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Chapter 5 Plasticity of neuroendocrine mechanisms regulating seasonal reproduction in sheep

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Abstract
The sheep is not only a valuable animal model in which to investigate the mechanisms underlying seasonality, it is a commercially important species where reproduction of many breeds is profoundly seasonal. This chapter will describe how studies in sheep have revealed plasticity in the hypothalamus that likely underlies seasonal changes in neuroendocrine function. This includes changes in the kisspeptin neuronal system that provides a key drive to the gonadotrophin releasing hormone (GnRH) secretory system, seasonal fluctuations in neurogenesis and cell fate in the hypothalamus, morphological reorganizations as revealed by changes in polysialylated neural cell adhesion molecule immunoreactivity, and changes in permeability of the blood-brain barrier.
Keywords: male effect, metabolic status, blood brain barrier, neuropeptides, tanycytes

5.1 Introduction

Numerous species adapt to their constantly changing environment and its seasonal fluctuations by modulating specific physiological functions and behaviours. In sheep and many other domestic species such as goats and horses, reproduction is a seasonal function that will allow the birth of offspring at the optimal time of year for their survival i.e. spring and early summer, when food availability is maximal and the climate temperate. In sheep, this means that gamete production and sexual behavior occur only for a few months every year in the so-called breeding season which is followed by many months of sexual quiescence ("seasonal anestrus"). This pattern of seasonal reproduction results from innate rhythmicity (Chapter 1) interacting with the perception of natural changes in daylength and its translation into a hormonal signal, the release of melatonin from pineal gland during night. Therefore, in winter, the duration of melatonin secretion is longer than that one observed in summer nights (Malpaux, 2006). The melatonin signal is then read by several brain and pituitary structures, among which the *pars tuberalis* allows its transmission to hypothalamic nuclei through a mechanism involving central production of bioactive thyroid hormone (Dardente, 2012; Wood et al., 2015). The cellular mechanisms induced by melatonin to synchronize the activity of gonadotrophin releasing hormone (GnRH) neurons and the timely expression of sexual behavior are not yet fully elucidated. This chapter will summarize the recent results obtained in sheep regarding the contribution of seasonal brain plasticity to this process.

5.2 Secondary modulators of seasonal reproduction

5.2.1 Social factors

Although changes in photoperiod and thus nocturnal melatonin secretion are the primary regulators of seasonal reproduction, a phenomenon reported since the XVIIIth century can result in the induction of out of season estrus cycles in small ruminants. It is called "the male effect" or colloquially as the "ram effect" and consists of introducing a sexually active male into a flock of sexually inactive females during seasonal anestrus (Figure 1). This introduction of the mature male will activate multiple neuronal circuits resulting in a rapid increase of GnRH/LH pulsatile secretion, leading eventually to an LH surge triggering ovulation (Figure 1). This male effect relies on the integration of multiple stimuli among which the odor of male seems to be prominent. Recent evidence pinpoints an involvement of kisspeptin (KP) neurons in this effect (Fabre-Nys et al., 2017), as the percentage of activated KP neurons increases in anestrous female sheep (ewes) exposed to sexually active males (rams) in contrast with results in anestrous females exposed to sexually active females (Figure 1). This male effect method has been developed and standardized to produce hormone-free out-of-season reproduction.

5.2.2 Metabolic status

Flushing is a method commonly used by breeders to improve reproduction in small ruminants. It consists into bringing an additional supply of energy for several consecutive weeks before and after mating in ovine species to improve ovulation rate and therefore fecundity. Not only does gonadal activity rely on metabolic status, but also on the duration and depth of seasonal anestrus. This was demonstrated by following two groups of ewes over consecutive years, one with a mid-high body mass index and one with a low index. Lean animals displayed longer anestrus seasons than controls with higher adiposity, and the percentage of ewes without ovulation during the breeding season was greater in lean animals (Menassol et al., 2011). These data support a role for regulation of the hypothalamo-gonadal axis by metabolic factors that interact with the seasonal status of sheep.

BOX 1 Sheep as an integrated model of seasonal processes in mammals

Sheep as experimental models have numerous advantages compared to rodents as their large size allows for long periods of frequent blood and/or cerebrospinal fluid (CSF) sampling. Moreover, this can be done on freely moving awake animals. The size of their brain also allows the accurate placement through a stereotaxic neurosurgical approach of cannula into the brain sites. This allows the infusion of experimental test compounds, tracers and viruses into delineated regions without spreading to neighboring regions. The sheep has also been widely studied regarding its energy requirements and food intake, and the consequences of changes of nutrition on physiology are understood well. Due to the long luteal phase where ewes produce high progesterone levels, the length of the estrous cycle in the ewe relates to the length of the ovarian cycle in woman better than the ovarian cycle observed in rodents where luteal function does not develop post-ovulation in the absence of cervical stimulation and fertilization. As a consequence, information about central control of reproduction obtained in sheep may be more physiologically relevant related to humans than that obtained in models with shorter ovarian cycles. Results obtained in domestic mammals are also of economic significance for farmers. As in any other experimental model, there are also limitations for the use of sheep, as the tools such as antibodies and genetic manipulations to study the different

components of the neuroendocrine system may not as be readily available as they are for laboratory rodents. The physical size of the sheep brain also requires more time for laboratory processing.

