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Review

Circadian rhythms in the fetus

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ABSTRACT

Throughout gestation, the close relationship between mothers and their progeny ensures adequate development and a successful transition to postnatal life. By living inside the maternal compartment, the fetus is inevitably exposed to rhythms of the maternal internal milieu such as temperature; rhythms originated by maternal food intake and maternal melatonin, one of the few maternal hormones that cross the placenta unaltered. The fetus, immature by adult standards, is however perfectly fit to accomplish the dual functions of living in the uterine environment and developing the necessary tools to "mature" for the next step, i.e. to be a competent newborn. In the fetal physiological context, organ function differs from the same organ's function in the newborn and adult. This may also extend to the developing circadian system. The information reviewed here suggests that the fetal circadian system is organized differently from that of the adult. Moreover, the fetal circadian rhythm is not just present simply as the initial immature expression of a mechanism that has function in the postnatal animal only. We propose that the fetal suprachiasmatic nucleus (SCN) of the hypothalamus and fetal organs are peripheral maternal circadian oscillators, entrained by different maternal signals. Conceptually, the arrangement produces internal temporal order during fetal life, inside the maternal compartment. Following birth, it will allow for postnatal integration of the scattered fetal circadian clocks into an adult-like circadian system commanded by the SCN.

Contents

1. Introduction

The Child is father of the Man
From "My heart leaps up when I behold";
William Wordsworth, 1802

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Wordsworth verses are very relevant for the fetal and neonatal physiologist, given the evidence that situations encountered *in utero* have long lasting consequences in adult life, known as programming. Throughout gestation, the close relationship between mothers and their progeny ensures adequate development and a successful transition to the postnatal life. The mother supplies oxygen, nutrients and hormones. The placenta, contributes to, regulates and also determines the rate of exchange of the above substrates. By living inside the maternal compartment, the fetus is unavoidably exposed to rhythms of the maternal internal milieu such as temperature, rhythms originated by maternal food intake and maternal melatonin, one of the few maternal hormones that cross the placenta unaltered. Even the effects of gravitation are decreased during gestation. The fetal-maternal arrangement changes abruptly at delivery. The newborn now depends on maternal behavior for care and feeding (and its own capacity to trigger appropriate maternal behavior). In addition it is left without a warm environment and without maternal hormones like melatonin and placental hormones.

An important consideration in trying to understand the fetal circadian system is that the fetus is not just a small and immature adult living inside a compartment provided by the mother. The fetus, is perfectly fit to accomplish the dual functions of living in the uterine environment while developing the necessary tools to "mature" into a competent newborn. A feat performed by the fetus is to live in a low PO_2 environment at a high external temperature. In such physiological context, fetal organs function differs from the same organ's function in the newborn and adult. Examples are seen in the cardiorespiratory system, (heart and lung), kidney, brown adipose tissue, liver and pineal gland among others. This may extend to the developing circadian system. The information to be reviewed here suggests that the fetal circadian system may be of functional significance for the fetus and not just present simply as the initial expression of a mechanism that has functions only in the postnatal animal.

2. The organization of the adult circadian system

Our current view is that in adult mammals, the circadian system is organized as a master clock: the suprachiasmatic nucleus of the hypothalamus (SCN) commanding peripheral circadian clocks in almost every organ of the body. At the molecular level, a transcriptional/translational feedback loop (TTFL) of the genes *Per1, Per2*, *Cry1, Cry2*, *Bmal1* and *Clock* drives the SCN and peripheral circadian clocks (Bass and Takahashi, 2010). This TTFL interacts with cellular metabolic processes to produce a 24 h oscillation in practically all cell functions. However, the clock gene TTFL is not an absolute requirement for cellular circadian rhythms, as shown by the recent evidence of autonomous circadian oscillation of physiological functions in red blood cells (O'Neill and Reddy, 2011). The SCN is a bilateral structure, and its function as a master clock is considered to be an emergent property of a complex neuronal arrangement. The SCN entrains to the light:dark cycle (LD) via the retinohypothalamic tract. Neurons of the SCN project to several hypothalamic nuclei and the combined nervous and humoral communication regulates overt circadian rhythms in physiological processes like thermoregulation, sleep, melatonin/ACTH/corticosteroid secretion and feeding.

Entraining signals for peripheral clocks are provided by the SCN through innervation by the autonomic nervous system as shown for lung, kidney, adrenal, pancreas and adipose tissue among others. Indirectly, SCN regulation of circadian rhythms like temperature, melatonin and cortisol affects all peripheral organs and circulating cells (reviewed by Bass and Takahashi, 2010). Additionally, liver and other peripheral clocks respond to an important time signal, restricted feeding time, which is independent from the SCN (Damiola et al., 2000). Relative importance of SCN dependent signals or feeding time may vary for a particular peripheral clock as shown by studying the effect of restricted feeding in denervated salivary glands (Vujovic et al., 2008).

