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Research report

Peri-pubertal gonadotropin-releasing hormone analog treatment affects hippocampus gene expression without changing spatial orientation in young sheep

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HIGHLIGHTS

- GnRHa treatment had sex and side specific effect on hippocampal gene expression.
- The affected genes are associated with endocrine function and plasticity.
- The mRNA expression changes did not reflect performance in spatial orientation.
- Females showed tendency to outperform males in spatial orientation.

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ABSTRACT

Background: Normal brain maturation is the result of molecular changes that can be modulated by endocrine variables associated with brain plasticity and results in sex- and age specific changes in cognitive performance. Using a sheep model, we have previously shown that peri-pubertal pharmacological blockade of gonadotropin releasing hormone (GnRH) receptors results in increased sex-differences in cognitive executive function and emotional control. In this study we explore effects of this treatment regime on hippocampal gene expression and spatial orientation.
Methods: The study was conducted with 30 same-sex twin lambs, half of which were treated with the GnRH analog (GnRHa) goserelin acetate every 4th week, beginning before puberty, until 50 weeks of age. Animals were tested in their spatial orientation ability at 48 weeks of age. Quantitative real time PCR analysis was conducted to examine effects of treatment on the expression of genes associated with synaptic plasticity and endocrine signaling.
Results: GnRHa treatment was associated with significant sex- and hemisphere specific changes in mRNA expression for some of the genes studied. The treatment had no significant effect on spatial orientation. However, there was a tendency that females performed better than males in spatial orientation.
Conclusion: Our results indicate that GnRH directly and/or indirectly, is involved in the regulation of sex- and side-specific expression patterns of genes. Hence, these results should be considered when long-term peri-pubertal GnRHa treatment is used in children.

1. Introduction

Brain development is characterized by sex-specific changes in cognitive performance, behavior and social ability [1,2]. Given the chronology and dynamic nature of such changes, for example the silencing of cognitive differences in late childhood, their

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Table 1
Target genes and real-time amplification products.

Gene symbol	Gene name	Forward primer	Reverse primer	Accession number
GnRHI	Gonadotropin-releasing hormone1	GCTGCGCCCTGGAGGAAAGAG	TCCAGAGCTGCCTTCAGGTCCC	U02517
GNRHII	Gonadotropin-releasing hormone2	GCAGCCCTGCTATGGGCCAC	GCCGTGGGACCGGTGTTGAG	XM_001255677
CYP19	Aromatase	CTGGCCTGGTGCGCATGGT	TGCGCCGCATGAGGGTCAAC	NM_001123000
AR	Androgen receptor	AAGGCCTTGCCTGGCTTCCG	TCCGGGACTTGTGCATGCGG	AF105713
GH	Growth hormone	CCGAAACCATCCCAGCCCCC	AGACACGGTCCGAGGTGCCA	NM_001009315
ESR1	Estrogen receptor alpha	TGCACGCCCCAGCCAACTTC	CGGGAGCCGGGGAACTCTCA	AY033393
ESR2	Estrogen receptor beta	TCAGGCCTGTCGACTGCGGA	GCTCAGGGCACTCAGCAGCA	NM_001009737
BDNF	Brain derived neurotrophic factor	GGGAGCTGAGCGTGTGCGAC	GCTGGCCTTTCGAGACGGGG	NM_001046607
VGF	VGF nerve growth factor inducible	AATCTCTGCTGACCGAGACC	CTCCTGAAGCAGGGAAGCTA	XM_870373
GRin1	Glutamate (NMDA) receptor, ionotropic	GACCACAAGCGCGGACCCAA	ACCAGCCACACGTACCCCGA	AY434689
NCAM1	Neural cell adheshion molecule 1	ACGTGAAACCCACAGCCAAACT	TGCCGGTTCGGCAACACCAA	NM_174399
Gria1	Glumate receptor AMPA1	TGCGGACCACAGAGGAGGGG	TCTCAGGGCGGACCCCTTGG	AY346122
LHX5	LIM homeobox 5	GCAGCAAAGTCTTCCACCTC	CAGGTAGTCGTCCTTGCACA	NM_001102061
Syn1	Synapsin1	GCAAAATTTCCCCAATCCTT	AAAACTTTGAGGGGCTTGGT	NM_174191
SNTA1	Syntrophin, alpha 1	GGTCACCCGTCTGGGGCTCT	GCAATCAGCGGAGGGCCAGG	NM_001075898
spn	spinophilin	CAAGGAGCTCCAGATCAAGC	ACCCCAGTAGCCTTCCAGTT	NM_001103102
GABRA4	Gamma-aminobutyric acid (GABA) A receptor, alpha 4	TTCCTGTGCCTGGCGGCTTG	CCCCCAAATCCAGGACGCAGC	NM_174543

reintroduction with puberty and the decline in cognitive performances during aging [3], it has been proposed that age-related endocrine changes may affect plasticity genes and modulate brain structure and function. Sex differences in hormone sensitivity of such plasticity genes might, therefore, underlie measured sex-specific cognitive differences [1,2].

