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RESEARCH ARTICLE

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Maternal deprivation and milk replacement affect the integrity of gray and white matter in the developing lamb brain

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Abstract

The psychoendocrine evaluation of lamb development has demonstrated that maternal deprivation and milk replacement alters health, behavior, and endocrine profiles. While lambs are able to discriminate familiar and non-familiar conspecifics (mother or lamb), only lambs reared with their mother develop such clear social discrimination or preference. Lambs reared without mother display no preference for a specific lamb from its own group. Differences in exploratory and emotional behaviors between mother-reared and mother-deprived lambs have also been reported. As these behavioural abilities are supported by the brain, we hypothesize that rearing with maternal deprivation and milk replacement leads to altered brain development and maturation. To test this hypothesis, we examined brain morphometric and microstructural variables extracted from in vivo T1-weighted and diffusion-weighted magnetic resonance images acquired longitudinally (1 week, 1.5 months, and 4.5 months of age) in mother-reared and mother-deprived lambs. From the morphometric variables the caudate nuclei volume was found to be smaller for mother-deprived than for mother-reared lambs. T1-weighted signal intensity and radial diffusivity were higher for mother-deprived than for mother-reared lambs in both the white and gray matters. The fractional anisotropy of the white matter was lower for mother-deprived than for mother-reared lambs. Based on these morphometric and microstructural characteristics we conclude that maternal deprivation delays and affects lamb brain growth and maturation.

KEYWORDS

artificial rearing, mothering, sheep, MRI, DTI, T1-weighted, white and gray matter

1 | INTRODUCTION

In both human and nonhuman primates, the quality of motherhood is important for an infant's development, especially for social personality development (Pryce, 1995). In nonhuman primates, the lack of mothering from birth to adolescence leads to anxiousness for novelty exploration, increased aggressiveness at puberty, and peer-reared primiparous females display disturbance of maternal behavior (Suomi, 1997). More generally, in pets, farm, or zoo animals, maternal deprivation at birth or early weaning leads to behavioral problems, such as reproductive or maternal deficits,

aggressiveness, and to stereotypic behaviors like nonnutritive suckling, head-banging, or self-harm in general (Latham & Mason, 2008). These impacts of mother deprivation can result simultaneously from the lack of a social model, that is, the mother (Fleming et al., 2002), and from a lack of maternal milk as a source of bioactive factors (Bernstein & Hinde, 2016). However, the challenge is not to isolate the impact of only one factor (mother or milk) but to consider "the dynamic interactions among the multitude of equally important maternal and non-maternal variables" (Tang et al., 2014).

In sheep (*ovis aries*), a precocial species, it is difficult to dissociate the impact of maternal deprivation from that of formula-milk feeding; each affecting the infants' development. The reciprocal mother–infant bond between an ewe and her lamb occurs in the first 12 h of life. It is based mainly on odorant cues and is characterized by an individual recognition of each partner and nursing exclusivity between a mother and her own infant (Nowak et al., 1997, 2011; Nowak & Poindron, 2006). In this context, it is not surprising that the mother is crucial for the development of the lamb. However, motherless rearing is commonly used in conventional dairy farming or in other situations if the mother is nonmaternal, has too many lambs or if she has mastitis. Despite its common practice in sheep farming, the impact of early rearing conditions involving mother-deprivation, milk replacement, or early weaning affect a wide range of functions and behaviors.

At birth, colostrum is important for lamb survival due to the passive immune transfer from mother to lamb (Hernández-Castellano et al., 2015; Khan & Ahmad, 1997; Nowak & Poindron, 2006). Lambs fed with commercial milk replacer have an altered immune response in comparison with mothered lambs or lambs fed with a mix of maternal and commercial milks (Sevi et al., 1999). However, being mother-deprived a few days after birth instead of at birth provides lambs access to colostrum and greatly reduces the impact on the immune response, especially if subsequently fed with ewe's milk (Napolitano, 2003; Napolitano et al., 1995).

During the first weeks of life, the mother plays a key role as a social demonstrator that influences the establishment of feeding (Black-Rubio et al., 2007; Saint-Dizier et al., 2007; Thorhallsdottir et al., 1990) and social preference between twins (Ligout & Porter, 2004) or appeasement induced by caregiver (Boivin et al., 2001; Guesdon et al., 2016). In the long term, the absence of the mother has a negative impact on the expression of sexual behavior of male lambs (Damián et al., 2015, 2018). Emotional reactivity, assessed in a social isolation context by cortisol plasma levels and behavioural responses, is also impacted by maternal deprivation (Napolitano, 2003; Napolitano et al., 2002; Sevi et al., 1999). In addition, endocrine imbalance is also reported, in regards to sexual behavior (Damián et al., 2015, 2018), infant attachment (Gaudin et al., 2018), or nutrition (Berry et al., 2016). These studies demonstrate how maternal deprivation, and consequently artificial feeding, have wide ranging, deleterious consequences, affecting the development of the immune system, behavior, endocrine development, growth, and cognitive capacities of lambs.

While the benefits of breastfeeding on brain development compared to formula-milk feeding have been reported in humans (Deoni et al., 2013, 2018; O'Muircheartaigh et al., 2014; Schack-Nielsen & Michaelsen, 2007) and nonhuman primates (Liu et al., 2019), very little information has been reported in sheep. Mainly used as a model of developmental disorders resulting from preterm birth (Castillo-Melendez et al., 2013; Chaillou et al., 2012; De Matteo et al., 2010; Dunlop et al., 1997; Huang et al., 2001; Malhotra et al., 2019), the impact of early postnatal experience on lamb brain development is largely unknown. The only reported data, concerning the hypothalamus, show that a short period of maternal deprivation (72 h at 2 weeks after birth) induces higher immunoreactivity in maternal-deprived lambs for adrenocorticotropic and gonadotrophin-releasing hormones (Wańkowska et al., 2006), and for somatostatin (Polkowska & Wańkowska, 2010). These findings are consistent with the endocrine perturbations described in similar maternal deprivation contexts (Damián et al., 2015, 2018; Sevi et al., 1999). However, in order to fully understand the consequences of adverse early postnatal experience on lamb neurobiological development a global, a dynamic view of brain maturation is required.

Magnetic resonance imaging (MRI) enables longitudinal investigation of various morphometric and microstructural indicators of brain maturation. In particular, T1-weighted (T1w) signal intensity (Flood et al., 2019; Luby et al., 2013) and diffusion tensor imaging (DTI) parameters (Dubois et al., 2006, 2014; Hüppi & Dubois, 2006) have been used to quantify maturation of the growing brain. Based on studies in humans (Deoni et al., 2013; Luby et al., 2013) and nonhuman primates (Liu et al., 2019), we examined the longitudinal impact of maternal deprivation on these morphometric and microstructural characteristics of the lamb brain.

To study the longitudinal impact of maternal deprivation, T1w and diffusion-weighted MR images were acquired longitudinally from mother-reared and mother-deprived lambs at three different stages of development: approximately 1 week, 1.5, and 4.5 months after birth. From T1w images, the total brain, gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) volumes were measured as well as the volumes of some specific GM structures involved in socioemotional behavior (olfactory bulbs [OB], periaqueductal gray [PAG], hippocampus [Hipp], and caudate nucleus [CN]). It was hypothesized that maternal deprivation would negatively affect the developmental increase of these volumes. Microstructural characteristics were also characterized by measuring T1w signal intensity, fractional anisotropy (FA) and radial diffusivity (RD) in total GM, WM and in specific WM areas (corpus callosum, optic chiasm and cere-

bellum, frontal, parietal, temporal, and occipital lobes). It was hypothesized that maternal deprivation would affect the maturation of these microstructural parameters especially in WM.

2 | MATERIAL AND METHODS

2.1 | Experimental animals

Twenty, Ile de France, female lambs (*Ovis aries*) were included in this study. Lambs were born and reared at one of the French National Research Institute for Agriculture, Food and Environment experimental units (UEPAO, INRAE, 2018. Animal Physiology Experimental Facility, DOI: 10.15454/1.5573896321728955E12). After parturition the lambs were allocated to one of two separate groups: 10 were kept with their mother for 24 h after birth, which gave them access to colostrum, before being separated from their mothers (mother-deprived group, MD); the other 10 were kept with their mothers and twins for the entire study (mother-reared group, MR). Mother-deprived lambs had free access to water and formula-milk composed of whey, skimmed milk powder, whey protein concentrate, palm oil, rapeseed oil, wheat gluten, extracts of yeast from inactive beer, selenium, lysine, and methionine (22% of crude protein, 23% of crude fat, 4400 kcal/kg; Agnodor Tradition plus, Univor, Chasseneuil-du-Poitou, France). Formula-milk was distributed via an automatic milk feeder connected to three rubber nipples, which the lambs were trained to use by caregivers during the first 72–96 h of life, 5 times per day. The mother-reared lambs and their mother and twins were visited by caregivers at the same frequency as the mother-deprived lambs. Concentrate pellets (net energy of 6.19 MJ/kg DM and metabolisable protein of 112 g/kg) were introduced about 10 days before weaning occurred at around 45 days of age for mother-deprived lambs and at around 90 days of age for mother-reared lambs. Both groups of lambs had free access to hay and straw.

To follow lambs' growth they were weighed at birth, before each of the three MRI acquisitions, and at around 2 months of age (Figure 1). Mother-deprived and mother-reared lambs were transported from their building to the MRI platform (less than 500 m) the same day as the first acquisition (T1) and the day before for the two last acquisitions (T2 and T3). Lambs were never alone and were always together with at least two familiar lambs (mother-deprived lambs) or with their mother and twin (mother-reared lambs).

During the experiment, three mother-deprived lambs died at 35, 42, and 45 days of age and one mother-reared lamb was excluded because of mother's mastitis. Final group sizes were $N = 7$ for mother-deprived and $N = 9$ for mother-reared.

2.2 | Ethics

All procedures were performed in accordance with the European directive 2010/63/EU for animal protection and welfare used for scientific purposes and approved by the local ethical committee for animal experimentation (CEEA VdL, Tours, France, authorization N° 00821.03 and 02272.01). At the end of the experiment all remaining animals returned to the UEPAO.

2.3 | MRI Acquisition

MRI of the brain was performed with a 3 Teslas Siemens Magnetom Verio® scanner (Erlangen, Germany) located at the CIRE (Chirurgie et Imagerie pour la Recherche et l'Enseignement) platform, INRAE Nouzilly, France. MRI was acquired for each lamb at three different timepoints. The first timepoint was collected approximately 1 week after birth (0.86–2.57 weeks, T1), the second at approximately 1.5 months of age (4.71–6.57 weeks, T2), and the third at approximately 4.5 months of age (17.57–20.5 weeks, T3). At each timepoint, individual ages (days old) were variable between lambs because they were born at term without induction of labor between September 1st and 12th and were submitted to MRI acquisitions at a rate of two animals per day. The mother-reared lambs ($n = 9$) were on average 1.6 ± 0.6, 5.5 ± 0.6, and 19.1 ± 0.65 weeks old and the mother-deprived lambs ($n = 7$) were on average 1.6 ± 0.5, 5.5 ± 0.4, and 18.6 ± 1 weeks old, respectively for the three timepoints.

Before each acquisition, lambs were placed under general anesthesia to prevent stress and avoid motion artifacts, despite its potentially detrimental effects on brain development (Andropoulos, 2018; Jevtovic-Todorovic, 2005; Olutoye et al., 2015). To prevent nausea and the associated risks of food entering the lungs during anesthesia, for the last two acquisitions (T2 and T3) lambs were fasted from the night before. Anesthesia was induced by an intravenous co-injection of rompun® and ketamine®. After this injection and the loss of muscular tonus, a tracheal tube was inserted to maintain the anesthesia with Isoflurane®. The drug doses and tracheal tube sizes were adapted to each animal based on age, body weight, and size (Table 1). The duration of anesthesia was limited to 2.5 h, during which cardiac rhythm and SpO_2 levels were continuously monitored. Due to their young age at the first timepoint, lambs were also perfused with a solution of Ringer-Glucose. At the end of the acquisition, Isoflurane® was stopped and the tracheal tube removed after the first signs of autonomous breathing.

