of 90% (p-value $\leq$ 0.011).

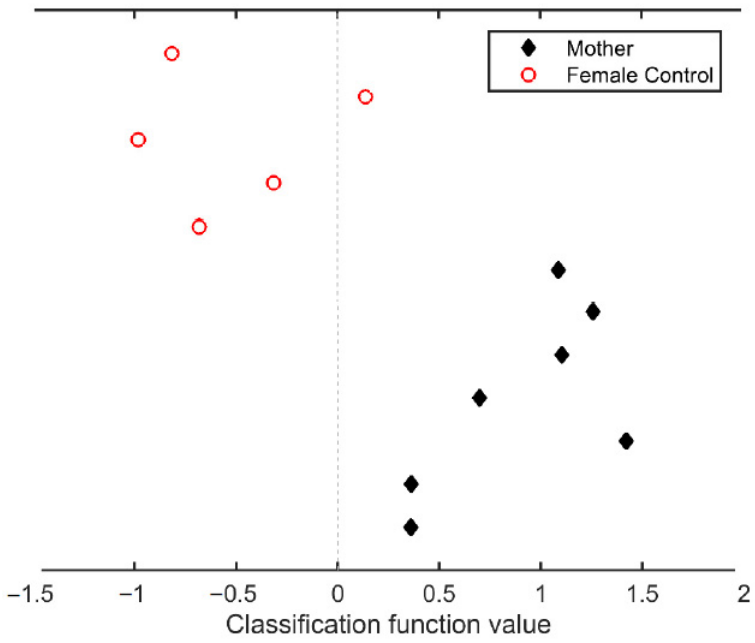

Figure 3. Classification analysis based on the gray matter volume changes between pre-pregnancy and six years after parturition sessions. Black diamonds represent the mothers, and white circles represent the nulliparous women. The dashed line is the cutoff function value between both groups. Classification function values (mean $\pm$ s.d.) for mothers and for nulliparous women are 0.899 $\pm$ 0.428 and $-$0.530 $\pm$ 0.448, respectively.

3.3.2. Regression

Regression analyses indicated that GM volume changes between the PRE and POST6y sessions significantly predicted postpartum scores of the MPAS subscale "Pleasure in Interaction" (Figure 4). Post hoc Spearman correlations indicated that the larger GM reductions at POST6y, the higher scores on the scale "Pleasure in Interaction" (Figure 5). No significant predictions were found for the MPAS subscales of "Absence of Hostility" and "Quality of Attachment".

3.4. Positive Predictive Value (PPV)

The estimated Cohen's d of the between-group differences in the GM volume change (POST6-PRE) of the "All ROIs" mask was 1.635, and the statistical power of the test was 0.768 (Supplementary Methods and Results 3). The large effect size counteracted the reduced PPV commonly associated with the reduced sample size. As indicated in Figure S3, the probability that the between-group difference in the "All ROIs" mask reflected a true effect, that is, the PPV, was 0.939.

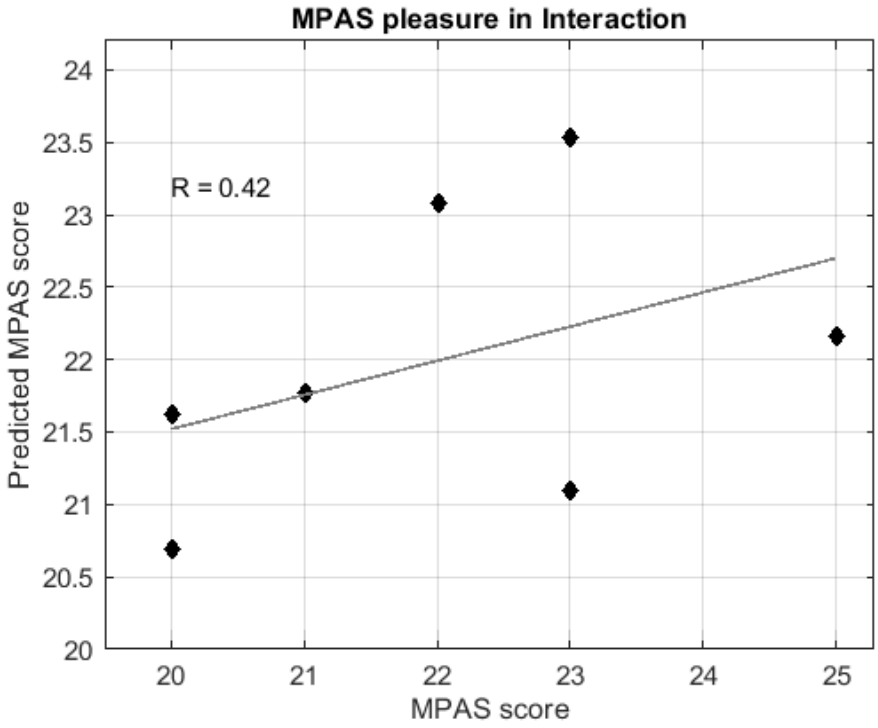

Figure 4. Regression analysis of the postpartum MPAS subscale of "Pleasure in Interaction" based on the gray matter volume changes between pre-pregnancy and six years after parturition sessions. The graph shows predicted (X-axis) versus actual "Pleasure in Interaction" MPAS score (Y-axis). The mean value (±s.d.) of the predicted score is 22.127 (±1.140), the correlation coefficient with the actual score is (R) = 0.65, *p*-value = 0.020, nMSE = 0.330, and pnMSE = 0.017. Abbreviations are as follows: MPAS = maternal postpartum attachment scale, s.d. = standard deviation, nMSE = normalized mean squared error, pnMSE = *p*-value of normalized mean squared error.

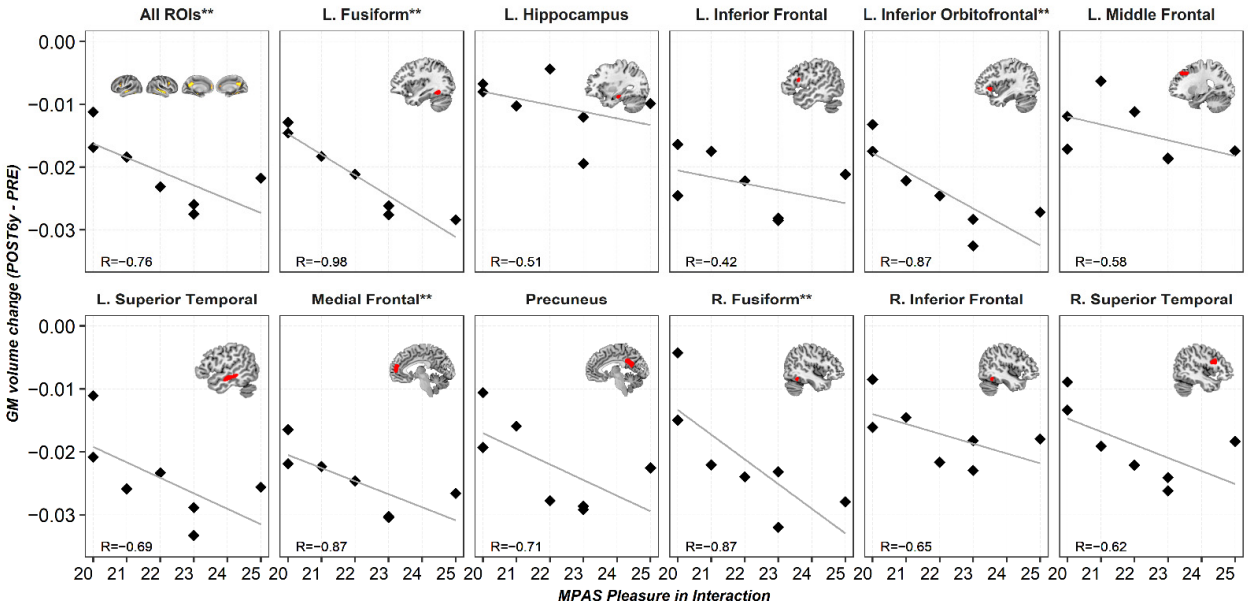

Figure 5. Scatter plots for the Spearman correlations: Y-axis represents gray matter volume changes (POST6y-PRE) for every region of interest, and X-axis represents postpartum scores in the MPAS subscale of "Pleasure in Interaction." Abbreviations are as follows: L. = left hemisphere, R. = right hemisphere, PRE = pre-pregnancy session, POST6y = six years after parturition session, MPAS = maternal postpartum attachment scale, R = Spearman correlation coefficient, and FDR = false discovery rate. ** Asterisks indicate correlations at $q < 0.05$ FDR-corrected for multiple comparisons.

4. Discussion

We found that most pregnancy-induced GM volume reductions in ToM brain regions persist at least six years after parturition. Based on GM volume changes at six years postpartum, we can classify women as having been pregnant or not with 91.67% of total accuracy. We also found that GM brain changes six years after parturition are associated with measurements of mother-to-infant attachment collected during early postpartum, supporting the hypothesis that the detected brain changes between the PRE and POST6y sessions are, indeed, related to early postpartum scores on the maternal attachment scale.

To put in perspective the following discussion, we would like to first address the main limitation of our study: the reduced sample size at POST6y. Small sample sizes are common in longitudinal studies tracking the effect of pregnancy on the brain. Aside from Hoekzema et al., 2017 [14], the other published study describing the impact of pregnancy on the human brain included data from only nine mothers, only two of whom had pre-pregnancy scans [13]. The reason behind such limitation is that scanning participants before and after pregnancy is challenging because it implies predicting the moment of conception and ensuring the viability of the pregnancies. For the current study, which was a continuation of Hoekzema et al., 2017 [14], we started with a sample of 25 primiparous women and followed them for six years. For the six-year, follow-up MRI session, we had to exclude mothers with second pregnancies, as well as female controls who became mothers. This, in addition to the typical dropout of such long longitudinal studies, led to a final sample of seven first-time mothers and five nulliparous women. To date, this sample represents the only longitudinal data set available to examine the long-term effects of pregnancy on the human brain. Statistically, we tried to minimize the caveats of small sample sizes by using more robust non-parametric tests and selecting a restrictive threshold corrected for multiple comparisons. Also, to determine the ratio of true "positive" findings, we calculated the PPV of our results. As our main effects were large, the PPV associated was also large. Specifically, the estimated probability that our significant findings indeed reflected a true effect was 0.939.

