Animal models for assessing impact of C-section delivery on biological systems

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A B S T R A C T

There has been a significant increase in Caesarean section (C-section) births worldwide over the past two decades and although it can be a life-saving procedure, the enduring effects on host physiology are now undergoing further scrutiny. Indeed, epidemiological data have linked C-section birth with multiple immune, metabolic and neuropsychiatric diseases. Birth by C-section is known to alter the colonisation of the neonatal gut microbiota (with C-section delivered infants lacking vaginal microbiota associated with passing along the birth canal), which in turn can impact the development and maintenance of many important biological systems. Appropriate animal models are key to disentangling the role of missing microbes in brain health and disease in C-section births. In this review of preclinical studies, we interrogate the effects of C-section birth on the development (and maintenance) of several biological systems and we discuss the involvement of the gut microbiome on C-section-related alterations.

1. Introduction

Caesarean section – also known as C-section - is the process of delivering babies through a surgical procedure. It is usually performed when natural delivery poses a life risk for the mother or the baby. Although C-section is a well-established practice, the number of babies born by this procedure has increased dramatically worldwide over recent decades, by far surpassing the World Health Organisation estimation of 15% of total births where it is implemented as a life-saving procedure (Betran et al., 2016; Dominguez Bello, 2019; Gibbons et al., 2010). This striking increase in C-section delivery mode is attributed to a compendium of social, scientific and cultural changes, such as an increase in older primiparous mothers (Franz and Husslein, 2010; Guichard and Blondel, 2001), the inclusion of psychological factors in the consideration of a C-section independent of medical reasons, as well as more subjective reasons (Barber et al., 2013), the mother’s request (Finger, 2003; Murray, 2000; Yazdizadeh et al., 2011) and the common scientifically inaccurate perspective of C-section being a “risk-free” procedure (Villar et al., 2007).

There is mounting evidence that C-section is linked to detrimental conditions including immune disorders (Bager et al., 2008; Sevelsted et al., 2015), asthma (Darabi et al., 2019; Thavagnanam et al., 2008), metabolic diseases such as coeliac disease (Decker et al., 2010; Mårild et al., 2012), diabetes type I (Algert et al., 2009; Cardwell et al., 2008), and obesity (Chu et al., 2018; Darmasseelane et al., 2014; Horta et al., 2013; Mueller et al., 2015).

The recent understanding that 1) gut microbiome influences neuronal and behavioural development and that 2) surgical delivery hampers the vertical transmission of gut microbiota during labour, has increased the focus placed on the neonatal microbiome in C-section investigations, as it is highly influenced by mode of birth (Wampach et al., 2018). Since C-section alters the normal acquisition and development of host microbiota it seems plausible therefore that certain microbial groups may influence physiological events in C-section born babies. In fact, gut microbiota is a decisive player in health and disease of the host, as it influences the development and maintenance of host physiology, in particular during early life (Codagnone et al., 2019; Dinan and Cryan, 2017). Thus, although the mechanisms linking C-section and a broad range of diseases are still unknown, the influence of the gut microbiota is beginning to receive a lot of attention. Nevertheless, although epidemiological studies have highlighted these associations, causality has not been proven so far.
The bidirectional communication between the gut microbiota and the central nervous system (CNS) has been described as the microbiota-gut-brain axis (Collins et al., 2012; Cryan et al., 2019; Martin et al., 2018; Morais et al., 2020a, 2020b). Available information on the microbiota-gut-brain axis has shown that alterations on the microbiome – due to microbiota depletion in germ-free animals or antibiotic-treated animals – can alter physiology and behaviour (Cryan and Dinan, 2012; Heijtz et al., 2011; Neufeld et al., 2011; O’Connor et al., 2020). As a consequence, the microbiota-gut-brain-axis is emerging as a potential therapeutic target for central nervous diseases (Long-Smith et al., 2020). The main four pillars by which the microbiota influences physiology and behaviour are 1) immune and inflammatory mechanisms, 2) chemical signalling through microbial metabolites to the enteric nervous system, 3) microbial neurotransmitters and 4) neuronal communications (such as vagal nerve) (reviewed in Cryan et al., 2019).

C-section deliveries have been associated with changes in the physiology of the offspring including neurotransmitter changes, immune readouts, endocrine outputs, metabolism and gut microbiota as well as in behaviour. The contributions of birth mode to physiological, behavioural and psychiatric alterations in humans have been mostly limited to longitudinal and observational studies (Sandall et al., 2018). Such studies have led to links between C-section birth and a range of diseases. However, when associations with disorders have been made, there is usually conflicting evidence.

One way to overcome the limitations of human research is to use animal models to mimic some of the facets of C-section delivery. Animal models are an invaluable tool to study the physiological and behavioural modifications induced by C-section and to examine the underlying mechanisms leading to those alterations. Moreover, animal models of C-section can be used to evaluate the potential benefits of early interventions, with appropriate statistical value and avoiding many confounding effects.

The aim of this review is to gather evidence of C-section mediated changes in physiology and behaviour from preclinical studies and to address the impact of delivery mode in these individual biological systems. Furthermore, we aim to expose how the altered acquisition of the gut microbiota after C-section birth is a key mediator for many of the biological changes observed with C-section delivery.

2. The biology of C-section

Being born is one of the most stressful events of life. During the transition to birth, many major physiological events take place in the neonate; the initiation of breathing air through the lungs, the feeding transition to birth, many major physiological events take place in the neonate or in elective/planned C-section deliveries where delivery occurs prior to the initiation of labour. This distinction between C-section deliveries is crucial as the presence or absence of labour will alter the resulting endocrine signalling in the neonate (Fig. 1).

Labour is a multifactorial physiological event that occurs as a result of endocrine, paracrine and autocrine crosstalk between the foetus and the mother (Kenkel, 2021). In all viviparous species, the activation of the foetal hypothalamic-pituitary-adrenal (HPA) axis is a common pathway leading to parturition. Thus, it is no wonder that the circumcision of labour by elective C-section practice has some implications on the endocrine system of C-section born offspring. For instance, babies born by vaginal delivery exhibit higher cortisol levels at birth than C-section babies (Goldkrand et al., 1976; Talbert et al., 1977) as birth is accompanied by a surge of foetal cortisol production and a stress response. Mode of delivery also influences neonatal stress reactivity as infants born through C-section did not show significant cortisol increases following a routine Hepatitis B inoculation and their overall cortisol reactivity was lower compared to infants born vaginally (Chiş et al., 2017).

Moreover, C-section is thought to affect the immune system of the offspring by a variety of mechanisms. For instance, alterations in immunity have been reported due to the lack of stress response before birth and also changes in immune priming due to a perturbed bacterial colonisation of the gut (Cho and Norman, 2013). Substantial epidemiological data supports that birth by C-section may be related to short and long-term immunological alterations. C-section has been linked as a risk factor for numerous immune function disorders such as asthma (Darabi et al., 2019; Huang et al., 2015; Thavagnanam et al., 2008), allergy (Bager et al., 2008; Eggesbo et al., 2003; Renz-Polster et al., 2005), and type I diabetes (Cardwell et al., 2008; Larsson et al., 2004). Furthermore, the neonatal microbiota of C-section born infants has been identified as a mediator of the association between C-section and immune disorders.

The impact of delivery mode on the infant

Fig. 1. Physiological effects of vaginal and C-section birth. Vaginal birth exposes the baby to maternal microbiota, long episodes of hypoxia and a surge of birth signalling and stress hormones. In C-section birth, these processes are altered, especially when it is performed before commencement of labour.
Babies born by C-section have an altered immune phenotype with reduced leucocyte count in umbilical cord blood (Nikischin et al., 1997). Not only have they a generalised reduction of immune cells, but also some alterations in function have been observed - with neutrophil granulocytes derived from cord blood of C-section babies exhibiting a lessened neutrophil activation in response to an Escherichia coli chalenge (Gessler and Dahinden, 2003). Furthermore, cord blood from babies born by C-section had lower levels of IL-1α and IL-6, tumour necrosis factor-α (TNF-α) and tumour necrosis factor-γ (TNF-γ) in comparison to babies born vaginally (Malamitsi-Puchner et al., 2005; Zanardo et al., 2006). However, general anaesthesia administered to the mother during the surgical procedure, might account for some of the differential effects seen on the immune system of C-section born babies in comparison to vaginally born babies.

The microbiome is a key regulator of the host physiology and therefore it is a critical factor in host health and disease (Lynch and Pedersen, 2016). The microbes that inhabit our gastrointestinal tract play a role in host nutrient metabolism, the maintenance of the integrity of the gut, protection against pathogens, and immune and endocrine modulation. By interrupting the evolutionarily-conserved vertical transmission of microbiota from mother to infant during labour, C-section delivery strongly impacts the acquisition and maturation of the offspring microbiota (Dominguez-Bello et al., 2010). Vaginally delivered babies harbour bacteria similar to the bacterial communities present in the mothers' vaginal environment, whereas C-section babies lack the consortium of bacteria from vaginal microbiota and harbour bacterial communities similar to the mothers' skin and the surgical environment (Dominguez-Bello et al., 2010; Shao et al., 2019). The gut microbiota of vaginally born babies is characterised by the presence of Lactobacillus, Prevotella, or Sneathia spp (Dominguez-Bello et al., 2010) whereas the gut microbiota of C-section babies is characterised by the presence of Clostridia and low abundance of Bifidobacterium, Bacteroides and Lactobacillus (Biasucci et al., 2010; Grönlund et al., 1999; Hill et al., 2017; Jakobsson et al., 2014; Penders et al., 2006). However, in emergency C-section births where labour has been initiated, the neonatal microbiota resembles more closely the composition of vaginally born babies (Azad et al., 2013), suggesting the presence of some vertical transmission of maternal microbiota in these cases. Interestingly, the neonate microbiome of overweight mothers is modulated differently due to mode of delivery (Mueller et al., 2016), demonstrating that maternal factors can also impact the neonate microbiota even in the absence of maternal transmission of bacteria. Labour-related aspects can have an impact as membrane rupture after C-section labour carried significantly higher risk of asthma later in life compared to membrane rupture before the C-section procedure or vaginal birth (Sevelsted et al., 2016). Thus, gut microbiota composition is affected by many perinatal factors, including mode of delivery during early life, and until infancy (Fouly et al., 2019; Hill et al., 2017; Shao et al., 2019; Stewart et al., 2018).