5.3 Seasonal fluctuations of blood brain barrier permeability

5.3.1 Blood brain barrier

The blood brain barrier (BBB) is made of a continuum of endothelial cells separated by tight junctions, found in brain capillaries and in choroid plexuses. Depending on their sizes and chemical nature, molecules found in the blood either diffuse freely through the BBB or are actively transported using energy-consuming cell membrane mechanisms. Passive diffusion takes place for some small and lipophilic molecules, but these attributes are not on their own predictive of the ability of a molecule to diffuse freely through the BBB. Large and hydrophilic molecules do not cross freely through the BBB; they always require specific transporters or receptors to attain entry to the cerebrospinal fluid (CSF) and/or brain parenchyma. Given that one of the key features of the BBB is its ability to give or restrain access of molecules to the brain, a key issue has been to determine whether access of molecules to the brain in sheep is modified across seasons or dependent upon photoperiodic status. This chapter will review the passage of gonadal steroids across the BBB, and then consider the seasonal variations of protein content within the CSF. The final part of this section will consider environmental endocrine disrupters such as polychlorinated biphenyls (PCBs) as these chemicals accumulate in the environment and are able to interact with steroid receptors which may affect the central control of reproduction.

5.3.2 Access of peripheral molecules to the brain

Gonadal steroid feedback is modified in sheep throughout the year. During the breeding season, estrogens and progesterone are released from the ovaries and acting through both positive and negative feedback result in the occurrence of 16-18 day long ovarian cycles until pregnancy occurs. During the non-breeding season, ovarian follicles do not develop, so estradiol levels in the plasma remain low and progesterone is not released as corpora lutea never form in the ovary. Whether or not these seasonal fluctuations in sex steroid production are associated with modulation of their passage to the brain was assessed in ovariectomized estradiol-replaced ewes treated with an intravaginal progesterone implant and exposed either to short photoperiod (16 hours of dark per 24h) or long photoperiod (8 hours of dark per 24h). Progesterone assays of extracts of brain tissue revealed a higher concentration of progesterone during long photoperiods, while progesterone metabolites were not different between groups (Thiery and Malpaux, 2003). Similarly, progesterone levels within the CSF were doubled in long photoperiod compared to short photoperiod, while blood concentrations of progesterone were similar between groups, suggesting a differentiated passage across the BBB between photoperiodic conditions (Figure 2). Similar results were obtained for estrogens, which seem to cross the BBB more readily during long photoperiod exposure (Thiery and Malpaux, 2003) (Figure 2). Of note, this modulation of BBB permeability to sex steroids is dependent upon melatonin, as pinealectomy abolished the difference between long and short photoperiod for CSF content of estrogen (Thiery et al.,

2006). These results of seasonal modulation of the access of gonadal steroids into CSF have been extended by the work of Adam et al (2006) demonstrating greater access of leptin, a protein hormone involved in food intake, to CSF in rams in long photoperiods (Adam et al., 2006). More recently, we hypothesized that CSF protein content would vary with photoperiod (Teixeira-Gomes et al., 2015). In order to test this hypothesis, CSF from ovariectomized estradiol-replaced ewes was collected from animals kept either in long photoperiod to induce seasonal anestrus, or in short photoperiod to mimic the situation encountered during breeding season. The concentrations of CSF metabolites and hormones were measured using Liquid Chromatography-Mass Spectrometry (LC-MS), allowing the identification of proteins with a mass > 10KDa. This lead to the identification of 103 proteins in total, among which 41 differed in concentration in the CSF between short and long photoperiod: 18 were more abundant in long photoperiod and 23 in short photoperiod (Teixeira-Gomes et al., 2015). Those proteins were involved in many diverse functions including hormone transport, metabolism, angiogenesis, and immune system activity. Proteins found in CSF can originate from the circulation via filtration by the choroid plexus, or from direct synthesis within the choroid plexus, or from drainage of brain-released compounds (Strazielle et al., 2004), so the precise origin of each protein in CSF varying with photoperiodic environment and its impact on seasonal physiology remain to be determined. Understanding these fluctuations in CSF may help to decipher the intricate cells mechanisms impinging on seasonal physiology.

4.3.3 Seasonal variation in CSF production

CSF is produced by choroid plexus, and experiments were done to measure CSF renewal rate by removing a given volume of CSF (between 0.5mL and 2mL) while measuring the intraventricular pressure and the time necessary to restore the initial ventricular pressure. This experimental approach allowed a calculation of the volume of CSF produced in groups of animals placed either in short or long photoperiod. It was established that CSF production was twice as low in long photoperiod compared to short photoperiod (Thiery et al., 2009), suggesting a larger dilution of molecules during short photoperiods in CSF (Figure 2).

4.3.4 Modulation of tight junction composition by photoperiod

The different concentrations of multiple proteins in CSF in ewes in long and short photoperiod led us to investigate whether the different levels of steroids and proteins found in CSF between may result from a seasonal regulation of BBB permeability. Choroid plexus tissues from ewes exposed to short or long photoperiods were collected, and after protein extraction, a quantitative analysis of specific proteins found in tight junctions was performed using Western blot analyses. Four proteins were expressed at lower levels in choroid plexus during long photoperiod (zona occludens 1, zona occludens 2, afadin 6, cadherin), suggesting that the passage of molecules between epithelial cells was probably enhanced during long photoperiod (Lagaraine et al., 2011) (Figure 2).
In addition to these mechanisms, it may be that a seasonal modulation of local synthesis of steroids occurs in the ovine brain as reported for songbirds (Schlinger and Remage-Healey, 2012) and frogs (Santillo et al., 2017), concurring with the difference of steroids concentrations reported earlier for progesterone and estrogens within brain parenchyma.

