Magnetic Resonance Imaging, a New Tool for Neuroendocrine Research in Sheep

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Keywords

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Abstract

Magnetic resonance imaging (MRI) brain analysis is used in rodents and for clinical investigation in humans, and it becomes also possible now for large animal models studies. Specific facilities are available with clinical scanners and benefit to neuroendocrine investigations in sheep. Sheep has a large gyrencephalic brain and its organization is very similar to primates and human, and among physiological regulations, oestrous cycle of the ewes is similar to women. Therefore, this animal is a good model for preclinical researches using MRI, as illustrated with steroids impact on the brain. New data were obtained concerning the effect of sexual steroids on neuronal networks involved in the control of reproduction and in the influence of sexual steroids on cognition. In addition to the importance of such data for understanding the role of these hormones on brain functions, they give new insights to consider the sheep as a powerful model for preclinical studies in the field of neuroendocrinology. These points are discussed in this short review.

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Introduction

For several years, brain imaging appears to be a promising approach to study neuroendocrine interactions for research purposes as illustrated in the control of reproduction in human. Magnetic resonance imaging (MRI) and 2-Deoxy Glucose positron electron tomography imaging were used to demonstrate the effect of oestrogen on the metabolic changes and cognition in menopausal and postmenopausal women [1, 2] and the oestrogen negative feedback in the mediobasal hypothalamus (MBH) in healthy women [3]. The effect of sex steroids in neuronal plasticity in the human hypothalamus were also studied during artificial menstrual cycle induced by oral contraceptive pills in young woman [4].

However, the MRI studies were mainly observational and, even though in vivo imaging is safe and non-invasive, the possibility of deeper experimental investigations remains limited in human for ethical reasons. Small individual sample size, heterogeneity of the groups of subjects according to age, hormonal status, etc., make difficult the interpretation of the data. Numerous studies were also performed on laboratory animals, mainly rodents, which present a wide range of genetically modified models.

Table 1. Comparison of some physiological features between sheep, human, and rodents

	Sheep	Human	Rodent
Body weight, kg	60–75	70	0.02–0.5
Brain weight, g	100–150	1,400	0.5–2.4
Cortical development	Gyrecephalic	Gyrecephalic	Lissencephalic
Pituitary morphology	Long pituitary stalk	Long pituitary stalk	Short pituitary stalk
Hormonal kinetics with large volume blood samples	Yes	Yes but with ethical concerns	No
Successive cerebral fluid sampling	Yes	Yes but with ethical concerns	No
Duration of oestrus cycle, days	18*	28	4
Newborns at birth	1–3	1	6–10
Weight at birth, kg	2.5–5	2.5–4.3	0.005–0.010

* Similar length of luteal phase

However, the physiological characteristics of these species are really different from human ones regarding reproduction and brain morphology. They have a gyrencephalic brain like human and a similar body size. Rodent reproduction is characterized by short oestrus cycles and a high ovulation rate, and has a smooth brain. Large animals like sheep are more suitable animal models for the neuroendocrine study of human reproduction. Ewes have a low ovulation rate (1–3 oocytes/oestrous cycle) and most of the breeds give birth to only 1–3 newborns. The length of the oestrus cycle of the ewe, especially the luteal phase, is very similar to those of woman (Table 1). This short review will thus focus on the use of MRI for studying neuroendocrine control of reproduction and cognition, in sheep, as a large animal model suitable for preclinical research.

MRI in Large Animals

MRI uses a high magnetic field that aligns the magnetic spin of hydrogen atoms in the tissue in a low energy configuration. The spins are then excited out of equilibrium by a radiofrequency pulse. When the magnetic field is switched off, the spin of hydrogen returns to equilibrium (relaxation phase), and the time constants T1 (longitudinal magnetization) and T2 (transverse magnetization) can be measured. These values are used to construct MR images, and because relaxation times differ across tissues, numerous details of the tissue can be observed with a high spatial resolution, similar to a histologic section. Therefore, this method is particularly suitable for rich water biological tissue like the brain which contains 75–80% of water.

MRI in large animal is now available according to the wide range of imaging facilities developed in the past de-

cade in most of European countries. Most of them are equipped with 3T MRI scanner similar to those used in clinics and suitable for animal such as sheep or pig, similar in size and weight to human [5].