3. Animal model of C-section

Animal models of C-section are essential in helping us understand the physiological mechanisms involved in C-section delivery, as well as the physiological and behavioural consequences for both the mother and offspring. Furthermore, they remain of vital importance to be able to investigate interventional therapeutic approaches to address the alterations seen in this procedure. The animal models of C-section resemble the surgical procedure used in humans; an incision is cut through the abdomen and uterus of the pregnant dam followed by quick manual retrieval of the pups from the uterus (see Morais et al., 2020). In rodents, this procedure can be performed with the administration of anaesthetics to mimic the human condition or in their absence (with previous sacrifice of the pregnant dam) to study the mechanisms involved in the procedure. In rodent studies where the development of the offspring is being investigated, the offspring is raised by a surrogate mother - a procedure known as cross-fostering (see Morais et al., 2020). Cross-fostering is a well-established research technique that is widely used to investigate early life manipulations of the gut microbiota, the immune system and epigenetics (McCarty, 2017; Moore and Stanley, 2016). Although it is a convenient procedure and does not bring substantial changes in behaviour, cross-fostering at birth also can modify metabolic and molecular outcomes in adulthood (Bartolomucci et al., 2004; Santangeli et al., 2016).

In this C-section/cross-fostering model, C-sections are performed after another contemporaneously mated pregnant dam has given birth vaginally, to avoid premature delivery of the C-section born pups (Fig. 2). In pigs, the offspring is separated from the mother and either cross-fostered or fed by researchers (Daniel et al., 1999; Sangild et al., 1993).

This C-section/cross-fostering model is not exempt of limitations. Firstly, C-section born pups are born slightly pre-term (although not premature), which constitutes a major difference from vaginally born pups. Secondly, C-section born pups are fostered by another dam and consequently do not receive feeding and nurture from their own mother. Moreover, by cross-fostering the C-section born pups to another dam, these pups are not exposed to the first form of milk, called colostrum. Indeed, colostrum is a concentrated liquid, rich in protein fat and immunomodulatory molecules like immunoglobulins, lactoferrin and growth factors (Ratsika et al., 2021). It is worth noting that the depivisation of colostrum has a significant role in early postnatal development of immunity in livestock (Clover and Zarkower, 1980; Ogawa et al., 2016).

3.1. C-section and antibiotic exposure model

According to clinical guidelines all parturient mothers undergoing a C-section surgery are given prophylactic antibiotic treatment to prevent postpartum infection (Kankuri et al., 2003). This procedure is considered safe for both mother and baby but the long-term effects on the neonate are still unclear. Consequently, the addition of maternal antibiotic exposure to the cross-foster dams after C-section pups have been born is an interesting and more translational model worth investigating. The combination of C-section birth with an antibiotic exposure is particularly interesting when studying the microbiome. Broad spectrum antibiotic treatment drastically alters gut microbial composition and leads to microbial alterations. Moreover, it induces further inherent physiological changes in the host (Pennycook and Scanlan, 2021; Xu et al., 2021). In particular, maternal antibiotic treatment has been shown to alter not only gut microbial composition but also brain cytokines and behaviour on the adult offspring (Leclercq et al., 2017; O’Connor et al., 2021).

The first studies using this C-section and antibiotic exposure model in rodents showed that adult offspring born by C-section and exposed to maternal antibiotic not only exhibited anxiety-like and social deficits typically seen in C-section born offspring but also had increased depressive-like behaviours and increased corticosterone levels in plasma when compared to C-section born offspring (O’Connor et al., 2022). Moreover, the maternal exposure to antibiotics translated into a greater reduction of gut microbial diversity in comparison to animals born by C-section and not exposed to antibiotics (O’Connor et al., 2022), indicating that antibiotics given to the lactating dam reach the neonate’s gut.

These investigations further highlight how stronger disruptions of the microbiome can alter host physiology and behaviour. Thus, given the clinical relevance of this model, this model should be used when assessing the implications of C-section on host biology.
4. The impact of C-section on biological systems

C-sections were initially performed in rodents to deliver pups aseptically to develop rodent colonies lacking all microorganisms, also known as germ-free rodents (Dubos and Schaedler, 1960). Before the 1990s, the C-section model was used to investigate the physiological differences between surgical and vaginal delivery in the offspring, by using C-section in Sprague-Dawley rats, rhesus monkeys, pigs and lambs (Meier and García-Rodríguez, 1966; Safwate et al., 1984; Smotherman et al., 1987). The pig has been used to investigate birth-related matters as it is an appropriate species to study metabolism-related disorders as its physiology, organ size and weight more closely resembles the human condition. Moreover, it is also a useful tool to study neuroscience as the pig brain is more similar to the human brain in terms of neurodevelopment and brain maturation than rodent species (Bassols et al., 2014). More recently, rodent models of C-section have been employed to investigate the relationship between mode of delivery and microbiota acquisition (Domínguez-Bello et al., 2010). The vertical transmission of maternal microbes to the offspring is a fundamental factor for the correct metabolic, immune and neuronal development of the offspring. Hence, we aim to compile all the compelling preclinical evidence gathered to date on the main C-section mediated changes in physiology, behaviour and development of both mother and offspring.

4.1. Physiological changes

C-section delivery is associated with numerous changes throughout the lifespan of the offspring in many physiological areas involving the CNS, endocrine, immune and metabolic systems (Kenkel, 2021; Lagercrantz, 2016). These changes will be thoroughly presented here to display the wide range of alterations incurred by C-section delivery as well as its potential consequences. The surgical delivery of animals – meaning the absence of many of the processes involved in “being born” such as the release of stress hormones and the exposure to long hypoxic periods during birth – has many consequences on the physiology of the new-born, and later in life (Table 1).
4.1.1. Neurobiology of C-Section

4.1.1.1. The dopaminergic system. In the CNS, the dopaminergic system seems to be very susceptible to alterations due to C-section birth. Dopamine is a brain neurotransmitter that regulates many brain pathways including the ones involved in reward-motivated behaviour and motor activity. The mesolimbic pathway transmits dopamine from the ventral tegmental area (VTA) in the midbrain to the ventral striatum, which includes both the nucleus accumbens (NAcc) and the olfactory tubercles and it regulates reward-related cognition. On the other side, the mesocortical pathway transmits dopamine from the VTA to the prefrontal cortex (PFC) and it regulates executive functions. Dysfunctions in these dopaminergic pathways are associated with addiction, schizophrenia and attention deficit hyperactivity disorder (ADHD) (Cai et al., 2021). Moreover, many of these disorders are also correlated with obstetric complications (Giannopoulou et al., 2018).

Seminal studies from the laboratory of Patricia Boksa at McGill University have shown that the dopaminergic system is subject to consistent modifications due to C-section delivery. In particular, the dopaminergic system in C-section born rat pups exhibited modifications in the levels of catecholamines in plasma and brain (El-Khodor and Boksa, 2003a; El-Khodor and Boksa, 1997) as well as in the products of glycolysis (e.g. lactate) at birth (El-Khodor and Boksa, 2003a). At adolescence, C-section born rats showed increased levels of dopamine D1-like receptors in the NAcc and the dorsal striatum (dSTR) (Juárez et al., 2005). These alterations are not transitory, as C-section delivery causes lasting neurochemical modifications in the catecholaminergic systems in rodent models as C-section delivered adult rats exhibited 50% increase in levels of tyrosine hydroxylase (TH) and roughly 40% increase of dopamine (DA) in NAcc and dSTR (El-Khodor and Boksa, 2003b; El-Khodor and Boksa, 1997) although other studies found no changes (Boksa and Zhang, 2008). Concurrently, excitatory amino acid receptors are also modified by birth mode as in rats born by C-section there is increased AMPA receptor binding in NAcc, NMDA receptor binding in cingulate cortex, and kainate receptor binding in the hippocampal CA1 region (El-Khodor et al., 2004).

In times of repeated stress exposure, adult rats originally born by C-section exhibited a greater dopaminergic response, with alterations in TH and DA activity in the NAcc, increased dopamine active transporter (DAT) receptor levels in dSTR (El-Khodor and Boksa, 2003b; El-Khodor and Boksa, 1997) although other studies found no changes (Boksa and Zhang, 2008). Concurrently, excitatory amino acid receptors are also modified by birth mode as in rats born by C-section there is increased AMPA receptor binding in NAcc, NMDA receptor binding in cingulate cortex, and kainate receptor binding in the hippocampal CA1 region (El-Khodor et al., 2004).