4.3.5 Impact of polychlorinated biphenyls (PCBs) on BBB permeability

PCBs that have been used widely as plasticizers are endocrine disruptors able to interact with steroid receptors that accumulate in the environment as they have a very long half-life. Their impact on choroid plexus permeability was investigated in ewes exposed either to short or long photoperiod. Animals were treated for 3 weeks with PCB153 as this is one of the most abundant congeners. As PCB153 was found to accumulate in CSF during short but not in long photoperiod, a Western blot analysis of proteins found in tight junctions in choroid plexus was carried out. In ewes in short photoperiods, PCB153 treatment induced a substantive decrease in claudin 1, afadin 6 and zona occludens 2 protein levels, while in long photoperiod animals no significant differences were observed (Szczepkowska et al., 2013). These observations suggested that seasonal regulation of the permeability of the choroid plexus was weakened by PCB153 absorption through the stomach, which could impact on animals' physiology. These findings have to be considered in the light that often PCBs act as a cocktail of congeners, and the "cocktail" effect may be more powerful than the effect induced by the short-term administration of a single congener.

4.4 Plasticity of neuropeptidergic systems involved in the control of seasonal reproduction

4.4.1 Kisspeptin neurons

KP is the most potent secretagogue of GnRH secretion found to date in mammals. The number of KP appositions on GnRH neurons in ewes is significantly greater during the breeding season as compared to the anestrus season (Smith et al., 2008). Several studies examined whether or not KP content in the ovine hypothalamus fluctuates with seasons (Smith et al., 2007) or photoperiod (Chalivoix et al., 2010a). Two populations of KP neurons exist in the ovine diencephalon, one in the preoptic area (POA) and the other more caudally in the arcuate nucleus (ARC) - median eminence (ME) region (Franceschini et al., 2006). It appears that photoperiod alone is able to modify the content of KP within the ARC, and to a more limited extent in the POA: indeed the number of neurons displaying KP immunoreactivity is significantly lower in animals in long photoperiod with an inactivated gonadotroph axis than in animals in short photoperiods with an activated gonadotroph axis (Chalivoix et al., 2010a). The factors responsible for this decrease in long photoperiod remain to be fully established, but good candidates are neurokinin B and dynorphin both co-expressed with KP neurons of the ARC (Goodman et al., 2007) that could exert a paracrine/autocrine regulation. It was recently demonstrated that neurokinin B content also decreased in the hypothalamus during the anestrus season (Weems et al., 2017), and that KP/neurokinin B content may be affected by the increase of dopaminergic appositions originating from the retrochiasmatic area in anestrus season (Weems et al., 2017). However other factors may also influence the content of KP neurons, for example neuromodulators involved in food intake, as it was demonstrated in sheep that the depth of anestrus and its duration is directly linked to metabolic status (Menassol et al., 2011). Moreover, KP expression is correlated with metabolic status, as evidenced by lean sheep showing lower KP expression than well-fed animals (Backholer et al., 2010).

4.4.2 Neurons involved in food intake

Two major systems driving food intake in sheep are located within the ARC. One system is orexigenic and is constituted of neurons synthesizing both neuropeptide Y and Agouti Related Protein (AgRP), while the second is anorexigenic and comprised neurons expressing both pro-opiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART). Both systems interact with each other and also with KP neurons, and are the recipient of peripheral hormone signals such as leptin produced by fat tissue and ghrelin from the stomach in sheep (Backholer et al., 2010). The action of neuropeptide Y on KP synthesis is more complex than the simple neuropeptide Y-induced stimulation of KP expression reported *in vitro* (Luque et al., 2007), as *in vivo* work demonstrated that NPY/KP regulation is dependent on metabolic status, since leptin inhibits NPY but stimulates KP expression (De Bond and Smith, 2014). CART may stimulate KP neurons and therefore help translate the energetic status to the reproductive axis (True et al., 2013). Melanocortin, a POMC-derived peptide, appears to have a dual action on KP expression depending on the site of action: being inhibitory in the ARC but stimulatory in the POA (Backholer et al., 2009). We investigated whether seasonal fluctuations of the interactions between KP and NPY neurons and between KP and POMC neurons occurred in ewes exposed either to short or long photoperiod treatment. We found similar numbers of NPY appositions on KP neurons in the ARC of ewes regardless of the photoperiod that they had been exposed to. In contrast, the appositions of β-endorphin, a peptide cleaved from the POMC precursor, on KP neurons were doubled in ewes having an activated gonadotroph axis as compared to ewes exposed to long photoperiod (Hellier and Dufourny, unpublished data). An intracerebroventricular infusion of β-endorphin (5µg) in ewes having high LH levels evoked an increase of the percentage of activated KP neurons within the ARC 2 hours later, as inferred from the number of KP neurons colocalizing c-Fos (Hellier and Dufourny, unpublished data). These observations suggest that β-endorphin modulates KP neuron activity and therefore influences the central control of reproduction.

4.5 Plasticity mechanisms involved in seasonal reproduction in sheep

4.5.1 Morphological reorganizations

Immunohistochemical analysis in sheep of polysialylated neural cell adhesion molecule (PSA-NCAM), a marker for brain synaptic plasticity, revealed that morphological reorganizations occurred in close proximity to GnRH neurons between the breeding season and anestrus season (Xiong et al., 1997) (Viguie et al., 2001) (Figure 3A). Further studies demonstrated that not only was the number of appositions on GnRH neurons greater during the breeding season (Xiong et al., 1997), but the chemical nature of these appositions was also different (Jansen et al., 2003; Sergeeva and Jansen, 2009; Smith et al., 2008). These morphological reorganizations also occurred elsewhere, for example in the retrochiasmatic area, where dopaminergic neurons (A15) relaying estrogen negative feedback receive two times more appositions on their dendrites and display an increased dendritic length during anestrus (Adams et al., 2006). This latter phenomenon is dependent on the presence of thyroid hormones (Adams et al., 2006).