During acquisition lambs laid on their stomachs (prone position) with a 4-channel Siemens FLEX coil wrapped around their head and, for their comfort and safety, were

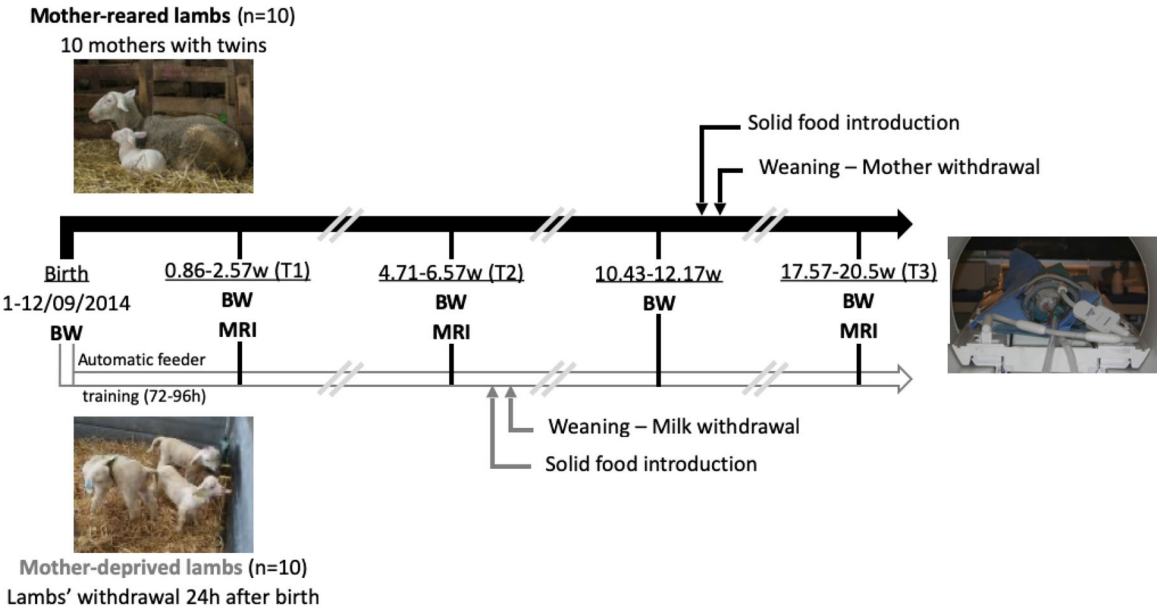

FIGURE 1 Timeline of the experimental design. (BW body weight; MRI magnetic resonance imaging; T1, T2, T3 timepoints of acquisition)

TABLE 1 Drug doses and Tracheal tube sizes used during the MRI acquisitions

Timepoint	Rompun (ml)	Ketamine (ml)	Size of the tracheal tube	Isoflurane (%)	0_2 / air (% / %)	Glucose (ml / 2.5h)
T1	0–0.03	1–1.5	3.5	1.5–2.5	1/1	100
T2	0.02–0.03	2.5	5.5	2.5–4	1/1–2/2	-
T3	0.05	3.5	6.5	2.8–3	1.5/1	-

Note that the lambs were fasted the day before timepoints T2 and T3.

covered with a cloth and wore ear plugs (Figure 1). All acquisitions (T1w and DTI) were performed within a single session, the animal being maintained in exactly the same position throughout the session.

T1w images were acquired with the 3D-MPRAGE (Magnetisation Prepared Rapid Acquisition with Gradient Echo) sequence with the following parameters at each timepoint: Repetition Time, TR = 2500 ms; Echo Time, *TE* = 3.44 ms; Bandwidth, *BW* = 150 Hz/pixel; Inversion Time, *TI* = 756 ms; Flip Angle = 9°; Slice Thickness = 0.4 mm; Field of View, FOV = 152 × 152 mm; matrix = 384 × 384; in plane resolution 0.4 × 0.4 mm; Number of Excitations, *NEX* = 4; Integrated Parallel Acquisition, iPAT = 2; acquisition time, *TA* = 44 m 32 s.

DTI-images were acquired with a single shot spin-echo echo sequence with the following parameters at all points of acquisition: *TR* = 13,000 ms, *TE* = 80 ms, *BW* = 1698 Hz/pixel, inter-echo spacing = 0.68 ms, *FOV* = 256 × 256mm, matrix = 128 × 128 mm, slice thickness = 2 mm, voxel size = 2 × 2 × 2 mm, iPAT = 2, *TA* = 8 min 29 s. Images were acquired in 30 directions with a *b*-value of 1000 s/mm^2 (similar to childhood studies, Mukherjee et al. 2002) and six images were acquired without diffusion weighting (*b*-value = 0 s/mm^2).

GRE (Gradient Echo) field mapping sequences were performed in magnitude and in phase with the following parameters: *TR* = 500 ms, *TE* short/long = 4.92/7.38 ms, flip angle = 55°, *BW* = 260 Hz/pixel, *FOV* = 192 × 192 mm, matrix = 64, voxel size = 3 × 3 × 3 mm, *TA* = 1 min 7 s.

2.4 | MRI Analysis

Native MR images were converted from DICOM to NIFTI with dcm2nii (mricron, http://www.mccauslandercenter.sc.edu/mricro/mricron).

2.4.1 | Morphometric variables

T1w images for volume calculation of brain, gray, and white matters and cerebrospinal fluid

T1w images were first denoised using the *DenoiseImage* function of the Advanced Normalization Tools (ANTs, Avants

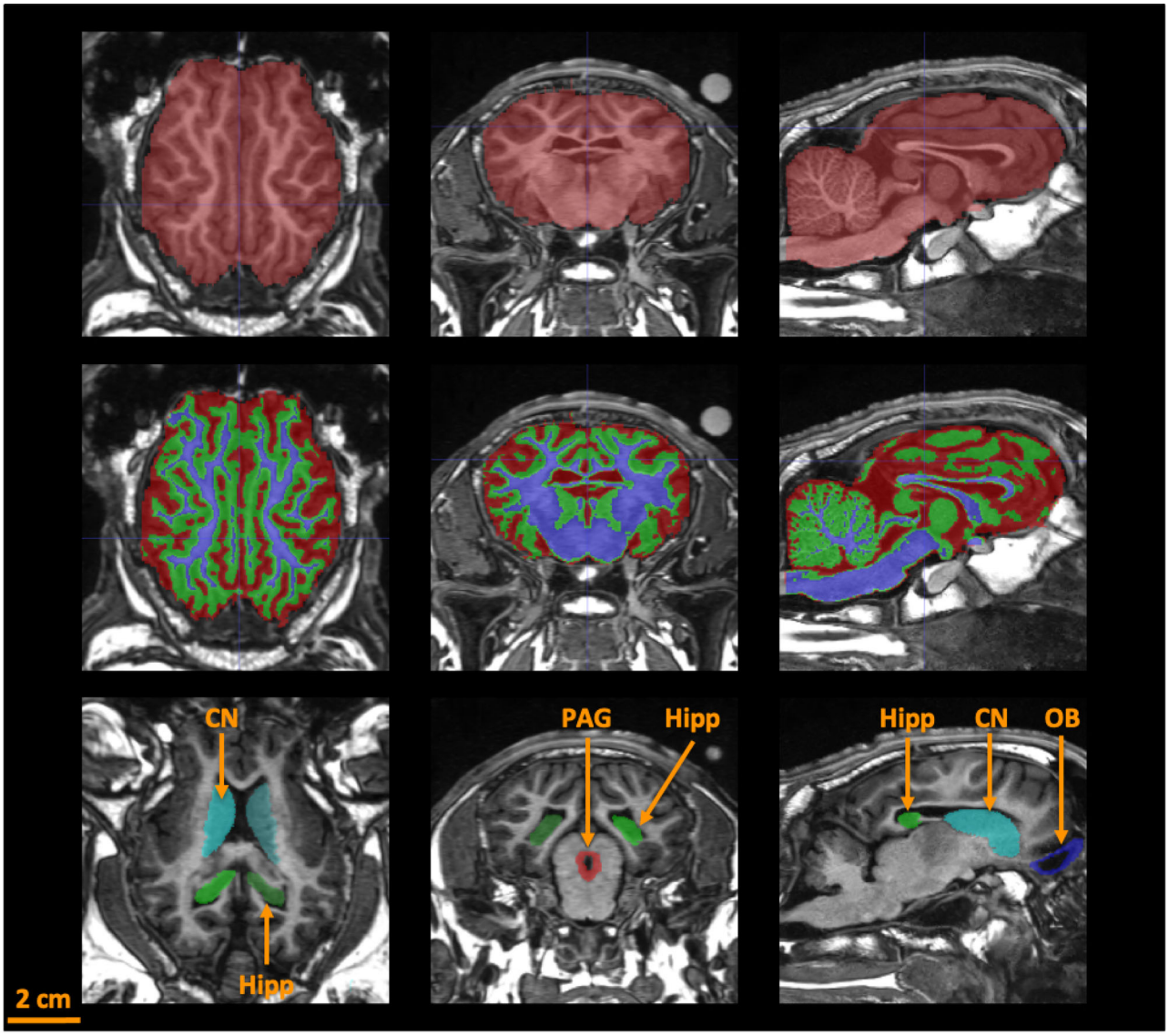

FIGURE 2 Axial (left), coronal (middle) and sagittal (right) slices of the T1w anatomical image of an individual lamb brain at T3 (around 4.5 months of age). The red overlay in the top row shows a manually segmented brain mask. The overlays in the middle row show the automatically segmented gray matter (green), white matter (blue) and cerebrospinal fluid (red). The overlays in the bottom row show the manually segmented regions-of-interest: olfactory bulbs (OB), periaqueductal gray (PAG), hippocampus (Hipp) and caudate nucleus (CN)

et al. 2014; Manjón et al. 2010). They were then cropped in all three dimensions to isolate the cerebral tissue as closely as possible from all nonbrain tissue. To reduce the time required for manual brain mask segmentation, the original resolution (0.4 mm^3 isotropic) of the T1w images was linearly subsampled to 0.8 mm^3 isotropic using the *resample-mm* function of the Convert3D (ITK-SNAP, www.itksnap.org, Yushkevich et al. 2006). The manual brain mask segmentation was performed with the ITK-SNAP software (Figure 2). The segmented brain mask was then upsampled to the original T1w resolution of 0.4 mm^3 isotropic before being manually inspected and corrected when necessary. Finally, all brain masks were controlled by experts in sheep neuroanatomy (EC, SAL). The automatic segmentation of gray matter, white matter and cerebrospinal fluid was conducted within each individual's brain mask using FAST (FMRIB Automated Segmentation Tool, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FAST, Jenkinson et al. 2012; Zhang et al. 2001; see Figure 2).

T1w images for volume calculation of specific brain structures

To calculate the volumes of specific brain structures involved in socioemotional behavior (OB, PAG, Hipp, CN) regions-of-interest (ROI) were manually segmented on the denoised T1w images for T2 and T3 using ITK-SNAP (Figure 2). The OB, Hipp, and CN were segmented bi-laterally and the mean volume was calculated. All ROI were controlled by experts in sheep neuroanatomy (EC, FL).

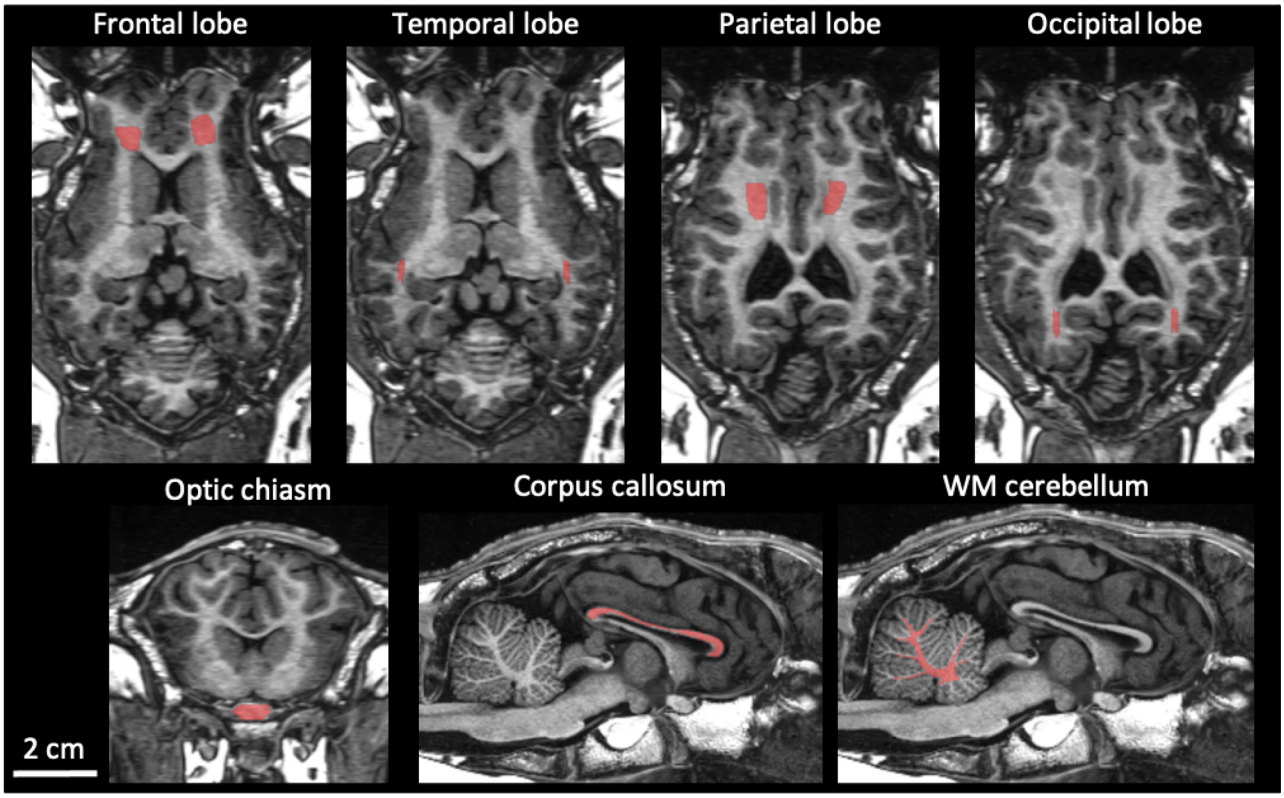

FIGURE 3 Regions-of-interest (red overlay) manually segmented in the white matter

2.4.2 | Microstructural variables

T1w signal intensity

The mean T1w signal intensity was calculated for each GM, WM, and CSF segmentation. The mean CSF value was used to normalise that of GM and WM, as previously done in a pediatric study (Flood et al., 2019).