3. The fetal circadian system

We know much less about the fetal circadian system. What we do know is that development of circadian rhythms is part of the offspring's development and proceeds normally in the absence of a functional maternal SCN as demonstrated by normal initiation of the postnatal circadian rhythms in rat pups of mothers devoid of circadian rhythms by SCN lesion or by double knockout $mPer1^{Brdn}/mPer2^{Brdn}/mPer2^{Brdn}$ mice or $mPer2^{Brdn}/mCry1$ mice (reviewed by Davis and Reppert, 2001; Jud and Albrecht, 2006). However, both experimental approaches demonstrate that maternal signals are required to synchronize these postnatal rhythms.

Studies in the human, non human primates and in sheep, demonstrate the presence of entrained 24 h rhythms of fetal heart rate, respiratory movements, fetal movements and hormones (reviewed in Serón-Ferré et al., 2007). In addition, in seasonal animals like sheep, hamsters, voles and deer the fetus program its physiology for the environment encountered after birth (Serón-Ferré et al., 1993; Ebling et al., 1989; Gorman et al., 2001; Adam et al., 1994). In adults, circadian and seasonal rhythms are dependent on the circadian system. Is there a circadian system akin to that of the adult operating in the fetus?

4. Fetal SCN development and expression of oscillatory function in rodents and other species

In rodents (rat and hamster) and in sheep, human and non-human primates, the SCN is recognizable by histology by mid-gestation and presents day/night differences in metabolic activity, vasopressin (AVP) mRNA and c-fos mRNA and protein before birth (Reppert and Schwartz, 1983, 1984; Davis and Gorski, 1985; Constandil et al., 1995; Nováková et al., 2010). However there are important differences between species in the age at which developmental landmarks, such as acquisition of the definitive number of neurons and innervation by the retinohypothalamic tract, are attained. Whilst these events are completed *in utero* in human, nonhuman primates and sheep, they take place postnatally in rodents (reviewed in Serón-Ferré et al., 2001a; Sumová et al., 2008; Weinert, 2005). Additional differences between species are also present in the age at which oscillatory expression of clock genes is detected in the fetal SCN of non-human primates and rodents.

In the capuchin monkey fetal SCN, *Bmal1* and *Per2* mRNA expression oscillate in antiphase at 90% gestation (Torres-Farfan et al., 2006). Compared to the oscillation of these genes in the adult capuchin SCN (Valenzuela et al., 2008), the amplitude of the oscillation is smaller in the fetal SCN than in the adult SCN and the interval between the increases of *Bmal1* and *Per2* is longer, reminiscent of the antiphase pattern described in most peripheral clocks in the adult capuchin and in other animals (Watanabe et al., 2006; Johnston et al., 2005; Lincoln et al., 2002; Zylka et al., 1998; Oishi et al., 1998). Metabolic activity data for the fetal capuchin SCN is not available. However, a possible clock controlled gene, the MT_1 receptor, showed 24 h oscillation in the fetal capuchin monkey SCN, sustaining the idea that it could be synchronized by the maternal circadian rhythm of melatonin. Melatonin crosses the placenta unaltered. As demonstrated in human, sheep and rats, the fetal pineal does not secrete melatonin, but passage of maternal melatonin generates a circadian rhythm of melatonin in the

fetal circulation, potentially conveying LD information to the fetus (Kennaway et al., 1992, 1996; McMillen et al., 1989; Deguchi, 1975). Indeed, suppression of maternal melatonin by chronic exposure to constant light from mid gestation shifted both clock gene and MT_1 receptor expression in the fetal capuchin SCN. Daily melatonin replacement to these mothers reversed the effect of constant light (Torres-Farfan et al., 2006), consistent with the fetal capuchin SCN being a circadian clock entrained by maternal melatonin. Of note, melatonin receptors are detected by midgestation in the human SCN (Thomas et al., 2002). Two pieces of information suggest functional development of the human fetal SCN by late gestation: 32–33 week pre-term newborns (80% gestation) show 24 h rhythms of temperature and of oxygen consumption, variables that are under SCN control in the adult (Mirmiran et al., 1990; Bauer et al., 2009).