Anatomical MRI scanning lasts for several minutes, usually between 15 and 20 min in human, a period during which the body must not move. As it is difficult to avoid any movement and stress induced by the procedure in awake animals, they have to be anaesthetised during scanning. This constraint of long scanning period becomes an advantage for anatomical and fMRI because it allows more signal acquisitions and an increase in the quality of the picture.

However, anaesthesia is known to alter both neuronal activity and hemodynamic state. During anaesthesia, perception of the stimulus may be reduced if not absent, and the response to the stimulus can be inhibited. The impairment of cellular physiology and neuronal connections could modify the expected physiological response like hormonal secretions in neuroendocrinology. Previous experiments have shown in the ewes that halothane anaesthesia reduced mean plasma Luteinizing Hormone (LH) levels by preventing pulsatile secretion of LH whereas it has no effect on Follicle-Stimulating Hormone or prolactin [6]. Different results were observed during anaesthesia with pentobarbital, which inhibited LH pulse frequency in luteal phase in intact and in ovariectomized ewes during the breeding season whereas it produced a dramatic, 3-fold increase in LH pulse frequency in intact animals during anoestrous [7]. The corticotrophic pituitary-adrenal axis is also stimulated by halothane and to a lesser extent by isoflurane in rabbits [8]. The choice of the anaesthetics is therefore important to study the pattern of hormonal secretion simultaneously with the analysis of brain activation by MRI under general anaesthesia.

Fig. 1. Atlas – Template and Associated Labels. Segmented atlas showing (1) ventricles, (2) motor cortices, (3) hippocampi, (4) thalami, (5) caudate (6) amygdala, and (7) cerebellum illustrating the transverse (**a**), parasagittal (**b**), dorsal planes (**d**), and 3d rendering of labels on a ventrolateral view with rostral to the left of the image (**c**). Red, ventricles; green right motor cortex; deep blue, left motor cortex; yellow, hippocampus; light blue, thalamus; purple, caudate nucleus; light pink, amygdala; grey, cerebellum. From Liyanage et al. [20].

Influence of Anaesthesia during MRI

Anaesthesia is also known to modify the cerebral hemodynamic, the glucose metabolic rate, the neuronal activity, and the neurovascular coupling, and it has an impact on BOLD fMRI usually used to detect and map brain activation [9]. Among the different anaesthetics used in sheep, inhaled ones like halothane, fluothane, and isoflurane are the most commonly used for long neurosurgery [10] because the doses are easily adapted from 1 to 3% under assisted ventilation. However, isoflurane induced-deep anaesthesia in rat decreases BOLD response but it has no effect if the animals were ventilated [9]. In spontaneously breathing rabbits, isoflurane and fentanyl alone decreased BOLD response magnitude, and the decrease is more important when both anaesthetics are used [11]. These data indicate that not only the type of anaesthesia is important but also the way they are administered, is important too. In large animals like sheep, the impact of inhaled anaesthesia was recently studied, and it was shown that low doses of isoflurane delivered through a respirator give reliable results on a model of BOLD fMRI under visual stimuli in lambs and ewes [12]. The authors observed robust BOLD response in the lateral geniculate nuclei after visual stimuli, and this effect is weaker in adults than in lambs. Telazol another anaesthetic, non-

narcotic, and nonbarbiturate injectable compound was also successfully used for BOLD fMRI in sheep submitted to external tactile and visual stimuli, to map the sensorimotor and visual areas of the sheep brain [13]. In the same study, DTI tractography was successfully used to visualize the corticospinal tracts and optic radiations in sheep, and the data were qualitatively comparable to those obtained from healthy human adults [13]. Isoflurane appears to be used in most of fMRI studies performed in sheep in various protocols like to measure perfusion deficit in a stroke model [14], to study progressive degenerative in Batten disease [15] or to investigate foetal cerebral lesions induced by long-term exposure to cocaine during sheep pregnancy [16].