These data strongly suggest that morphological rearrangements are essential for the central control of reproduction, we therefore chose to investigate morphological reorganizations induced by photoperiod in the ewe diencephalon (Chalivoix et al., 2013). For that, ovariectomized estradiol replaced (OVX+E2) ewes exposed to either short or long

photoperiod were switched to the alternate photoperiods. Blood samples were collected every two weeks until circulating LH levels reached the expected concentrations (0 ng/mL after a switch to long photoperiod and LH > 1ng/mL for at least 2 consecutives blood sampling after a switch to short photoperiod). PSA-NCAM levels were then quantified through Western blot analysis of microdissected hypothalamic areas. Following a transition to short photoperiod, as early as day 15, a sharp decrease of PSA-NCAM occurred in the organum vasculosum of lamina terminalis -POA region known to host most GnRH neuronal cell bodies in sheep. Later decreases in PSA-NCAM levels were observed in more caudal areas for example the ARC. Following the transition to long photoperiod, the rate of PSA-NCAM increased transiently in the same regions where decreases were reported following exposure to short photoperiod, including the ARC and POA. Of note, only one area displayed a decrease of PSA-NCAM subsequent to a transition to long photoperiod: the premammillary nucleus (Chalivoix et al., 2013).
In a second experiment (Chalivoix et al., 2013), endoneuraminidase N, an enzyme that cleaves polysialic acid chains from NCAM (Figure 3B), was injected within the POA (the region encompassing most GnRH somas in sheep, Figure 3C) before a transition to short photoperiod, and the time necessary to observe a rise in LH levels was measured in treated animals and controls (Figure 3D). Two thirds of treated animals showed delays 3 times longer than control ewes to develop an increase in their LH levels indicative of transition to the breeding state (>250 days vs 84 days). These data suggest that specific kinetics of morphological rearrangements take place depending on photoperiod and on the hypothalamic region examined. This plasticity may be a key component for the timing of seasonal reproduction.
In a parallel study (Chalivoix et al., 2010b), the association between GnRH neurons and PSA-NCAM in groups of OVX+E2 ewes submitted to a transition from long to short photoperiod was quantified. The association of GnRH with PSA-NCAM in animals with an activated gonadotrophin axis after a transition to short photoperiod was five times greater than in animals with an inactive axis. However, the numbers of synapses per GnRH neuron was not modified with photoperiodic status. This may reflect the fact that the morphological rearrangements suggested by PSA-NCAM variations on GnRH neurons consist of a change in the phenotype of inputs, rather than an overall increase or decrease in a specific input.
Cellular morphological rearrangements have also been described in the sheep *pars tuberalis* where the highest density of melatonin binding sites is found (Wood et al. 2015). Subsets of *pars tuberalis* cells producing thyroid stimulating hormone (TSH) are more numerous in summer, increase their junctional contacts and increase their rough endoplasmic reticulum compared to winter where those thyrotroph cells are separated from each other by folliculostellate cells (Wood et al. 2015). This plastic phenomenon may be a key factor for the transduction of melatonin signal to hypothalamic neurons for the synchronization of seasonal functions throughout year.

5.5.2 Seasonal fluctuations of neurogenesis and cell fate

Recent work performed by the team led by Martine Migaud used uptake of the thymidine analogue bromodeoxyuridine (BrdU) infused into the CSF to demonstrate that new cells emerge in the adult ovine brain (Fig. 4), most specifically in the thalamic and hypothalamic compartments. These newborn cells are more numerous when the BrdU injection is carried out

in sheep exposed to short photoperiod, suggesting that cell neogenesis may be enhanced by the long duration of melatonin secretion under this photoperiod (Migaud et al., 2011). Cell neogenesis also occurs in several brain areas in rams, including the hippocampus, where cell proliferation is greater in long photoperiod in feral sheep of the Soay breed (Hazlerigg et al., 2013). In this study, hypothalamic cell proliferation and survival at 28 days post BrdU infusion was dependent on season, with a higher survival of new cells during short photoperiods (Hazlerigg et al., 2013). In ewes, as highlighted by immunostaining for doublecortin, a marker for neuroblasts, immature neurons were found in the ARC and ME (Batailler et al., 2014). Regarding the fate of hypothalamic newborn cells, further studies demonstrated that a substantial proportion mature into glial cells, while only a restricted portion becomes neurons (Figure 4) (Butruille et al., 2018). Interestingly, immunostaining for markers associated with glial cells (GFAP, Iba1, CNPase, Olig2) did not vary significantly between short and long photoperiods, while the density of a neuronal marker (PSA-NCAM) was greater in short photoperiods (Butruille et al., 2018) in the ARC-ME area. This could suggest that emergence of new neurons is more abundant in short photoperiods in the mediobasal hypothalamus, independent of estradiol fluctuations as suggested initially (Migaud et al., 2011). The most recent findings demonstrated that infusion of the antimitotic drug AraC into the third ventricle resulted in a decrease of neurogenesis associated with an impaired timing of seasonal reproduction, specifically an earlier termination of the breeding season in treated animals (Batailler et al., 2018). Overall these data suggest that seasonal fluctuations of neurogenesis may play a significant role in the integration of melatonin signals and timing of seasonal reproduction.