Diffusion parameters

The diffusion-weighted images were corrected for geometric deformations due to B0 inhomogeneities in the magnetic field using the Fieldmap tool from the Statistical Parametric Mapping toolbox (Friston et al, 1994; Jezzard & Balaban, 1995). Eddy-current correction was performed with FSL (FMRIB Software Library v.5.0.4). The FA- and RD-maps were calculated using FSL. The mean FA- and RD-values were calculated for GM, WM and for specific WM-areas. These specific WM-areas were sampled by the segmentation of three (corpus callosum, optic chiasm and cerebellum) or four (frontal, parietal, temporal and occipital lobes) successive slices (Figure 3). This segmentation was done manually on the T1w images and was controlled by experts in sheep neuroanatomy (EC, FL). The ROI were then resampled to the DTI-image resolution ($2 \times 2 \times 2$ mm^3) and the mean FA- and RD-values were calculated.

2.5 | Statistical analysis

Body weight data was not collected for three mother-reared lambs at T1. Due to a lack of T1w image contrast in a few individuals, the OB were only segmented for $n = 6$ mother-reared and $n = 6$ mother-deprived lambs and the Hipp for $n = 7$ mother-reared lambs (see Table 2). At the resolution of the DTI-images, some specific WM-areas, that had been segmented on T1w images, contained very few or even zero voxels for some individuals. We only include WM-areas of three voxels or more in the statistical analysis.

To investigate the impact of early experience on lamb growth, the coefficient of correlation between age (in days) and body weight (in kg), measured at five timepoints during the experiment (Figure 1), were compared between the mother-reared and mother-deprived groups using a *t*-test.

To investigate the impact of early experience and maturation, each measure (the different morphological and structural parameters and body weight) was analyzed independently using analysis of variance (ANCOVA) with Type II Sum of Squares. The models included early experience (mother-reared or mother-deprived) and timepoint (T1, T2, and T3) as fixed effects and age (days old) at acquisition as a covariate of no interest. Post hoc analyses were conducted with Bonferroni correction. Summary statistics for the different

TABLE 2 Mean volumes (mm^3) of the olfactory bulbs, the caudate nucleus, the hippocampus, and the periaqueductal grey

Brain structure	Timepoint	Mother-reared lambs	Mother-deprived lambs	ANCOVAanalyses results
Caudate nucleus	*n*	9	7	**p_{EE}<.001 ($F_{(1,27)}$=14.86)**
	T2	675.47 ± 72.17	600.64 ± 29.33	**p_T=.014 ($F_{(1,27)}$=6.97)**
	T3	859.87 ± 102.11	763.91 ± 48.03	p_{EExT}=.41 ($F_{(1,27)}$=0.69)
Hippocampus	*n*	7	7	p_{EE}=.89 ($F_{(1,23)}$=0.02)
	T2	519.36 ± 38.26	518.13 ± 14.59	**p_T=.001 ($F_{(1,23)}$=39.09)**
	T3	616.65 ± 56.88	629.49 ± 64.05	p_{EExT}=.56 ($F_{(1,23)}$=0.35)
Olfactory bulb	*n*	6	6	p_{EE}=.42 ($F_{(1,19)}$=0.68)
	T2	221.54 ± 49.98	246.58 ± 33.73	p_T=.88 ($F_{(1,19)}$=0.68)
	T3	346.27 ± 52.19	354.23 ± 54.93	p_{EExT}=.73 ($F_{(1,19)}$=0.73)
PAG	*n*	9	7	p_{EE}=.13 ($F_{(1,27)}$=2.45)
	T2	223.88 ± 21.54	199.96 ± 15.74	p_T=.79 ($F_{(1,27)}$=0.07)
	T3	250.63 ± 21.11	244.51 ± 35.64	p_{EExT}=.23 ($F_{(1,27)}$=1.36)

Note: PAG, periaqueductal gray; EE, early experience; T, timepoint; EExT, interaction; statistical significant effects are indicated in bold.

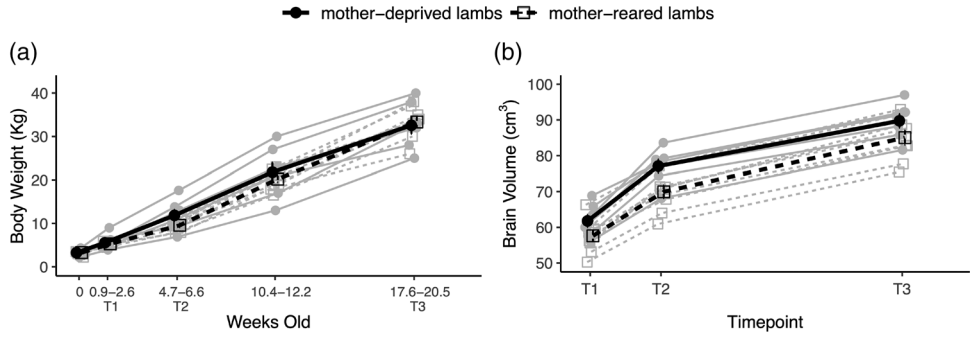

FIGURE 4 Body weights (a) and total brain volumes (b) are presented as group means ± standard error in black and as individual data in gray

measures are presented as means ± standard deviation. All statistical analyses were conducted using JASP (version 0.13.0, https://jasp-stats.org/, JASP Team, 2020).

3 | RESULTS

3.1 | Body weight

Body weight and age of lambs were positively correlated in mother-reared (r^2 = .995 ± 0.005, n = 9) and in mother-deprived lambs (r^2 = .993 ± 0.003, n = 7). There was no significant effect of early experience on the coefficient of correlation (t = −0.97, p = .38).

The ANCOVA investigating the impact of early experience & timepoint on body weight after controlling for age at acquisition highlighted no significant effect of early experience (F(1,52) = 0.81, p = .37, η^2 = 0.013), timepoint (F(3,52) = 2.20, p = .10, η^2 = 0.108), or their interaction (F(3,52) = 0.63, p = .6, η^2 = 0.031) (Figure 4a).

3.2 | Morphometric data

Examination of the morphometric data highlighted higher total brain, gray, white, and CSF volumes in mother-deprived lambs than in mother-reared lambs, all increasing throughout the experiment. However, differences associated with the early experience were not observed for the proportions of GM, WM, and CSF in the brain. OB, CN, Hipp, and PAG volumes increased between 1 and 4 months, with a delayed increase in the CN of mother-deprived lambs. Detailed statistical analyses are described below.

3.3 | Brain volumes

The total brain volume ANCOVA highlighted effects of early experience (F(1,41) = 12.20, p = .001, η^2 = 0.161) and timepoint (F(2,41) = 10.85, p < .001, η^2 = 0.287) but not for their interaction (F(2,41) = 0.38, p = .69, η^2 = 0.01). Brain volume increased with timepoint and it was greater

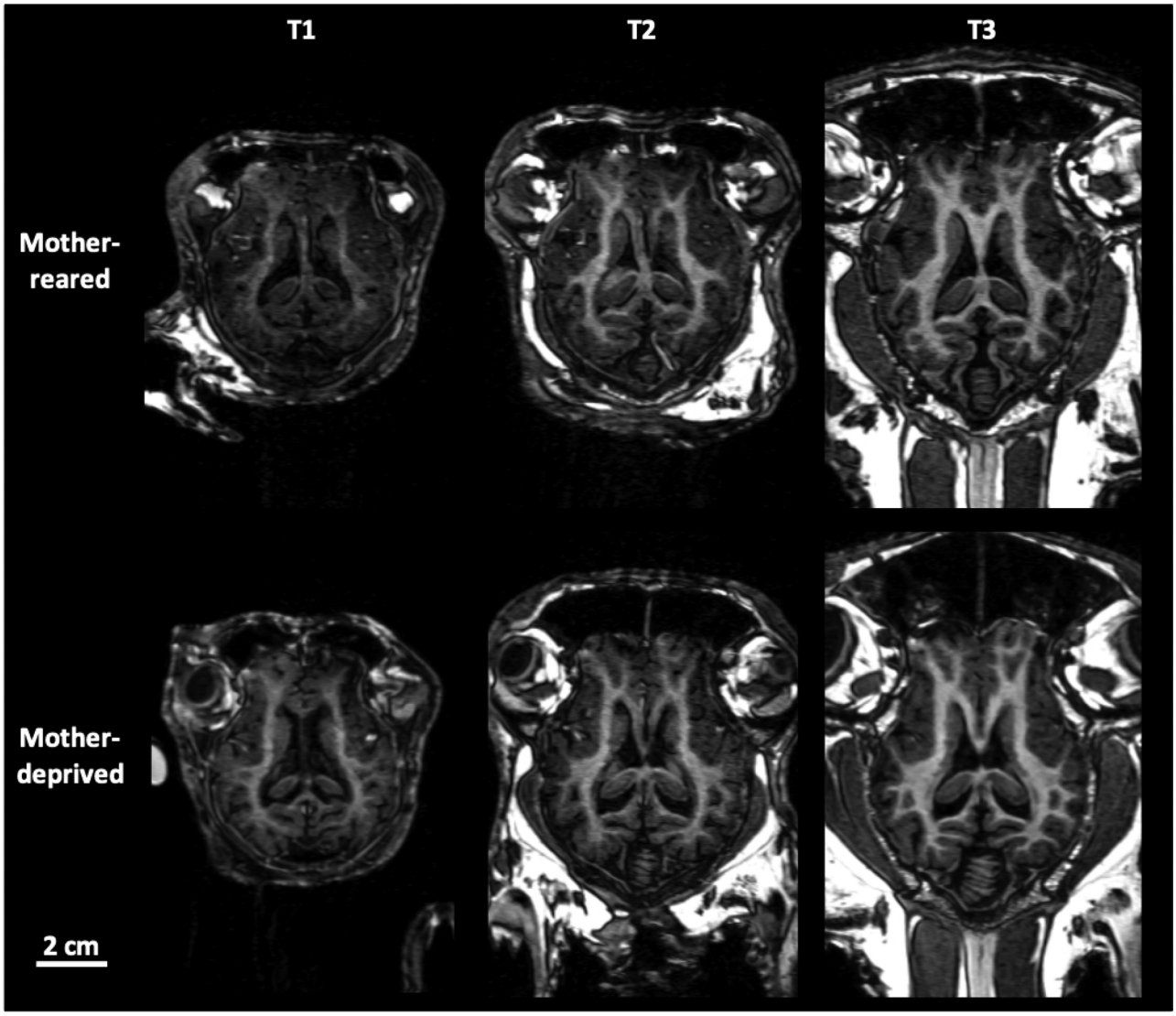

FIGURE 5 Representative horizontal slices of T1-weighted images at each timepoint (T1, T2, T3) of one mother-reared lamb (#14738) and one mother-deprived lamb (#14627)

for mother-deprived than for mother-reared lambs: T1 *MD* = 61.79 ± 4.57 cm^3, *MR* = 57.63 ± 4.57 cm^3; T2 *MD* = 77.08 ± 4.84 cm^3, *MR* = 69.95 ± 5.32 cm^3; T3 *MD* = 89.75 ± 5.01 cm^3, and *MR* = 85.05 ± 5.89 cm^3 (Figures 4b and 5).

3.4 | Gray, white matters, and CSF volumes

The GM volume ANCOVA highlighted effects of early experience ($F(1,41) = 9.05$, $p = .004$, $\eta^2 = 0.143$) and timepoint ($F(2,41) = 6.15$, $p = .005$, $\eta^2 = 0.194$) but not for their interaction ($F(2,41) = 0.29$, $p = .75$, $\eta^2 = 0.009$). GM volume increased with timepoint and mother-deprived GM volume was greater than that of mother-reared: T1 *MD* = 30.15±2.05 cm^3, *MR* = 28.60±2.09 cm^3; T2 *MD* = 35.36 ± 2.43 cm^3, *MR* = 32.48 ± 2.84 cm^3; T3 *MD* = 39.59 ± 2.32 cm^3, *MR* = 36.59 ± 2.88 cm^3 (Figure 6a).