Ontogeny of oscillatory expression of clock genes in the fetal SCN has been extensively studied in rats, mice and hamsters by measuring *in vivo* expression of the full complement of clock genes and also by measuring *in vitro* expression of a single reporter gene (*Per1* or *Per2*) in fetal SCN slices. The information provided by the two approaches differs. All *in vivo* studies have shown that expression of clock genes in the fetal SCN is low and an antiphase oscillatory expression of the core clock genes is reached by postnatal day 10 (Shimomura et al., 2001; Sladek et al., 2004; Li and Davis, 2005; Sumová et al., 2008; Ansari et al., 2009). In rats, as discussed by Sumová et al. (2008), manifestation of circadian clock gene oscillation parallels the development of synapses in the fetal SCN. *In vitro* studies in fetal and newborn rat SCN slices present a discrepancy with the *in vivo* data. At 22 days' gestational age (term 23 days) strong circadian expression of *Per1* in fetal SCN slices of transgenic rats was detected by Ohta et al. (2008). It is not clear whether culture conditions uncovered the potential for *Per1* oscillation in the fetal SCN as demonstrated by Dolatshad et al. (2010) for fetal organs in mice. However, despite the absence of the canonical clockwork, 24 h rhythms in metabolic activity and c-fos and AVP mRNA expression are present in the fetal rodent SCN *in vivo* (Reppert and Schwartz, 1983, 1984; Davis and Gorski, 1985; Nováková et al., 2010). As suggested by Sumová et al. (2008), these fetal SCN rhythms may not be endogenous fetal SCN rhythms and could arise in response to cyclic maternal signals. Alternatively, an intriguing possibility is that during development, these functions may be initially driven by a non TTFL oscillator as in red blood cells (O'Neill and Reddy, 2011), and as postnatal neural organization of the rodent SCN is completed the TTFL becomes active.

5. Circadian rhythms in fetal peripheral organs and overt circadian rhythms

5.1. Rodents

Ontogeny of the expression of clock genes in fetal and newborn peripheral organs has been studied extensively in rodents. Circadian clocks may be involved in early development as mRNA of the six canonical clock genes (*Per1, Per2, Cry1, Cry2*, *Clock* and *Bmal1*) are expressed in the unfertilized mice oocyte. After fertilization, expression of these mRNA decreases between 2 cell and 16 cell stage to be reinitiated at the blastocyst stage (Johnson et al., 2002; Ko et al., 2000). Upon implantation and thereafter clock genes are detected in whole fetuses and several peripheral organs. *In vivo* measurements by imaging techniques in pregnant rats carrying transgenic *Per1*:luc fetuses, showed luciferase expression in the pregnant uterus at 10 days of gestation and a dawn/dusk difference in luminiscence intensity at 12 days. Overall, there was an exponential increase in *Per1*:luc expression in the uterus from day 10 to the end of gestation and in the day 1 newborn (Saxena et al., 2007). Other researchers have addressed the expression of clock genes in whole fetuses and in selected fetal organs *in vivo* around the clock in mice (Dolatshad et al., 2010) and rat fetuses (Torres-Farfan et al., unpublished). In whole mice fetuses, expression of the clock genes *Per2*, *Cry1*, *Bmal1* and *Clock* was detected from day 10 of gestation to postnatal day 1. However, measurements of the expression of *Per2* and *Bmal1* at 4 h intervals did not show a 24 h pattern in the whole fetus at 10, 14 and 19 days of gestational age. Similar negative results were found in isolated fetal liver, kidney and heart at the two latter ages. The same question was explored in the rat fetus at 16 days of gestational age, with the difference that clock genes were quantified separately in whole head and whole body (Fig. 1). As shown in the figure, an anti-phase 24 h oscillation of *Per2* and *Bmal1* was observed in both compartments. These oscillations were hidden when the data from head and body were combined. Potential brain structures and organs contributing to 24 h clock gene oscillation in the mice and rat fetal head and in the rat fetal body at 18 days of gestational age are summarized in Fig 2. In mice, the fetal *pars tuberalis* shows oscillatory expression of the clock proteins BMAL1, mPER2 and mCRY1 (Ansari et al., 2009). In the rat, day–night differences in *Per2* and *Bmal1* mRNA expression in anti-phase were present in the fetal hippocampus and in the fetal pineal gland. Other brain structures were not tested. At the body level, no oscillation was found in the rat fetal liver, in agreement with findings of a more detailed study of Sladek et al. (2007). A suggestion of oscillation is found in the rat fetal heart. In contrast, a strong oscillation in anti-phase of *Per2* and *Bmal1* was found in the rat fetal adrenal gland (Torres-Farfan et al., 2011). Other authors have detected circadian postnatal expression of *Per1*:luc at day of birth in rat the pineal gland, liver, thyroid, and adrenal gland (Yamazaki et al., 2009). Altogether, the data available supports the presence of peripheral circadian clocks in fetal rodents at a gestational age at which clock gene oscillation of the fetal SCN is absent.

5.2. Non-human primates

Only two studies to date have explored expression of clock genes in peripheral organs of non-human primate fetuses (Fig 3). In the fetal capuchin monkey (Torres-Farfan et al., 2006), at 90% gestation, *Bmal1*, *Per2*, *Cry2*, and *Clock* were expressed in SCN, adrenal, pituitary, thyroid, and brown adipose tissue. However, there were some differences in clock gene expression between these tissues. Expression of *Bmal1* and *Per2* was higher in the SCN and adrenal than in the other tissues. The fetal pineal did not express *Per2* but expressed *Bmal1* and *Clock* and had a significantly higher expression of *Cry2* than the other tissues tested. A recent study shows expression of *Npas2* (a paralog of the clock gene *Clock*), *Per1* and *Reverbα* in fetal Japanese macaque liver at 75% gestation. Although a single clock time was studied, the relative proportion of these genes is consistent with a circadian oscillation (Suter et al., 2011).