Despite the abovementioned sample size limitations, the insights provided from this unique data set indicate that pregnancy leads to GM volume reductions that are still detectable six years after parturition. This finding supports previous literature and provides new insights about the trajectories and the temporality of the brain changes accompanying motherhood. Before the current study, only six longitudinal MRI studies were designed to test the impact of motherhood on the anatomy of a woman's brain [13,15,17,26]. Whereas Kim et al., 2010 [16], Lisofsky et al., 2019 [17], and Luders et al., 2018 [26], approached the topic of motherhood by analyzing how the brain changes during the postpartum period, only Oatridge et al., 2002 [13], and Hoekzema et al., 2017 [14], included the pregnancy period. Together, these studies suggest that there are GM volume decreases during pregnancy [13,14], followed by increases after parturition [13,17,26].

There is controversy on whether GM reductions fully recover during the postpartum. According to Oatridge et al., 2002 [13], there is a reduction in brain size during pregnancy with an inflection point at parturition and a recovery at six months postpartum. On the contrary, Hoekzema et al., 2017 [14], found that the GM volume reductions detected during pregnancy persisted at least two years after parturition. As opposed to Oatridge et al., 2002 [13], in our previous study we included a control group and restricted the sample to primiparous women [14]. The inclusion of a group of nulliparous women is crucial to control for brain changes induced by aging, as well as by other possibly unknown confounding factors. Besides, it is essential to control for the effect of previous pregnancies since, according to rodent literature, the number of pregnancies affects behavior, neurobiology, and hormonal sensitivity [27]. Here, we showed that most of the GM volume reductions observed when comparing pre-pregnancy with postpartum brains are still present six years after parturition. Likewise, whole-brain analyses revealed that GM volume decreases, while no significant increases with respect to the PRE session were detected. Our results also revealed that women were classified as being mothers or not with a 91.67% total

accuracy based on GM volume changes. Every mother was correctly classified, and only one nulliparous woman was misclassified. Altogether, our data suggest that the magnitude of GM volume decreases associated with pregnancy exceeds potential increases during early postpartum and that, even six years after parturition, pregnancy-induced GM volume changes have not remitted. Literature in rodents indicates that some of the behavioral and neural changes induced by pregnancy are maintained after the weaning period [28]. Behaviorally, the dams of post-weaned rats are less anxious and fearful than virgin rats [29] and have better foraging and spatial memory skills [30–33]. Also, primiparous rats initiate maternal behavior faster when grouped with donors' pups [28], and multiparous mice retrieve pups faster than primiparous dams [34], thus illustrating the long-term priming effects of previous pregnancies. Neurally, the previous reproductive experience is known to cause long-term changes in crucial areas of the maternal brain circuit, especially in the hippocampus and hypothalamus. Specifically, the hippocampus of post-weaning primiparous rats differs from that of virgin rats in several aspects such as dendritic structure [35,36], amount of neural aging [31], and estrogens' sensitivity [37]. Besides, in mice, post-weaning primiparous dams exhibit altered hypothalamic gene expression compared to virgin mice [38].

In humans, recent cross-sectional studies also support the long-term effects of parenthood on the brain. In particular, they indicate that elderly subjects who were parents differ anatomically from those who did not have children [39,40]. Those long-term effects may be mediated by hormonal factors. Other periods of acute exposure to estrogens, such as adolescence or hormone therapy in male-to-female transgender subjects, lead to reductions in GM volume that extend beyond the period of hormonal exposure [41,42]. Indeed, some of these GM volume reductions, such as those observed during adolescence, are considered life-lasting. We recently showed that the profile of neuroanatomical changes induced by pregnancy resemble those occurring during adolescence [15]. There, we discussed the potential biological mechanisms behind these GM volume reductions [15]. Here, we showed that at least some pregnancy-induced brain changes remain several years after parturition. Findings open the possibility that brain changes induced by pregnancy are, indeed, permanent.

Besides hormonal factors, another plausible mediator of the enduring effects of motherhood on the brain is the day-to-day caring of the infant. Research has shown that the interaction with the baby during the postpartum period can impact the anatomy and functionality of the caregiver's brain, either in mothers [16,43], fathers [44,45], or foster parents [46]. The long-term maternal commitment forces women to continuously stay alert for the infants' needs and to engage multitasking strategies to take care of them. In fact, enhanced working memory has been reported long after the weaning in female rodents that both gestated and mothered the pups, but not in females that had only undergone pregnancy [47], suggesting that some of the brain changes in mothers are long-term maintained by the ongoing relationship with the child.

In addition, it is plausible that factors not directly related to pregnancy and motherhood could account for the observed brain changes. However, we believe this is unlikely. To minimize the possible effect of confounding factors, we used three strategies. First, we restricted the analysis to the ROIs that, according to the results of Hoekzema et al., 2017 [14], underwent a GM volume reduction between the pre-pregnancy and post-pregnancy sessions. Importantly, quantification analyses with all the functional brain networks (seven-network parcellation by Yeo et al., 2011 [48]) showed that these ROIs overlap with regions involved in ToM [14], a brain network that has been extensively related to maternal behavior [49]. Second, we performed a whole-brain analysis of GM volume changes (between the pre-pregnancy and the six years after parturition sessions), in which we included age as a nuisance covariate. The results indicated that the GM volume reductions detected in the current study are not driven by age variability. Finally, we tested the association between the gray matter volume reductions in the "All ROIs" mask observed at six years postpartum and a key feature of maternal care: mother-to-infant attachment. Specifically,

we analyzed whether the observed brain changes were related to the scores obtained during the early postpartum on the MPAS, a scale that measures maternal attachment. Both prediction models and correlation analysis suggest that the observed brain changes are related to maternal attachment. Specifically, the more pleasure the mother reported while interacting with her child, the greater the decrease in GM volume between sessions. Hence, although we cannot entirely discard the influence of other unknown factors, a plausible interpretation is that the brain changes found at six years postpartum are related to pregnancy-related factors.

Although more research is needed, our findings are consistent with animal research, which indicates that gray matter modifications are triggered by pregnancy and peripartum hormonal fluctuations and play a critical role in maternal care. Indeed, a recent study suggests that pregnancy might represent another time of a woman's lifespan akin to puberty, where hormonal fluctuations might have organizational effects on the brain structure [15].

5. Conclusions

In conclusion, we used a unique data set to study the long-term effects of pregnancy on the human brain. Our results illustrate that pregnancy-induced brain changes are detectable even at six years after parturition; at this period, the brain of a mother is still different from that of a nulliparous woman. In fact, based exclusively on GM volume changes, we can correctly classify women as having undergone pregnancy or not with 91.67% of total accuracy. Those brain changes seem to be related to maternal behavior, as they predict the measure of mother-to-infant attachment collected during the early postpartum. These findings open the possibility that the brain changes induced by pregnancy are lifelong and enduring.

Supplementary Materials: The following are available online at https://www.mdpi.com/2076-3425/11/2/168/s1, Figure S1: Regions where gray matter volume decreases more in the mothers' group (n = 25) than in the nulliparous control group (n = 20), Figure S2: Image processing and statistical analysis, Figure S3: Gray matter volume changes for every region of interest at every time point without software correction, Figure S4: Positive Predictive Value (PPV) of the main, and Figure S5: Gray matter volume changes for every region of interest at every time point excluding the radiofrequency head coil Table S1: Clinical and parturition data, Table S2: Group differences in cognitive tests, and Table S3: Group differences in gray matter changes.

Author Contributions: Conceptualization, M.M.-G., M.P.-D., Ó.V., E.H., and S.C.; formal analysis, M.M.-G., M.P.-D., D.M.d.B., and S.C.; funding acquisition, M.D., Ó.V., and S.C.; investigation, M.M.-G. and S.C.; methodology, E.B.-M., L.B., R.C., C.P., M.P., E.B.-P., A.M.-R., and A.B.; supervision, S.C.; visualization, M.P.-D.; writing—original draft, M.M.-G., M.P.-D., D.M.d.B., and S.C.; writing—review and editing, M.M.-G., M.P.-D, D.M.d.B., L.M.-V., A.F.-P., M.D., Ó.V., E.H., and S.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Ministerio de Ciencia, Innovación y Universidades project RTI2018-093952-B-100 and by Instituto de Salud Carlos III projects CP16/00096 and PI17/00064, and co-funded by European Regional Development Fund (ERDF), "A way of making Europe." The project exAScale ProgramIng models for extreme Data procEssing (ASPIDE) has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 801091. M.M.-G. and S.C. were funded by Ministerio de Ciencia, Innovación y Universidades, Instituto de Salud Carlos III, Predoctorales de Formación en Investigación en Salud (PFIS), contract FI18/00255 and Miguel Servet Type I research contract CP16/00096, respectively) and co-funded by European Social Fund "Investing in your future." M.P.-D. was supported by Consejería de Educación e Investigación, Comunidad de Madrid, co-funded by European Social Fund "Investing in your future" (PEJD-2018-PRE/BMD-9401). The project leading to these results has received funding from "la Caixa" Foundation under the project code LCF/PR/HR19/52160001. The Centro Nacional de Investigaciones Cardiovasculares (CNIC) is supported by the Ministerio de Ciencia, Innovación y Universidades, and the Pro CNIC Foundation, and is a Severo Ochoa Center of Excellence (SEV-2015-0505).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the local ethic committee of *l'Institut Municipal d'Assistència Sanitària* in Barcelona, Spain (CEEAH UAB 3787, 26 July 2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author, Susanna Carmona. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