The WM volume ANCOVA highlighted effects of early experience ($F(1,41) = 13.66$, $p < .001$, $\eta^2 = 0.169$) and timepoint ($F(2,41) = 11.98$, $p < .001$, $\eta^2 = 0.296$) but not for their interaction ($F(2,41) = 0.52$, $p = 0.60$, $\eta^2 = 0.013$). WM volume increased with timepoint and mother-deprived WM volume was greater than that of mother-reared: T1 *MD* = 15.94 ± 1.46 cm^3, *MR* = 14.61 ± 1.32 cm^3; T2 *MD* = 21.15 ± 1.53 cm^3, *MR* = 19.03 ± 1.26 cm^3; T3 *MD* = 26.98 ± 1.61 cm^3, *MR* = 26.05 ± 1.46 cm^3 (Figure 6b).

The CSF volume ANCOVA highlighted effects of early experience ($F(1,41) = 8.1$, $p = .007$, $\eta^2 = 0.114$) and timepoint ($F(2,41) = 10.67$, $p < .001$, $\eta^2 = 0.3$) but not for their interaction ($F(2,41) = 0.31$, $p = .74$, $\eta^2 = 0.009$). CSF volume increased with timepoint and mother-deprived CSF volume was greater than that of mother-reared: T1 *MD* = 15.70 ± 1.96 cm^3, *MR* = 14.41 ± 1.56 cm^3; T2 *MD* = 20.58 ±

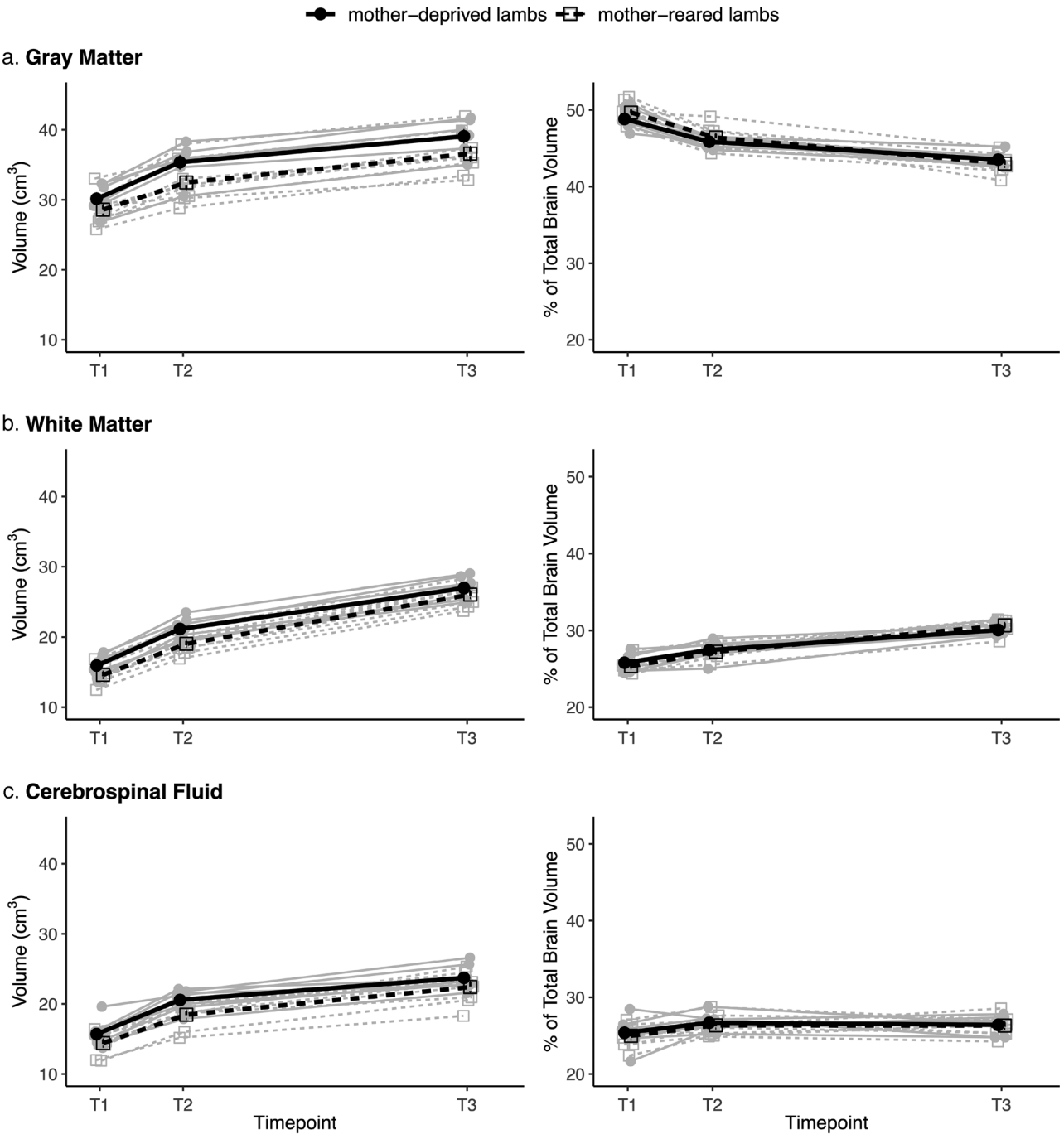

FIGURE 6 Volumes (left) and proportion of total brain volumes (right) of the gray matter (a), white matter (b), and cerebrospinal fluid (c) are presented as group means ± standard error in black and as individual data in gray

1.53 cm^3, *MR* = 18.45 ± 1.77 cm^3; T3 *MD* = 23.71 ± 1.78 cm^3, *MR* = 22.41 ± 2.16 cm^3 (Figure 6C).

However, examination of the proportions of GM, WM and CSF in the brain showed that they evolved in the same way in mother-reared and mother-deprived lambs during their growth (Figure 6, right column).

For the proportion of GM in the brain, the ANCOVA highlighted an effect of timepoint ($F(2,41) = 11.18$, $p < .001$, $\eta^2 = 0.321$) but not of early experience ($F(1,41) = 1.39$ $p = .245$, $\eta^2 = 0.02$) or their interaction ($F(2,41) = 0.95$, $p = .39$, $\eta^2 = 0.027$). Regardless of early experience the proportion of GM in the brain decreased with timepoint.

For the proportion of WM in the brain, the ANCOVA highlighted an effect of timepoint ($F(2,41) = 4.64$, $p = .015$, $\eta^2 = 0.153$) but not of early experience ($F(1,41) = 0.37$ $p = .55$, $\eta^2 = 0.006$) or their interaction ($F(2,41) = 0.73$, $p = .49$, $\eta^2 = 0.024$). Regardless of early experience the proportion of WM in the brain increased with timepoint.

For the proportion of CSF in the, brain the ANCOVA highlighted no significant effects: early experience ($F(1,41) = 0.35$ $p = .56$, $\eta^2 = 0.007$), timepoint ($F(2,41) = 2.89$, $p = .067$, $\eta^2 = 0.122$), or their interaction ($F(2,41) = 0.07$, $p = .93$, $\eta^2 = 0.003$).

3.5 | Volumes of GM structures

For the CN volume, the ANCOVA highlighted effects of early experience ($F(1,27) = 14.86$, $p < .001$, $\eta^2 = 0.276$) and timepoint ($F(1,27) = 6.97$, $p = .014$, $\eta^2 = 0.129$) but not for their interaction ($F(1,27) = 0.69$, $p = .41$, $\eta^2 = 0.013$). The volume of the CN increased with timepoint and was smaller for mother-deprived than for mother-reared lambs (Table 2).

For the hippocampal volume, the ANCOVA highlighted a significant effect of timepoint ($F(1,23) = 39.09$, $p = .001$, $\eta^2 = 0.422$) but not of early experience ($F(1,23) = 0.02$, $p = .89$, $\eta^2 < 0.001$) or their interaction ($F(1,23) = 0.35$, $p = .56$, $\eta^2 = 0.004$). The volume of the Hipp increased with timepoint.

For the OB volume, the ANCOVA highlighted no significant effects: early experience ($F(1,19) = 0.68$, $p = .42$, $\eta^2 = 0.034$), timepoint ($F(1,19) = 0.02$, $p = .88$, $\eta^2 = 0.001$), and their interaction ($F(1,19) = 0.12$, $p = .73$, $\eta^2 = 0.006$).

For the PAG volume, the ANCOVA highlighted no significant effects: early experience ($F(1,27) = 2.45$, $p = .13$, $\eta^2 = 0.077$), timepoint ($F(1,27) = 0.07$, $p = .79$, $\eta^2 = 0.002$), and their interaction ($F(1,27) = 1.36$, $p = .23$, $\eta^2 = 0.044$).

3.6 | Microstructural data

Examination of the microstructural data highlighted an impact of early experience in both gray and white matter, with more parameters being impacted in white matter (Figure 7). Indeed, T1w signal intensity and RD were higher in the gray and white matter of mother-deprived lambs than in mother-reared lambs. FA was also impacted in white matter with higher values observed in mother-reared than in mother-deprived lambs. Moreover, the microstructural data changed between the first two timepoints in white matter (increased T1w signal intensity and FA) and in gray matter (increased FA). FA and RD in different white matter areas indicate an impact of early experience and/or timepoint in a restricted number of areas. In the frontal white matter, there was higher FA in mother-reared than in mother-deprived lambs, and in parietal white matter, there was increased FA throughout the experiment. Detailed statistical results are described below.

3.7 | Total gray matter

For the T1w signal intensity of GM relative to CSF, the ANCOVA highlighted a significant effect of early experience ($F(1,41) = 20.88$, $p < .001$, $\eta^2 = 0.034$) but not of timepoint ($F(2,41) = 2.09$, $p = .136$, $\eta^2 = 0.058$) or their interaction ($F(2,41) = 2.89$, $p = .067$, $\eta^2 = 0.08$). GM intensity was higher for mother-deprived than for mother-reared lambs.

For FA in GM, the ANCOVA highlighted a significant effect of timepoint ($F(2,41) = 10.72$, $p < 0.001$, $\eta^2 = 0.319$) but not of early experience ($F(1,41) = 0.59$, $p = .45$, $\eta^2 = 0.009$) or of their interaction ($F(2,41) = 1.91$, $p = .16$, $\eta^2 = 0.057$). The FA values in GM increased significantly only between T1 and T2.

For RD in GM, the ANCOVA highlighted a significant interaction between early experience and timepoint ($F(2,41) = 4.41$, $p = .018$, $\eta^2 = 0.103$) and a significant effect of early experience ($F(1,41) = 32.68$, $p < .001$, $\eta^2 = 0.38$) but not of timepoint ($F(2,41) = 1.68$, $p = .198$, $\eta^2 = 0.039$). The main effect of early experience was driven by higher RD values for mother-deprived lambs than mother-reared lambs. Investigating the interaction effect by observing the data however, showed that the higher RD values for mother-deprived lambs were present at T1 and T2 but not T3; however, none of the Bonferroni corrected post hoc tests to investigate this interaction were significant.

3.8 | Total white matter

For the T1w signal intensity of WM relative to CSF, the ANCOVA highlighted significant effects of early experience ($F(1,41) = 4.67$, $p = .037$, $\eta^2 = 0.086$), timepoint ($F(2,41) = 3.92$, $p = .028$, $\eta^2 = 0.144$) but not their interaction ($F(2,41) = 0.53$, $p = .59$, $\eta^2 = 0.02$). The T1w signal intensity in WM was higher for mother-deprived than mother-reared lambs. Observation of the data showed that the T1w signal intensity in WM increased between T1 and T2 but not between T2 and T3; however, none of the Bonferroni corrected post-hoc tests to investigate the effect of timepoint were significant.

For FA in WM, the ANCOVA highlighted significant effects of early experience ($F(1,41) = 5.78$, $p = .021$, $\eta^2 = 0.065$), timepoint ($F(2,41) = 12.02$, $p < .001$, $\eta^2 = 0.271$) and their interaction ($F(2,41) = 4.47$, $p = .018$, $\eta^2 = 0.101$). Observation of the data showed that mother-reared lambs had higher FA values in WM than mother-deprived lambs for T1 and T2 but not T3; however, none of the Bonferroni corrected post hoc tests to investigate this interaction were significant.

For RD in WM, the ANCOVA highlighted a significant interaction between early experience and timepoint ($F(2,41) = 5.68$, $p = .007$, $\eta^2 = 0.157$) and a significant effect of early experience ($F(1,41) = 14.9$, $p < .001$, $\eta^2 = 0.206$) but not of timepoint ($F(2,41) = 0.27$, $p = .76$, $\eta^2 = 0.007$). Observation of the data showed that mother-deprived lambs had higher RD values in WM than mother-reared lambs for T1 and T2 but not T3; however, none of the Bonferroni corrected post-hoc tests to investigate this interaction were significant.