In times of repeated stress exposure, adult rats originally born by C-section exhibited a greater dopaminergic response, with alterations in TH and DA activity in the NAcc, increased dopamine active transporter (DAT) receptor levels in dSTR and NAcc, compared to vaginally born controls (Boksa and Zhang, 2008). The dopaminergic response to stress in C-section born adult animals is well documented, with dopaminergic deficits in the PFC (Brake et al., 2000; El-Khodor and Boksa, 1997), reduced DAT receptor binding and D3 receptor levels in the NAcc (El-Khodor and Boksa, 2002), and increased D4-like receptors in dSTR and NAcc, in comparison to vaginally born counterparts (El-Khodor and Boksa, 2003b).
### Table 1
CNS alterations in C-section born offspring.

| Species | Developmental stage | Gender | Results | References |
|---------|---------------------|--------|---------|------------|
| CB57BL/6J mice | Birth | N/S | Monoamine levels: VB mice > ↑NE, DA, DOPAC, HVA, 5-HIAA levels than CS born mice. 5-HT was also higher but not significant. VB mice > ↑turnover ratios (DOPAC/DA, HVA/DA, DOPAC+HVA/DA and 5-HIAA/5-HT).<br> C-fos expression: VB mice > ↑c-fos expression in the piriform cortex, caudate nucleus and putamen and cerebellar cortex than CS born mice.<br> Synaptic transmission protein levels: CS born mice > ↑synaptic transmission proteins (Na pump α3 subunit, KCC2, the inwardly rectifying K+ channel protein and aquaporin) than VB mice.<br> Both AC3 quantification: VB mice > ↑acetylcholine cell death between E19 and 3 h postnatally that was absent in CS born pups. CS born mice > ↑ cell death density in the PVN at 3 h postnatally.<br> Vasopressin cell quantification: No difference due to mode of delivery in total PVN cell numbers at PND21 but there was a significant 20% reduction in the number of cells immunoreactive for vasopressin in the CS born mice.<br> Vasopressin neurons activation: No differences due to mode of delivery. VB mice > ↑vasopressin neurons activated than oxytocin neurons, this effect is not seen in CS born mice. | (Ikeda et al., 2019) |
| NIH Swiss mice | Adulthood | Both | mRNA expression in PVM: Oxtr and Avpr1a mRNA expression in the PVN were not changed between VB and CS born mice. | (Morais et al., 2021) |
| ICR mice | Infancy | Both | Monoamine levels: CS born mice > ↑HVA/DA and 5-HIAA/5-HT. | (Castillo-Ruiz et al., 2018a) |
| CD1 mice | Birth | N/S | Monoamine levels: CS born mice > ↑NE, DA, DOPAC, HVA, 5-HIAA levels than CS born mice. 5-HT was also higher but not significant. VB mice > ↑turnover ratios (DOPAC/DA, HVA/DA, DOPAC+HVA/DA and 5-HIAA/5-HT).<br> C-fos expression: VB mice > ↑c-fos expression in the piriform cortex, caudate nucleus and putamen and cerebellar cortex than CS born mice.<br> Synaptic transmission protein levels: CS born mice > ↑synaptic transmission proteins (Na pump α3 subunit, KCC2, the inwardly rectifying K+ channel protein and aquaporin) than VB mice.<br> Both AC3 quantification: VB mice > ↑acetylcholine cell death between E19 and 3 h postnatally that was absent in CS born pups. CS born mice > ↑ cell death density in the PVN at 3 h postnatally.<br> Vasopressin cell quantification: No difference due to mode of delivery in total PVN cell numbers at PND21 but there was a significant 20% reduction in the number of cells immunoreactive for vasopressin in the CS born mice.<br> Vasopressin neurons activation: No differences due to mode of delivery. VB mice > ↑vasopressin neurons activated than oxytocin neurons, this effect is not seen in CS born mice. | (Ikeda et al., 2019) |
| GCE Rosa UCP2–/– KO mice and GCE Rosa UCP-2 +/+ mice | Adulthood | Both | Mitochondrial and synaptic density: WT CS mice had lower mitochondrial density in layer 3 of the PFC and higher synaptic density in layer 3 of the PFC than WT VB. No differences were seen between KO VB and KO CS. | (Taylor-Giorlando et al., 2019) |
| Sprague-Dawley rats | Birth | Males | Whole brain lactate: At 1 h after birth, brain lactate > CS born rats – VB rats but ↑ CS + anoxic rats at 10–30 min after birth. Plasma catecholaminines: CS born rats > ↓ plasma epinephrine and ↑ DA levels in plasma. CS + anoxic rats > ↑ NE, epinephrine, and DA. Monoamine levels: CS > ↑ DA in VTA and SN and ↓ DA and Glu in STR. CS + anoxic (21–22 min) > ↓ DA, ↑ Glu levels in SN and CS + anoxic (10–11 min) > ↑ anoxic rats, | (El-Khodor and Boksa, 1997) |
| | | | ↑ brain lactate at 1, 5, and 12 h after birth. CS + anoxic rats, ↑ brain lactate at 1 h after birth. Plasma catecholaminines: CS + anoxic rats > ↑ epinephrine and NE. CS born + rats > ↓ plasma epinephrine monoamine levels. | (El-Khodor and Boksa, 2003a) |
| | Infancy | Males | Monoamine levels: CS born rats > ↓DA levels in the PFC and ↑ DA levels in the NAcc and STR and ↑ [DOPAC/DA] ratio in the PFC but ↓ in the NAcc. CS + anoxic > ↓ DA in the NAcc. CS + anoxic (19–21 min) > ↓ DOPAC in DA, ↑ DA in VTA and ↑ DA, DOPAC, HVA in NAcc. Dopaminergic cell bodies: CS born rats > ↑TH cell bodies in the SN and VTA. Dopaminergic system: At PND35 CS born rats > ↑ DA D1-like receptors in NAcc and dSTR. At PND60, CS born rats > ↑ DA D2-like receptors in the NAcc. Catecholamines and metabolites levels: anoxia > ↓ DA, Glu and Asp levels in STR, HVA, GABA and Asp levels in the SN. After AMPH administration, CS + anoxic > ↓ DA and compared to levels in CS born rats. Dopaminergic cell bodies: CS > + anoxic rats > ↑ TH + cell bodies in the SN and VTA. Electrochemical measurements: In stress responses following apomorphine > no birth mode effect. PFC dopamine response to stress: CS and CS + anoxic rats > ↑ right PFC DA stress responses. Dopaminergic system: No differences in PFC D1-like and D2-like receptor binding due to mode of birth. ↑D1-like receptor binding in left NAcc shell in all birth groups, both before and after stress. CS born rats > ↑ D1-like receptor binding in the infralimbic cortex and anterior olfactory nucleus, in the NAcc shell and core and olfactory bulb. | (Loidl et al., 1994) |
| | Adolescence | Males | Astrocytic bFGF expression: CS born rats > ↑ bFGF- immunoreactive cells in both VTA and SN. | (Flores et al., 2002) |
| | | | Dopaminergic system: At PND35 CS born rats > ↑ DA D1-like receptors in NAcc and dSTR. At PND60, CS born rats > ↑ DA D2-like receptors in the NAcc. | (Juarez et al., 2005) |
| | | | Catecholamines and metabolites levels: anoxia > ↓ DA, Glu and Asp levels in STR, HVA, GABA and Asp levels in the SN. After AMPH administration, CS + anoxic > ↓ DA and compared to levels in CS born rats. Dopaminergic cell bodies: CS > + anoxic rats > ↑ TH + cell bodies in the SN and VTA. Electrochemical measurements: In stress responses following apomorphine > no birth mode effect. PFC dopamine response to stress: CS and CS + anoxic rats > ↑ right PFC DA stress responses. Dopaminergic system: No differences in PFC D1-like and D2-like receptor binding due to mode of birth. ↑D1-like receptor binding in left NAcc shell in all birth groups, both before and after stress. CS born rats > ↑ D1-like receptor binding in the infralimbic cortex and anterior olfactory nucleus, in the NAcc shell and core and olfactory bulb. | (Loidl et al., 1994) |

(continued on next page)
| Species          | Developmental stage | Gender | Results                                                                                                                                                                                                 | References          |
|------------------|--------------------|--------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
|                  |                    |        | **Dopaminergic system after stress:** After stress, CS born rats > D3 receptors in NAcc and D4-like receptors in STR, NAcc and olfactory tubercles.                                                                 |                     |
|                  |                    |        | CS born rats > ↑TH in NAcc and ↑DAT in dSTR and NAcc.                                                                                                                                                   |                     |
|                  |                    |        | **DAT Binding:** CS + anoxia rats ↑ DAT binding in the right PFC, in CS rats there was a tendency for ↑ DAT binding in the right PFC. In non-stressed animals, adult CS rats > ↑DAT binding in the dSTR and NAcc core and there was a tendency in the NAcc-shell. In the CS + anoxia rats > ↑DAT binding in the cingulate and infralimbic regions of the PFC. |                     |
|                  |                    |        | **DAT binding after stress:** In VB rats > ↑DAT binding in the NAcc shell and core and in the cingulate cortex. In CS born rats > ↑DAT binding in the STR, NAcc shell and core, and olfactory tubercles, but ↑DAT binding in the cingulate cortex. In CS + anoxia rats > ↑DAT binding only in the infralimbic cortex. |                     |
|                  |                    |        | In repeatedly stressed adults, CS born and CS + anoxia rats > ↑DAT binding in the NAcc and the left olfactory tubercle.                                                                                   |                     |
|                  |                    |        | **Astrocytic bFGF expression:** CS + anoxia born rats > ↑bFGF-immunoreactive cells in the VTA. With repeated stress, CS + anoxia born rats > ↑bFGF immunoreactivity. CS + anoxia rats > ↑bFGF-immunoreactive cells in NAcc. | (Flores et al., 2002) |
|                  |                    |        | **EAA receptors:** CS born rats > ↑AMPA receptor binding in NAcc, ↑NMDA receptor binding in cingulate cortex, ↑kainate receptor binding in the hippocampal CA1 region. Adult CS + anoxic rats ↑CA1 kainate receptor and ↑ anterior olfactory NMDA receptor binding. | (El-Khodor et al., 2004) |
|                  |                    |        | **EAA receptors after stress:** ↑AMPA receptor binding in several regions of PFC and ↑NMDA receptor binding in infralimbic cortex and dentate gyrus, across all birth groups. Stress in CS born rats > ↑ NMDA receptor binding in cingulate cortex, ↑kainate receptor binding in the olfactory tubercle. |                     |
|                  |                    |        | **Both TH activity:** rats born by CS or by CS + anoxic ↑TH activity in the prefrontal cortex, NAcc, and hypothalamus.                                                                                 | (El-Khodor and Boksa, 2003b) |
|                  |                    |        | Stress ↑TH activity in the NAcc in VB rats and ↓TH activity in the hypothalamus of CS and CS + anoxic rats.                                                                                              | (El-Khodor and Boksa, 2003a) |
|                  |                    |        | Repeated stress ↑tryptophan hydroxylase activity in the dSTR in all birth groups.                                                                                                                      |                     |
|                  |                    |        | **Brain catecholamines:** CS born♂ rats > ↑DA in NAcc and STR. Adult CS born♂ rats > ↑NE in the amygdala. Adult CS born♀ rats > ↑NE levels in the thalamus and right ventral Hipp. No differences due to birth group, region or sex were found for 5-HT levels. No birth group differences in levels of HVA or DOPAC, or in 5-HIAA, in NAcc, STR and amygdala in both sexes. CS + anoxia born♂ rats > ↑5-HIAA in the left dorsal hippoc. CS + anoxia born♀ rats > ↑5-HIAA in the thalamus. |                     |