Atlases Development

In parallel to the development of suitable protocols for anaesthesia, specific sheep brain templates and atlases have been built for analysing the MRI images of sheep brain and properly identified brain structure [17–22]. Identification and segmentation of brain structures were manually or automatically performed using tools previously prepared for human analysis pictures and adapted to sheep as illustrated on Figure 1. When compared to classical brain atlases built with histological tools, the an-

atomical resolution is lower, in a millimetre range. The voxel size is about $0.5 \times 0.5 \times 0.5$ mm with the scanner used (3T) and such anatomical resolution make difficult the identification of small hypothalamic nuclei like the arcuate nucleus. Higher resolution will be possible with 7T or 11T scanner when they will be available for large animals.

Sheep Model in Neuroendocrinology

In sheep, the pituitary is large, and the stalk is long like in human which makes that the pituitary is not closed to the hypothalamus like in rodents but clearly separated like in human. The anterior part of the gland and the pituitary stalk is surrounded by a particularly dense network of small blood vessels. These morphological characteristics make blood sampling available and several millilitres of blood samples can be collected for several hours, allowing the study of neurohormone kinetic release. This approach was developed by Caraty et al. [23] to demonstrate for the first time that Gonadotrophin Releasing Hormone (GnRH) was released in a pulsatile manner in the hypophyseal portal blood. Using this technique in parallel with peripheral blood sampling in rams, the authors showed that GnRH release in the hypophyseal portal blood mirrors the LH release in the general circulation [24]. Other hypothalamic neurohormones are available with this approach such as Corticotrophin Releasing Hormone [25], vasopressin [25, 26], somatostatin [27], Growth Hormone Releasing Hormone [28], Thyrotrophin Releasing Hormone [29] and more recently discovered peptides like Kisspeptide [30, 31] and Gonadotrophin Inhibiting Hormone [32]. It is noteworthy that all these experiments were performed on conscious and unrestrained animals. For GnRH, Corticotrophin Releasing Hormone, and vasopressin, the portal neuropeptide release mirrors the peripheral release of the LH and adrenocorticotrophic hormone, respectively [24, 25]. For TRH, somatostatin, Growth Hormone Releasing Hormone, and Gonadotrophin Inhibiting Hormone, the studies show a more complex regulation that initially hypothesized.

According to the large size of the sheep brain compared to rodents or small nonhuman primates it is easy to identify neuronal nuclei and to characterize the neuronal target of peripheral hormones. For example, the presence of androgen receptors has been carefully described in the brain of sheep [33] in different neuronal population of hypothalamus and adjacent areas, more particularly in

the somatostatin containing neurones of ventromedial hypothalamic nucleus [34]. Estradiol action sites were identified using 17 beta-estradiol microimplants positioned in the medial preoptic area or the MBH of ovariectomized ewes. Only the microimplants in the MBH were able to induce a normal LH surge [35]. Later on, the presence of estradiol receptor alpha and receptor beta was described using immunohistochemistry in the hypothalamus of ewes [36–38], and in the brainstem in noradrenergic neurones [39, 40]. Using immunohistochemistry, the presence of glucocorticoids receptors was demonstrated in the hippocampus [41] and in the hypothalamus of ewes [42] but not in GnRH neurones [43].

Because sheep are seasonal breeders, melatonin is another key regulator of the gonadotrop axis. The central nuclei sensitive to this hormone were identified in the premammillary area of the hypothalamus using autoradiography with ^{125}I -labelled melatonin and the stimulatory effect on LH secretion induced by microimplant of melatonin placed in the same area [44, 45]. All these areas have a millimetre size allowing easier experimental and analytical approach than in rodents.

In the field of neuroendocrine control of reproduction, a pioneering MRI analysis were made in castrated ram treated with zeranol, a non-steroidal oestrogen analogue previously used in cattle to stimulate growth through the secretion of GH [46]. Using a 0.5T scanner, the authors observed that the pituitary gland increased 3 time greater in zeranol treated animals than in controls. More recently, additional studies were developed to investigate the role of sexual steroids on the brain using more powerful scanners.