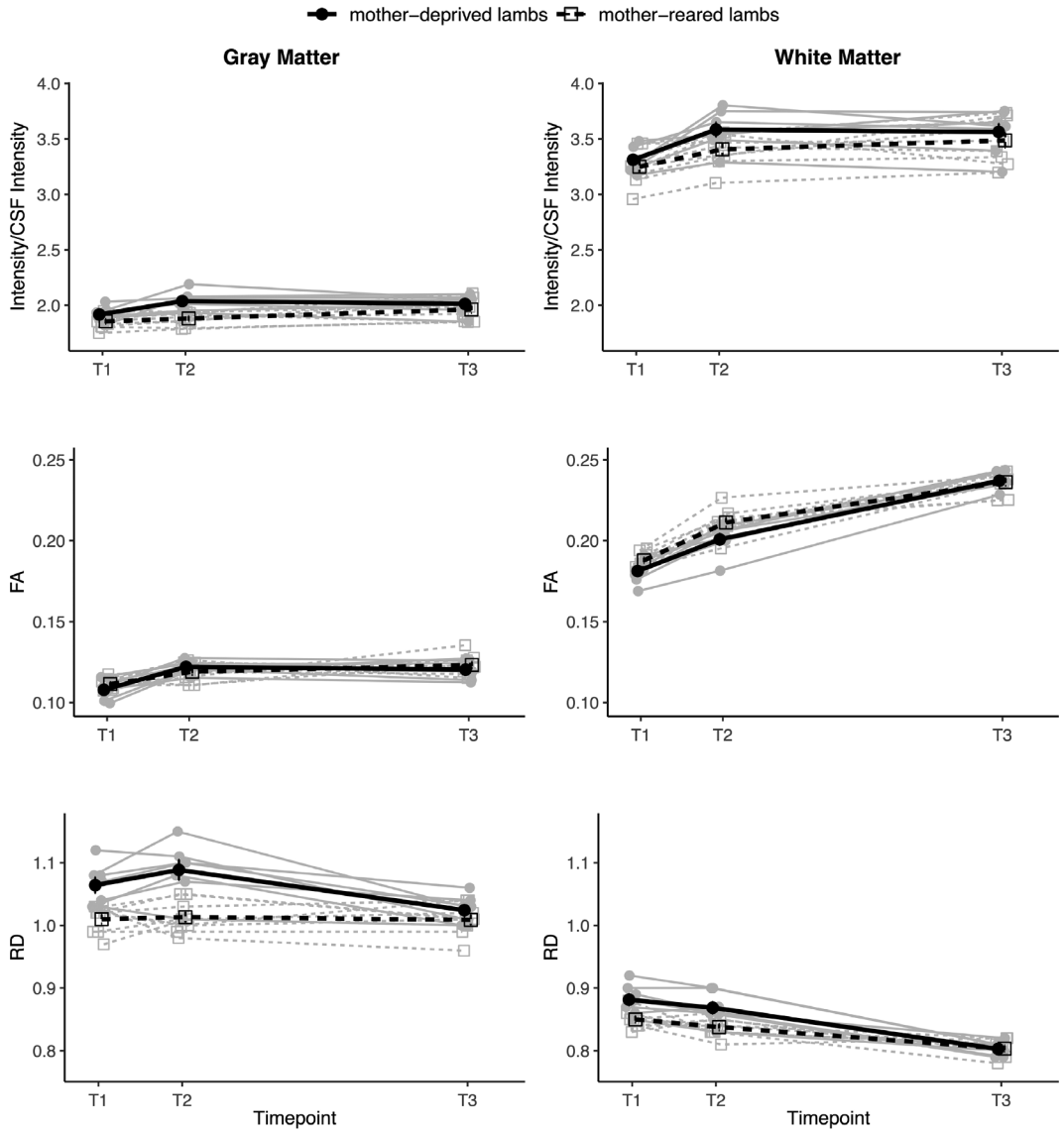

FIGURE 7 T1w signal intensity (top), fractional anisotropy (FA, middle) and radial diffusivity (RD, bottom) are presented as group means ± standard error in black and as individual data in gray for both the gray (left column) and white (right column) matters

3.9 | WM-areas

FA and RD-values calculated in each WM-area (Figure 3) are reported in Tables 3 and 4, respectively.

3.9.1 | Frontal WM

For FA, the ANCOVA highlighted a significant effect of early experience ($F(1,36) = 5.05$, $p = .031$, $n^2 = 0.117$) but not of timepoint ($F(2,36) = 0.80$, $p = .46$, $n^2 = 0.037$) or their interaction ($F(2,36) = 0.26$, $p = .77$, $n^2 = 0.012$). Mother-reared lambs had higher FA values than mother-deprived lambs in the WM-brain area of the frontal lobe. Because variance of RD in frontal WM equals 0 after grouping on timepoint and early experience, we did not present the statistical analysis results.

3.9.2 | Parietal WM

For FA, the ANCOVA highlighted a significant effect of timepoint ($F(2,41) = 4.91$, $p = .031$, $n^2 = 0.012$) but not of early experience ($F(1,41) = 0.02$, $p = .89$, $n^2 = 0.002$) or their interaction ($F(2,41) = 0.85$, $p = .44$, $n^2 = 0.025$). Observation of the data showed that the FA values in this area were higher at T2 than at T1 and T3; however, none of the Bonferroni corrected post hoc tests to investigate the effect were significant. For RD in the WM-brain area of the parietal lobe, the ANCOVA highlighted no significant effects: early experience

TABLE 3 Mean fractional anisotropy values calculated in different WM-area

WM areas	Timepoint	Mother-reared lambs	Mother-deprived lambs	ANCOVAanalyses results
Frontal lobe	T1	0.254 ± 0.026 ($n = 7$)	0.225 ± 0.031 ($n = 6$)	$\boldsymbol{p_{EE}}$**=.031 (**$\mathbf{F_{(2,36)}}$**=5.05)**
	T2	0.252 ± 0.030 ($n = 8$)	0.240 ± 0.033 ($n = 6$)	p_T=.46 ($F_{(1,36)}$=0.80)
	T3	0.328 ± 0.035 ($n = 9$)	0.303 ± 0.032 ($n = 7$)	p_{EExT}=.77 ($F_{(2,36)}$=0.26)
Parietal lobe	T1	0.127 ± 0.023 ($n = 9$)	0.123 ± 0.035 ($n = 7$)	p_{EE}=.89 ($F_{(1,41)}$=0.02)
	T2	0.139 ± 0.028 ($n = 9$)	0.131 ± 0.019 ($n = 7$)	$\boldsymbol{p_T}$**=.012 (**$\mathbf{F_{(2,41)}}$**=4.91)**
	T3	0.181 ± 0.015 ($n = 9$)	0.183 ± 0.035 ($n = 7$)	p_{EExT}=.44 ($F_{(2,41)}$=0.85)
Temporal lobe	T1	0.245 ± 0.053 ($n = 9$)	0.290 ± 0.024 ($n = 7$)	*p_{EE}=0.059 ($F_{(1,41)}$=3.78)*
	T2	0.257 ± 0.050 ($n = 9$)	0.264 ± 0.054 ($n = 7$)	p_T=.70 ($F_{(2,41)}$=0.37)
	T3	0.249 ± 0.068 ($n = 9$)	0.291 ± 0.048 ($n = 7$)	p_{EExT}=.57 ($F_{(2,41)}$=0.58)
Occipital lobe	T1	0.205 ± 0.031 ($n = 9$)	0.202 ± 0.036 ($n = 7$)	p_{EE}=.41 ($F_{(1,41)}$=0.69)
	T2	0.251 ± 0.046 ($n = 9$)	0.239 ± 0.018 ($n = 7$)	p_T=.21 ($F_{(2,41)}$=1.64)
	T3	0.307 ± 0.059 ($n = 9$)	0.285 ± 0.035 ($n = 7$)	p_{EExT}=.89 ($F_{(2,41)}$=0.12)
Corpus callosum	T1	0.194 ± 0.032 ($n = 4$)	0.171 ± 0.041 ($n = 6$)	p_{EE}=.5 ($F_{(1,32)}$=0.47)
	T2	0.209 ± 0.036 ($n = 7$)	0.223 ± 0.051 ($n = 7$)	$\boldsymbol{p_T}$**=.05 (**$\mathbf{F_{(2,32)}}$**=3.29)**
	T3	0.186 ± 0.018 ($n = 9$)	0.207 ± 0.025 ($n = 6$)	p_{EExT}=.29 ($F_{(2,32)}$=1.28)
Optic chiasm	T1	0.209 ± 0.011 ($n = 5$)	0.191 ± 0.034 ($n = 5$)	*p_{EE}=0.096 ($F_{(1,29)}$=2.96)*
	T2	0.264 ± 0.059 ($n = 7$)	0.185 ± 0.048 ($n = 5$)	p_T=.64 ($F_{(2,29)}$=0.46)
	T3	0.261 ± 0.043 ($n = 7$)	0.270 ± 0.057 ($n = 7$)	*p_{EExT}=0.074 ($F_{(2,29)}$=2.86)*
Cerebellum	T1	0.217 ± 0.048 ($n = 9$)	0.215 ± 0.048 ($n = 7$)	p_{EE}=.75 ($F_{(1,41)}$=0.104)
	T2	0.263 ± 0.058 ($n = 9$)	0.262 ± 0.028 ($n = 7$)	*p_T=0.057 ($F_{(2,41)}$=3.07)*
	T3	0.298 ± 0.038 ($n = 9$)	0.300 ± 0.051 ($n = 7$)	p_{EExT}=.892 ($F_{(2,41)}$=0.12)

Note: EE, early experience; T, timepoint; EExT, interaction; statistical significant effects are indicated in bold.

($F(1,41) = 0.83$, $p = .37$, $n^2 = 0.016$), timepoint ($F(2,41) = 2.74$, $p = .077$, $n^2 = 0.104$), and their interaction ($F(2,41) = 0.79$, $p = .46$, $n^2 = 0.03$).

3.9.3 | Temporal WM

For FA, the ANCOVA highlighted no significant effects: early experience ($F(1,41) = 3.78$, $p = .059$, $n^2 = 0.08$), timepoint ($F(2,41) = 0.37$, $p = .70$, $n^2 = 0.016$), and their interaction ($F(2,41) = 0.58$, $p = .57$, $n^2 = 0.025$). Similarly, for RD in the WM-brain area of the temporal lobe, the ANCOVA highlighted no significant effects: early experience ($F(1,41) = 3.51$, $p = .068$, $n^2 = 0.07$), timepoint ($F(2,41) = 0.57$, $p = .58$, $n^2 = 0.022$), and their interaction ($F(2,41) = 1.57$, $p = .22$, $n^2 = 0.063$).

3.9.4 | Occipital WM

For FA, the ANCOVA highlighted no significant effects: early experience ($F(1,41) = 0.69$, $p = .41$, $n^2 = 0.015$), timepoint ($F(2,41) = 1.64$, $p = .21$, $n^2 = 0.07$), and their interaction ($F(2,41) = 0.12$, $p = .89$, $n^2 = 0.005$). Similarly, for RD in the WM-brain area of the occipital lobe, the ANCOVA highlighted no significant effects: early experience ($F(1,41) = 1.15$, $p = .29$, $n^2 = 0.023$), timepoint ($F(2,41) = 2.94$, $p = .06$, $n^2 = 0.118$), and their interaction ($F(2,41) = 0.07$, $p = .93$, $n^2 = 0.003$).

3.9.5 | Corpus callosum

For FA, the ANCOVA highlighted a significant effect of timepoint ($F(2,32) = 3.29$, $p = .05$, $n^2 = 0.157$) but not of early experience ($F(1,32) = 0.47$, $p = .5$, $n^2 = 0.011$) or their interaction ($F(2,32) = 1.28$, $p = .29$, $n^2 = 0.061$). None of the Bonferroni corrected post-hoc tests investigating the effect of timepoint were significant. For RD in the corpus callosum, the ANCOVA highlighted no significant effects: early experience ($F(1,32) = 0.44$, $p = .51$, $n^2 = 0.012$), timepoint ($F(2,32) = 1.12$, $p = .34$, $n^2 = 0.059$), and their interaction ($F(2,32) = 0.37$, $p = .69$, $n^2 = 0.02$).