Acknowledgments: We acknowledge the participants for their contribution to this study. We thank Darby Saxbe for her recommendations and careful revision of the article. Also, we thank Francisco Javier Navas for his advice on the image processing and statistical analyses.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Lambert, K.G.; Kinsley, C.H. Brain and behavioral modifications that accompany the onset of motherhood. *Parenting* **2012**, *12*, 74–88. [CrossRef]
2. Brunton, P.; Russell, J. Maternal brain adaptations in pregnancy. In *Knobil and Neill's Physiology of Reproduction: Two-Volume Set*; Plant, T., Zeleznik, A., Eds.; Elsevier: Amsterdam, The Netherlands, 2015; Volume 2, ISBN 978-0-12-397175-3.
3. Numan, M.; Young, L.J. Neural mechanisms of mother-infant bonding and pair bonding: Similarities, differences, and broader implications. *Horm. Behav.* **2016**, *77*, 98–112. [CrossRef] [PubMed]
4. Numan, M.; Woodside, B. Maternity: Neural mechanisms, motivational processes, and physiological adaptations. *Behav. Neurosci.* **2010**, *124*, 715–741. [CrossRef] [PubMed]
5. Kim, P.; Strathearn, L.; Swain, J.E. The maternal brain and its plasticity in humans. *Horm. Behav.* **2016**, *77*, 113–123. [CrossRef]
6. Atzil, S.; Hendler, T.; Zagoory-Sharon, O.; Winetraub, Y.; Feldman, R. Synchrony and specificity in the maternal and the paternal brain: Relations to oxytocin and vasopressin. *J. Am. Acad. Child Adolesc. Psychiatry* **2012**. [CrossRef]
7. Wan, M.W.; Downey, D.; Strachan, H.; Elliott, R.; Williams, S.R.; Abel, K.M. The neural basis of maternal bonding. *PLoS ONE* **2014**, *9*, e88436. [CrossRef]
8. Feldman, R. The adaptive human parental brain: Implications for children's social development. *Trends Neurosci.* **2015**, *38*, 387–399. [CrossRef]
9. Saxbe, D.; Rossin-Slater, M.; Goldenberg, D. The transition to parenthood as a critical window for adult health. *Am. Psychol.* **2018**, *73*, 1190–1200. [CrossRef]
10. Barba-Müller, E.; Craddock, S.; Carmona, S.; Hoekzema, E. Brain plasticity in pregnancy and the postpartum period: Links to maternal caregiving and mental health. *Arch. Women's Ment. Health* **2019**, *22*, 289–299. [CrossRef]
11. Cárdenas, E.F.; Kujawa, A.; Humphreys, K.L. Neurobiological changes during the peripartum period: Implications for health and behavior. *Soc. Cogn. Affect. Neurosci.* **2019**, 1–14. [CrossRef]
12. Duarte-Guterman, P.; Leuner, B.; Galea, L.A.M. The long and short term effects of motherhood on the brain. *Front. Neuroendocrinol.* **2019**, *53*, 100740. [CrossRef] [PubMed]
13. Oatridge, A.; Holdcroft, A.; Saeed, N.; Hajnal, J.V.; Puri, B.K.; Fusi, L.; Bydder, G.M. Change in brain size during and after pregnancy: Study in healthy women and women with preeclampsia. *AJNR Am. J. Neuroradiol.* **2002**, *23*, 19–26. [PubMed]
14. Hoekzema, E.; Barba-Müller, E.; Pozzobon, C.; Picado, M.; Lucco, F.; García-García, D.; Soliva, J.C.; Tobeña, A.; Desco, M.; Crone, E.A.; et al. Pregnancy leads to long-lasting changes in human brain structure. *Nat. Neurosci.* **2017**, *20*, 287–296. [CrossRef] [PubMed]
15. Carmona, S.; Martínez-García, M.; Paternina-Die, M.; Barba-Müller, E.; Wierenga, L.M.; Alemán-Gómez, Y.; Pretus, C.; Marcos-Vidal, L.; Beumala, L.; Cortizo, R.; et al. Pregnancy and adolescence entail similar neuroanatomical adaptations: A comparative analysis of cerebral morphometric changes. *Hum. Brain Mapp.* **2019**, *40*, 2143–2152. [CrossRef]
16. Kim, P.; Leckman, J.F.; Mayes, L.C.; Feldman, R.; Wang, X.; Swain, J.E. The plasticity of human maternal brain: Longitudinal changes in brain anatomy during the early postpartum period. *Behav. Neurosci.* **2010**, *124*, 695–700. [CrossRef]
17. Lisofsky, N.; Gallinat, J.; Lindenberger, U.; Kühn, S. Postpartal neural plasticity of the maternal brain: Early renormalization of pregnancy-related decreases? *Neurosignals* **2019**, *27*, 12–24. [CrossRef]
18. Victora, C.G.; de Onis, M.; Hallal, P.C.; Blossner, M.; Shrimpton, R. Worldwide timing of growth faltering: Revisiting implications for interventions. *Pediatrics* **2010**, *125*, e473–e480. [CrossRef]
19. Kim, P.; Dufford, A.J.; Tribble, R.C. Cortical thickness variation of the maternal brain in the first 6 months postpartum: Associations with parental self-efficacy. *Brain Struct. Funct.* **2018**, *223*, 3267–3277. [CrossRef]
20. Ashburner, J. Symmetric diffeomorphic modeling of longitudinal structural MRI. *Front. Neurosci.* **2013**, *6*. [CrossRef]
21. Ashburner, J.; Friston, K.J. Unified segmentation. *NeuroImage* **2005**, *26*, 839–851. [CrossRef]
22. Ashburner, J. A fast diffeomorphic image registration algorithm. *NeuroImage* **2007**, *38*, 95–113. [CrossRef] [PubMed]

23. Benjamini, Y.; Hochberg, Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B (Methodol.)* **1995**, *57*, 289–300. [CrossRef]
24. Ioannidis, J.P.A. Why most published research findings are false. *PLoS Med.* **2005**, *2*, e124. [CrossRef] [PubMed]
25. Button, K.S.; Ioannidis, J.P.A.; Mokrysz, C.; Nosek, B.A.; Flint, J.; Robinson, E.S.J.; Munafò, M.R. Power failure: Why small sample size undermines the reliability of neuroscience. *Nat. Rev. Neurosci.* **2013**, *14*, 365–376. [CrossRef] [PubMed]
26. Luders, E.; Gingnell, M.; Poromaa, I.S.; Engman, J.; Kurth, F.; Gaser, C. Potential brain age reversal after pregnancy: Younger brains at 4–6 weeks postpartum. *Neuroscience* **2018**, *386*, 309–314. [CrossRef]
27. Maupin, A.N.; Roginiel, A.C.; Rutherford, H.J.V.; Mayes, L.C. A Preliminary review of whether prior reproductive experience influences caregiving. *New Dir. Child Adolesc. Dev.* **2016**, *2016*, 73–86. [CrossRef]
28. Scanlan, V.F.; Byrnes, E.M.; Bridges, R.S. Reproductive experience and activation of maternal memory. *Behav. Neurosci.* **2006**, *120*, 676–686. [CrossRef]
29. Wartella, J.; Amory, E.; Macbeth, A.; McNamara, I.; Stevens, L.; Lambert, K.G.; Kinsley, C.H. Single or multiple reproductive experiences attenuate neurobehavioral stress and fear responses in the female rat. *Physiol. Behav.* **2003**, *79*, 373–381. [CrossRef]
30. Kinsley, C.H.; Madonia, L.; Gifford, G.W.; Tureski, K.; Griffin, G.R.; Lowry, C.; Williams, J.; Collins, J.; McLearie, H.; Lambert, K.G. Motherhood improves learning and memory. *Nature* **1999**, *402*, 137–138. [CrossRef]
31. Gatewood, J.D.; Morgan, M.D.; Eaton, M.; McNamara, I.M.; Stevens, L.F.; Macbeth, A.H.; Meyer, E.A.A.; Lomas, L.M.; Kozub, F.J.; Lambert, K.G.; et al. Motherhood mitigates aging-related decrements in learning and memory and positively affects brain aging in the rat. *Brain Res. Bull.* **2005**, *66*, 91–98. [CrossRef]
32. Love, G.; Torrey, N.; McNamara, I.; Morgan, M.; Banks, M.; Hester, N.W.; Glasper, E.R.; DeVries, A.C.; Kinsley, C.H.; Lambert, K.G. Maternal experience produces long-lasting behavioral modifications in the rat. *Behav. Neurosci.* **2005**, *119*, 1084–1096. [CrossRef] [PubMed]
33. Lemaire, V.; Billard, J.M.; Dutar, P.; George, O.; Piazza, P.V.; Epelbaum, J.; Moal, M.L.; Mayo, W. Motherhood-induced memory improvement persists across lifespan in rats but is abolished by a gestational stress. *Eur. J. Neurosci.* **2006**, *23*, 3368–3374. [CrossRef] [PubMed]
34. Lopatina, O.; Inzhutova, A.; Pichugina, Y.A.; Okamoto, H.; Salmina, A.B.; Higashida, H. Reproductive experience affects parental retrieval behaviour associated with increased plasma oxytocin levels in wild-type and cd38-knockout mice. *J. Neuroendocrinol.* **2011**, *23*, 1125–1133. [CrossRef] [PubMed]
35. Kinsley, C.H.; Trainer, R.; Stafisso-Sandoz, G.; Quadros, P.; Marcus, L.K.; Hearon, C.; Meyer, E.A.A.; Hester, N.; Morgan, M.; Kozub, F.J.; et al. Motherhood and the hormones of pregnancy modify concentrations of hippocampal neuronal dendritic spines. *Horm. Behav.* **2006**, *49*, 131–142. [CrossRef]
36. Pawluski, J.L.; Galea, L.A.M. Hippocampal morphology is differentially affected by reproductive experience in the mother. *J. Neurobiol.* **2006**, *66*, 71–81. [CrossRef]
37. Barha, C.K.; Galea, L.A.M. Motherhood alters the cellular response to estrogens in the hippocampus later in life. *Neurobiol. Aging* **2011**, *32*, 2091–2095. [CrossRef]
38. Arbeitman, M.N. Maternal experience leads to lasting gene expression changes in some regions of the mouse brain. *G3 Genes Genomes Genet.* **2019**, *9*, 2623–2628. [CrossRef]
39. De Lange, A.-M.G.; Kaufmann, T.; Van de Meer, D.; Maglanoc, L.; Alnæs, D.; Moberget, T.; Douaud, G.; Andreassen, O.A.; Westlye, L.T. Population-based neuroimaging reveals traces of childbirth in the maternal brain. *bioRxiv* **2019**, *116*, 22341–22346. [CrossRef]
40. Orchard, E.R.; Ward, P.G.; Sforazzini, F.; Storey, E.; Egan, G.F.; Jamadar, S.D. Cortical changes associated with parenthood are present in late life. *bioRxiv* **2019**. [CrossRef]
41. Sisk, C.L.; Zehr, J.L. Pubertal hormones organize the adolescent brain and behavior. *Front. Neuroendocrinol.* **2005**, *26*, 163–174. [CrossRef]
42. Zubiaurre-Elorza, L.; Junque, C.; Gómez-Gil, E.; Guillamon, A. Effects of cross-sex hormone treatment on cortical thickness in transsexual individuals. *J. Sex. Med.* **2014**, *11*, 1248–1261. [CrossRef] [PubMed]
43. Parsons, C.E.; Young, K.S.; Petersen, M.V.; Jegindoe Elmholdt, E.-M.; Vuust, P.; Stein, A.; Kringelbach, M.L. Duration of motherhood has incremental effects on mothers' neural processing of infant vocal cues: A neuroimaging study of women. *Sci. Rep.* **2017**, *7*, 1727. [CrossRef] [PubMed]
44. Abraham, E.; Hendler, T.; Shapira-Lichter, I.; Kanat-Maymon, Y.; Zagoory-Sharon, O.; Feldman, R. Father's brain is sensitive to childcare experiences. *Proc. Natl. Acad. Sci. USA* **2014**. [CrossRef] [PubMed]
45. Kim, P.; Rigo, P.; Mayes, L.C.; Feldman, R.; Leckman, J.F.; Swain, J.E. Neural plasticity in fathers of human infants. *Soc. Neurosci.* **2014**. [CrossRef] [PubMed]
46. Grasso, D.J.; Moser, J.S.; Dozier, M.; Simons, R. ERP correlates of attention allocation in mothers processing faces of their children. *Biol. Psychol.* **2009**, *81*, 95–102. [CrossRef] [PubMed]
47. Pawluski, J.L.; Galea, L.A.M. Reproductive experience alters hippocampal neurogenesis during the postpartum period in the dam. *Neuroscience* **2007**, *149*, 53–67. [CrossRef]