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, AMPH: Amphetamine, Asp: Aspartate, Avpr1a: vasopressin receptor 1a gene, bFGF: basic fibroblast growth factor, CS: C-section, DA: Dopamine, DAT: Dopamine transporter, DOPAC: 3,4-Dihydroxyphenylacetic acid, E: Embryonic day, Fkbp5: FK506 binding protein 5, Glu: Glutamate, Hipp: Hippocampus, HVA: Homovanillic Acid, NMDA: N-methyl-D-aspartate receptor, NE: Norepinephrine, N/S: Not Specified, Nr3c1: glucocorticoid receptor gene, NAc: Nucleus accumbens, NRF: Nuclear Respiratory Factor 1, PVN: Paraventricular Nucleus, PND: Postnatal day, PFC: Prefrontal cortex, Oxt: oxytocin receptor gene, STR: Striatum, SN: Substantia Nigra, TFAM: Mitochondrial transcription factor A, TH: tyrosine hydroxylase, UCP: Uncoupling protein, VB: Vaginally born, VTA: Ventral tegmental area, 5-HIAA: 5-hydroxyindoleacetic acid, 5-HT: Serotonin.
Interestingly, these C-section induced changes in the catecholaminergic system under stressful situations could be prevented with an injection of epinephrine at birth (Boksa and Zhang, 2008).

Since alterations in the catecholaminergic system are present in the pathophysiology of schizophrenia and ADHD, some conjectures have been made about the possible implications of C-section and birth complications to these diseases (Boksa and El-Khodor, 2003; Vaillancourt and Boksa, 2000). A modified version of the C-section model, where an asphyctic episode is experienced before birth, generates schizophrenic-like alterations in the catecholaminergic system (Bjellie et al., 1991). By using this model of prenatal hypoxia, a time-dependent loss of pyramidal cells in the CA1 and CA3 regions of the hippocampus and a time-dependent proliferation of dopaminergic neurons in the substantia nigra pars compacta (SNpc) was reported in rats born by C-section with an added episode of anoxia (Bjellie et al., 1991). These results highly correlate with the schizophrenic condition, where hippocampal lesions are observed after a hypoxic event (Graham, 1977; Nakamura et al., 1986). Moreover, these alterations persist into adulthood as rats born by C-section with an added period of global anoxia exhibited hyperdopaminergic responses to amphetamine-induced locomotion and stress stimuli (Brake et al., 2000; El-Khodor and Boksa, 2002, 2003). These findings suggest that C-section born asphyctic rats may suffer from an imbalance in the dopaminergic system. In addition to the dopaminergic alterations, adult asphyctic rats had a decreased number of neurons in the hippocampus, expressed with mild deficits in spatial tasks (Bjellie et al., 1991; Boksa et al., 1995). Thus, it is now well established how the combination of C-section and an episode of acute anoxia can result in long-term neuro-behavioural changes in this animal model. In conclusion, extensive experimental data has shown that the dopaminergic system is particularly sensitive to birthing events such as C-section delivery or a hypoxic episode in rodents.

4.1.1.2. Neurodevelopment. Birth is a critical period for brain structural development and alterations in this process may lead to alterations in short- and long-term consequences (Ben-Ari, 2015). The physiological changes experienced during delivery are well documented and summarised here but our understanding of neuronal development during this crucial period is still limited. As a result, C-section rodent models have been used to analyse the impact of birth mode in neurodevelopment and consequently their relationship with neurodevelopmental disorders such as autism spectrum disorder. C-section delivery is associated with a mild increased risk of autism spectrum disorder in humans (Curran et al., 2015).

Investigations into understanding how environmental factors such as mode of birth impact neural plasticity and neurodevelopment are scarce even though it is becoming increasingly accepted that gut microbial modulation impacts on neurocognitive development (Borre et al., 2014; Cowan et al., 2020). One of these studies reported that C-section birth induced transient developmental delays in hippocampal pyramidal neurons and communicative deficits in infancy in mice, but did not lead to changes in the GABAergic system nor long-term consequences (Chiesa et al., 2018). Furthermore, lower dendritic spine densities in neurons from the PFC and NAcc as well as in hippocampal CA1 pyramidal neurons were observed in C-section born adolescent rats but not in adulthood (Juarez et al., 2008). C-section birth reduced the expression of c-Fos, a neural activity marker, in many regions of the brain when compared to vaginal birth and the levels of many synaptic transmission related proteins were also altered at birth (Ikeda et al., 2019).

A range of structural and functional changes in the brain have been documented in microbiota-depleted animals (Castillo-Ruiz et al., 2018a; Cowan et al., 2020), but to our knowledge they have not been extensively investigated in C-section microbiota-altered animals.

4.1.2. The endocrine system

When studying the endocrine system, special attention should be given to the distinction between unplanned C-section vs planned or elective C-section, as the former are scheduled well before the initiation of labour and will avoid the activation of the HPA axis. This fact limits the application of many human studies in endocrinology as often the type of C-section is not recorded. For this reason, larger mammals such as the pig have been used to investigate the alterations seen in the HPA axis due to mode of delivery and are summarised in Table 2. Pigs born by C-section exhibited 2-fold decrease of cortisol concentrations and a 2-fold increase of adrenocorticotropic hormone (ACTH) levels in blood serum at birth in comparison to piglets born vaginally (Carroll et al., 2000; Daniel et al., 2008, 1999; Sangild et al., 1995, 1993), although others found no changes in the cortisol levels at birth due to mode of delivery (Carroll et al., 2000). Furthermore, adrenal weights of C-section born piglets at birth were smaller in some studies (Sangild et al., 1993), but not in others (Sangild et al., 1995).

The transition from intrauterine to extrauterine life requires many maturational changes in the neonate that are reflected in hormone profiles. In the post-natal period, pigs born by C-section showed higher levels of cortisol (Carroll et al., 2000; Sangild et al., 1995) and growth hormone in plasma as well as reduced levels of IGF-1 and IGF-2 (Carroll et al., 2000). Hence, mode of delivery highly impacts the HPA axis during the postnatal period in pigs. Upon HPA axis stimulation, C-section born piglets showed increased levels of cortisol in comparison to vaginally born piglets (Carroll et al., 2000). The same response was observed when C-section-born adult rats were subjected to a 20-min restraint stress (Boksa et al., 1996). These results suggest a disruption of the HPA that lasts well beyond birth.

Intriguingly, the HPA axis is not only activated by labour but also modulated by the microbiota-gut-brain axis. The implication of the microbiota in immune development is well documented (Zheng et al., 2020). Thus, it may not be surprising that many changes in cytokine levels have been documented in the endocrine-immune system of neonates born by C-section in comparison to vaginally born babies at birth (Gasparoni et al., 2002; Zanardo et al., 2006). Also, increased levels of TNF-α have been recorded in piglets born by C-section at 2 weeks of age (Daniel et al., 2008). Therefore, it is now apparent that birth is not a passive mechanical event where the foetus gets expelled, but a complex process that triggers the maturation of several mechanisms for the normal physiological and immunological development of the baby, and that the avoidance of labour appears to have consequences long after birth.

Later in life, C-section has been associated as a risk factor for diabetes mellitus type I in childhood, however results are conflicting (Cardwell et al., 2008; Daalsgard Clausen et al., 2016; Samuelsson et al., 2015; Tanoey et al., 2019). In a porcine study, the insulin levels in blood at birth did not differ due to mode of delivery, but at 7 days of age, C-section born pigs were hyperinsulinaemic compared to vaginally born piglets (Hyde et al., 2010). Nonetheless, the long-term effects of delivery mode on the development of diabetes have not been fully investigated in preclinical studies. Most animal studies investigating the relationship between diabetes and offspring development focus primarily on gestational diabetes and its consequences in the offspring.

4.1.3. The immune system

The preclinical investigations studying the role of delivery mode on immunity are limited and summarised in Table 2. Since the immune system is regulated by other systems that are altered due to mode of delivery (dopaminergic, endocrine and microbiome) it is plausible that the immune system could be modulated directly, via mode of delivery, or indirectly through the other biological systems. Of particular interest is the gut microbiome, which is known to help prime the immune system in the neonate.