3.9.6 | Optic chiasm

For FA, the ANCOVA highlighted no significant effects: early experience ($F(1,29) = 2.96$, $p = .096$, $n^2 = 0.077$), timepoint

TABLE 4 Mean radial diffusivity values (x10^{-3}) calculated in different WM-area

WM areas	Timepoint	Mother-reared lambs	Mother-deprived lambs	ANCOVAanalyses results
Frontal lobe	T1	0.779 ± 0.027 (n = 7)	0.808 ± 0.038 (n = 6)	Variance equals 0 after grouping on timepoint, early experience
	T2	0.769 ± 0.037 (n = 8)	0.775 ± 0.082 (n = 6)	
	T3	0.706 ± 0.046 (n = 9)	0.700 ± 0.000 (n = 7)	
Parietal lobe	T1	0.872 ± 0.044 (n = 9)	0.871 ± 0.049 (n = 7)	p_{EE}=.37 ($F_{(1,41)}$=0.83) p_T=*0.077* ($F_{(2,41)}$=*2.74*) p_{EExT}=.46 ($F_{(2,41)}$=0.79)
	T2	0.800 ± 0.025 (n = 9)	0.843 ± 0.079 (n = 7)	
	T3	0.756 ± 0.039 (n = 9)	1.286 ± 1.374 (n = 7)	
Temporal lobe	T1	0.828 ± 0.103 (n = 9)	0.736 ± 0.056 (n = 7)	p_{EE}=*0.068* ($F_{(1,41)}$=*3.51*) p_T=.58 ($F_{(2,41)}$=0.56) p_{EExT}=.22 ($F_{(2,41)}$=1.57)
	T2	0.789 ± 0.060 (n = 9)	0.771 ± 0.064 (n = 7)	
	T3	0.761 ± 0.065 (n = 9)	0.743 ± 0.061 (n = 7)	
Occipital lobe	T1	0.794 ± 0.058 (n = 9)	0.807 ± 0.079 (n = 7)	p_{EE}=.29 ($F_{(1,41)}$=1.15) p_T=*0.064* ($F_{(2,41)}$=*2.69*) p_{EExT}=.89 ($F_{(2,41)}$=0.12)
	T2	0.733 ± 0.050 (n = 9)	0.750 ± 0.050 (n = 7)	
	T3	0.700 ± 0.050 (n = 9)	0.736 ± 0.056 (n = 7)	
Corpus callosum	T1	1.175 ± 0.222 (n = 4)	1.183 ± 0.133 (n = 6)	p_{EE}=.51 ($F_{(1,32)}$=0.44) p_T=.34 ($F_{(2,32)}$=1.12) p_{EExT}=.69 ($F_{(2,32)}$=0.37)
	T2	1.243 ± 0.237 (n = 7)	1.214 ± 0.241 (n = 7)	
	T3	1.378 ± 0.148 (n = 9)	1.233 ± 0.137 (n = 6)	
Optic chiasm	T1	1.140 ± 0.167 (n = 5)	1.140 ± 0.182 (n = 5)	p_{EE}=.13 ($F_{(1,29)}$=2.40) p_T=.58 ($F_{(2,29)}$=0.56) p_{EExT}=.25 ($F_{(2,29)}$=1.44)
	T2	1.086 ± 0.234 (n = 7)	1.360 ± 0.297 (n = 5)	
	T3	1.129 ± 0.138 (n = 7)	1.157 ± 0.199 (n = 7)	
Cerebellum	T1	0.681 ± 0.063 (n = 7)	0.683 ± 0.055 (n = 7)	p_{EE}=.76 ($F_{(1,41)}$=0.098) p_T=.13 ($F_{(2,41)}$=2.17) p_{EExT}=.78 ($F_{(2,41)}$=0.25)
	T2	0.643 ± 0.036 (n = 9)	0.648 ± 0.038 (n = 7)	
	T3	0.641 ± 0.060 (n = 9)	0.631 ± 0.063 (n = 7)	

Note: EE, early experience; T, timepoint; EExT, interaction.

($F(2,29) = 0.46$, $p = .64$, $n^2 = 0.024$), and their interaction ($F(2,29) = 2.86$, $p = .074$, $n^2 = 0.148$). Similarly, for FA in the optic chiasm, the ANCOVA highlighted no significant effects: early experience ($F(1,29) = 2.40$, $p = .132$, $n^2 = 0.067$), timepoint ($F(2,29) = 0.56$, $p = .58$, $n^2 = 0.031$), and their interaction ($F(2,29) = 1.44$, $p = .25$, $n^2 = 0.081$).

3.9.7 | Cerebellum WM

For FA, the ANCOVA highlighted no significant effects: early experience ($F(1,41) = 0.10$, $p = .75$, $n^2 = 0.002$), timepoint ($F(2,41) = 3.07$, $p = .057$, $n^2 = 0.119$), and their interaction ($F(2,41) = 0.12$, $p = .89$, $n^2 = 0.004$). Similarly for RD in the cerebellum, the ANCOVA highlighted no significant effects: early experience ($F(1,41) = 0.10$, $p = .76$, $n^2 = 0.002$), timepoint ($F(2,41) = 2.17$, $p = .13$, $n^2 = 0.089$) and their interaction ($F(2,41) = 0.25$, $p = .78$, $n^2 = 0.01$).

4 | DISCUSSION

The present study characterizes for the first time the developing brain of lambs reared either with or without their mother. As the mother generally plays a crucial role in development, we first discuss the growth of the mother-reared lambs' brain before comparing it to that of mother-deprived lambs.

4.1 | Brain development and maturation in mother-reared lambs

When investigating brain development using MRI there is a potential problem due to the change of tissue contrast that occurs with maturation. In particular, GM and WM intensities in the developing brain change as the brain matures, which could affect their segmentation. In very young human infants, WM can even be represented by a binomial distribution of intensity values. That is, in the exaggerated case WM can be represented by both white and black intensity values in the same MR brain image (for an example see the Graphical Abstract in Li et al., 2019). This clearly prevents the use of intensity-based segmentation techniques, such as the one used here, for very young human infants (Li et al., 2019). Since sheep are a precocial species their WM does not display the same binomial distribution as in human newborns. In this case, the available automatic intensity-based

segmentation techniques allowed to evaluate accurately the GM, WM, and CSF volumes.

We expected increases in total brain, GM, and WM volumes during the growth of lambs. In humans, typical pediatric brain development from early childhood to adolescence is characterized by an increasing WM volume trajectory and inverted U-shaped trajectories for the total brain and GM volumes (Giedd & Rapoport, 2010). Increased total brain volume has also been observed in rhesus macaques between 2 and 6 months of age (Liu et al., 2019). In our study, the volumes of brain, GM, WM, and CSF increase with similar trajectories from about 1–2 weeks to 4.5 months of age. Even though these increases appear to slow down after approximately 1.5 months of age, especially for the CSF, the development of the brain, GM and WM are likely incomplete at the end of our experiment, around 4.5 months of age. Further longitudinal studies extending beyond 4.5 months of age are required to investigate the potential U-shaped trajectories of total brain and GM volumes in sheep. However, focusing on specific brain structures, we can see that the volumes of OB and PAG were not statistically different between 1.5 and 4.5 months of age suggesting that the growth of these, and potentially other, specific structures may be almost complete. Similar regionalized brain growth has also been observed in other species (e.g., Giedd and Rapoport, 2010; Scott et al., 2016).

Examination of the relative proportions of GM, WM, and CSF in the brain revealed that the relative CSF growth trajectory was relatively flat throughout the experiment whereas those of GM and WM went in opposite directions. This reflects the fact that WM growth occurs faster than that of the total brain volume whereas GM growth is slower, as previously described in rhesus macaques between 2 and 6 months of age (Liu et al., 2019).

As suggested by several studies performed in primates (Dubois et al., 2006, 2014; Hüppi & Dubois, 2006; Liu et al., 2019; Mukherjee et al., 2002), examining MRI parameters extracted from different types of MRI sequences provides complementary information on brain development. First, we examined T1w signal intensity. This is a relevant indicator of early GM and WM maturation because changes in T1w signal intensity can reflect a decrease in water content and an increase in macromolecules in the brain (Barkovich et al., 1988; Shroff et al., 2010). Based on data reported in humans (Barkovich et al., 1988; Flood et al., 2019; Serag et al., 2011; Westlye et al., 2010), we hypothesized higher values in WM than in GM and increasing values in both brain tissues throughout development of mother-reared lambs. We found the highest values of T1 signal intensity in the WM, which were 1.5 times greater than those measured in GM whatever the timepoint of acquisition. In both brain tissues, the increase of T1 signal intensity was essentially observed between about 1–2 weeks and 1.5 months of age suggesting that this period corresponds to an important maturational period in lambs. Second, we hypothesized an increasing FA and decreasing RD throughout the experiment in both GM and WM, due to the water decrease during brain maturation and white fiber tract development (Dubois et al., 2014). Moreover, we predicted higher FA and lower RD in the WM than in the GM (Mukherjee et al., 2002). Our data confirmed our hypothesis with concomitant FA increase and RD decrease in WM, the variation being less notable for the GM. These observations are coherent with reported data in primates (Liu et al., 2019; Mukherjee et al., 2002).

In summary, our study described for the first time brain development and maturation of mother-reared lambs, showing its similarity to both human and nonhuman primates. The most prominent changes in the sheep brain appear to occur between about 1–2 weeks and 1.5 months of age but the brain's growth and development is still not complete by 4.5 months.

4.2 | Impact of maternal deprivation and milk replacement

Total brain, WM, GM, and CSF volumes were all lower in mother-reared than in mother-deprived lambs. The total brain volume finding is in contrast to a study of Rhesus macaques in which no impact of maternal deprivation on total brain volume was found (Liu et al., 2019). While Liu and colleagues did not present WM, GM, or CSF volumes to compare to they, like in the current results, did not find any difference between the groups in the proportion of brain occupied by WM or GM. These results raise the issue of the best parameter to evaluate brain development. In our opinion, it is more appropriate to follow the proportions of different brain matter since brain volume is not tightly linked to body weight in children (Giedd & Rapoport, 2010), even if the question of the best method to evaluate the brain size is questioned (Hasboun et al., 1996).

Because of their role in the socioemotional neuronal network (Grahn et al., 2008; Guesdon et al., 2016; Menant et al., 2016; White, 2009; Zelena et al., 2018), we examined the impact of early experience on OB, CN, Hipp, and PAG volumes and revealed two important results. First, whatever the early experience, brain growth is heterogeneous according to the different examined structures, the OB and the Hipp are the only brain structures with a volume increase between 1.5 and 4 months of age. Second, maternal deprivation and milk replacement affected the CN since it was smaller in mother-deprived than in mother-reared lambs. Due to the roles of the CN in motor processes and cognitive functions (Grahn et al., 2008; White, 2009) and to the importance of the mother in lambs' cognitive development and motor activities we expected a negative impact of maternal deprivation and milk replacement on this brain structure. This result is in accordance with observations in humans in which social

as well as nutritional causes have been reported. Concerning the social cause, early-life social stress (loss of parent or primary family member, witnessed domestic violence, sexual abuse) is associated with smaller CN in adulthood (Cohen et al., 2006). Concerning nutritional cause, adolescents of preterm birth nourished with a high-nutrient regime present higher volume of CN than adolescents of preterm birth nourished with a standard-nutrient regime (Isaacs et al., 2008). Based on the reported observations in humans, we could suspect a cumulative effect of maternal deprivation and milk-replacement to explain the impact we observed on the CN of mother-deprived lambs. In our study, the lack of impact of maternal deprivation on the volume of Hipp is similar to previous data reported in rhesus monkeys reared with their mother or in maternally deprived conditions (Sánchez et al., 1998; Spinelli et al., 2009) and in humans submitted to early-life social stress (Cohen et al., 2006). However, lower hippocampal volumes have been reported in children exposed to poverty (Luby et al., 2013).

In addition to these morphometric alterations, we hypothesized that maternal deprivation would affect brain maturation, evaluated by T1w signal intensity and diffusion parameters. The negative impact of maternal deprivation on GM and WM maturation is particularly important between about 1.5 weeks and 1.5 month of age. Concerning GM, higher T1w signal intensity and RD in mother-deprived lambs compared to mother-reared lambs suggests a deficit in brain maturation, especially before 4.5 months of age. This is supported by the fact that typical brain maturation is associated with decreased T1w signal intensity and decreased RD due to the incorporation of macromolecules into membranes of neural cells and the decrease of free water (Geeraert et al., 2019; Mukherjee et al., 2002). The WM seems to be the most impacted compartment of the brain since all structural parameters are affected by maternal deprivation: T1w signal intensity and RD were higher while FA was lower in mother-deprived than in mother-reared lambs. Similar results have been reported in formula-fed children (Deoni et al., 2013, 2018), in bonnet macaques born from stressed mothers (Coplan et al., 2010) or in formula-fed rhesus macaques (Liu et al., 2019). Based on description of T1w signal intensity, FA and RD changes in the typical human brain (Barkovich et al., 1988; Flood et al., 2019; Mukherjee et al., 2002; Westlye et al., 2010) and our observations in mother-reared lambs, we conclude that maternal deprivation and milk replacement leads to delay in brain maturation in lambs. As mentioned in the introduction, the impact on white matter may result from imbalanced nutrition from the milk replacer. But it may also result from chronic stress caused by the absence of the mother. Similar to results that show in utero exposure to stress in sheep modifies neural cells in corticolimbic structures (Petit et al., 2015) and alters neuronal myelination in corpus callosum (Huang et al., 2001) of new-born lambs. Specifically concerning myelination, it is debated whether the myelination process, when measured by lipid composition, is completed or not at birth in lambs (Barlow, 1969; Finnie, 2012; McIntosh et al., 1979; Patterson et al., 1971; Turley et al., 1996). In this context, it would be necessary to investigate postnatal myelination by studying oligodendrocyte progenitor cells (Singh et al., 2018). Indicators of the myelination process that are regularly explored to clearly describe this process in mammals such as humans (Williamson & Lyons, 2018).