48. Yeo, B.T.T.; Krienen, F.M.; Sepulcre, J.; Sabuncu, M.R.; Lashkari, D.; Hollinshead, M.; Roffman, J.L.; Smoller, J.W.; Zollei, L.; Polimeni, J.R.; et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *J. Neurophysiol.* **2011**, *106*, 1125–1165. [CrossRef]
49. Schurz, M.; Radua, J.; Aichhorn, M.; Richlan, F.; Perner, J. Fractionating theory of mind: A meta-analysis of functional brain imaging studies. *Neurosci. Biobehav. Rev.* **2014**, *42*, 9–34. [CrossRef]

7. GENERAL DISCUSSION

This final chapter discusses the impact of the research of the present thesis on the parental brain field, health system and society as a whole. A detailed discussion of the specific results of each study can be found in the articles. The section begins by providing a broader view of the significance of the findings. Then, it discusses the potential neuroplasticity mechanisms operating in the human maternal brain. Finally, the chapter describes the limitations of the current thesis and proposes several future lines of research.

7.1. Significance and impact of the findings

In Study 1, a detailed surface-based morphometric analysis unveiled, for the first time, the effects of pregnancy on the cortical mantle and sulcal anatomy of the human cerebral cortex (Carmona et al., 2019). The exact brain morphological measures are depicted in Figure 1 of the article. The study analyzed the MRI prospective data of a group of 25 female adolescents who had never been pregnant, a group of 25 first-time adult mothers, and a group of 20 adult females who had never been pregnant. The results reveal that the pre-to-post pregnancy gray matter reductions initially found by Hoekzema et al., (2017) reflect a flattening of the cortex similar to that taking place during adolescence, that is, a thinner and less gyrified cortex with shorter, narrower, and shallower sulci (Figure 2, Study 1). These results have two major implications in the field of parenting and neuroplasticity. First, this study suggests that the phenomenon “matrescence”, coined by the anthropologist Dana Raphael in the 1970s, might indeed have a neurobiological basis. Just as adolescence describes a teenager's transition to adulthood, “matrescence” describes a woman's transition to motherhood and all the psychological and cognitive changes that it brings (Raphael, 2011). Study 1 shows that the similarities between the adolescence and motherhood transitions are also evident at the neuroanatomic level; that both periods involve an alike flattening of the cortex. Many studies propose that gray matter volume reductions during adolescence reflect a pruning or selective elimination of synaptic boutons (Huttenlocher, 1979; Huttenlocher & Dabholkar, 1997) and the surrounding glial cells and vasculature elements (Mills & Tamnes, 2014). Whether the neural mechanisms that govern the cortical flattening of adolescence can also apply to pregnancy is still under debate (see section 7.2 for further information about the plasticity

mechanisms that have been proposed to subserve human maternal brain changes). Second, Study 1 reports similar neuroanatomic changes during two periods of significant increases in steroid hormones, which are key regulators of neuroplasticity, memory, and behavior (Galea et al., 2017). These findings suggest that pregnancy, like adolescence, may be a "sensitive period" when steroid hormonal priming triggers an augmented state of neuroplasticity that serves an adaptive purpose for the cognitive and emotional challenges posed by motherhood.

While the results of Study 1 support the view that steroid hormones are the primary mediators of maternal brain changes, experience-dependent factors associated with approaching parenthood can also induce brain plasticity. Among these experience factors is the interaction with the baby after birth. Study 2 aimed to isolate the experience-induced effects on the parental brain by following the brains of two overseas samples of first-time fathers participating in parenting, since they experience the cognitive and emotional demands of caring for a newborn without going through pregnancy (Martínez-García et al., 2022b). Two samples of first-time fathers, one in Spain and the other in California, were scanned before and after the delivery of their partners and were compared with a control group of childless men. This study demonstrates that the parental brain structural adaptations in mothers and fathers share common bases but also differ in some ways. On the one hand, the results of the study indicate that, similarly to mothers, both samples of first-time fathers show significant cortical reductions across fatherhood. These changes are, however, less marked than those previously seen in mothers after their first pregnancy. While first-time mothers display a 2.6% reduction in total cortical volume (Study 1), Californian and Spanish fathers display a 1.14 % and 0.76% reduction, respectively (Study 2). These findings suggest that both gestational factors and the postpartum experience as a parent can trigger structural neuroplasticity in the parental brain. Fathers' cortical reductions are located in brain networks linked to social cognition and visual processing. One interpretation is that such reductions reflect a fine-tuning of brain areas that control behaviors required for childrearing such as sustained attention and mentalizing. In this article, there were no specific measures of participants' paternal involvement to indicate if there was any relationship between father-infant interaction and parental brain plasticity, but other studies have suggested that the caregiving practice and father-to-infant feelings can help develop the paternal brain both at the structural

(Kim et al., 2014) and functional levels (Abraham et al., 2014). On the other hand, Study 2 finds unique brain structural adaptations in fathers. In mothers, literature has consistently reported structural changes in cortical circuits involved in social cognition, as well as in subcortical circuits associated with motivation and reward processing (Martínez-García et al., 2021a). Conversely, according to Study 2 fathers exhibit changes in cortical networks but preservation of subcortical structures. According to these findings, postpartum parenting may induce plasticity primarily in cortical networks that facilitate the cognitive and emotional demands of parenting, while subcortical circuits may be more sensitive to pregnancy-related factors. The findings suggest that fathers who spend time at home after birth become more attuned fathers, which has tremendous implications for family policies and parental leave plans. In fact, this study, and others from our team (Paternina-Die et al., 2020), have appeared in opinion articles of prestigious magazines (New York Times, Medscape) to explain why parenting responsibilities and paid family leave is not just a women's issue, but one that concerns all caregivers. The article derived from Study 2 has received high online interest, ranking among the top 5% of all research outputs monitored by Altmetrics.

Lastly, Study 3 tracked the durability of the maternal brain changes beyond the period of maximal maternal investment (Martínez-García et al., 2021b). It is becoming increasingly apparent from brain aging studies that motherhood may have a long-lasting impact on the brain (de Lange, Barth, Kaufmann, Anatürk, et al., 2020; de Lange et al., 2019). Yet longitudinal studies have not explored the maternal brain beyond the first two years postpartum. Trying to fill this gap of knowledge, Study 3 re-contacted primiparous mothers of the study of Hoekzema., (2017) for a follow-up MRI scanning session at six years postpartum. Despite its small sample size (seven mothers and five controls), this study represents the only longitudinal available dataset exploring the long-term effects of pregnancy on the human brain. Results indicate that most pregnancy-induced gray matter volume reductions have not remitted six years after parturition. In combination with the results of Study 1, these findings suggest that pregnancy is another time of a woman's life similar to adolescence, when hormones might have profound organizational effects on the brain structure that extend far beyond the period of hormonal exposure. Besides hormonal factors, changes may be long-term maintained by the ongoing relationship and interaction with the child. In any case, the long-term effects of motherhood on the brain

suggest that parenting-related variables should be routinely included in neuroimaging studies, especially when studying healthy and disrupted brain aging trajectories. As of now, the UK Biobank is the only publicly available large-scale MRI database that collects the reproductive history of middle-aged participants. This resource has led to numerous findings of the female-specific traits of brain aging (de Lange, Barth, Kaufmann, Anatürk, et al., 2020; de Lange, Barth, Kaufmann, Maximov, et al., 2020; de Lange et al., 2019; Voldsbekk et al., 2021). Initiatives like this will advance our understanding of women's health, which has been overlooked in the past.