Sex-specific differences in brain organization and function are seen in numerous species [4]. In humans, the temporal sequence of brain maturation and the formation of functional circuits are sexually differentiated; the subcortical (e.g. striatum) and prefrontal cortical regions develop at different times in boys and girls [5]. These temporal and organizational differences in brain development are thought to result in sex-specific behaviors [5] and it has been proposed that they may also underlie sex-associated differences in the risk of developing some neuropsychiatric diseases [6]. The observation that the time of onset of neuropsychiatric disorders such as schizophrenia, autism and Alzheimer's disease (AD) correlates with major endocrine changes during puberty and menopause [7–9] would also support this hypothesis.

While the primary target of the neuropeptide gonadotropin releasing hormone (GnRH) has historically been considered to be in the pituitary gland, to facilitate the central regulation of reproductive function, recent studies have identified non-reproductive effects of GnRH, including a role in cognitive function [10]. In this regard, GnRH receptors are expressed in regions of the brain not associated with the regulation of reproduction [11] and recent reports have also indicated that therapy with GnRH analogs (GnRHa) may reduce adult neuropsychiatric disease symptoms [12]. Consequently, their use in the treatment of Alzheimer's disease (AD) has been discussed [10]. While it was originally proposed that the cognitive effects of GnRH were indirect and mediated by luteinizing hormone (LH) or gonadal steroids [9], recent findings support a direct GnRH receptor (GnRHR) mediated effect [10].

This study is part of a major project, characterizing the effects of peri-pubertal GnRHa treatment in sheep. Using this novel sheep model, we have demonstrated significant effects of GnRHa treatment on sex-specific cognitive functions and emotional control in young sheep [13,14]. Specifically, GnRHR blockage led to pronounced avoidance behavior in females and exaggerated risk-taking approach behavior in males.

The aim of the present study was two-fold; first to investigate whether pharmacological blockade of GnRH action affected expression of hippocampal genes associated with endocrine signaling and synaptic plasticity and second, to explore the effects of peripubertal GnRHa treatment on a hippocampus dependent cognitive task, namely spatial orientation. We investigated effects of GnRHa treatment on a selection of genes (Table 1) known to be involved in the synaptic transmission (*Grin1*, *Gria1*, *GABRA4*, *syn1*, *spn*, *BDNF*, *VGF*), proliferation and differentiation (*LHX5*) and structuring (*NCAM1*) that underlie synaptic plasticity and genes associated with endocrine signaling (*GnRH I*, *GnRH II*, *CYP19*, *AR*, *GH*, *ESR1* and *ESR2*).[1]

2. Materials and methods

2.1. Animals

All animal procedures were conducted at the University of Glasgow's Cochno Research Centre (55°55′N) following review by the University's Welfare and Ethics Committee and in accordance with Home Office regulations (PPL 60/3826). To eliminate the possible developmental effects of steroid transfer between siblings of different sexes, the study was conducted using 46 pairs of same-sex twin lambs (Scottish Mule Texel Cross, 22 female and 24 male). The study presented here is based on a subsample of 30 animals (14 female and 16 male) that had been tested in their spatial orientation ability and that had their hippocampal gene expression analyzed. Lambs were born between 17th March and 1st April 2008 and remained with their dams until weaned at about 12 weeks of age. Males and females were maintained separately during the entire study period. Within each set of twins, one was randomly assigned, at birth, to the control (C) and the other to the treatment (T) group. Animals in the treatment group received subcutaneous implants of the GnRH analog goserelin acetate (Zoladex, kindly donated by Astra Zeneca; Macclesfield, UK 3.6 mg) every 4 weeks from 8 weeks of age in males and 28 weeks of age in females. The differences in the timing of treatment initiation between males and females, was instigated as puberty in sheep, as in humans, is sexually dimorphic. Opposite to humans, in sheep, male pubertal transition begins earlier than female. This treatment paradigm was designed so that the pharmacological inhibition of GnRH action should begin approximately 2 weeks before the predicted time of onset of pubertal development in both rams and ewes [15]. Blood serum analyses were performed regularly during the animals' life. The analyses confirmed that treatment prevented puberty by complete suppression of the hypothalamus–pituitary–gonadal axis (data not shown).

2.2. Behavioral test: spatial orientation and learning

Animals were tested at about 48 weeks of age, approximately 2 weeks before they were euthanized and tissues, including the brains collected for laboratory analysis. The spatial maze used in this study (Fig. 1) was an adaptation of that used by Lee and colleagues [16]. The dividing walls of the maze were made of metal penning that was familiar to the animals and through which the test animals could see the audience pen. The outer walls of the maze arena were solid. For testing, animals were separated into smaller groups and sequentially placed in the 'audience' pen. Individual 'test' animals were removed from the audience pen by a trained and familiar handler and calmly led to the entrance of the maze. Each animal was given 300 s to traverse the maze and the test was deemed completed when the animal passed the finish line (Fig. 1). One of the T males effectively completed the task but stopped for a while before crossing the finish line increasing his total time to 274 s. After all animals had been tested once, the process was repeated a 2nd time on the same day and for a 3rd time the following day.

[1] All genes symbols and full names are explained in Table 1.