6. The fetal adrenal gland is a peripheral circadian clock in primates and rats

Our group studied in detail the presence of circadian oscillatory function in the fetal adrenal in the capuchin monkey and in the rat. In both species, the fetal adrenal gland is an active steroid secreting gland that at the end of gestation through glucocorticoid production, orchestrates maturational processes important for the transition to newborn (Liggins, 1994). The fetal adrenal develops early in fetal life in primates and rats. In both species there is evidence for a 24 h rhythm of fetal adrenal function. In term human fetuses, a

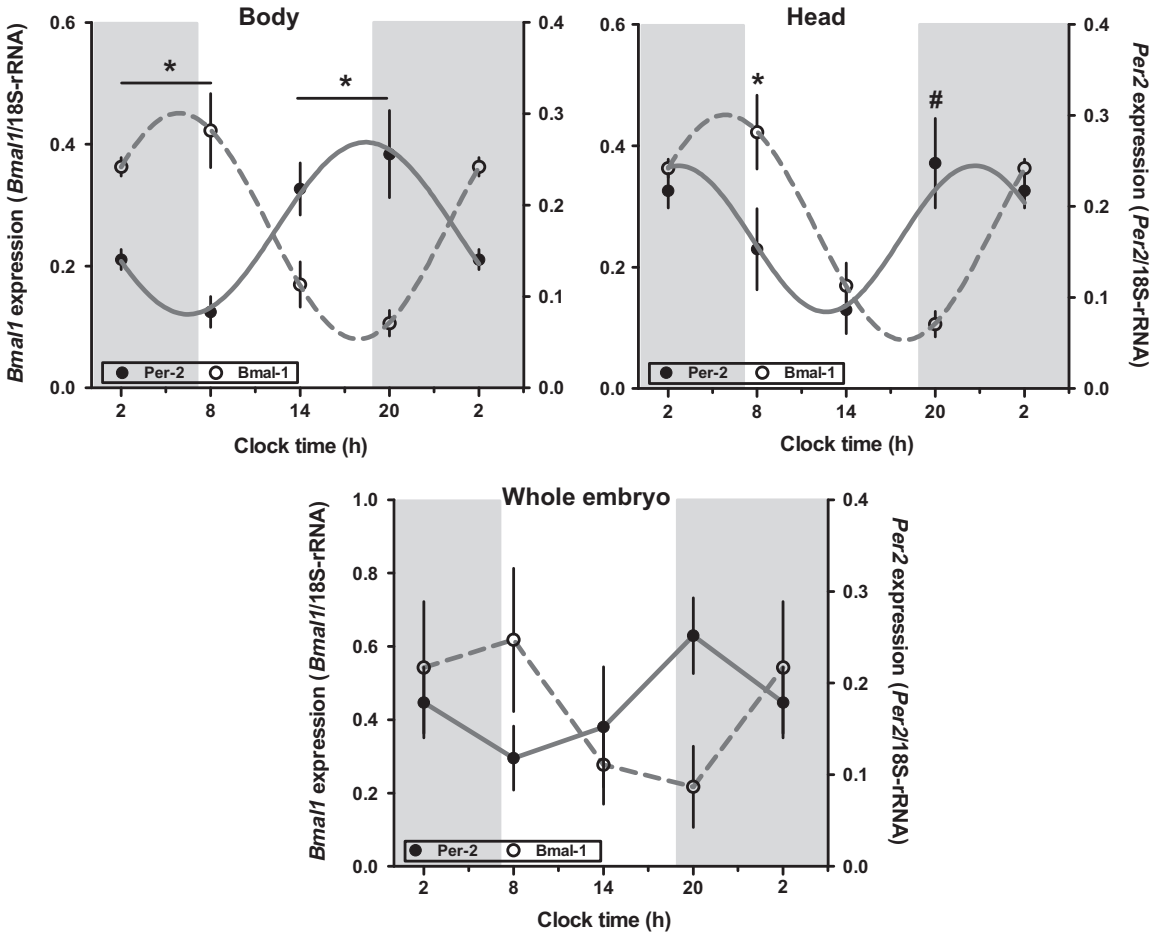

Fig. 1. Mean ± SEM expression of *Per2* and *Bmal1* in head and body and in the whole rat embryo at 16 days' gestation. Results are expressed as mean ± SEM of clock gene to 18S-rRNA. Pregnant rats (n = 6/clock time) were euthanized at by thiopental overdose at 8, 14, 20 and 2 h. Fetuses (n = 3/dam) were delivered by histerotomy and the head (whole fetal central nervous system) and the body (peripheral tissues) were separated. Tissues were preserved in Trizol until RNA extraction using a commercial kit (Promega). In each fetal compartment we measured expression of the clock genes *Per2* and *Bmal1* by real time PCR as reported (Torres-Farfan et al., 2011). Expression in the whole embryo was calculated by adding expression in the head and body at each clock time. *: different from other clock times ($P < 0.05$, ANOVA). #: different from 8 and 14-h ($P < 0.05$, ANOVA). The gray bars indicate lights-off hours (Torres-Farfan et al., unpublished).