5 | CONCLUSION

This study characterizes for the first time the developing brain of lambs reared with their mother. Increased volumes of brain, gray and white matter were observed together with increased values of FA and decreased values of RD, and these changes are particularly significant before 4.5 months of age. In addition, the current findings indicate that early maternal deprivation, currently practised in sheep production, delays brain maturation as early as 1.5 weeks of age. These alterations observed may help to explain the behavioral, endocrine and health alterations reported in artificially reared lambs.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

AUTHORS CONTRIBUTION

S. Love: post-processing: statistical analysis, results discussion, secondary writer. E. Haslin, L. Leroy, M. Bellardie, Cs L. Fazekas: internship (supervised by E. Chaillou), segmentation, bibliography. M. Morisse: technician, segmentation. D. Zelena: PHC Balaton (research exchange with Csilla Fazekas) F. Andersson, L. Barantin: MRI protocol, MRI acquisition. I. Filipiak: Diffusion MRI sequences and analysis. H. Adriaensen: MRI protocol, MRI acquisition. F. Elleboudt, C. Moussu: animal preparation, anesthesia, caregivers. F. Lévy: protocol discussion, interpretation, article reviewing. R. Nowak: coordinator of the Ovin2A project, protocol discussion, interpretation, article reviewing.

E. Chaillou: coordinator of the "brain development and maturation" task in the project, supervisor of the study, lead author.

DATA AVAILABILITY STATEMENT

The data that support the statistical findings of this study are openly available in Zenodo at http://doi.org/10.5281/zenodo.4714660.

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REFERENCES

Andropoulos, D. B. (2018). Effect of anesthesia on the developing brain: Infant and fetus. *Fetal Diagnosis and Therapy*, *43*(1), 1–11. https://doi.org/10.1159/000475928

Avants, B. B., Tustison, N. J., Stauffer, M., Song, G., Wu, B., & Gee, J. C. (2014). The Insight ToolKit image registration framework. *Frontiers in Neuroinformatics*, *8*, 44. https://doi.org/10.3389/fninf.2014.00044

Barkovich, A. J., Kjos, B. O., Jackson, D. E., & Norman, D. (1988). Normal maturation of the neonatal and infant brain: MR imaging at 1.5 T. *Radiology*, *166*(1), 173–180. https://doi.org/10.1148/radiology.166.1.3336675

Barlow, R. M. (1969). The foetal sheep: Morphogenesis of the nervous system and histochemical aspects of myelination. *The Journal of Comparative Neurology*, *135*(3), 249-261. https://doi.org/10.1002/cne.901350302

Bernstein, R. M., & Hinde, K. (2016). Bioactive factors in milk across lactation: Maternal effects and influence on infant growth in rhesus macaques (*Macaca mulatta*): Macaque milk bioactive factors. *American Journal of Primatology*, *78*(8), 838–850. https://doi.org/10.1002/ajp.22544

Berry, M. J., Jaquiery, A. L., Oliver, M. H., Harding, J. E., & Bloomfield, F. H. (2016). Neonatal milk supplementation in lambs has persistent effects on growth and metabolic function that differ by sex and gestational age. *British Journal of Nutrition*, *116*(11), 1912–1925. https://doi.org/10.1017/S0007114516004013

Black-Rubio, C. M., Cibils, A. F., & Gould, W. R. (2007). Maternal influence on feeding site avoidance behaviour of lambs. *Applied Animal Behaviour Science*, *105*(1–3), 122–139. https://doi.org/10.1016/j.applanim.2006.06.004

Boivin, X., Nowak, R., & Garcia, A. T. (2001). The presence of the dam affects the efficiency of gentling and feeding on the early establishment of the stockperson–lamb relationship. *Applied Animal Behaviour Science*, *72*(2), 89–103. https://doi.org/10.1016/S0168-1591(00)00201-X

Castillo-Melendez, M., Baburamani, A. A., Cabalag, C., Yawno, T., Witjaksono, A., Miller, S. L., & Walker, D. W. (2013). Experimental modelling of the consequences of brief late gestation asphyxia on newborn lamb behaviour and brain structure. *PLoS ONE*, *8*(11), e77377. https://doi.org/10.1371/journal.pone.0077377

Chaillou, E., Tillet, Y., & Andersson, F. (2012). MRI Techniques and new animal models for imaging the brain. In T. Mantamadiotis (Ed.), *When things go wrong—diseases and disorders of the human brain*. InTech. https://doi.org/10.5772/35834

Cohen, R. A., Grieve, S., Hoth, K. F., Paul, R. H., Sweet, L., Tate, D., Gunstad, J., Stroud, L., McCaffery, J., Hitsman, B., Niaura, R., Clark, C. R., MacFarlane, A., Bryant, R., Gordon, E., & Williams, L. M. (2006). Early life stress and morphometry of the adult anterior cingulate cortex and caudate nuclei. *Biological Psychiatry*, *59*, 975–982. https://doi.org/10.1016/j.biopsych.2005.12.016

Coplan, J. D., Abdallah, C. G., Tang, C. Y., Mathew, S. J., Martinez, J., Hof, P. R., Smith, E. L. P., Dwork, A. J., Perera, T. D., Pantol, G., Carpenter, D., Rosenblum, L. A., Shungu, D. C., Gelernter, J., Kaffman, A., Jackowski, A., Kaufman, J., & Gorman, J. M. (2010). The role of early life stress in development of the anterior limb of the internal capsule in nonhuman primates. *Neuroscience Letters*, *480*(2), 93–96. https://doi.org/10.1016/j.neulet.2010.06.012

Damián, J. P., Beracochea, F., Hötzel, M. J., Banchero, G., & Ungerfeld, R. (2015). Reproductive and sexual behaviour development of dam or artificially reared male lambs. *Physiology & Behavior*, *147*, 47–53. https://doi.org/10.1016/j.physbeh.2015.04.004

Damián, J. P., Beracochea, F., Machado, S., Hötzel, M. J., Banchero, G., & Ungerfeld, R. (2018). Growing without a mother results in poorer sexual behaviour in adult rams. *Animal*, *12*(1), 98–105. https://doi.org/10.1017/S1751731117001574

De Matteo, R., Blasch, N., Stokes, V., Davis, P., & Harding, R. (2010). Induced preterm birth in sheep: A suitable model for studying the developmental effects of moderately preterm birth. *Reproductive Sciences*, *17*(8), 724–733. https://doi.org/10.1177/1933719110369182

Deoni, S. C. L., Dean, D. C., Piryatinsky, I., O'Muircheartaigh, J., Waskiewicz, N., Lehman, K., Han, M., & Dirks, H. (2013). Breastfeeding and early white matter development: A cross-sectional study. *NeuroImage*, *82*, 77–86. https://doi.org/10.1016/j.neuroimage.2013.05.090

Deoni, S., Dean, D., Joelson, S., O'Regan, J., & Schneider, N. (2018). Early nutrition influences developmental myelination and cognition in infants and young children. *NeuroImage*, *178*, 649–659. https://doi.org/10.1016/j.neuroimage.2017.12.056

Dubois, J., Dehaene-Lambertz, G., Kulikova, S., Poupon, C., Hüppi, P. S., & Hertz-Pannier, L. (2014). The early development of brain white matter: A review of imaging studies in fetuses, newborns and infants. *Neuroscience*, *276*, 48–71. https://doi.org/10.1016/j.neuroscience.2013.12.044

Dubois, J., Hertz-Pannier, L., Dehaene-Lambertz, G., Cointepas, Y., & Le Bihan, D. (2006). Assessment of the early organization and maturation of infants' cerebral white matter fiber bundles: A feasibility study using quantitative diffusion tensor imaging and tractography. *NeuroImage*, *30*(4), 1121–1132. https://doi.org/10.1016/j.neuroimage.2005.11.022

Dunlop, S. A., Quinlivan, J. A., Beazley, L. D., & Newnham, J. P. (1997). Repeated prenatal corticosteroids delay myelination in the ovine central nervous system. *The Journal of Maternal-Fetal Medicine*, *6*(6), 309–313. https://doi.org/10.1002/(SICI)1520-6661(199711/12)6:6<309::AID-MFM1>3.0.CO;2-S

Finnie, J. W. (2012). Comparative approach to understanding traumatic injury in the immature, postnatal brain of domestic animals. *Australian Veterinary Journal*, *90*(8), 301–307. https://doi.org/10.1111/j.1751-0813.2012.00955.x

Friston, K. J., Holmes, A. P., Worsley, K. J., Poline, J.-P., Frith, C. D., & Frackowiak R. S. J. (1994). Statistical parametric maps in functional imaging: A general linear approach. *Human brain mapping*, *2*(4), 189–210. https://onlinelibrary.wiley.com/doi/10.1002/hbm.460020402

Fleming, A. S., Gonzalez, A., Lovic, V., Rees, S., & Melo, A. (2002). Mothering begets mothering: The transmission of behavior and its neurobiology across generations. *Pharmacology Biochemistry*

and Behavior, *73*(1), 61–75. https://doi.org/10.1016/S0091-3057(02)00793-1

Flood, T. F., Bhatt, P. R., Jensen, A., Maloney, J. A., Stence, N. V., & Mirsky, D. M. (2019). Age-dependent signal intensity changes in the structurally normal pediatric brain on unenhanced T1-weighted MR imaging. *American Journal of Neuroradiology*, *40*(11), 1824–1828. https://doi.org/10.3174/ajnr.A6254

Gaudin, S., Chaillou, E., Wycke, M.-A., Cornilleau, F., Moussu, C., Calandreau, L., Lainé, A.-L., & Nowak, R. (2018). All bonds are not alike: A psychoendocrine evaluation of infant attachment. *Developmental Psychobiology*, *60*(1), 90–103. https://doi.org/10.1002/dev.21552

Geeraert, B. L., Lebel, R. M., & Lebel, C. (2019). A multiparametric analysis of white matter maturation during late childhood and adolescence. *Human Brain Mapping*, *40*(15), 4345–4356. https://doi.org/10.1002/hbm.24706

Giedd, J. N., & Rapoport, J. L. (2010). Structural MRI of pediatric brain development: what have we learned and where are we going? *Neuron*, *67*(5), 728–734. https://doi.org/10.1016/j.neuron.2010.08.040

Grahn, J. A., Parkinson, J. A., & Owen, A. M. (2008). The cognitive functions of the caudate nucleus. *Progress in Neurobiology*, *86*(3), 141–155. https://doi.org/10.1016/j.pneurobio.2008.09.004

Guesdon, V., Nowak, R., Meurisse, M., Boivin, X., Cornilleau, F., Chaillou, E., & Lévy, F. (2016). Behavioral evidence of heterospecific bonding between the lamb and the human caregiver and mapping of associated brain network. *Psychoneuroendocrinology*, *71*, 159–169. https://doi.org/10.1016/j.psyneuen.2016.05.020

Hasboun, D., Chantôme, M., Zouaoui, A., Sahel, M., Baulac, M., Marsault, C., Duyme, M., & Dormont, D. (1996). Évaluation du volume cérébral: Reproductibilité et précision d'une technique 3D IRM. *Bulletins et Mémoires de la Société d'anthropologie de Paris*, *8*(1), 43–56. https://doi.org/10.3406/bmsap.1996.2427

Hernández-Castellano, L. E., Suárez-Trujillo, A., Martell-Jaizme, D., Cugno, G., Argüello, A., & Castro, N. (2015). The effect of colostrum period management on BW and immune system in lambs: From birth to weaning. *Animal*, *9*(10), 1672–1679. https://doi.org/10.1017/S175173111500110X

Huang, W. L., Harper, C. G., Evans, S. F., Newnham, J. P., & Dunlop, S. A. (2001). Repeated prenatal corticosteroid administration delays myelination of the corpus callosum in fetal sheep. *International Journal of Developmental Neuroscience*, *19*(4), 415–425. https://doi.org/10.1016/S0736-5748(01)00026-0

Hüppi, P. S., & Dubois, J. (2006). Diffusion tensor imaging of brain development. *Seminars in Fetal and Neonatal Medicine*, *11*(6), 489–497. https://doi.org/10.1016/j.siny.2006.07.006

Isaacs, E. B., Gadian, D. G., Sabatini, S., Chong, W. K., Quinn, B. T., Fischl, B. R., & Lucas, A. (2008). The effect of early human diet on caudate volumes and IQ. *Pediatric Research*, *63*(3), 308–314. https://doi.org/10.1203/PDR.0b013e318163a271

Jenkinson, M., Beckmann, C. F., Behrens, T. E. J., Woolrich, M. W., & Smith, S. M. (2012). FSL. *NeuroImage*, *62*(2), 782–790. https://doi.org/10.1016/j.neuroimage.2011.09.015

Jevtovic-Todorovic, V. (2005). General anesthetics and the developing brain: Friends or foes? *Journal of Neurosurgical Anesthesiology*, *17*(4), 204–206. https://doi.org/10.1097/01.ana.0000178111.26972.16

Jezzard, P. & Balaban R. S. (1995). Correction for geometric distorsion in echo planar images from B0 field variation. *Magnetic Resonance in Medicine 34*(1), 65–73. https://doi.org/10.1002/mrm.1910340111

Khan, A., & Ahmad, R. (1997). Maternal immunoglobulins transfer and neonatal lamb mortality—A review. *Pakistan Veterinary Journal*, *17*(4), 161–167.