In piglets, no effects were seen due to mode of birth on the levels of mRNAs expression of several cytokines in many organs, at birth, and at 2 weeks of age (Daniel et al., 2008). In another study, IFN-γ gene expression was decreased two-fold seven days after birth (Hyde et al.,
### Table 2
Metabolic, endocrine and immune alterations in C-section born offspring.

| Species                  | Developmental stage | Gender | Results                                                                                                                                                                                                 | References                                                                 |
|--------------------------|---------------------|--------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| NIH Swiss mice           | Adulthood           | Males  | Gut permeability: No differences due to mode of birth. Gastrointestinal transit: CS born mice > faster gastrointestinal transit time. Cytokine release in LPS stimulated splenocytes: splenocytes from CS born mice > TNF-α than the splenocytes from VB mice. Body fat: CS born mice > marginal increase in body fat at 15w. | (Morais et al., 2021)                                                    |
| CB57BL/6J mice           | Birth               | Both   | Plasma osmolality: VB mice > plasma osmolality, this effect is not seen in CS born mice. Corticosteroid receptor binding: CS born rats > type I corticosteroid receptor binding in Hipp and Hyp than VB group. | (Hoffiz et al., 2021)                                                   |
| Sprague-Dawley rats      | Adolescence         | Both   | Basal corticosterone secretion: No mode of birth effect but CS + anoxia rats > a tendency for corticosterone in plasma. Organ weights: No mode of birth effect in several organ weights. Stress-induced Corticosterone and ACTH secretion: CS born rats (with or without anoxia) > a blunting of the corticosterone response to a 20-min restraint stress test. CS + anoxia born rats > plasma corticosterone levels compared to VB controls at 0, 60 and 120 min after the stress test. | (Castillo-Ruiz et al., 2018a)                                            |
| Wistar rats              | Infancy             | Both   | Plasma osmolality: VB mice > plasma osmolality, this effect is not seen in CS born mice. Corticosteroid receptor binding: CS born rats > type I corticosteroid receptor binding in Hipp and Hyp than VB group. | (Ioksa et al., 1996)                                                   |
| Pigs (Large White x Landrace) | Birth              | N/S    | BW: No differences due to mode of birth. Centralized clot-blood ratio: CS born pigs > clot/blood ratio. Somatotropic hormones: CS born pigs > adrenal weight and cortisol and glucose in plasma at birth. | (Daniel et al., 2008, 1999; Sangild et al., 1993) |
| Pigs (Large White x Landrace) | Infanty            | Both or N/S | No differences in IL-6, IL-1β, TNF-α due to birth mode in hypothalamus, pituitary, thymus, spleen, liver, and adrenal tissue. CS born piglets > pituitary GH receptor mRNA at birth. | (Daniel et al., 2008)                                                   |
| Pigs (Large White x Landrace) | Infanty            | Both or N/S | BW: CS born pigs > weight than VB pigs at 2w. | (Carroll et al., 2000; Daniel et al., 1999)                          |
| Pigs (25%Meishan, 12.5%Duroc, 62.5% Large White × Landrace) | Infanty            | Both   | BW: At PND7, no differences in BW or liver weight but CS born pigs > iα2. Glucosehomeostasis: CS born pigs > iα2 than VB born pigs. Thyroid hormones: CS born pigs > iTH in plasma at birth, no differences at PND7. Hepatic lipid metabolism: After 7 days of parenteral feeding, CS born pigs > hepatic lipids than VB born pigs. | (Carroll et al., 2000; Daniel et al., 1999) |
|                          |                     |        | Immunedeficiency: no differences due to mode of birth. | (Hoffiz et al., 2021)                                                   |

ACTH: adrenocorticotropic hormone, BW: Body weight, CRH: Corticotropin-releasing hormone, CS: C-section, GH: Growth hormone, Hipp: Hippocampus, Hyp: Hypothalamus, IGF: Insulin-like growth factor, N/S: Not Specified, PND: Postnatal day, T3: Triiodothyronine, VB: Vaginally born, w- weeks.
models and particular developmental stages (Table 2). Body weight and mode of delivery can only be drawn in certain animal studies, as noted that these alterations in metabolism have only been reported in a small study of C-section delivered piglets at 14 days after birth (Daniel et al., 2008). Interestingly, when 14-day old piglets were faced with an immune LPS challenge, the levels of inflammatory agents such as cytokines or cortisol of both vaginally and C-section born animals did not differ (Daniel et al., 2008), suggesting that mode of delivery did not alter the immune system functionality in an acute response test.

Germ-free animal studies have demonstrated that the gut microbiota participates in the development and maturation of the immune system and that bacterial colonisation at a critical time window during early development is necessary to achieve normal immune development in the adult (El Aidy et al., 2013). Moreover, a transfer of gut microbiota can modulate the immune system of C-section born animals (Jasarevic et al., 2018). In a recent preclinical study, inoculation of C-section delivered pups at birth with vaginal microbes isolated from humans, resulted in altered population of innate and adaptive immune cells during neonatal and adult periods, when compared to vaginally delivered age-matched mice (Jasarevic et al., 2021a). These data suggest that early-life colonisation of birth-related microbes has a lasting impact on circulating immune cell composition ranging from the neonatal period and all the way to adulthood (Jasarevic et al., 2021a). These important findings from preclinical studies should be used as a starting point in employing the existing knowledge to deliver favourable products and practices with clinical implications for human health.

A recent study of healthy male participants, suggests that individuals born by C-section have significantly higher concentration of baseline anti-inflammatory cytokines IL-10 and IL-10 in their circulation, compared to people born vaginally, in early adulthood (Dinan et al., 2022). The individuals born by C-section, displayed higher levels of circulating IL-10 compared to vaginally born counterparts while under a naturalistic stressor of exams period (Dinan et al., 2022). Even though the microbiota profiles between C-section and vaginally born individuals are very similar in adulthood, this small study suggests that delivery mode might result in an altered, possibly hyper-responsive immune system in humans (Dinan et al., 2022).

In summary, although short-term alterations in the immune system due to Caesarean birth have been disclosed in the past, further investigations should explore the potential long-term alterations in the immune system functionality due to the abnormal acquisition of gut microbiota in C-section born individuals.

4.1.4. Metabolism

Preclinical investigations assessing the impact of delivery mode on metabolism are outlined in Table 2. Delivery mode has been shown to impact appetite regulation as piglets born by C-section showed reduced levels of orexigenic peptides at 7 days of age (Hyde et al., 2010). Moreover, piglets born by C-section had lower levels of gastrin at birth, a peptide hormone that is involved in digestion, which further supports that idea (Sangild et al., 1995). Interestingly, cortisol stimulates the secretion of gastrin during foetal development in piglets (Sangild et al., 1994) and this could partially explain why C-section born animals presented these metabolic alterations. Furthermore, since hepatic metabolism is linked to glucose homeostasis, alterations in the liver may contribute to obesity or other metabolic diseases. In this direction, C-section born piglets were reported to have lower plasma glucose at birth, but more importantly these alterations persisted further than the post-partum period since these piglets exhibited reduced activation of gluconeogenesis from lipid-derived sources, evolving into an excess of hepatic lipids at 7 days of age (Hyde et al., 2010). However, it has to be noted that these alterations in metabolism have only been reported during early-life in pigs and that these associations between changes in body weight and mode of delivery can only be drawn in certain animal models and particular developmental stages (Table 2).

Emerging data support a key role of the microbiome in host metabolism both in health and disease, and gut dysbiosis has been involved in metabolic diseases (van de Wouw et al., 2017). However, the influence of the gut microbiota in metabolic disease is still being elucidated. In mice, C-section birth has been associated with increased body weight gain in adulthood due to abnormalities in the early development of gut microbiota (Martinez et al., 2017). Although an increasing interest in examining the links between the gut microbiota and host metabolism has been seen in microbiome science, very little attention has been put on investigating the effects of mode of delivery in metabolic disease and the evidence to date is limited.

4.2. Behavioural changes

Birth mode not only alters physiological parameters, but it can also modify the behaviour of the mother and offspring during many stages of life. In humans, C-section has been linked to many social, anxiety and stress related disorders (Zhang et al., 2019). In rodents, a compendium of neuro-behavioural alterations have been reported in C-section born mice, both in early life and adulthood (Morais et al., 2020). In this section, we are going to review the impact of C-section on several behaviours including maternal and neonate behaviours, and those occurring later in life such as sociability, anxiety, stress and learning and memory. To date, some differences in behaviour elicited by birth mode such as dopamine-related behaviours are well established, but there is a paucity of data on other behaviours.

4.2.1. Maternal behaviours

The lack of parturition in C-section procedures carries many consequences for the pregnant mother, as labour is a complex biochemical event that triggers a cascade of signals for other biological processes such as lactation. Human epidemiological research investigating the effects of C-section delivery on breastfeeding outcomes such as breastfeeding initiation and duration gathered conflicting results (Hobbs et al., 2016; Pérez-Escamilla et al., 1996; Pérez-Ríos et al., 2008). However, mode of delivery (either elective or emergency C-section) was shown to modify the milk microbiome (Cabrera-Rubio et al., 2012; Hermansson et al., 2019). Other human studies assessing the peculiarities of mother-to-infant bonding in C-section deliveries have shown that these mothers’ brains are less responsive to the sound of their own babies crying (Swain et al., 2008) and show lower scores in maternal attachment scales (Cetisli et al., 2018). Nonetheless, in a small cohort study, no differences in mother-to-infant bonding were found according to the type of C-section delivery (elective vs unplanned) at 10–12 weeks post-partum (Azul Forti-Buratti et al., 2017) and a more recent study found that maternal-infant attachment in C-section deliveries increased over time (Rookesh et al., 2021).

Interestingly, C-section born lactating rats showed a more hyperactive behaviour, being quicker in their pup retrieving behaviour, and exhibited more licking behaviour than vaginally born lactating rats, suggesting a more anxious profile (Bonsignore et al., 2006). Consistent evidence supports the involvement of dopamine in maternal behaviours, and alterations in dopaminergic activity negatively impact maternal behaviour (Keer and Stern, 1999; Kinsley et al., 1994). Thus, it is not surprising that C-section born animals, that have a long-term alteration in the dopaminergic system, exhibit these slight disruptions in maternal behaviours. Moreover, rats born by C-section after an asphyctic period, known to have major alterations in the dopaminergic system, exhibited exacerbated maternal care disfunctions in comparison to C-section born lactating rats (Bonsignore et al., 2006; Cirulli et al., 2003). Maternal and neonate behaviour alterations are summarised in Table 3.