24-rhythm of cortisol production was detected by measuring arterio-venous differences in the umbilical cord (Serón-Ferré et al., 2001b). The primate fetal adrenal gland is endowed with a fetal zone, that secretes large amounts of dehydroepiandrosterone sulphate (DHEAS) generating a 24 h rhythm in the fetal circulation. DHEAS is the precursor for the elevated placental estrogen production characteristic of primate pregnancy; and in human and non-human primates, including the capuchin monkey, plasma maternal estrogens show a circadian rhythm (Serón-Ferré et al., 2007). In the rat, the fetal adrenal presents a circadian rhythm of corticosterone content and of steroidogenic enzymes expression at 18 days of gestation (Torres-Farfan et al., 2011).

In the rat, at 18 days of gestational age, the fetal adrenal gland showed circadian expression of *Bmal1* and *Per2* in anti-phase as well as of MT_1 and of the early gene *Egr1* and of *StAR* (Torres-Farfan et al., 2011). Circadian oscillation of these genes persisted during 48-h in culture; however the anti-phase between *Per2* and *Bmal1* was lost, being restored by a pulse of melatonin. These results strongly support that in the rat, the fetal adrenal is a peripheral clock potentially amenable to direct regulation by maternal melatonin at a gestational age at which clock gene oscillation of the fetal SCN is absent.

In the capuchin, at 90% gestation, we explored whether the fetal adrenal is a peripheral clock commanded by the fetal SCN, which presents 24 h clock gene oscillation at this gestational age and is entrained by maternal melatonin. The capuchin fetal adrenal expressed *Bmal1* and *Per2* in anti-phase. Adrenal clock gene oscillation was accompanied with a circadian output of DHEAS. In addition, we found 24 h rhythms of expression of the MT_1 melatonin receptor and the steroidogenic enzyme 3β-HSD. Two observations support that the capuchin fetal adrenal rhythms are not under fetal SCN control. One is that the temporal pattern of clock gene oscillation in the fetal adrenal gland was identical to that found for the fetal SCN, in contrast with the phase delay between the SCN and peripheral circadian clocks reported in the adult. (Valenzuela et al., 2008; Watanabe et al., 2006; Johnston et al., 2005; Lincoln et al., 2002; Zylka et al., 1998; Oishi et al., 1998). The second observation is that suppression of maternal melatonin shifted clock gene expression in the fetal capuchin SCN, but not in the fetal adrenal gland. These findings suggest that fetal primate SCN and fetal adrenal gland are under separated maternal circadian control, the SCN by maternal melatonin and the fetal adrenal by an unknown maternal signal.

Overall, the previous findings led us to suggest that the circadian arrangement during fetal life in primates and rodents is such that the fetal organs (one of them the primate fetal SCN) are peripheral maternal circadian oscillators, which could be entrained by different maternal circadian signals (Serón-Ferré et al., 2007).