The studies comprising this thesis are unique in their well-controlled design, which provides valuable insight into the impact of motherhood on the female brain. First, in contrast to the vast majority of cross-sectional studies comparing parents and nonparents at a single postpartum time-point, the study designs used in this thesis are longitudinal, following the same subjects at different time-points during the parental transition. Longitudinal designs provide more accurate information about brain changes and require far fewer participants than cross-sectional designs, since they control for natural inter-individual variations (Mills & Tamnes, 2014). Second, while most longitudinal studies have scanned parents during the postpartum, the studies of this thesis capture the entire parental transition by scanning participants before conception and then again at different postpartum stages. This strategy is especially challenging because it depends on the success of conception and pregnancy, which inevitably leads to a slow recruitment and dropouts. Third, to minimize the noise caused by image acquisition, age, and other confounding variables, these longitudinal studies included a comparison group of non-parents, which although necessary is unusual in the parental brain literature. Fourth, this thesis has restricted the sample of study to first-time parents, which adds another layer of complexity to recruitment. Lastly, some of these studies derive from international collaborations that have enabled the inclusion of longitudinal samples with additional valuable information, such as the sample of female adolescents (Study 1) and the sample of first-time Californian fathers (Study 2). All in all, it has been costly and time-consuming to implement these studies because they require the collection of large amounts of initial data and long periods of time between acquisitions. Yet, these unique naturalistic observational experiments in humans have paved the way for understanding the structural neuroplasticity of the human parental brain.

In the last years, the articles of the present thesis have been present in the public debate of motherhood and perinatal mental health. They have been broadcasted in high-profile media outlets such as The New York Times, The Atlantic, The Economist and The Conversation. In Spain, these articles have been promoted via the "Instituto Europeo de Salud Mental Perinatal", high-reach podcasts such as "Carne Cruda" and "A vivir que son dos días", and social media channels such as Instagram and Twitter. The audience our findings have reached is diverse, from parents and parents-to-be to professionals such as gynecologists, obstetricians, psychiatrists, midwives, nurses, or psychologists. While some media have misread our findings as reflecting a negative "shrinkage" and cognitive impairments (Norrmén-Smith et al., 2021), most have seen them as proof of the many benefits of motherhood (The Atlantic).

7.2. Proposed neuroplasticity mechanisms

In all three articles reviewed by this thesis, reported brain changes point in the same direction of reductions in gray matter volume. These structural brain changes have been raised to reflect a neural adjustment and a hallmark for neuroplasticity (Been et al., 2021, p. 202). Perhaps it is unexpected that gray matter reductions would lead to a functional improvement. In fact, media have often misinterpreted maternal brain findings (Norrmén-Smith et al., 2021), mistakenly linking volume reductions to the popular perception of decreased cognitive abilities in pregnancy, colloquially referred to as 'mommy brain' or 'baby brain' (Pownall et al., 2021). These headlines were written under the assumption that neuroplasticity is only supported by proliferative events. However, contrary to popular belief, structural decreases within the nervous system are not always linked to behavioral deficits such as those observed in aging or neurodegenerative disorders (Pawluski et al., 2021). As explained in section 3.1 of the Introduction, pruning synapses, and surrounding glial and vascular elements promotes healthy behavioral outcomes during developmental stages such as adolescence. Similarly, the observed gray matter volume reductions observed during the parental transition have been found coupled with behavioral changes that improve the subjects' ability to deal with the challenges ahead. In particular, in both human mothers and fathers, gray matter is associated with greater

responsiveness and attunement towards the newborn during the postpartum period (Hoekzema et al., 2020; Paternina-Die et al., 2020).

Currently, researchers are unable to determine the microscopic mechanisms underlying the changes in mothers' human brain structures, but ex vivo non-human animal research can provide some hypotheses. Typical MRI anatomical images of the human brain have a spatial resolution of approximately 1 cubic millimeter (mm^3), a volume that contains thousands of neurons and glial cells, as well as neuronal processes, blood vessels, intracortical myelin, and dendritic spines (Mills & Tamnes, 2014). While this resolution is adequate for capturing major neuroanatomical trends, it is not enough for capturing molecular and cellular processes. Histological experiments conducted on rodents are better suited to examine neuroplasticity.

Rodent studies indicate that the parental transition comprises multiscale neuroplasticity changes that reflect a ‘fine tuning’ of the brain (Pawluski et al., 2021). At the molecular level, synaptic plasticity is enhanced with motherhood (Hillerer et al., 2014; Pereira, 2016). Neuroplastic changes include morphological modifications such as increased dendritic arborization (Haim et al., 2014; Hillerer et al., 2018; Pawluski et al., 2012; Salmaso et al., 2011; Stern & Armstrong, 1998) and spine density (Haim et al., 2014; Hillerer et al., 2018; Kinsley et al., 2006; Opala et al., 2019; Salmaso et al., 2011), which enable neuronal circuits to establish new synapses. Plasticity in the rodent maternal brain also involves larger scale changes in the number of neuronal and glial brain cells, namely decreases in neurogenesis (Leuner & Sabihi, 2016) and microglial proliferation (Haim et al., 2017), as well as increased myelin production and repair (Gregg et al., 2007; Kalakh & Mouihate, 2019). Among all these indicators of neuroplasticity, changes in neuronal and glial cell number are likely to translate directly into the large-scale gray matter reductions observed in human mothers, given the scale of change they entail compared to molecular and morphological modifications. Microglial and neurogenesis decreases would directly translate into gray matter reductions, whereas myelination would cause an apparent decrease in gray matter by misclassifying voxels at tissue interfaces as white matter. As in rodents, it is possible that the transition to motherhood also leads to molecular and morphological brain changes, but these may contribute very little to macroscopically visible structural changes.

7.3. Limitations and future directions

The longitudinal studies that compose this doctoral thesis offer a unique lens into the structural plasticity of the human maternal brain. Findings of Study 1 (Carmona et al., 2019), 2 (Martínez-García et al., 2022b) and 3 (Martínez-García et al., 2021b) provide valuable insight into the morphological details, mediating factors, and durability of brain changes in human mothers. Still, these studies share a number of caveats that difficult to entirely resolve these questions. This section discusses the main limitations of the studies and how current and future projects overcome these constraints.

First, the sample sizes of the three current studies are relatively small. This reduced sample size is especially notable in Study 3, which includes seven first-time mothers at six years postpartum and five nulliparous women (Martínez-García et al., 2021b). This follow-up study needs to be replicated with larger samples. In addition, the candidate is co-coordinating a multimodal MRI study (hereafter referred as "the multimodal project") that currently includes more than 400 primiparous women evaluated at different time points of their motherhood transition. These larger samples will increase the sensitivity to detect meaningful changes in the maternal brain not captured by the current studies.

One key research line in the parental brain is determining the temporal course of the motherhood-induced brain changes. Study 1 (Carmona et al., 2019) and 3 (Martínez-García, et al., 2021b) of this thesis scanned mothers before pregnancy and after delivery, thus cannot disentangle with certainty whether the gray matter volume reductions were triggered during pregnancy, delivery or postpartum. Other scholars have scanned mothers at two times during the postpartum (Kim et al., 2010; Lisofsky et al., 2019; Luders et al., 2020; Zhang et al., 2020), leaving a critical gap of knowledge during pregnancy and parturition. In a recent published review article, the candidate combined findings from longitudinal studies that had tracked the brain anatomy of mothers thus far and inferred a dynamic trajectory of initial gray matter decline followed by an increase during postpartum (Martínez-García, et al., 2021a). For researchers to be able to confirm these proposed brain trajectories, it is crucial to scan participants multiple times during pregnancy and postpartum. For this purpose, the multimodal project is scanning

participants five times across the motherhood transition: before pregnancy, during the second and third trimesters, and at one and six months postpartum.

It is also imperative to clarify the gestational factors that coordinate the brain remodeling of the human maternal brain. Murine models indicate that maternal brain changes are facilitated by pregnancy-related hormonal fluctuations (Numan, 2020, Chapter 3). However, the studies of this thesis were unable to evaluate the hormonal correlates of the maternal brain changes, since there were no assessments of pregnancy hormone levels. In order to fill this gap in humans, the multimodal project will evaluate the inter-subject variability in brain changes associated with peripheral hormonal. Also, recent findings point to immune activity fluctuations during pregnancy as an additional potential trigger of maternal brain remodeling (Haim et al., 2017). The pregnancy-related immune adaptations seem to also extend to the central nervous system, populated by immunocompetent macrophage glial cells known as microglia (Prinz et al., 2021). Future studies need to explore the contribution of peripheral and central immune markers on maternal brain plasticity. As for experiential factors, the studies of this thesis lack measures of parenting (Carmona et al., 2019; Martínez-García et al., 2021b; Martínez-García et al., 2022b). The multimodel project is collecting more precise quantitative and qualitative measures of maternal investment, which will shed light on the amount of brain plasticity induced by the parenting experience. At the same time, the sleep quality and stress levels will be considered as potential contributors of the maternal brain changes, especially during the postpartum. Finally, the addition of a group of non-gestational mothers in future projects will allow to capture the influence of extrinsic postpartum factors while also excluding potential influences of sex and gender, complementing the findings of Study 2.

Of note, this thesis focuses on gray matter tissue changes, but white matter integrity, myelination, and functional connectivity among maternal brain regions of interest are also likely to be affected during the motherhood transition. The multimodal MRI sequences of the project (anatomical, resting-state, diffusion, and spectroscopy) will allow to characterize the motherhood transition in terms of brain morphometry, white matter microstructure, functional and structural connectivity. Furthermore, multimodal MRI acquisitions will reveal potential neuroplasticity mechanisms behind the macroscopic

MRI brain changes observed in mothers, which cannot be detected by the T1-weighted anatomical images of the current thesis. For instance, spectroscopy will quantify markers of neuronal and glial activity and processes such as cell proliferation or apoptosis, diffusion MRI will be used to estimate white matter integrity, fiber orientation, myelin density, and axon diameters, and resting-state fMRI will inform about different patterns of functional connectivity.

Lastly, the studies of this thesis focused on delineating the healthy pattern of brain adaptations in mothers. The multimodal project will be novel in evaluating how the deviations of these brain adaptations are related to the emergence of postpartum mental health disorders. Specifically, the project will compare the profile of brain changes in mothers with and without postpartum mental health disorders. Postpartum mental health will also be explored in relation to different birth courses and birth experiences, since the degree of intervention and the mother's psychological and physiological experience during labor can all affect the mother's mental health postpartum.