4.2.2. Neonatal behaviour

Delay or interference in the initiation of the mother-child attachment experience may have long-term social and affection effects on the newborn (Olza-Fernández et al., 2014). The avoidance of parturition in C-section deliveries leads to a compendium of neurohormonal disruptions, known to affect the establishment of mother-child attachment behaviours. For instance, neonates born by C-section cried much less on
Table 3
Maternal and neonate behaviour in C-section offspring.

| Species   | Developmental stage | Gender | Results                                                                 | References |
|-----------|---------------------|--------|-------------------------------------------------------------------------|------------|
| Wistar rats | Adulthood           | Female dams | Maternal behaviour in home cage: CS + anoxia ♀ > ↑ self-grooming and ↑ out-of-nest behaviours than CS ♂ but no differences in arched-back nursing. | (Cirulli et al., 2003) |
|           |                     |        | Maternal behaviour in novel cage: No birth effect on pup-retrieving latencies. |            |
|           |                     |        | Maternal separation test: At PPD4, no differences in first pup retrieved due to mode of birth. CS + anoxia ♀ > ↓ retrieving the last pup than CS ♀. CS ♀ > ↑ licking behaviour in first 10 min of the trial vs CS + anoxia or VB ♂. |            |
|           |                     |        | EPM: At PPD7, no differences due to mode of birth. Frequency to enter centre zone > ↑ VB vs CS and CS + anoxia ♀. Frequency to enter closed arms > ↑ VB vs CS + anoxia groups. Self-grooming > ↑ (CS + anoxia vs VB ♀). | (Bonsignore et al., 2006) |
| Infancy   | Both                |        | Fear-conditioning: At PPD7, lower latency to freeze in CS + anoxia lactating rats. USVs: CS + anoxia ♀ > ↑ USVs emissions than ♀. CS group > ↑ USVs than VB rats. | (Venerosi et al., 2006) |
| Adolescence | Both              |        | Novelty seeking behaviour: No differences due to birth insult at PND35. Social interaction: At PND35, VB ♂ > ↑ Play Soliciting behaviour than CS ♂ in the first 5 min of the test. No differences were seen between CS and CS + anoxia groups. Fear-conditioning task: CS + anoxia rats > ↑ USVs than CS rats. CS ♂ + anoxia rats > ↑ emissions. CS ♂ + asphyxia rats > ↓ active during the first tone trial than VB group. No differences were observed in rearing behaviours. |            |
| Adulthood | Both                |        | OF: No differences due to birth insult at PND70. OF with D1 agonist: ↑ locomotion and ↓ stereotyped behaviour in CS + anoxia rats after D1 agonist administration vs CS alone. |            |

CS: C-section, D1: D1-like dopamine receptors, EPM: Elevated plus maze, OF: Open field, PND: Postnatal day, PPD: Postpartum day, VB: Vaginally born, USV: Ultrasound vocalisations.

a maternal separation test than vaginally delivered neonates, indicative of an alteration of attachment behaviours (Olza Fernández et al., 2013). Similarly, it has been reported that C-section born rat pups emit more ultrasound vocalisations (USV) during infancy when isolated from the conspecific animals. For example, an increase in social interactions was observed in C-section delivered adolescent rats versus vaginally born counterparts (Venerosi et al., 2006). This increase in social behaviours in adolescent animals has often been interpreted as social anxiety and consequently, indicative of anxiety-like disorders.

Another widely used technique of rodent social behaviour is the three-chamber sociability test, which brings insights on rodent sociability and social cognition. In adulthood, C-section delivered mice have been recorded to exhibit a comparable sociability to vaginally born subjects in the three-chamber sociability test, but were also found to display a reduced social-novelty preference in the same test compared to vaginally born controls, when a novel mouse was placed in the arena (Morais et al., 2020; Zachariassen et al., 2021). Strikingly, this social-novelty preference deficit was reversed when C-section born mice were co-housed with vaginally born counterparts. One possible explanation for this may be due to the coprophagic nature of rodents, where vaginally born microbes were ingested by C-section born mice. Once more, these results highlight the important link between microbiota and mode of birth.

4.2.3.2. Anxiety. Anxiety is an evolutionarily-preserved emotion that translates into a state of alertness upon undefined triggers. Anxiety behaviours in laboratory animals are quite often investigated by placing the animal in a novel unconditioned environment that will cause both curiosity and fear (Campos et al., 2013). In addition to social-novelty changes, C-section born adult rats manifested an anxious phenotype with exaggerated responses in many behavioural anxiety tests such as marble burying test (MBT), elevated plus maze (EPM) and open field (OF) test (Morais et al., 2020). In the OF in particular, several studies have reported a reduction in locomotion or other measures indicative of anxiety in C-section born adult mice (Ikeda et al., 2019; Morais et al., 2020; Zachariassen et al., 2021). For example, C-section born mice exhibited more grooming behaviour in the OF than their vaginally born counterparts (Xie et al., 2021). Moreover, alterations in the dopaminergic system of C-section born adult rats translated to increased locomotor activity after administration of dopamine agonists such as amphetamine, which has been typically seen as a measure of anxiety (Boksa et al., 2002; El-Khodor and Boksa, 1998; Vaillancourt and Boksa, 1998); the same effect has also been reported in C-section born pigs (Vaillancourt and Boksa, 2000). However, changes in locomotion can be influenced by genetic factors, since C-section birth increased amphetamine-induced locomotion in adult Sprague Dawley rats but had the opposite effect in adult Lewis rats (Berger et al., 2000). Furthermore, anoxic C-section born rats showed exacerbated anxious effects in these drug-induced dopamine-mediated behavioural outcomes (El-Khodor and Boksa, 1998; Juárez et al., 2005; Venerosi et al., 2004), and they were even greater under stressful conditions (El-Khodor and Boksa,
### Table 4

Behavioural changes in C-section born offspring.