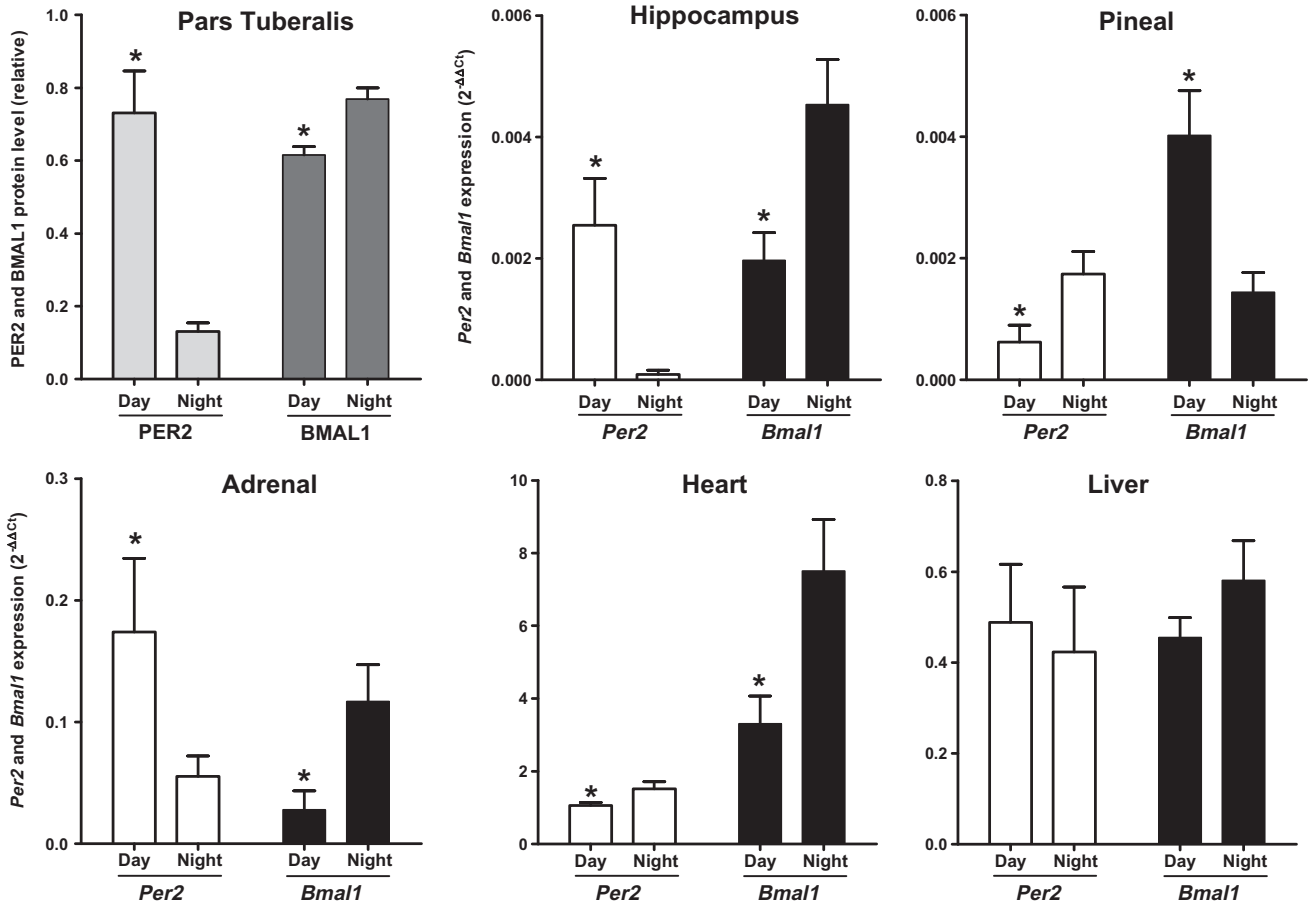

Fig. 2. Mean ± SEM day and night Per2 and Bmal1 expression in rodent fetal tissues at 18 days' gestation. The protein levels of PER2 and BMAL1 in mice *pars tuberalis* were modified from Ansari et al. (2009) and the *Per2* and *Bmal1* mRNA expression in rat fetal adrenal from Torres-Farfan et al. (2011). *Per2* and *Bmal1* mRNA expression in heart, liver, pineal and hippocampus was measured in fetuses from six groups of 5 pregnant rats; three groups were euthanized at day time (between 0800 and 1400) and the other three under red light at nighttime (between 2000 and 0200), Torres-Farfan et al., unpublished. Fetuses (n = 9/dam) were delivered by histerotomy and tissues were dissected and preserved in Trizol until RNA extraction. *Per2* and *Bmal1* expression was measured by real time PCR as reported (Torres-Farfan et al., 2011). *: different from nighttime ($P < 0.05$, T test).

7. Entrainment of fetal rhythms by circadian maternal signals

An unknown aspect of circadian clocks is entrainment. The adult SCN entrains to the LD cycle via the retinohypothalamic tract, but the molecular mechanisms by which neurotransmitters acting on SCN neurons shift the molecular clock are unknown. The clock gene rhythms in the fetal capuchin monkey SCN and *AVP* mRNA and *c-Fos* mRNA rhythms in the fetal rat SCN are entrained to the external LD cycle (Torres-Farfan et al., 2006; El-Hennamy et al., 2008; Nováková et al., 2010). The evidence discussed above indicates for the capuchin fetal SCN entrainment may be provided by the maternal melatonin rhythm. To date two maternal entraining signals, melatonin and restricted feeding time have been demonstrated in rodents. A recent experiment investigated the role of the LD cycle vs. restricted feeding in the rat fetal SCN rhythms of *AVP* and *c-Fos*, finding no effect of restricted feeding on these rhythms when the LD cycle was maintained (Nováková et al., 2010). However, exposure to constant light, which disrupts the maternal activity rhythm (and possibly other rhythms, including that of melatonin) flattened the rhythms of *AVP* and *c-Fos* in the fetal SCN, suggesting suppression or desynchronization of these rhythms. Under this situation, maternal restricted feeding restored the fetal SCN rhythms, but the amplitude was lower and the phase was shifted compared to that of fetuses of mothers kept in LD (Nováková et al., 2010). The authors conclude that maternal derived LD signals prevail over food signals for these specific fetal SCN rhythms.