5.6 Perspectives

Many questions remain regarding the functional consequences of seasonal morphological rearrangements on the start and termination of breeding season in sheep. For example, is the fluctuation of CSF content a consequence or a component of the modifications that occur in the brain parenchyma throughout the year? What is the fate of newborn cells? Do they migrate and if they do, into which brain regions, and what are their peptidergic and/or monoaminergic phenotypes? How will these new cells integrate into existing networks and interact with others populations involved in the central control of reproduction, namely GnRH and KP neurons? Future studies will also focus on studying the different genetic, endocrine, metabolic and social factors responsible for the induction of plastic phenomenon in line with the seasonal regulation of reproduction.

Recommended Further Reading

Neurogenesis is not only observed in the hypothalamus but also in other brains areas in sheep and this review summarizes recent data about neurogenesis potential involvement in several physiological functions and behaviors:

Lévy F., Batailler M., Meurisse M., Migaud M., 2017. Adult neurogenesis in sheep : Characterization and contribution to Reproduction and Behavior. Front Neurosci 11, 570.

This review summarizes the most recent data on molecular pathways involved in the translation of photoperiod to the hypothalamus and also expand the discussion by addressing the possible involvement of RFRP-3 neurons in the central control of reproduction.
Dardente H., Wood S., Ebling F., Saenz de Miera C., 2019. An integrative view of mammalian seasonal neuroendocrinology. J. Neuroendocrinol. 31: e12729.

Cited References

Adam, C.L., Findlay, P.A., Miller, D.W., 2006. Blood-brain leptin transport and appetite and reproductive neuroendocrine responses to intracerebroventricular leptin injection in sheep: influence of photoperiod. Endocrinology 147, 4589-4598.
Adams, V.L., Goodman, R.L., Salm, A.K., Coolen, L.M., Karsch, F.J., Lehman, M.N., 2006. Morphological plasticity in the neural circuitry responsible for seasonal breeding in the ewe. Endocrinology 147, 4843-4851.
Backholer, K., Smith, J., Clarke, I.J., 2009. Melanocortins may stimulate reproduction by activating orexin neurons in the dorsomedial hypothalamus and kisspeptin neurons in the preoptic area of the ewe. Endocrinology 150, 5488-5497.
Backholer, K., Smith, J.T., Rao, A., Pereira, A., Iqbal, J., Ogawa, S., Li, Q., Clarke, I.J., 2010. Kisspeptin cells in the ewe brain respond to leptin and communicate with neuropeptide y and proopiomelanocortin cells. Endocrinology 151, 2233-2243.
Batailler, M., Chesneau, D., Derouet, L., Butruille, L., Segura, S., Cognie, J., Dupont, J., Pillon, D., Migaud, M., 2018. Pineal-dependent increase of hypothalamic neurogenesis contributes to the timing of seasonal reproduction in sheep. Sci Rep 8, 6188.
Batailler, M., Droguerre, M., Baroncini, M., Fontaine, C., Prevot, V., Migaud, M., 2014. DCX-expressing cells in the vicinity of the hypothalamic neurogenic niche: a comparative study between mouse, sheep, and human tissues. J Comp Neurol 522, 1966-1985.
Butruille, L., Batailler, M., Mazur, D., Prevot, V., Migaud, M., 2018. Seasonal reorganization of hypothalamic neurogenic niche in adult sheep. Brain Struct Funct 223, 91-109.
Chalivoix, S., Bagnolini, A., Caraty, A., Cognie, J., Malpaux, B., Dufourny, L., 2010a. Effects of photoperiod on kisspeptin neuronal populations of the ewe diencephalon in connection with reproductive function. J Neuroendocrinol 22, 110-118.
Chalivoix, S., Guillaume, D., Cognie, J., Thiery, J.C., Malpaux, B., Dufourny, L., 2013. Photoperiodic variations of the polysialylated form of neural cell adhesion molecule within the hypothalamus and related reproductive output in the ewe. Cell Tissue Res 352, 387-399.
Chalivoix, S., Malpaux, B., Dufourny, L., 2010b. Relationship between polysialylated neural cell adhesion molecule and beta-endorphin- or gonadotropin releasing hormone-containing neurons during activation of the gonadotrope axis in short daylength in the ewe. Neuroscience 169, 1326-1336.
Dardente, H., 2012. Melatonin-dependent timing of seasonal reproduction by the pars tuberalis: pivotal roles for long daylengths and thyroid hormones. J Neuroendocrinol 24, 249-266.
De Bond, J.A., Smith, J.T., 2014. Kisspeptin and energy balance in reproduction. Reproduction 147, R53-63.