8. CONCLUSION

In the last 20 years, our understanding of the human maternal brain has grown considerably. The neuroendocrinology of maternal behavior in rodents was established before the 20th century. A new generation of maternal brain researchers has demonstrated groundbreaking evidence that becoming a mother impacts a woman's brain structurally and functionally. Through longitudinal neuroimaging designs with first-time parents, the present thesis contributed to characterize brain changes in first-time mothers in terms of cortical morphometry, mediating factors, and durability. The main findings of the studies are summarized below:

- **Study 1:** The pre-to-post pregnancy gray matter reductions initially found by Hoekzema et al., (2017) reflect a flattening of the cortex similar to that taking place during adolescence, that is, a thinner and less gyrified cortex with shorter, narrower, and shallower sulci (Carmona et al., 2019).
- **Study 2:** First-time fathers show cortical reductions across fatherhood which are less marked than those seen in mothers after their first pregnancy. Brain adaptations in first-time parents can be triggered both by gestational factors and by postpartum experiences, but gestational factors play a greater role (Martínez-García, et al., 2022b).
- **Study 3:** Most gray matter reductions in first-time mothers do not remit six years after delivery, raising the possibility that they may be permanent (Martínez-García, et al., 2021b).

Altogether, these findings suggest that becoming a mother leads to a prolonged state of neuroplasticity that supports the pending challenges of motherhood. This thesis aligns with the recent tradition of rewriting the history of motherhood as a period of gains rather than losses.

9. REFERENCES

Abraham, E., & Feldman, R. (2018). The neurobiology of human allomaternal care; implications for fathering, coparenting, and children's social development. *Physiology and Behavior*. https://doi.org/10.1016/j.physbeh.2017.12.034

Abraham, E., & Feldman, R. (2022). The Neural Basis of Human Fatherhood: A Unique Biocultural Perspective on Plasticity of Brain and Behavior. *Clinical Child and Family Psychology Review*, *25*(1), 93–109. https://doi.org/10.1007/s10567-022-00381-9

Abraham, E., Hendler, T., Shapira-Lichter, I., Kanat-Maymon, Y., Zagoory-Sharon, O., & Feldman, R. (2014). Father's brain is sensitive to childcare experiences. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.1402569111

Aleknaviciute, J., Evans, T. E., Aribas, E., de Vries, M. W., Steegers, E. A. P., Ikram, M. A., Tiemeier, H., Kavousi, M., Vernooij, M. W., & Kushner, S. A. (2022). Long-term association of pregnancy and maternal brain structure: The Rotterdam Study. *European Journal of Epidemiology*, *37*(3), 271–281. https://doi.org/10.1007/s10654-021-00818-5

Almey, A., Milner, T. A., & Brake, W. G. (2015). Estrogen receptors in the central nervous system and their implication for dopamine-dependent cognition in females. *Hormones and Behavior*, *74*, 125–138. https://doi.org/10.1016/j.yhbeh.2015.06.010

American Psychiatric Association. (2013). *Dsm-v Manual 5th Edition*.

Anderson, S. M., MacLean, P. S., McManaman, J. L., & Neville, M. C. (2015). Chapter 46—Lactation and its Hormonal Control. In T. M. Plant & A. J. Zeleznik (Eds.), *Knobil and Neill's Physiology of Reproduction (Fourth Edition)* (pp. 2055–2105). Academic Press. https://doi.org/10.1016/B978-0-12-397175-3.00046-6

Attwell, D., & Iadecola, C. (2002). The neural basis of functional brain imaging signals. *Trends in Neurosciences*, *25*(12), 621–625. https://doi.org/10.1016/S0166-2236(02)02264-6

Backhausen, L. L., Herting, M. M., Tamnes, C. K., & Vetter, N. C. (2021). Best Practices in Structural Neuroimaging of Neurodevelopmental Disorders. *Neuropsychology Review*. https://doi.org/10.1007/s11065-021-09496-2

Bak, Y., Nah, Y., Han, S., Lee, S.-K., & Shin, N.-Y. (2020). Altered neural substrates within cognitive networks of postpartum women during working memory process and

resting-state. *Scientific Reports*, *10*(1), 9110. https://doi.org/10.1038/s41598-020-66058-x

Banks, W. A. (2012). Brain meets body: The blood-brain barrier as an endocrine interface. *Endocrinology*, *153*(9), 4111–4119. https://doi.org/10.1210/en.2012-1435

Barth, C., & de Lange, A.-M. G. (2020). Towards an understanding of women's brain aging: The immunology of pregnancy and menopause. *Frontiers in Neuroendocrinology*, *58*, 100850. https://doi.org/10.1016/j.yfrne.2020.100850

Been, L. E., Sheppard, P. A. S., Galea, L. A. M., & Glasper, E. R. (2021). Hormones and neuroplasticity: A lifetime of adaptive responses. *Neuroscience & Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2021.11.029

Beeri, M. S., Rapp, M., Schmeidler, J., Reichenberg, A., Purohit, D. P., Perl, D. P., Grossman, H. T., Prohovnik, I., Haroutunian, V., & Silverman, J. M. (2009). Number of children is associated with neuropathology of Alzheimer's disease in women. *Neurobiology of Aging*, *30*(8), 1184–1191. https://doi.org/10.1016/j.neurobiolaging.2007.11.011

Bethlehem, R. A. I., Seidlitz, J., White, S. R., Vogel, J. W., Anderson, K. M., Adamson, C., Adler, S., Alexopoulos, G. S., Anagnostou, E., Areces-Gonzalez, A., Astle, D. E., Auyeung, B., Ayub, M., Bae, J., Ball, G., Baron-Cohen, S., Beare, R., Bedford, S. A., Benegal, V., … VETSA. (2022). Brain charts for the human lifespan. *Nature*, *604*(7906), 525–533. https://doi.org/10.1038/s41586-022-04554-y

Bjertrup, A. J., Friis, N. K., & Miskowiak, K. W. (2019). The maternal brain: Neural responses to infants in mothers with and without mood disorder. *Neuroscience & Biobehavioral Reviews*, *107*, 196–207. https://doi.org/10.1016/j.neubiorev.2019.09.011

Blakemore, S.-J. (2008). The social brain in adolescence. *Nature Reviews Neuroscience*, *9*(4), 267–277. https://doi.org/10.1038/nrn2353

Bridges, R. S. (1984). A quantitative analysis of the roles of dosage, sequence, and duration of estradiol and progesterone exposure in the regulation of maternal behavior in the rat. *Endocrinology*, *114*(3), 930–940. https://doi.org/10.1210/endo-114-3-930

Brinton, R. D. (2009). Estrogen-induced plasticity from cells to circuits: Predictions for cognitive function. *Trends in Pharmacological Sciences*, *30*(4), 212–222. https://doi.org/10.1016/j.tips.2008.12.006

Busnelli, M., & Chini, B. (2018). Molecular Basis of Oxytocin Receptor Signalling in the Brain: What We Know and What We Need to Know. *Current Topics in Behavioral*

Neurosciences, *35*, 3–29. https://doi.org/10.1007/7854_2017_6

Carmona, S., Martínez-García, M., Paternina-Die, M., Barba-Müller, E., Wierenga, L. M., Alemán-Gómez, Y., Pretus, C., Marcos-Vidal, L., Beumala, L., Cortizo, R., Pozzobon, C., Picado, M., Lucco, F., García-García, D., Soliva, J. C., Tobeña, A., Peper, J. S., Crone, E. A., Ballesteros, A., … Hoekzema, E. (2019). Pregnancy and adolescence entail similar neuroanatomical adaptations: A comparative analysis of cerebral morphometric changes. *Human Brain Mapping*, *40*(7), 2143–2152. https://doi.org/10.1002/hbm.24513

Chechko, N., Dukart, J., Tchaikovski, S., Enzensberger, C., Neuner, I., & Stickel, S. (2021). The Expectant Brain–Pregnancy Leads to Changes in Brain Morphology in the Early Postpartum Period. *Cerebral Cortex*, bhab463. https://doi.org/10.1093/cercor/bhab463

Colucci, M., Cammarata, S., Assini, A., Croce, R., Clerici, F., Novello, C., Mazzella, L., Dagnino, N., Mariani, C., & Tanganelli, P. (2006). The number of pregnancies is a risk factor for Alzheimer's disease. *European Journal of Neurology*, *13*(12), 1374–1377. https://doi.org/10.1111/j.1468-1331.2006.01520.x

Crapser, J. D., Arreola, M. A., Tsourmas, K. I., & Green, K. N. (2021). Microglia as hackers of the matrix: Sculpting synapses and the extracellular space. *Cellular & Molecular Immunology*, *18*(11), 2472–2488. https://doi.org/10.1038/s41423-021-00751-3

de Lange, A.-M. G., Barth, C., Kaufmann, T., Anatürk, M., Suri, S., Ebmeier, K. P., & Westlye, L. T. (2020). The maternal brain: Region-specific patterns of brain aging are traceable decades after childbirth. *Human Brain Mapping*, *41*(16), 4718–4729. https://doi.org/10.1002/hbm.25152

de Lange, A.-M. G., Barth, C., Kaufmann, T., Maximov, I. I., van der Meer, D., Agartz, I., & Westlye, L. T. (2020). Women's brain aging: Effects of sex-hormone exposure, pregnancies, and genetic risk for Alzheimer's disease. *Human Brain Mapping*, *41*(18), 5141–5150. https://doi.org/10.1002/hbm.25180

de Lange, A.-M. G., Kaufmann, T., van der Meer, D., Maglanoc, L. A., Alnæs, D., Moberget, T., Douaud, G., Andreassen, O. A., & Westlye, L. T. (2019). Population-based neuroimaging reveals traces of childbirth in the maternal brain. *Proceedings of the National Academy of Sciences*, *116*(44), 22341–22346. https://doi.org/10.1073/pnas.1910666116.