| Species              | Experimental groups | Gender | Results                                                                                   | References                                      |
|----------------------|---------------------|--------|-------------------------------------------------------------------------------------------|------------------------------------------------|
| **Sprague-Dawley rats** |                     |        | Capsaicin-induced nociception test: CS born rats > VB rats. MWM: VB rats > latency and swim distance to find the platform vs CS born rats both with/without pain. Passsive avoidance (Shuttle box): CS born rats > VB rats. Electrophysiology: VB rats > induction and maintenance of LTP vs CS rats with/without pain. | (Mohamadi-Jorjafki et al., 2020) |
| Adulthood            | Males               |        | Adolescence FST: A significant effect of birth group and of trial was found. CS born rats differed significantly from CS + anoxia born rats. CS born rats were not different from VB rats. | (Boksa et al., 1998) |
|                      |                     |        | Other behaviours: No group differences in rates of spontaneous alternation or in measures of orientation, head scans, limb clasping, limb placing, or time to turn on an inclined grid, but rats born by CS + anoxia (15 min) > latency to respond to nociceptive stimuli. | (Boksa et al., 1995) |
|                      |                     |        | OF: No mode of birth differences on latency to first approach or on number of approaches during the first 3 min following introduction of the novel object. No differences in latency to sniff the food for the first time or to begin eating, when animals were food restricted for 24 h. | (Boksa et al., 1998) |
|                      |                     |        | MBT: CS born mice > buried marbles than VB mice. EPM: CS born mice > entries in the open arms than VB mice. |                                                   |
|                      |                     |        | OF: CS born mice > locomotion than VB mice. Social behaviour: During the sociability trial in the 3CST, no differences were seen between VB and CS born mice. In the social cognition trial of the 3CST, CS born mice did not have a preference for the novel mouse vs the familiar mouse. MBT: CS born mice > buried marbles than VB mice. EPM: CS born mice > entries in the open arms than VB mice. OF: CS born mice > locomotion than VB mice. Social behaviour: During the sociability trial in the 3CST, no differences were seen between VB and CS born mice. In the social cognition trial of the 3CST, CS born mice did not have a preference for the novel mouse vs the familiar mouse. MBT: CS born mice > buried marbles than VB mice. EPM: CS born mice > entries in the open arms than VB mice. | (Morais et al., 2020) |
|                      |                     |        | N/S Locomotor activity: CS born mice > locomotor activity than VB mice, as measured by the total moving distance in the OF, EPM, LD, and BB tests. | (Ikeda et al., 2019) |
|                      |                     |        | Other behaviours: Fear/anxiety, motor coordination, muscle strength and spontaneous motor activity were not significantly different between VB and CS born mice. |                                                   |
| NIH Swiss mice       |                     |        | USVs: At PND9, no differences due to mode of birth. Maternal attachment: At PND10, CS born mice > time in maternal bedding than VB pups. | (Morais et al., 2021) |
| Infancy              | Both                |        | Males USVs: At PND9, CS born mice > calls than VB mice. Maternal attachment: At PND10, CS born mice > time in maternal bedding than VB pups. |                                                   |
|                      |                     |        | Adolescence Social behaviour: During the sociability trial in the 3CST, no differences were seen between VB and CS born mice. In the social cognition trial of the 3CST, CS born mice did not have a preference for the novel mouse vs the familiar mouse. MBT: CS born mice > buried marbles than VB mice. EPM: CS born mice > entries in the open arms than VB mice. OF: CS born mice > locomotion than VB mice. Social behaviour: During the sociability trial in the 3CST, no differences were seen between VB and CS born mice. In the social cognition trial of the 3CST, CS born mice did not have a preference for the novel mouse vs the familiar mouse. MBT: CS born mice > buried marbles than VB mice. EPM: CS born mice > entries in the open arms than VB mice. | (Morais et al., 2020) |
|                      |                     |        | N/S Locomotor activity: CS born mice > locomotor activity than VB mice, as measured by the total moving distance in the OF, EPM, LD, and BB tests. | (Ikeda et al., 2019) |
| CB57BL/6J mice       |                     |        | USVs: CS born ♂ mice > emitting softer calls. | (Castillo-Ruiz et al., 2018a) |
| Infancy              | Both                |        | BW: CS born mice > BW than VB mice at PND21. |                                                   |
|                      |                     |        | Adolescence Social behaviour: CS born ♂ mice > locomotor activity, defecation. | (Zachariasen et al., 2021) |
|                      |                     |        | Adulthood Social behaviour: CS born ♂ mice > locomotor activity than VB mice, as measured by the total moving distance in the OF, EPM, LD, and BB tests. |                                                   |
|                      |                     |        | MBT: No changes due to mode of delivery. | (Ikeda et al., 2019) |
|                      |                     |        | Other behaviours: Fear/anxiety, motor coordination, muscle strength and spontaneous motor activity were not significantly different between VB and CS born mice. |                                                   |
|                      |                     |        | N/S Locomotor activity: CS born mice > locomotor activity than VB mice, as measured by the total moving distance in the OF, EPM, LD, and BB tests. | (Ikeda et al., 2019) |
|                      |                     |        | Other behaviours: Fear/anxiety, motor coordination, muscle strength and spontaneous motor activity were not significantly different between VB and CS born mice. |                                                   |
|                      |                     |        | MBT: CS born mice > buried marbles than VB mice. EPM: CS born mice > entries in the open arms than VB mice. | (Morais et al., 2020) |
|                      |                     |        | OF: CS born mice > locomotion than VB mice. Social behaviour: During the sociability trial in the 3CST, no differences were seen between VB and CS born mice. In the social cognition trial of the 3CST, CS born mice did not have a preference for the novel mouse vs the familiar mouse. MBT: CS born mice > buried marbles than VB mice. EPM: CS born mice > entries in the open arms than VB mice. OF: CS born mice > locomotion than VB mice. Social behaviour: During the sociability trial in the 3CST, no differences were seen between VB and CS born mice. In the social cognition trial of the 3CST, CS born mice did not have a preference for the novel mouse vs the familiar mouse. MBT: CS born mice > buried marbles than VB mice. EPM: CS born mice > entries in the open arms than VB mice. | (Morais et al., 2020) |
|                      |                     |        | N/S Locomotor activity: CS born mice > locomotor activity than VB mice, as measured by the total moving distance in the OF, EPM, LD, and BB tests. | (Ikeda et al., 2019) |
|                      |                     |        | Other behaviours: Fear/anxiety, motor coordination, muscle strength and spontaneous motor activity were not significantly different between VB and CS born mice. |                                                   |
|                      |                     |        | MBT: No changes due to mode of delivery. | (Ikeda et al., 2019) |
| ICR mice             |                     |        | OF: No differences due to delivery mode at PND39 (Huang et al., 2019) or at PND44 (Xie et al., 2021). CS born mice > grooming behaviour at PND44 (Xie et al., 2021). | (Huang et al., 2019) |
| Adolescence          | Both                |        | MWM: No differences due to delivery mode at PND42-47. Spatial Probe: At PND47, CS born mice > lower target quadrant entries and ratio of time spent in target quadrant vs VB mice. Sucrose preference: CS born mice > preference for sucrose at PND45. EPM: No differences in immobility time due to mode of delivery at PND45. FST: No differences in open arm duration due to mode of delivery at PND45. | (Xie et al., 2021) |
|                      |                     |        | MBT: No changes due to mode of delivery at PND60 (Xie et al., 2021). CS born mice > grooming behaviour at PND60 (Xie et al., 2021). |                                                   |
|                      |                     |        | Adulthood OF: No differences due to delivery mode at PND70-75. Spatial Probe: At PND70-75, CS born mice > lower target quadrant entries and ratio of time spent in target quadrant vs VB group. No differences at PND75. Sucrose preference: CS born mice > preference for sucrose at PND75. |                                                   |

(continued on next page)
4.2.3.3. Stress. C-section creates subtle long-term alterations in the CNS function, in particular to the dopaminergic system. Under stressful conditions, dopamine transmission in the mesolimbic pathway is stimulated, and with repeated exposure, dopaminergic neurons will get sensitised to the effect. Although initial response to stress is not changed by birth mode, after mild repeated-stressful stimuli, C-section birth resulted in sensitisation of the dopaminergic neurons in the NAcc in adult rats (Brake et al., 1997) as well as increased TH activity in NAcc and increased DAT in dSTr and NAcc, compared to vaginally born controls (Boksa and Zhang, 2008). Not surprisingly, these animals also showed decreased DAT in the NAcc (El-Khodor and Boksa, 2002), decreased D3-like receptors in NAcc and increased D4-like receptors in dSTr, NAcc and olfactory tubercles, compared to vaginally born controls after mild repeated stress (El-Khodor and Boksa, 2001). Furthermore, under conditions of minimal stress, C-section born adult rats showed increased TH activity in comparison to vaginally delivered rats (El-Khodor and Boksa, 2003). In a recent study of healthy male participants, young adults born by C-section have reported higher levels of perceived stress compared to vaginally born individuals, while exposed to the naturalistic stressor of stress during exams period (Dinan et al., 2022). However, the HPA response in the periphery as measured by cortisol levels in blood was not different among C-section and vaginally born individuals (Dinan et al., 2022).

4.2.3.4. Pain. In the context of C-section delivery in humans, pain is mostly studied in relation to the parturient mother. However, a human study found that physiological and behavioural responses to pain stimuli were exacerbated in C-section born neonates as they lacked the high pain threshold conferred to the foetus by labour (Bergqvist et al., 2009).

Experimental data analysing the long-lasting effects of delivery mode on pain nociception is limited. El-Khodor and Boksa (2000) reported no differences in nociceptive responses in adult rats born vaginally, by C-section or C-section with an added period of anoxia, suggesting that mode of delivery does not modify pain sensitivity in adult rats. However, capsaicin-induced inflammatory pulpal pain produced more significant nociceptive behaviour in vaginally delivered adult rats compared to C-section born rats, indicating that C-section delivery reduced the cognitive impairment and consequently the nociceptive response produced by sensitisation of capsaicin (Mohamadi-Jorjafki et al., 2020). Therefore, there is limited evidence that mode of delivery generates long-lasting alterations in the sensitisation or perception of pain.

4.2.3.5. Learning & memory. The perinatal environment is a critical window for brain development; any disruption in the microbiota during these critical windows can have a massive impact on specific functions such as neurocognitive development (Cowan et al., 2020). The relationship between C-section delivery and long-term effects on cognition has been investigated by measuring the academic performance, with many studies reporting an increased risk of lower academic performance in children and adolescents born by C-section (Bentley et al., 2016; Curran et al., 2017; Poldián et al., 2017) but not in others (Slykerman et al., 2020). This evidence has limited value as academic performance is not equivalent to cognition.
Surprisingly, few preclinical investigations have addressed the short- and long-term effects of mode of delivery on cognition. There is mounting evidence coming from germ-free rodents that disturbances in the microbiota can lead to long-term negative cognitive outcomes (Luczynski et al., 2016). Dopamine can also modulate many hippocampal functions, such as long-term memory (Lisman and Grace, 2005; Rossato et al., 2009). Castillo-Ruiz et al. (2018a) systematically analysed neuronal cell death before and after birth in many brain regions in mice born vaginally or by C-section to discover that C-section delivered mice had greater neuronal cell loss in the paraventricular nucleus (PVN) of the hypothalamus but also in other brain areas that regulate birth-related physiological functions. Consequently, these results indicate that vaginal delivery inhibited cell death in many brain areas, thus having a neuroprotective effect likely to be explained by one or more of the complex processes co-occurring during birth. More importantly, this time-specific cell death has implications well after birth as weaned C-section born mice had less vasopressin-immunoactive neurons in the PVN compared to vaginally born counterparts (Castillo-Ruiz et al., 2018). Intriguingly, vasopressin is involved in the immune response, the HPA axis as well as in pain and social behaviour (Mavani et al., 2015). Moreover, in another study using conventionally colonised and germ-free mice, gut microbiota modified cell death in the hypothalamus and hippocampus (Castillo-Ruiz et al. 2018b).

The hippocampus has an important role in short- and long-term memory formation and consolidation and it considered a corollary for memory and learning. Natural birth triggers the mitochondrial uncoupling protein 2 (UCP2) (which is known to be involved in neuroprotection and synapticogenesis) in hippocampal neurons in the adult brain, and its expression was reduced in C-section born mice (Simón-Areces et al., 2012). Also in the hippocampus, a higher expression of the glucocorticoid receptor gene in C-section born mice was observed, followed by transitory impairments in learning and memory in adolescence Huang et al., (2019). These results are in line with human studies that associate C-section birth with a delay in cognitive development (Al Khalaf et al., 2015). Nonetheless, no differences were observed in the performance of spatial learning tasks by adult rats born either vaginally or by C-section (Boks et al., 1995) nor in mice (Huang et al., 2019; Taylor-Giorlando et al., 2019). All this information together further supports the hypothesis of a transitory effect of mode of delivery in cognition during adolescence.

4.2.3.6. Attention. Early life events, including birth mode can influence cognitive development, including attention. For example, in three-month old babies, C-section delivered infants showed a reduction in stimulus-driven reflexive spatial attention but not in voluntary visual attention in comparison to naturally born babies (Adler and Wong-Kee-You, 2015). Others have reported that C-section delivery was associated with a visuo-spatial cognitive delay in childhood (Hanrahan et al., 2019), which is in line with cognition outputs mentioned in the previous section. However, although C-section birth has frequently been associated with attention disorders such as ADHD (Curran et al., 2015; Zhang et al., 2019), preclinical investigations have addressed this issue, assessing hyperactivity in relation to dopaminergic dysfunctions rather than directly investigating alterations in attention.