In rodents, entrainment exerted during fetal life has a long postnatal duration. It is well established that the phase of postnatal behavioral circadian rhythms that appear at 2–3 weeks of age in hamsters and rats are entrained by maternal signals experienced during pregnancy. A compelling demonstration that the melatonin rhythm of the pregnant dam is involved in pup's entrainment is the finding that maternal pinealectomy during gestation desynchronize the pup's rhythm of drinking. Synchronization of this rhythm between pups is restored by daily maternal melatonin replacement during late gestation (Bellavia et al., 2006). The mechanisms by which this entrainment is exerted are unknown; however there is growing concern on the long term effects in the human offspring of interference with feeding and the maternal melatonin rhythm by lifestyle and shift work during pregnancy.

8. The maternal circadian system during pregnancy

From our review it becomes clear that maternal circadian signals during pregnancy are important for entrainment of fetal and newborn circadian rhythms. However, there is limited information on the physiological adaptations of the maternal circadian system to pregnancy and the response of this system to environmental perturbations (food restriction, stress, shifts in the LD cycle among others). In undisturbed conditions, studies in human and rats have shown an increase in the amplitude of the maternal rhythm of

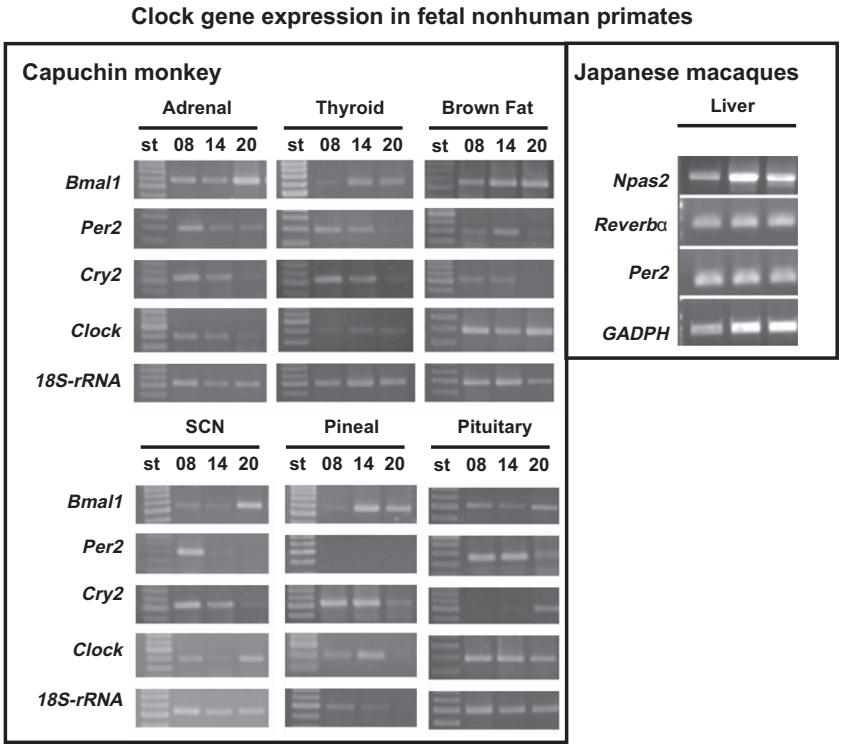

Fig. 3. Clock gene expression in fetal organs of two non human primates: capuchin monkey and Japanese macaque (redrawn from Torres-Farfan et al., 2006 and from Suter et al., 2011, respectively).

melatonin (reviewed by Tamura et al., 2008). In addition, in both species there is an increase in the concentration of maternal plasma glucocorticoid whilst the plasma glucocorticoid rhythm is maintained and the hypothalamic–adrenal axis response to stress is decreased (reviewed by Weerth and Buitelaar, 2005; Brunton, 2010). Other maternal rhythms like activity and temperature show species specific changes during pregnancy. In the diurnal Nile grass rat; the phase of the activity and temperature rhythms were similar to those in non-pregnant animals, but the amplitude was decreased (Schrader et al., 2009). In contrast, in the nocturnal rat there were differences in both amplitude and phase of the temperature and activity rhythm (Kittrell and Satinoff, 1988). In line with normal pregnancy affecting the maternal circadian system, a recent study comparing rhythms in *Fos* and *Per2* protein expression in the SCN and ventral subparaventricular zone (vSPZ) of early pregnant and diestrus rats, detected several functional differences. Of note, peak *Per2* expression was phase-advanced by 4 h in the SCN of pregnant rats and there were differences in response to light of the core and shell SCN and of the vSPZ. These findings were interpreted as indication of a functional reorganization of these regions during pregnancy (Schrader et al., 2010).

Maternal response to circadian perturbation may also differ between species. Exposure of pregnant capuchin and rhesus to constant light suppresses the maternal melatonin rhythm but has no effect over the maternal cortisol, progesterone, estradiol and temperature rhythms (Matsumoto et al., 1991; Torres-Farfan et al., 2004). In contrast, exposure to constant light of the pregnant rat results in a free running of the activity rhythm and eventually in the disappearance of this rhythm (Nováková et al., 2010). Effects on other maternal rhythms have not been studied.