Latham, N. R., & Mason, G. J. (2008). Maternal deprivation and the development of stereotypic behaviour. *Applied Animal Behaviour Science*, *110*(1–2), 84–108. https://doi.org/10.1016/j.applanim.2007.03.026

Li, G., Wang, L., Yap, P., Wang, F., Wu, Z., Meng, Y., Dong, P., Kim, J., Shi, F., Rekik, I., Lin, W., & Shen, D., (2019). Computational neuroanatomy of baby brains: A review. *NeuroImage*, *185*, 906–925. https://doi.org/10.1016/j.neuroimage.2018.03.042

Ligout, S., & Porter, R. H. (2004). Effect of maternal presence on the development of social relationships among lambs. *Applied Animal Behaviour Science*, *88*(1–2), 47–59. https://doi.org/10.1016/j.applanim.2004.03.010

Liu, Z., Neuringer, M., Erdman, J. W., Kuchan, M. J., Renner, L., Johnson, E. E., Wang, X., & Kroenke, C. D. (2019). The effects of breastfeeding versus formula-feeding on cerebral cortex maturation in infant rhesus macaques. *NeuroImage*, *184*, 372–385. https://doi.org/10.1016/j.neuroimage.2018.09.015

Luby, J., Belden, A., Botteron, K., Marrus, N., Harms, M. P., Babb, C., Nishino, T., & Barch, D. (2013). The effects of poverty on childhood brain development: The mediating effect of caregiving and stressful life events. *JAMA Pediatrics*, *167*(12), 1135. https://doi.org/10.1001/jamapediatrics.2013.3139

Malhotra, A., Sepehrizadeh, T., Dhollander, T., Wright, D., Castillo-Melendez, M., Sutherland, A. E., Pham, Y., Ditchfield, M., Polglase, G. R., de Veer, M., Jenkin, G., Pannek, K., Shishegar, R., & Miller, S. L. (2019). Advanced MRI analysis to detect white matter brain injury in growth restricted newborn lambs. *NeuroImage: Clinical*, *24*, 101991. https://doi.org/10.1016/j.nicl.2019.101991

Manjón, J. V., Coupé, P., Martí-Bonmatí, L., Collins, D. L., & Robles, M. (2010). Adaptive non-local means denoising of MR images with spatially varying noise levels: Spatially adaptive nonlocal denoising. *Journal of Magnetic Resonance Imaging*, *31*(1), 192–203. https://doi.org/10.1002/jmri.22003

McIntosh, G. H., Baghurst, K. I., Potter, B. J., & Hetzel, B. S. (1979). Foetal brain development in the sheep. *Neuropathology and Applied Neurobiology*, *5*(2), 103–114. https://doi.org/10.1111/j.1365-2990.1979.tb00664.x

Menant, O., Andersson, F., Zelena, D., & Chaillou, E. (2016). The benefits of magnetic resonance imaging methods to extend the knowledge of the anatomical organisation of the periaqueductal gray in mammals. *Journal of Chemical Neuroanatomy*, *77*, 110–120. https://doi.org/10.1016/j.jchemneu.2016.06.003

Mukherjee, P., Miller, J. H., Shimony, J. S., Philip, J. V., Nehra, D., Snyder, A. Z., Conturo, T. E., Neil, J. J., & McKinstry, R. C. (2002). Diffusion-tensor MR imaging of gray and white matter development during normal human brain maturation. *American Journal of Neuroradiology*, *23*(9), 1445–1456.

Napolitano, F. (2003). Lambs prevented from suckling their mothers display behavioral, immune and endocrine disturbances. *Physiology & Behavior*, *78*(1), 81–89. https://doi.org/10.1016/S0031-9384(02)00892-2

Napolitano, F., Cifuni, G. F., Pacelli, C., Riviezzi, A. M., & Girolami, A. (2002). Effect of artificial rearing on lamb welfare and meat quality. *Meat Science*, *60*(3), 307–315. https://doi.org/10.1016/S0309-1740(01)00140-1

Napolitano, F., Marino, V., De Rosa, G., Capparelli, R., & Bordi, A. (1995). Influence of artificial rearing on behavioral and immune response of lambs. *Applied Animal Behaviour Science*, *45*(3–4), 245–253. https://doi.org/10.1016/0168-1591(95)00637-8

Nowak, R., Keller, M., & Lévy, F. (2011). Mother-young relationships in sheep: A model for a multidisciplinary approach of the study of attachment in mammals. *Journal of Neuroendocrinology*, *23*(11), 1042–1053. https://doi.org/10.1111/j.1365-2826.2011.02205.x

Nowak, R., Murphy, T. M., Lindsay, D. R., Alster, P., Andersson, R., & S-Moberg, K. U. (1997). Development of a preferential relationship with the mother by the newborn lamb: Importance of the sucking activity. *Physiology and Behavior*, *62*(4), 681–688. https://doi.org/10.1016/s0031-9384(97)00079-6

Nowak, R., & Poindron, P. (2006). From birth to colostrum: Early steps leading to lamb survival. *Reproduction Nutrition Development*, *46*(4), 431–446. https://doi.org/10.1051/rnd:2006023

Olutoye, O. A., Lazar, D. A., Akinkuotu, A. C., Adesina, A., & Olutoye, O. O. (2015). Potential of the ovine brain as a model for anesthesia-induced neuroapoptosis. *Pediatric Surgery International*, *31*(9), 865–869. https://doi.org/10.1007/s00383-015-3751-7

O'Muircheartaigh, J., Dean, D. C., Ginestet, C. E., Walker, L., Waskiewicz, N., Lehman, K., Dirks, H., Piryatinsky, I., & Deoni, S. C. L. (2014). White matter development and early cognition in babies and toddlers: White matter development and early cognition. *Human Brain Mapping*, *35*(9), 4475–4487. https://doi.org/10.1002/hbm.22488

Patterson, D. S., Sweasey, D., & Hebert, C. N. (1971). Changes occurring in the chemical composition of the central nervous system during foetal and post-natal development of the sheep. *Journal of Neurochemistry*, *18*(11), 2027–2040. https://doi.org/10.1111/j.1471-4159.1971.tb05062.x

Petit, B., Boissy, A., Zanella, A., Chaillou, E., Andanson, S., Bes, S., Lévy, F., & Coulon, M. (2015). Stress during pregnancy alters dendritic spine density and gene expression in the brain of new-born lambs. *Behavioural Brain Research*, *291*, 155–163. https://doi.org/10.1016/j.bbr.2015.05.025

Polkowska, J., & Wańkowska, M. (2010). Effects of maternal deprivation on the somatotrophic axis and neuropeptide Y in the hypothalamus and pituitary in female lambs. The histomorphometric study. *Folia Histochemica et Cytobiologica*, *48*(2), 299–305. https://doi.org/10.2478/v10042-010-0024-0

Pryce, C. R. (1995). Determinants of motherhood in human and nonhuman primates. A biosocial model. *Motherhood in Humans and Nonhuman Primates*, 1–15. https://doi.org/10.1159/000424482

Saint-Dizier, H., Lévy, F., & Ferreira, G. (2007). Influence of the mother in the development of flavored-food preference in lambs. *Developmental Psychobiology*, *49*(1), 98–106. https://doi.org/10.1002/dev.20197

Sánchez, M. M., Hearn, E. F., Do, D., Rilling, J. K., & Herndon, J. G. (1998). Differential rearing affects corpus callosum size and cognitive function of rhesus monkeys. *Brain Research*, *812*(1–2), 38–49. https://doi.org/10.1016/S0006-8993(98)00857-9

Schack-Nielsen, L., & Michaelsen, K. F. (2007). Advances in our understanding of the biology of human milk and its effects on the offspring. *The Journal of Nutrition*, *137*(2), 503S–510S. https://doi.org/10.1093/jn/137.2.503S

Scott, J. A., Grayson, D., Fletcher, E., Lee, A., Bauman, M. D., Schumann, C. M., Buonocore, M. H., & Amaral, D. G. (2016). Longitudinal analysis of the developing rhesus monkey brain using magnetic resonance imaging: Birth to adulthood. *Brain Structure and Function*, *221*(5), 2847–2871. https://doi.org/10.1007/s00429-015-1076-x

Serag, A., Aljabar, P., Counsell, S., Boardman, J., Hajnal, J. V., & Rueckert, D. (2011). Tracking developmental changes in subcortical structures of the preterm brain using multi-modal MRI. *2011 IEEE International Symposium on Biomedical Imaging: From nano to macro*, 349–352. https://doi.org/10.1109/ISBI.2011.5872421

Sevi, A., Napolitano, F., Casamassima, D., Annicchiarico, G., Quarantelli, T., & De Paola, R. (1999). Effect of gradual transition from maternal to reconstituted milk on behavioural, endocrine and immune responses of lambs. *Applied Animal Behaviour Science*, *64*(4), 249–259. https://doi.org/10.1016/S0168-1591(99)00042-8

Shroff, M. M., Soares-Fernandes, J. P., Whyte, H., & Raybaud, C. (2010). MR Imaging for diagnostic evaluation of encephalopathy in the newborn. *RadioGraphics*, *30*(3), 763–780. https://doi.org/10.1148/rg.303095126

Singh, D. K., Ling, E.-A., & Kaur, C. (2018). Hypoxia and myelination deficits in the developing brain. *International Journal of Developmental Neuroscience: The Official Journal of the International Society for Developmental Neuroscience*, *70*, 3–11. https://doi.org/10.1016/j.ijdevneu.2018.06.012

Spinelli, S., Chefer, S., Suomi, S. J., Higley, J. D., Barr, C. S., & Stein, E. (2009). Early-life stress induces long-term morphologic changes in primate brain. *Archives of General Psychiatry*, *66*(6), 658–665. https://doi.org/10.1001/archgenpsychiatry.2009.52

Suomi, S. J. (1997). Early determinants of behaviour: Evidence from primate studies. *British Medical Bulletin*, *53*(1), 170–184. https://doi.org/10.1093/oxfordjournals.bmb.a011598

Tang, A. C., Reeb-Sutherland, B. C., Romeo, R. D., & McEwen, B. S. (2014). On the causes of early life experience effects: Evaluating the role of mom. *Frontiers in Neuroendocrinology*, *35*(2), 245–251. https://doi.org/10.1016/j.yfrne.2013.11.002

Thorhallsdottir, A. G., Provenza, F. D., & Balph, D. F. (1990). Ability of lambs to learn about novel foods while observing or participating with social models. *Applied Animal Behaviour Science*, *25*(1–2), 25–33. https://doi.org/10.1016/0168-1591(90)90066-M

Turley, S. D., Burns, D. K., & Rosenfeld, C. R. (1996). Brain does not utilize low density lipoprotein-cholesterol during fetal and neonatal development in the sheep. *Journal of Lipid Research*, *37*, 1953–1961.

Wańkowska, M., Starzec, A., Counis, R., & Polkowska, J. (2006). Effects of maternal deprivation on the adrenocorticotrophic and gonadotrophic axes in the hypothalamo–pituitary unit of preweanling female sheep: The histomorphometric approach. *Journal of Chemical Neuroanatomy*, *31*(1), 51–58. https://doi.org/10.1016/j.jchemneu.2005.08.006

Westlye, L. T., Walhovd, K. B., Dale, A. M., Bjørnerud, A., Due-Tønnessen, P., Engvig, A., Grydeland, H., Tamnes, C. K., Østby, Y., & Fjell, A. M. (2010). Differentiating maturational and aging-related changes of the cerebral cortex by use of thickness and signal intensity. *NeuroImage*, *52*(1), 172–185. https://doi.org/10.1016/j.neuroimage.2010.03.056

White, N. M. (2009). Some highlights of research on the effects of caudate nucleus lesions over the past 200 years. *Behavioural Brain Research*, *199*(1), 3–23. https://doi.org/10.1016/j.bbr.2008.12.003

Williamson, J. M., & Lyons, D. A. (2018). Myelin dynamics throughout life: an ever-changing landscape? *Frontiers in Cellular Neuroscience*, *12*, 424. https://doi.org/10.3389/fncel.2018.00424

Yushkevich, P. A., Piven, J., Hazlett, H. C., Smith, R. G., Ho, S., Gee, J. C., & Gerig, G. (2006). User-guided 3D active contour segmentation of anatomical structures: Significantly improved efficiency and reliability. *NeuroImage*, *31*(3), 1116–1128. https://doi.org/10.1016/j.neuroimage.2006.01.015

Zelena, D., Menant, O., Andersson, F., & Chaillou, E. (2018). Periaqueductal gray and emotions: The complexity of the problem and the light at the end of the tunnel, the magnetic resonance imaging. *Endocrine Regulations*, *52*(4), 222–238. https://doi.org/10.2478/enr-2018-0027

Zhang, Y., Brady, M., & Smith, S. (2001). Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Transactions on Medical Imaging*, *20*(1), 45–57. https://doi.org/10.1109/42.906424

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