5. Implications for the microbiome

In recent years, preclinical studies have been questioning the involvement of the microbiome in each step of development including during pregnancy and labour. First of all, the idea that pregnancy and the foetal environment are completely sterile has been questioned, as microbial DNA has been found in the placenta (Mancino et al., 2019) and amniotic fluid (Jiménez et al., 2005). Moreover, the implications of maternal microbiota in offspring development is increasingly being studied. Recently it has been reported that bifidobacterial inheritance is highly determined by mother-offspring vertical transmission (Mancino et al., 2019). In another example, maternal microbiota transplantation from stressed dams into C-section born pups resulted in a similar profile of prenatally-stressed offspring, thus emphasising the importance that maternal microbiota has to modulate specific behaviours in the offspring (Jatarević et al., 2018).

Some human epidemiological data suggest that the early alteration of the microbiota by C-section delivery is linked to many diseases later in life (Zhang et al., 2021) such as obesity (Goldani et al., 2011; Huh et al., 2012; Mueller et al., 2015; Yuan et al., 2016). In rodents, C-section born pups without antibiotic treatment presented an abnormal development of microbiota as well as an increased body weight at adolescence, compared to vaginally born counterparts (Martinez et al., 2017). In this study, the differences in diversity indexes and taxonomic composition due to mode of delivery were apparent during weaning but reduced during adulthood (Martinez et al., 2017). Once more, preclinical studies were able to reflect what has been previously detected in humans. However, it has not been yet shown that the microbiome can directly influence the central regulation of food intake in the CNS (Cryan and Dinan, 2012).

Delivery mode and perinatal antibiotics influence gut microbiome composition in children but also its metabolic functionality (Vanni et al., 2021). A recent systematic review reported that infants born by C-section had a lower abundance of Bifidobacterium and Bacteroides spp. up to 6 months of age (Princival et al., 2021). Similarly, it is now known that C-section born offspring have shifts in the normal gut microbial composition (Jatarević et al., 2018; Zachariassen et al., 2021), present a lower microbial diversity at weaning and consequently have many underrepresented microbial taxa in comparison to vaginally born counterparts (Jatarević et al., 2018; Martinez et al., 2017). Nevertheless, microbial composition alterations resolve in adulthood, and diet has a higher influence in the microbial colonisation than mode of delivery, at least when assessed in piglets (Wang et al., 2013).

It has been hypothesised that these early life shifts in gut microbial colonisation skew the immune system homeostasis as the microbiome is involved in shaping the immune system in the new-born. Germ-free mice display altered microglial phenotypes, suggesting the involvement of host microbiota to microglial homeostasis (Erny et al., 2015). In fact, C-section birth correlated with a reduced tolerogenic immune response (Hansen et al., 2014; Zachariassen et al., 2019). More importantly, gut microbiota are responsible for these immune changes, thus establishing the causality of the microbiome in immune alterations in mice (Zachariassen et al., 2019). A series of microbiome-focused interventional strategies have been identified to potentially minimise the negative effects of C-section on the microbiota (Moya-Pérez et al., 2017). For example, the administration of human milk with a rifampicin-resistant bifidobacterial mutant (RBBM) in humanised germ-free mice colonised with C-section born infants’ microbiota induced changes in the gut microbiome of mice, with reduced counts of Clostridia (Musilova et al., 2017). Moreover, by using this C-section model, it was possible to study the effects of bifidobacterial colonisation and to observe that a differential bifidobacterial inheritance was achieved when rats were treated with a single strain or a mix of Bifidobacterium strains (Mancino et al., 2019). Interestingly, low levels of Bifidobacterium in early-life have been related to the later development of allergies (Negele et al., 2004).

Faecal microbiota transplantation (FMT) is another microbiome modulatory intervention under investigation to ameliorate C-section alterations. The concept behind this approach is that FMT could shift the microbiome of C-section born infants to a similar microbiome of those born vaginally. In a recent proof-of-concept study, babies born by elective C-section were given an oral administration of their own mother’s microbes (Korpela et al., 2020). The FMT modulated microbiota development to resemble that of infants born by vaginal delivery with no apparent complications at 3 months post-intervention (Korpela et al., 2020). FMT is increasingly being proposed as a therapeutic
manipulation as FMT protocols for C-section babies have been recently published (Helve et al., 2021). Similarly, vaginal seeding — a procedure where maternal vaginal microbiota is inoculated to the neonate just after the C-section procedure — also looks to restore the neonate microbiota (Moya-Pérez et al., 2017). However, further studies are required that assess the long-lasting efficacy and safety of such procedures. Although these procedures are far from being implemented in the clinic, microbiota transplants are a useful scientific tool to examine the effects of microbiome modulation in C-section born animals (Jasarevic et al., 2021b, 2018).

Hence, C-section birth model has been proven to be a very useful tool to study the changes in the establishment and development of the microbiome in surgically delivered new-born as well as to evaluate the potential benefits of interventions that could minimise these alterations.

6. Conclusions

Over the last decades, C-section deliveries have increased dramatically in both developed and developing countries (Barber et al., 2013; Finger, 2003; Gibbons et al., 2010). There is no evidence of any health benefits of C-section birth when there are no threatening risks. However, the surgical delivery of babies prevents natural birth and the vertical transmission of microbiota (Dominguez-Bello et al., 2016, 2010), which are associated with long term health benefits (Hyde et al., 2012).

C-section birth bypasses natural birth, a complex biological event that triggers the maturation of many physiological systems to prepare the foetus for extraterrene life. C-section delivery comes with the absence of many biologically meaningful processes such as long hypoxic periods, the physical compression of the foetus through the vaginal canal, or the surge of birth-related hormones. On top of these direct effects that birth carries, C-section delivery hampers the vertical transmission of microbiota from the mother to neonate, directly altering the acquisition and maturation of the new-born microbiota (Bokulich et al., 2016; Dominguez-Bello et al., 2010). These changes in the new-born gut microbiota during a critical period have been shown to disturb many other systems known to be connected to the microbiome; further, C-section born babies have different hormonal, microbial and immune profiles. These subtle changes in physiology have been linked to an increased risk of immune and metabolic diseases. This knowledge has shifted the common belief that C-section delivery had no impact in the new-born later in life and it has highlighted the importance of limiting C-section procedures to when it is an imperative medical need. For this reason, there is a need to investigate the alterations induced by C-section surgeries to be able to address them in those cases where C-section is a life-saving procedure.

Human epidemiological studies have associated C-section birth with an increased risk of numerous disorders, but the data extracted from these investigations are usually conflicting and even contradictory. One of the main limitations of epidemiological studies regarding mode of birth is the heterogeneity of the samples and the lack of proper controls, as these studies tend to include a broad range of subjects that involve many confounding variables. For instance, although most of these studies consider variables such as the mother’s age, BMI and socioeconomic background, quite often the type of C-section (planned vs elective) is not acknowledged.

Animal models of C-section have been an invaluable tool to evaluate the effects produced by C-section in physiology and behaviour. As summarised throughout this piece, animal model manipulations offer the possibility to conduct mechanistic research — unable to be performed in humans — and also to preliminarily scan the benefits of potential treatments. The number of investigations assessing interventions for C-section alterations are still sparse, but an increasing interest is being captured in the microbiome field (Moya-Pérez et al., 2017).

Rodent studies have highlighted a degree of gender-dependent vulnerability (Chiera et al., 2020), that may help explain the disparities seen in epidemiological human data. Although these dissimilar deficits have also been observed in many human epidemiological studies, animal models offer a non-skewed, controlled and more homogeneous sample to study gender differences in health and pathological conditions.

The growing realisation that the microbiota-gut-brain axis is a key regulator of neurodevelopmental, immune and metabolic diseases coupled with the compelling evidence that early life microbiota has dramatic impact on both health and disease have shifted the focus on C-section derived research. Although mechanisms by which microbiota exerts functions on the various systems are still being unravelled, it seems undeniable that the alterations observed in C-section born individuals could be explained, at least partially, by the disrupted microbiome. A new era of microbiome-centred investigations in C-section could help improve the current knowledge on the microbiota-gut-brain axis while assessing potential therapies through modulation of the microbiome.

Nevertheless, all these benefits of animal models come with some limitations that need to be considered. Rodent animal models have many limitations, as the usage of a different species comes with differences almost in every aspect of biology: brain and gut anatomy, genetics, behaviour, reproduction, gestation etcetera (Nguyen et al., 2015). For instance, although many similarities exist between mammal species, the gastrointestinal systems from rodents and humans have many differences due to differential diets, feeding regimes and metabolisms (Nguyen et al., 2015), which should be taken into account when investigating the microbiome. Similarly, from a neurodevelopmental perspective, the human brain at birth is more mature than in other mammals (Clancy et al., 2001, 2007), and consequently caution should be taken when comparing rodent and human brains at developmental stages. Not only the anatomical characteristics has to be taken into consideration but also the genetic background as, for example, the effects of C-section delivery upon behaviour were highly variable depending on the rat strain (Berger et al., 2000). The scientific community needs to be cautious when extrapolating this animal-based data to the human condition.

Taken together it is clear that C-section birth encompasses many changes in several biological systems that are now well documented. However, there are still unexplored effects of C-section delivery that should be addressed in order to be able to fully understand its impact and how these could be minimised. Undoubtedly, the joint efforts of the microbiome and neuroscience fields have the potential to unravel the mechanisms of action behind the alterations in C-section individuals and to determine novel, efficacious and safe strategies to address them and that animal models will be key to such approaches.

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