The long term consequences for the offspring of possible perturbations of the fetal circadian system are beginning to be explored. In human and experimental animals, malnutrition during pregnancy increases the risk of cardiovascular disease and metabolic syndrome in the offspring generating the concept of developmental origins of health and disease (DOHAD). Of great interest is the increase in obesity already starting in children (Entringer et al., 2010). Studies feeding pregnant Japanese macaques a high fat diet show an increase in offspring adiposity. A target was the fetal liver, showing lipid accumulation, increased expression of genes in the gluconeogenic pathway and disrupted expression of the clock genes *Per1* and *Npas2*, due to increased occupancy by H3K14ac histone acetylase sites in the *Npas2* promoter (McCurdy et al., 2009; Suter et al., 2011). A different dietary perturbation, feeding pregnant mice with a low protein diet induces insulin resistance and increased adiposity in the offspring by 20 weeks of age. Of note, circadian behavioral abnormalities in timing of activity and eating behavior and an alteration of day/night *Bmal1*, *Per2* and *Clock* mRNA expression in the cortical forebrain and liver were already present at 8 weeks of age, prior to the appearance of metabolic abnormalities. Interestingly, the treatment did not affect clock gene expression in the hypothalamus, containing the SCN (Sutton et al., 2010). Another situation receiving attention is the effect on the offspring of shift work during pregnancy. Epidemiological studies in women show that shift work increases the risks of spontaneous abortion, premature delivery and low birth weight babies (Zhu et al., 2004). Shift work may disrupt the maternal melatonin rhythm and impose abnormal maternal sleep and feeding patterns. Varcoe et al., 2011, studied the long term effects on the rat offspring of simulated shift work during pregnancy. At 3 months of age, rats showed increased adiposity, hyperleptinemia and by 12 months of age showed the altered glucose tolerance and insulin resistance of metabolic syndrome. In contrast, there were no effects on the circadian rhythm of temperature, suggesting that chronic LD phase shifts experienced during pregnancy did not result in long term effects in the offspring SCN function. The important health issues involved require establishing whether an alteration in the developing peripheral circadian clocks plays a causal role in the manifestation of the metabolic syndrome.

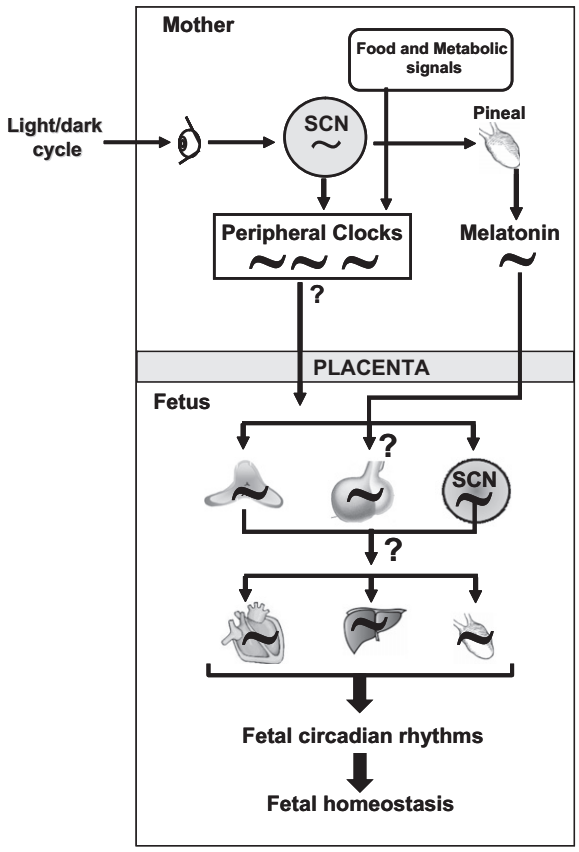

Fig. 4. Schematic representation of the proposed entrainment pathways of the fetal circadian system. The rat fetal adrenal and the primate fetal SCN could be entrained by the rhythms of maternal melatonin whereas other fetal peripheral clocks are phase entrained by: (1) the maternal SCN through humoral or metabolic signals that cross the placenta or (2) a fetal peripheral circadian clock. We propose that a potential candidate for this task is the fetal adrenal gland through circadian glucocorticoid production.

9. Concluding remarks

We propose that the circadian arrangement during fetal life is such that the fetal SCN and fetal organs are peripheral maternal circadian oscillators entrained by different maternal signals (Fig 4). Conceptually, this arrangement produces internal temporal order during fetal life, inside the maternal compartment. Following birth, it will allow for postnatal integration of the scattered fetal peripheral circadian clocks into an adult-like circadian system commanded by the SCN and entrained to the LD cycle. Whether particular situations during pregnancy (diet composition, maternal shift work) may impinge on specific fetal peripheral clocks leading to long term metabolic effects in the offspring is just beginning to be explored.

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