Fabre-Nys, C., Cognie, J., Dufourny, L., Ghenim, M., Martinet, S., Lasserre, O., Lomet, D., Millar, R.P., Ohkura, S., Suetomi, Y., 2017. The Two Populations of Kisspeptin Neurons Are Involved in the Ram-Induced LH Pulsatile Secretion and LH Surge in Anestrous Ewes. Endocrinology 158, 3914-3928.
Franceschini, I., Lomet, D., Cateau, M., Delsol, G., Tillet, Y., Caraty, A., 2006. Kisspeptin immunoreactive cells of the ovine preoptic area and arcuate nucleus co-express estrogen receptor alpha. Neurosci Lett 401, 225-230.
Goodman, R.L., Lehman, M.N., Smith, J.T., Coolen, L.M., de Oliveira, C.V., Jafarzadehshirazi, M.R., Pereira, A., Iqbal, J., Caraty, A., Ciofi, P., Clarke, I.J., 2007. Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. Endocrinology 148, 5752-5760.
Hazlerigg, D.G., Wyse, C.A., Dardente, H., Hanon, E.A., Lincoln, G.A., 2013. Photoperiodic variation in CD45-positive cells and cell proliferation in the mediobasal hypothalamus of the Soay sheep. Chronobiol Int 30, 548-558.
Jansen, H.T., Cutter, C., Hardy, S., Lehman, M.N., Goodman, R.L., 2003. Seasonal plasticity within the gonadotropin-releasing hormone (GnRH) system of the ewe: changes in identified GnRH inputs and glial association. Endocrinology 144, 3663-3676.
Lagaraine, C., Skipor, J., Szczepkowska, A., Dufourny, L., Thiery, J.C., 2011. Tight junction proteins vary in the choroid plexus of ewes according to photoperiod. Brain Res 1393, 44-51.
Luque, R.M., Kineman, R.D., Tena-Sempere, M., 2007. Regulation of hypothalamic expression of KiSS-1 and GPR54 genes by metabolic factors: analyses using mouse models and a cell line. Endocrinology 148, 4601-4611.
Malpaux, B., 2006. Seasonal regulation of reproduction in mammals., in: Neill, J.D. (Ed.), Knobil and Neill's Physiology of Reproduction, Third Edition. Elsevier, New York, pp. 2231-2281.
Menassol, J.B., Collet, A., Chesneau, D., Malpaux, B., Scaramuzzi, R.J., 2011. The interaction between photoperiod and nutrition and its effects on seasonal rhythms of reproduction in the ewe. Biol Reprod 86, 52.
Migaud, M., Batailler, M., Pillon, D., Franceschini, I., Malpaux, B., 2011. Seasonal changes in cell proliferation in the adult sheep brain and pars tuberalis. J Biol Rhythms 26, 486-496.
Santillo, A., Falvo, S., Di Fiore, M.M., Chieffi Baccari, G., 2017. Seasonal changes and sexual dimorphism in gene expression of StAR protein, steroidogenic enzymes and sex hormone receptors in the frog brain. Gen Comp Endocrinol 246, 226-232.
Schlinger, B.A., Remage-Healey, L., 2012. Neurosteroidogenesis: insights from studies of songbirds. J Neuroendocrinol 24, 16-21.
Sergeeva, A., Jansen, H.T., 2009. Neuroanatomical plasticity in the gonadotropin-releasing hormone system of the ewe: seasonal variation in glutamatergic and gamma-aminobutyric acidergic afferents. J Comp Neurol 515, 615-628.
Smith, J.T., Clay, C.M., Caraty, A., Clarke, I.J., 2007. KiSS-1 messenger ribonucleic acid expression in the hypothalamus of the ewe is regulated by sex steroids and season. Endocrinology 148, 1150-1157.
Smith, J.T., Coolen, L.M., Kriegsfeld, L.J., Sari, I.P., Jaafarzadehshirazi, M.R., Maltby, M., Bateman, K., Goodman, R.L., Tilbrook, A.J., Ubuka, T., Bentley, G.E., Clarke, I.J., Lehman, M.N., 2008. Variation in kisspeptin and RFamide-related peptide (RFRP) expression and terminal

connections to gonadotropin-releasing hormone neurons in the brain: a novel medium for seasonal breeding in the sheep. Endocrinology 149, 5770-5782.
Strazielle, N., Khuth, S.T., Ghersi-Egea, J.F., 2004. Detoxification systems, passive and specific transport for drugs at the blood-CSF barrier in normal and pathological situations. Adv Drug Deliv Rev 56, 1717-1740.
Szczepkowska, A., Lagaraine, C., Robert, V., Dufourny, L., Thiery, J.C., Skipor, J., 2013. Effect of a two-week treatment with a low dose of 2,2'4,4',5,5'-hexachlorobiphenyl (PCB153) on tight junction protein expression in ovine choroid plexus during long and short photoperiods. Neurotoxicol Teratol 37, 63-67.
Teixeira-Gomes, A.P., Harichaux, G., Gennetay, D., Skipor, J., Thiery, J.C., Labas, V., Dufourny, L., 2015. Photoperiod affects the cerebrospinal fluid proteome: a comparison between short day- and long day-treated ewes. Domest Anim Endocrinol 53, 1-8.
Thiery, J.C., Lomet, D., Bougoin, S., Malpaux, B., 2009. Turnover rate of cerebrospinal fluid in female sheep: changes related to different light-dark cycles. Cerebrospinal Fluid Res 6, 9.
Thiery, J.C., Lomet, D., Schumacher, M., Liere, P., Tricoire, H., Locatelli, A., Delagrange, P., Malpaux, B., 2006. Concentrations of estradiol in ewe cerebrospinal fluid are modulated by photoperiod through pineal-dependent mechanisms. J Pineal Res 41, 306-312.
Thiery, J.C., Malpaux, B., 2003. Seasonal regulation of reproductive activity in sheep: modulation of access of sex steroids to the brain. Ann N Y Acad Sci 1007, 169-175.
True, C., Verma, S., Grove, K.L., Smith, M.S., 2013. Cocaine- and amphetamine-regulated transcript is a potent stimulator of GnRH and Kisspeptin cells and may contribute to negative energy balance-induced reproductive inhibition in females. Endocrinology.
Viguie, C., Jansen, H.T., Glass, J.D., Watanabe, M., Billings, H.J., Coolen, L., Lehman, M.N., Karsch, F.J., 2001. Potential for polysialylated form of neural cell adhesion molecule-mediated neuroplasticity within the gonadotropin-releasing hormone neurosecretory system of the ewe. Endocrinology 142, 1317-1324.
Weems, P., Smith, J., Clarke, I.J., Coolen, L.M., Goodman, R.L., Lehman, M.N., 2017. Effects of Season and Estradiol on KNDy Neuron Peptides, Colocalization With D2 Dopamine Receptors, and Dopaminergic Inputs in the Ewe. Endocrinology 158, 831-841.
Wood, S.H., Christian, H.C., Miedzinska, K., Saer, B.R., Johnson, M., Paton, B., Yu, L., McNeilly, J., Davis, J.R., McNeilly, A.S., Burt, D.W., Loudon, A.S., 2015. Binary Switching of Calendar Cells in the Pituitary Defines the Phase of the Circannual Cycle in Mammals. Curr Biol 25, 2651-2662.
Xiong, J.J., Karsch, F.J., Lehman, M.N., 1997. Evidence for seasonal plasticity in the gonadotropin-releasing hormone (GnRH) system of the ewe: changes in synaptic inputs onto GnRH neurons. Endocrinology 138, 1240-1250.