Brain Imaging and Sex Steroids Effects on the Sheep Brain

The effect of gonadal steroids on the morphological and functional brain sexual dimorphism has been studied in adult sheep using in vivo MRI [47]. Scanning was performed during the breeding season first on intact animals and then after gonadal removal on the same animals. Using Voxel-based Morphometry data processing, the authors identified 37 sexual dimorphic structures in intact animals, which are also dimorphic in human, such amygdala, hippocampus, hypothalamus, and pituitary [48, 49]. Gray matter volume is higher in the hypothalamus of male as observed in men [50], but the pituitary is smaller in female as observed in women [51]. After removal of the gonads, 22 structures were still dimorphic, but the gray

Fig. 2. Effect of gonadectomy on sexually dimorphic structures. The mean values of GMC were evaluated bilaterally in hippocampus (a), hypothalamus (b), pituitary gland (c) and ectolateralis gyrus for both male and female sheep before and after gonadectomy. Data were compared using a two-way ANOVA followed by Holm-

Sidak multiple comparisons test and expressed as mean $\pm$ SEM; * $p < 0.05$; ** $p < 0.01$, and *** $p < 0.001$. $N = 20$ animals (8 males and 12 females) from Barrière et al. [47]. GMC, grey matter concentration.

matter concentration of several structure decreased and were no more different between ewes and ram like the hypothalamus and the amygdala (Fig. 2). This shows clearly the impact of sexual steroids on these structures as observed in human [52, 53]. According to previous neuroanatomical studies at the neuronal level, the effect of these steroids on the hypothalamus was expected because most of these nuclei are involved in the control of reproduction and contains steroids receptors in sheep [33, 36–38] like in human [54, 55].

Although gonadectomy does not influence the sexual dimorphic networks including the hypothalamus, steroids removal induced an increase in functional connectivity of this network in both sexes [47]. In human, estradiol, and progesterone are also able to modify connectivity in sexual dimorphic structures including the hippocampal-frontostriatal network as demonstrated for cognitive tasks in women during the menstrual cycle [56].

According to the sex differences on physiological and cognitive parameters observed in sheep [57, 58], the role of reproductive axis on brain structures related to emotion were investigated in sheep brain using MRI. The experiments were performed in young male and female sheep treated with a GnRH agonist to stop the production of gonadal hormones and to block pubertal development [59]. Previous studies in sheep shown that this compound has also a sex specific effect on behaviour and emotion regulation [60]. Fixed brain were analysed ex vivo with a 3T scanner, focusing on the volume of amygdala, hippocampus, and total brain. The authors showed that blockade of gonadal axis and puberty induced an increase of the size of amygdala in treated animals, the effect being stronger in female compared to male, no effect was observed in the hippocampus. The sexual dimorphism observed in the amygdala in sheep by Nuruddin et al. [59] was also observed by Barrière et al. [47] and in human [49]. Sexual dimorphism of the hippocampus was observed by Barri-

ère et al. [61] in sheep but remains controversial in human, it would be larger in girls compared to boys only during adolescence [62]. The authors observed in sheep a global gray matter volume larger in male than in female as observed in human by Peper et al. [63]. These results underlined that the lamb treated with GnRH agonist is a suitable model to study more deeply the role of this analogue used to treat precocious puberty in children [64].

Conclusion

This short review underlines that MRI analysis with 3T clinical MRI scanner is now possible in sheep, a good model for neuroendocrine control of reproduction and cognition and that it gives new and original data in the field of neuroendocrinology as already shown in the field of neuropathology [65–70]. In the field of neuroendocrinology, MRI will strengthen the use of ewes as a model of neuroendocrine aspect of PCOS, the most important disorder of woman reproduction for which ewes would be a suitable animal model. Androgenisation of ewes during gestation gives birth to females that present PCOS traits [71–73]. Using MRI, it will be now possible to study the central aspect of the syndrome as previously described in mice [74] and for which woman studies remains limited for ethical reasons [75]. According to its physiological and developmental features, sheep are also suitable model for the study of endocrine disruptor, and their impact

on foetal development and on brain development [76]. MRI is now available to study in sheep these emergent disorders and emphasize the importance of this model.

The limits of these studies are the availability of complete anatomical atlas of the sheep brain and appropriate templates for the identification of brain areas, especially for cortical and subcortical structures. Some atlases were already prepared by various laboratories [17–22] and are suitable for the identification of numerous brain areas even though they are less accurately described than in rodents and human. Additional anatomical studies of the sheep brain will be necessary. MRI analysis will give new opportunities for using sheep as a model for studying neuroendocrine interactions in the field of human pathology of reproduction in addition to academic research.

Conflict of Interest Statement

The author has no conflicts of interest to declare.

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Author Contributions

Yves Tillet analysed the literature and wrote the manuscript.

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