Deems, N. P., & Leuner, B. (2020). Pregnancy, postpartum and parity: Resilience and vulnerability in brain health and disease. *Frontiers in Neuroendocrinology*, *57*, 100820. https://doi.org/10.1016/j.yfrne.2020.100820

Dinç, H., Esen, F., Demirci, A., Sari, A., & Resit Gümele, H. (1998). Pituitary dimensions and volume measurements in pregnancy and post partum. MR assessment. *Acta Radiologica (Stockholm, Sweden: 1987)*, *39*(1), 64–69. https://doi.org/10.1080/02841859809172152

Dufford, A. J., Erhart, A., & Kim, P. (2019). Maternal Brain Resting-State Connectivity in the Postpartum Period. *Journal of Neuroendocrinology*, *31*(9), e12737. https://doi.org/10.1111/jne.12737

Duthie, L., & Reynolds, R. M. (2013). Changes in the Maternal Hypothalamic-Pituitary-Adrenal Axis in Pregnancy and Postpartum: Influences on Maternal and Fetal Outcomes. *Neuroendocrinology*, *98*(2), 106–115. https://doi.org/10.1159/000354702

Fahrbach, S. E., Morrell, J. I., & Pfaff, D. W. (1985). Possible role for endogenous oxytocin in estrogen-facilitated maternal behavior in rats. *Neuroendocrinology*, *40*(6), 526–532. https://doi.org/10.1159/000124125

Feldman, R. (2015). The adaptive human parental brain: Implications for children's social development. *Trends in Neurosciences*, *38*(6), 387–399. https://doi.org/10.1016/j.tins.2015.04.004

Galea, L. A. M., Frick, K. M., Hampson, E., Sohrabji, F., & Choleris, E. (2017). Why estrogens matter for behavior and brain health. *Neuroscience and Biobehavioral Reviews*, *76*(Pt B), 363–379. https://doi.org/10.1016/j.neubiorev.2016.03.024

García-Segura, L. M. (2009). 3Hormones and the Mutable Brain. In *Hormones and Brain Plasticity*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780195326611.003.0001

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., Paus, T., Evans, A. C., & Rapoport, J. L. (1999). Brain development during childhood and adolescence: A longitudinal MRI study. *Nature Neuroscience*, *2*(10), 861–863. https://doi.org/10.1038/13158

Grattan, D. R., Pi, X. J., Andrews, Z. B., Augustine, R. A., Kokay, I. C., Summerfield, M. R., Todd, B., & Bunn, S. J. (2001). Prolactin receptors in the brain during pregnancy and lactation: Implications for behavior. *Hormones and Behavior*, *40*(2), 115–124. https://doi.org/10.1006/hbeh.2001.1698

Gregg, C., Shikar, V., Larsen, P., Mak, G., Chojnacki, A., Yong, V. W., & Weiss, S. (2007). White Matter Plasticity and Enhanced Remyelination in the Maternal CNS. *Journal of Neuroscience*, *27*(8), 1812–1823. https://doi.org/10.1523/JNEUROSCI.4441-06.2007

Haim, A., Julian, D., Albin-Brooks, C., Brothers, H. M., Lenz, K. M., & Leuner, B. (2017). A survey of neuroimmune changes in pregnant and postpartum female rats. *Brain, Behavior, and Immunity*, *59*, 67–78. https://doi.org/10.1016/j.bbi.2016.09.026

Haim, A., Sherer, M., & Leuner, B. (2014). Gestational stress induces persistent depressive-like behavior and structural modifications within the postpartum nucleus accumbens. *The European Journal of Neuroscience*, *40*(12), 3766–3773. https://doi.org/10.1111/ejn.12752

Hillerer, K. M., Jacobs, V. R., Fischer, T., & Aigner, L. (2014). The Maternal Brain: An Organ with Peripartal Plasticity. *Neural Plasticity*, *2014*, 1–20. https://doi.org/10.1155/2014/574159

Hillerer, K. M., Woodside, B., Parkinson, E., Long, H., Verlezza, S., & Walker, C.-D. (2018). Gating of the neuroendocrine stress responses by stressor salience in early lactating female rats is independent of infralimbic cortex activation and plasticity. *Stress (Amsterdam, Netherlands)*, *21*(3), 217–228. https://doi.org/10.1080/10253890.2018.1434618

Hoekzema, E., Barba-Müller, E., Pozzobon, C., Picado, M., Lucco, F., García-García, D., Soliva, J. C., Tobeña, A., Desco, M., Crone, E. A., Ballesteros, A., Carmona, S., & Vilarroya, O. (2017). Pregnancy leads to long-lasting changes in human brain structure. *Nature Neuroscience*, *20*(2), 287–296. https://doi.org/10.1038/nn.4458

Hoekzema, E., Tamnes, C. K., Berns, P., Barba-Müller, E., Pozzobon, C., Picado, M., Lucco, F., Martínez-García, M., Desco, M., & Ballesteros, A. (2020). Becoming a mother entails anatomical changes in the ventral striatum of the human brain that facilitate its responsiveness to offspring cues. *Psychoneuroendocrinology*, *112*, 104507.

Huttenlocher, P. R. (1979). Synaptic density in human frontal cortex—Developmental changes and effects of aging. *Brain Research*, *163*(2), 195–205. https://doi.org/10.1016/0006-8993(79)90349-4

Huttenlocher, P. R., & Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *Journal of Comparative Neurology*, *387*(2), 167–178. https://doi.org/10.1002/(SICI)1096-9861(19971020)387:2<167::AID-CNE1>3.0.CO;2-

Ikegami, A., Haruwaka, K., & Wake, H. (2019). Microglia: Lifelong modulator of neural circuits: Microglial machinery wires synapses. *Neuropathology*. https://doi.org/10.1111/neup.12560

Jang, H., Bae, J. B., Dardiotis, E., Scarmeas, N., Sachdev, P. S., Lipnicki, D. M., Han, J. W., Kim, T. H., Kwak, K. P., Kim, B. J., Kim, S. G., Kim, J. L., Moon, S. W., Park, J. H., Ryu, S.-H., Youn, J. C., Lee, D. Y., Lee, D. W., Lee, S. B., … Kim, K. W. (2018). Differential effects of completed and incomplete pregnancies on the risk of Alzheimer disease. *Neurology*, *91*(7), e643–e651. https://doi.org/10.1212/WNL.0000000000006000

Kalakh, S., & Mouihate, A. (2019). Enhanced remyelination during late pregnancy: Involvement of the GABAergic system. *Scientific Reports*, *9*(1), Article 1. https://doi.org/10.1038/s41598-019-44050-4

Kim, P., Leckman, J. F., Mayes, L. C., Feldman, R., Wang, X., & Swain, J. E. (2010). The plasticity of human maternal brain: Longitudinal changes in brain anatomy during the early postpartum period. *Behavioral Neuroscience*, *124*(5), 695–700. https://doi.org/10.1037/a0020884

Kim, P., Rigo, P., Mayes, L. C., Feldman, R., Leckman, J. F., & Swain, J. E. (2014). Neural plasticity in fathers of human infants. *Social Neuroscience*. https://doi.org/10.1080/17470919.2014.933713

Kinsley, C. H., Bardi, M., Karelina, K., Rima, B., Christon, L., Friedenberg, J., & Griffin, G. (2008). Motherhood induces and maintains behavioral and neural plasticity across the lifespan in the rat. *Archives of Sexual Behavior*, *37*(1), 43–56. https://doi.org/10.1007/s10508-007-9277-x

Kinsley, C. H., Trainer, R., Stafisso-Sandoz, G., Quadros, P., Marcus, L. K., Hearon, C., Meyer, E. A. A., Hester, N., Morgan, M., Kozub, F. J., & Lambert, K. G. (2006). Motherhood and the hormones of pregnancy modify concentrations of hippocampal neuronal dendritic spines. *Hormones and Behavior*, *49*(2), 131–142. https://doi.org/10.1016/j.yhbeh.2005.05.017

Knudsen, E. I. (2004). Sensitive periods in the development of the brain and behavior. *Journal of Cognitive Neuroscience*, *16*(8), 1412–1425. https://doi.org/10.1162/0898929042304796

Kolb, B., & Gibb, R. (2011). Brain plasticity and behaviour in the developing brain.

Journal of the Canadian Academy of Child and Adolescent Psychiatry = Journal de l'Academie Canadienne de Psychiatrie de l'enfant et de l'adolescent, *20*(4), 265–276.
Kuo, K., Hackney, D., & Mesiano, S. (2016). The Endocrine Control of Human Pregnancy. In E. Bonora & R. DeFronzo (Eds.), *Diabetes. Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment* (pp. 1–33). Springer International Publishing. https://doi.org/10.1007/978-3-319-27318-1_26-1
Leuner, B., & Sabihi, S. (2016). The birth of new neurons in the maternal brain: Hormonal regulation and functional implications. *Frontiers in Neuroendocrinology*, *41*, 99–113. https://doi.org/10.1016/j.yfrne.2016.02.004
Lisofsky, N., Gallinat, J., Lindenberger, U., & Kühn, S. (2019). Postpartal Neural Plasticity of the Maternal Brain: Early Renormalization of Pregnancy-Related Decreases? *Neuro-Signals*, *27*(1), 12–24. https://doi.org/10.33594/000000105
Luders, E., Kurth, F., Gingnell, M., Engman, J., Yong, E.-L., Poromaa, I. S., & Gaser, C. (2020). From baby brain to mommy brain: Widespread gray matter gain after giving birth. *Cortex*, *126*, 334–342. https://doi.org/10.1016/j.cortex.2019.12.029
Martínez-García, M., Cardenas, S. I., Pawluski, J., Carmona, S., & Saxbe, D. E. (2022a). Recent Neuroscience Advances in Human Parenting. In G. González-Mariscal (Ed.), *Patterns of Parental Behavior: From Animal Science to Comparative Ethology and Neuroscience* (pp. 239–267). Springer International Publishing. https://doi.org/10.1007/978-3-030-97762-7_8
Martínez-García, M., Paternina-Die, M., Barba-Müller, E., Martín de Blas, D., Beumala, L., Cortizo, R., Pozzobon, C., Marcos-Vidal, L., Fernández-Pena, A., & Picado, M. (2021b). Do Pregnancy-Induced Brain Changes Reverse? The Brain of a Mother Six Years after Parturition. *Brain Sciences*, *11*(2), 168.
Martínez-García, M., Paternina-Die, M., Cardenas, S. I., Vilarroya, O., Desco, M., Carmona, S., & Saxbe, D. E. (2022b). First-time fathers show longitudinal gray matter cortical volume reductions: Evidence from two international samples. *Cerebral Cortex*. https://doi.org/10.1093/cercor/bhac333
Martínez-García, M., Paternina-Die, M., Desco, M., Vilarroya, O., & Carmona, S. (2021a). Characterizing the Brain Structural Adaptations Across the Motherhood Transition. *Frontiers in Global Women's Health*, *2*, 76. https://doi.org/10.3389/fgwh.2021.742775
Mateos-Aparicio, P., & Rodríguez-Moreno, A. (2019). The Impact of Studying Brain