Figure Legends

Figure 1 The ram effect. Following introduction of a male (ram) in a flock of seasonally anestrous females, a rapid increase of LH pulsatile secretion is observed in females (**A**) that will eventually lead to a surge of LH (**B**). In contrast, in females exposed to females, LH levels remains stable (**C**). **D** 12 hours after exposure to a ram, numerous neurons in the arcuate nucleus (ARC) and medial preoptic area (mPOA) display c-Fos-ir, a nuclear marker for cell activation (red) in KP-ir neurons (green) (scale bar 40 µm). **E** significantly more dual-labelled neurons occurred within the ARC and mPOA of females exposed to rams for 2 hours and

12 hours than in the same regions of females exposed to females. (Adapted from Fabre-Nys et al, 2017).

Figure 2 Photoperiod regulates the passage of steroids and proteins from blood to brain, the renewal rate of CSF, and the structure of tight junctions in the ovine brain. Each rectangle depicts a compartment, the size of blue rounded arrows is proportional to the rate of CSF renewal as well as the size of red arrows that represent the passage of molecules through tight junctions (represented as green bars). The size of characters is also representative of the different concentrations for molecules found in CSF and/or brain and in blood under the different photoperiods. BBB: blood brain barrier, CSF: cerebrospinal fluid, P_4: progesterone, E_2: estradiol.

Figure 3 Polysialylated neural cell adhesion molecule (PSA-NCAM) and morphological rearrangements at the membrane level. In a stable status, cells develop interactions through homophilic liaisons between proteins anchored in cytoplasmic membrane (**A**).
When polysialic acid (PSA) chains are added on NCAM protein chain, it results in cell plasticity stemming from repulsion between membranes (**A**). In order to evaluate the physiological importance of PSA-NCAM for seasonal reproduction, we used an enzyme that cleaves PSA residues from NCAM (**B**): endoneuraminidase N (EndoN). We injected it at the level of the preoptic area (POA) where most GnRH neurons are observed in ewes (**C**), and transferred the animals to a short photoperiod. A delay in the LH rise in short photoperiod occurred in two thirds of treated animals (**D**), suggesting that morphological plasticity involving PSA-NCAM is an important mechanism for the synchronization of seasonal reproduction. Adapted from the work of Chalivoix et al. (2013).

Figure 4 Neurogenesis in ovine hypothalamus depends on photoperiod. Top: in short photoperiod, the number of neural progenitors is greater than in long photoperiod. These progenitors differentiate into glial progenitors and into a limited number of immature neurons but similar levels of both populations appeared under each photoperiod. In contrast, the markers associated with mature neurons and more specifically with neural plasticity were more abundant in long photoperiod than in short photoperiod. Below: examples of new hypothalamic cells having incorporated BrdU and expressing either a glial marker (GFAP) or a neuronal marker (NeuN) as shown (adapted from Migaud et al., 2011).

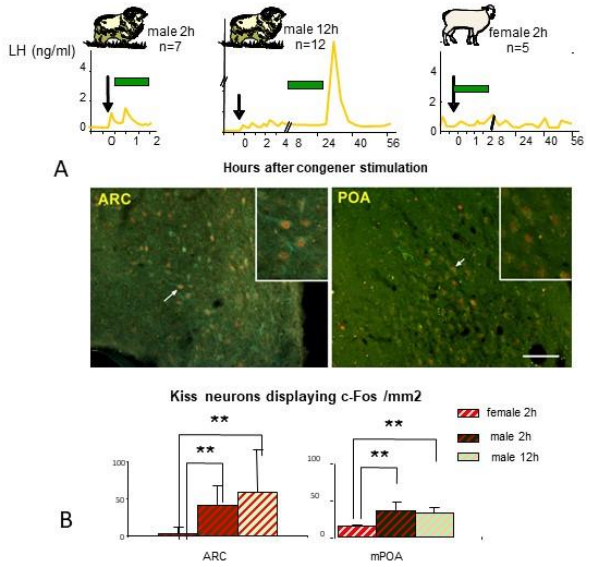

Figure 1

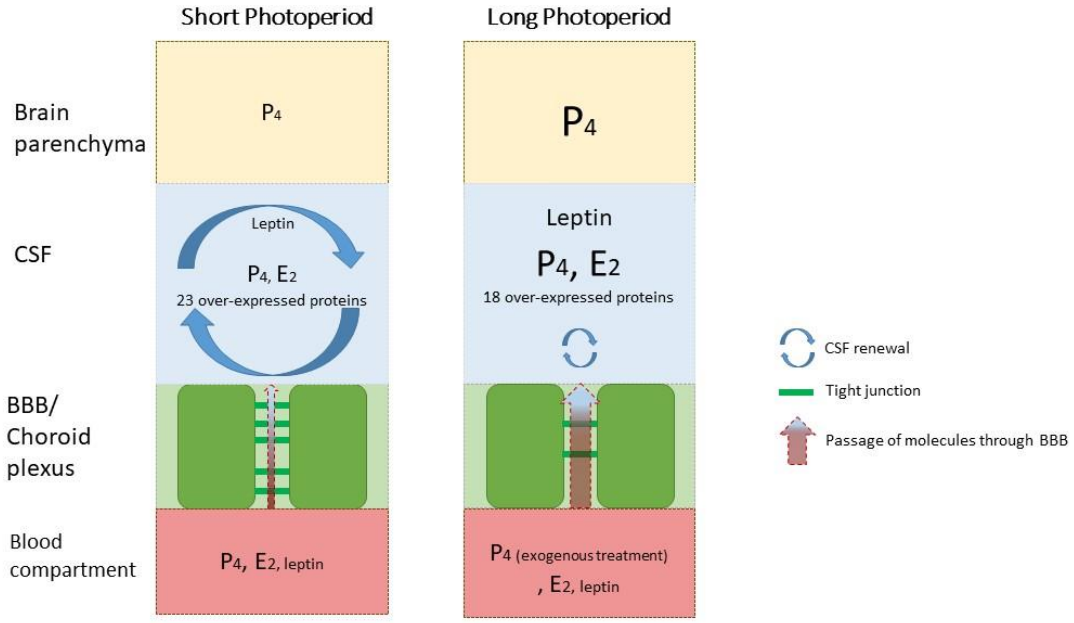

Figure 2

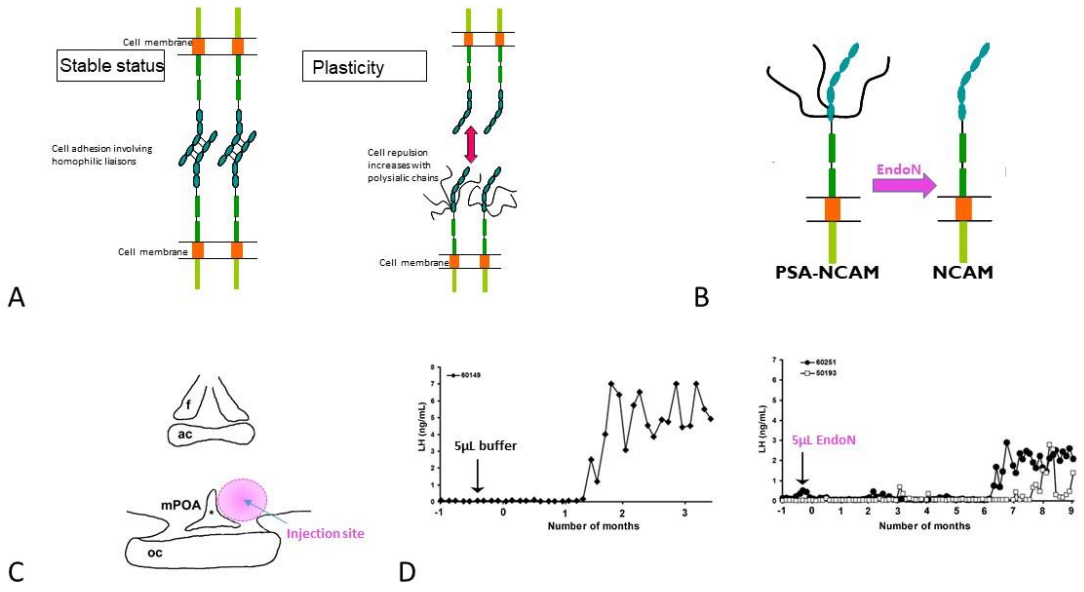

Figure 3

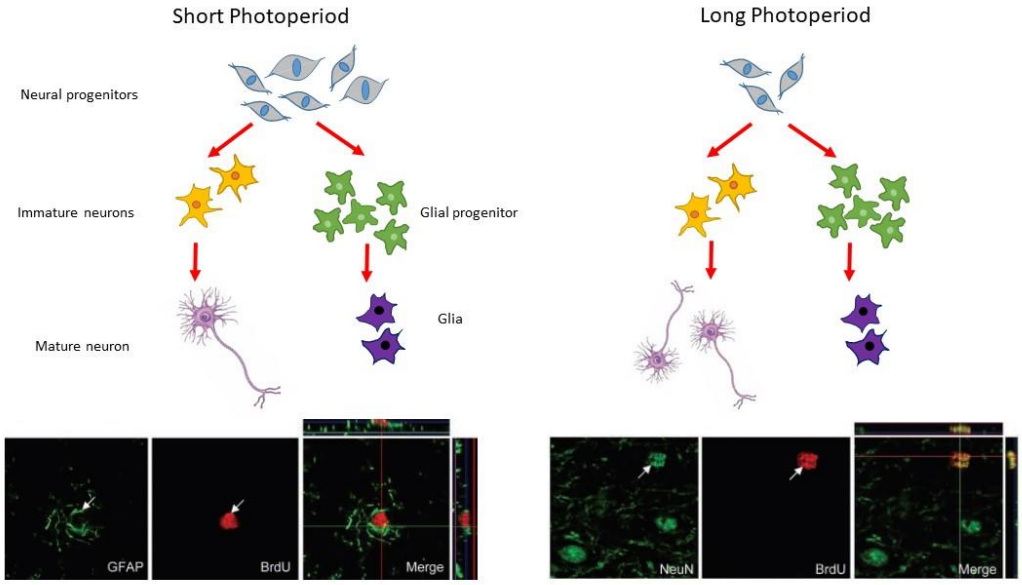

Figure 4