Plasticity. *Frontiers in Cellular Neuroscience*, *13*.
https://doi.org/10.3389/fncel.2019.00066
Mills, K. L., & Tamnes, C. K. (2014). Methods and considerations for longitudinal structural brain imaging analysis across development. *Developmental Cognitive Neuroscience*, *9*, 172–190. https://doi.org/10.1016/j.dcn.2014.04.004
Moltz, H., Lubin, M., Leon, M., & Numan, M. (1970). Hormonal induction of maternal behavior in the ovariectomized nulliparous rat. *Physiology & Behavior*, *5*, 1373–1377. https://doi.org/10.1016/0031-9384(70)90122-8
Norrmén-Smith, I. O., Gómez-Carrillo, A., & Choudhury, S. (2021). "Mombrain and Sticky DNA": The Impacts of Neurobiological and Epigenetic Framings of Motherhood on Women's Subjectivities. *Frontiers in Sociology*, *6*, 77.
https://doi.org/10.3389/fsoc.2021.653160
Numan, M. (2020). *The Parental Brain: Mechanisms, Development, and Evolution*.
Oatridge, A., Holdcroft, A., Saeed, N., Hajnal, J. V., Puri, B. K., Fusi, L., & Bydder, G. M. (2002). Change in brain size during and after pregnancy: Study in healthy women and women with preeclampsia. *AJNR. American Journal of Neuroradiology*, *23*(1), 19–26.
Opala, E. A., Verlezza, S., Long, H., Rusu, D., Woodside, B., & Walker, C.-D. (2019). Experience of Adversity during a First Lactation Modifies Prefrontal Cortex Morphology in Primiparous Female Rats: Lack of Long Term Effects on a Subsequent Lactation. *Neuroscience*, *417*, 95–106.
https://doi.org/10.1016/j.neuroscience.2019.08.022
Orchard, E. R., Voigt, K., Chopra, S., Thapa Rana, T., Ward, P. G., Egan, G. F., & Jamadar, S. D. (2022). The maternal brain is more flexible and responsive at rest: Effective connectivity of the parental caregiving network in postpartum mothers. *BioRxiv*. https://doi.org/10.1101/2022.09.26.509524
Orchard, E. R., Ward, P. G. D., Sforazzini, F., Storey, E., Egan, G. F., & Jamadar, S. D. (2020). Relationship between parenthood and cortical thickness in late adulthood. *PLOS ONE*, *15*(7), e0236031. https://doi.org/10.1371/journal.pone.0236031
Paternina-Die, M., Martínez-García, M., Pretus, C., Hoekzema, E., Barba-Müller, E., Martín de Blas, D., Pozzobon, C., Ballesteros, A., Vilarroya, Ó., & Desco, M. (2020). The Paternal Transition Entails Neuroanatomic Adaptations that are Associated with the Father's Brain Response to his Infant Cues. *Cerebral Cortex Communications*, *1*(1),

tgaa082.
Paul, S., Austin, J., Elliott, R., Ellison-Wright, I., Wan, M. W., Drake, R., Downey, D., Elmadih, A., Mukherjee, I., Heaney, L., Williams, S., & Abel, K. M. (2019). Neural pathways of maternal responding: Systematic review and meta-analysis. *Archives of Women's Mental Health*, *22*(2), 179–187. https://doi.org/10.1007/s00737-018-0878-2
Pawluski, J. L., Hoekzema, E., Leuner, B., & Lonstein, J. S. (2021). Less Can Be More: Fine Tuning the Maternal Brain. *Neuroscience & Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2021.11.045
Pawluski, J. L., Valença, A., Santos, A. I. M., Costa-Nunes, J. P., Steinbusch, H. W. M., & Strekalova, T. (2012). Pregnancy or stress decrease complexity of CA3 pyramidal neurons in the hippocampus of adult female rats. *Neuroscience*, *227*, 201–210. https://doi.org/10.1016/j.neuroscience.2012.09.059
Pedersen, C. A., Ascher, J. A., Monroe, Y. L., & Prange, A. J. J. (1982). Oxytocin induces maternal behavior in virgin female rats. *Science (New York, N.Y.)*, *216*(4546), 648–650. https://doi.org/10.1126/science.7071605
Pereira, M. (2016). Structural and Functional Plasticity in the Maternal Brain Circuitry. *New Directions for Child and Adolescent Development*, *2016*(153), 23–46. https://doi.org/10.1002/cad.20163
Pereira, M., Smiley, K. O., & Lonstein, J. S. (2022). Parental Behavior in Rodents. *Advances in Neurobiology*, *27*, 1–53. https://doi.org/10.1007/978-3-030-97762-7_1
Perez-Catalan, N. A., Doe, C. Q., & Ackerman, S. D. (2021). The role of astrocyte-mediated plasticity in neural circuit development and function. *Neural Development*, *16*(1), 1. https://doi.org/10.1186/s13064-020-00151-9
Pownall, M., Conner, M., & Hutter, R. R. C. (2021). The effects of activating a "baby brain" stereotype on pregnant women's cognitive functioning. *Journal of Applied Social Psychology*, *51*(8), 809–824. https://doi.org/10.1111/jasp.12802
Quintana, D. S., Rokicki, J., van der Meer, D., Alnæs, D., Kaufmann, T., Córdova-Palomera, A., Dieset, I., Andreassen, O. A., & Westlye, L. T. (2019). Oxytocin pathway gene networks in the human brain. *Nature Communications*, *10*(1), 668. https://doi.org/10.1038/s41467-019-08503-8
Raphael, D. (2011). Matrescence, Becoming a Mother, A "New/Old" Rite de Passage. In D. Raphael (Ed.), *Reproduction, Power, and Change* (pp. 65–72). De Gruyter Mouton. https://doi.org/doi:10.1515/9783110813128.65

Rigo, P., Kim, P., Esposito, G., Putnick, D. L., Venuti, P., & Bornstein, M. H. (2019). Specific maternal brain responses to their own child's face: An fMRI meta-analysis. *Developmental Review*, *51*, 58–69. https://doi.org/10.1016/j.dr.2018.12.001

Rosenblatt, J. S. (1967). Nonhormonal Basis of Maternal Behavior in the Rat. *Science*, *156*(3781), 1512–1513. https://doi.org/10.1126/science.156.3781.1512

Rosenblatt, J. S. (2003). Outline of the evolution of behavioral and nonbehavioral patterns of parental care among the vertebrates: Critical characteristics of mammalian and avian parental behavior. *Scandinavian Journal of Psychology*, *44*(3), 265–271. https://doi.org/10.1111/1467-9450.00344

Salmaso, N., Quinlan, M. G., Brake, W. G., & Woodside, B. (2011). Changes in dendritic spine density on layer 2/3 pyramidal cells within the cingulate cortex of late pregnant and postpartum rats. *Hormones and Behavior*, *60*(1), 65–71. https://doi.org/10.1016/j.yhbeh.2011.03.002

Shih, H.-C., Kuo, M.-E., Wu, C. W., Chao, Y.-P., Huang, H.-W., & Huang, C.-M. (2022). The Neurobiological Basis of Love: A Meta-Analysis of Human Functional Neuroimaging Studies of Maternal and Passionate Love. *Brain Sciences*, *12*(7). https://doi.org/10.3390/brainsci12070830

Stern, J. E., & Armstrong, W. E. (1998). Reorganization of the Dendritic Trees of Oxytocin and Vasopressin Neurons of the Rat Supraoptic Nucleus during Lactation. *The Journal of Neuroscience*, *18*(3), 841–853. https://doi.org/10.1523/JNEUROSCI.18-03-00841.1998

Stolzenberg, D. S., & Champagne, F. A. (2016). Hormonal and non-hormonal bases of maternal behavior: The role of experience and epigenetic mechanisms. *Hormones and Behavior*, *77*, 204–210. https://doi.org/10.1016/j.yhbeh.2015.07.005

Taylor, C. M., Pritschet, L., Yu, S., & Jacobs, E. G. (2019). Applying a Women's Health Lens to the Study of the Aging Brain. *Frontiers in Human Neuroscience*, *13*, 224. https://doi.org/10.3389/fnhum.2019.00224

Terkel, J., & Rosenblatt, J. S. (1972). Humoral factors underlying maternal behavior at parturition: Corss transfusion between freely moving rats. *Journal of Comparative and Physiological Psychology*, *80*(3), 365–371. https://doi.org/10.1037/h0032965

Voldsbekk, I., Barth, C., Maximov, I. I., Kaufmann, T., Beck, D., Richard, G., Moberget, T., Westlye, L. T., & de Lange, A.-M. G. (2021). A history of previous childbirths is linked to women's white matter brain age in midlife and older age. *Human*

Brain Mapping. https://doi.org/10.1002/hbm.25553

Voltolini, C., & Petraglia, F. (2014). Neuroendocrinology of pregnancy and parturition. *Handbook of Clinical Neurology*, *124*, 17–36. https://doi.org/10.1016/B978-0-444-59602-4.00002-2

Wang, Z., Liu, J., Shuai, H., Cai, Z., Fu, X., Liu, Y., Xiao, X., Zhang, W., Krabbendam, E., Liu, S., Liu, Z., Li, Z., & Yang, B. X. (2021). Mapping global prevalence of depression among postpartum women. *Translational Psychiatry*, *11*(1), 543. https://doi.org/10.1038/s41398-021-01663-6

Zhang, K., Wang, M., Zhang, J., Du, X., & Chen, Z. (2020). Brain Structural Plasticity Associated with Maternal Caregiving in Mothers: A Voxel- And Surface-Based Morphometry Study. *Neurodegenerative Diseases*, *361005*, 1–12. https://doi.org/10.1159/000506258

Zheng, J.-X., Ge, L., Chen, H., Yin, X., Chen, Y.-C., & Tang, W.-W. (2020). Disruption within brain default mode network in postpartum women without depression. *Medicine*, *99*(18), e20045. https://doi.org/10.1097/MD.0000000000020045