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Effects of peripubertal gonadotropin-releasing hormone agonist on brain development in sheep— A magnetic resonance imaging study

The Sex On Brain European Research Group — SOBER
Syed Nuruddin [a], Muriel Bruchhage [b], Erik Ropstad [a], Anette Krogenæs [a], Neil P. Evans [c], Jane E. Robinson [c], Tor Endestad [d], Lars T. Westlye [d,e], Cindee Madison [f], Ira Ronit Hebold Haraldsen [b,*]

[a] Norwegian School of Veterinary Science, PB 8146 Dep, 0033 Oslo, Norway
[b] Department of Neuropsychiatry and Psychosomatic Medicine, Division of Surgery and Clinical Neuroscience, Oslo University Hospital — Rikshospitalet, Sognsvannsveien 20, 0027 Oslo, Norway
[c] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Bearsden Road, Glasgow G61 1QH, Scotland, UK
[d] Center for the Study of Human Cognition, Department of Psychology, University of Oslo, PB 1094 Blindern, 0317 Oslo, Norway
[e] KG Jebsen Centre for Psychosis Research, Division of Mental Health and Addiction, Oslo University Hospital Ullevål, Kirkeveien 166, PO Box 4956 Nydalen, 0424 Oslo, Norway
[f] Department of Psychology, University of California, Berkeley, CA, USA

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Summary In many species sexual dimorphisms in brain structures and functions have been documented. In ovine model, we have previously demonstrated that peri-pubertal pharmacological blockade of gonadotropin releasing hormone (GnRH) action increased sex-differences of executive emotional behavior. The structural substrate of this behavioral alteration however is unknown. In this magnetic resonance image (MRI) study on the same animals, we investigated the effects of GnRH agonist (GnRHa) treatment on the volume of total brain, hippocampus and amygdala.

In total 41 brains (17 treated; 10 females and 7 males, and 24 controls; 11 females and 13 males) were included in the MRI study. Image acquisition was performed with 3-T MRI scanner. Segmentation of the amygdala and the hippocampus was done by manual tracing and total gray and white matter volumes were estimated by means of automated brain volume segmentation of the individual T2-weighted MRI volumes. Statistical comparisons were performed with general linear models.

* Corresponding author. Tel.: +47 92011533; fax: +47 23074920.

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Highly significant GnRHa treatment effects were found on the volume of left and right amygdala, indicating larger amygdalae in treated animals. Significant sex differences were found for total gray matter and right amygdala, indicating larger volumes in male compared to female animals. Additionally, we observed a significant interaction between sex and treatment on left amygdala volume, indicating stronger effects of treatment in female compared to male animals. The effects of GnRHa treatment on amygdala volumes indicate that increasing GnRH concentration during puberty may have an important impact on normal brain development in mammals. These novel findings substantiate the need for further studies investigating potential neurobiological side effects of GnRHa treatment on the brains of young animals and humans.

1. Introduction

Pronounced neurobiological differences exist between males and females beyond the traditional domain of reproduction, including brain anatomy and function (Keller and Menon, 2009; Tomasi and Volkow, 2011). In humans, sex differences have been documented in the limbic system (Filipek et al., 1994; Caviness et al., 1996; Goldstein et al., 2001), and sex differences in the brain correlate with emotional and facial processing, working memory and spatial learning ability (George et al., 1996; Speck et al., 2000; Killgore et al., 2001; Andreano and Cahill, 2009).

To gain an understanding of the hormonal mechanisms that might underlie sex differences in cognitive functions, studies have investigated the modulatory effects of gonadotropin-releasing hormone (GnRH) and its agonist (GnRHa) (Grigorova et al., 2006; Bryan et al., 2010; Wojniusz et al., 2011) on physiological and cognitive parameters (Skinner et al., 2009; Wojniusz et al., 2011; Evans et al., 2012). GnRH is a decapeptide neurohormone that plays a key role in the reproductive axis, ultimately modulating the release of gonadal steroid hormones. Its receptor (GnRHR) has been found in various brain regions, including the frontal cortex, the olfactory bulb and the limbic system (Albertson et al., 2008; Skinner et al., 2009; Chu et al., 2010; Rosati et al., 2011; Schang et al., 2011). Importantly, not all of these brain regions have roles in the control of reproductive function, suggesting that GnRH is involved in other processes and, as such, may modulate sex differences in emotional processing and cognitive functions.

In this regard, GnRHa treatment has been shown to influence visuospatial and higher-order executive control functions (Casadesus et al., 2006; Bryan et al., 2010). To date, effects of GnRHa on cognition have only been reported in adults and studies that delineate the possible effects of peri-pubertal GnRHa treatment on brain development in children and adolescents are lacking. During critical stages of development, structural and functional neuronal circuits that follow sex-specific spatiotemporal patterns are built (Casey and Jones, 2010) and it has been proposed that disturbances of this maturational process might cause abnormal behavioral and cognitive function in later life (Sisk and Foster, 2004; Casey and Jones, 2010). Despite the widespread use of GnRHa for treatment purposes, ranging from central precocious puberty, gonadal protection for children undergoing chemotherapy, congenital adrenal hyperplasia to autism (Carel et al., 2009), the mechanisms by which GnRHa treatment may interfere with the maturation of fronto-temporal-limbic circuits and corresponding cognitive functions are not understood.

In order to investigate possible sex specific effects of peripubertal GnRHa treatment on brain development, we have developed a novel ovine model, chosen because of its relatively long period of brain maturation compared to other model species, e.g. rodents. To assess possible effects of GnRHa treatment on brain morphology, we combined this model with postmortem magnetic resonance imaging (MRI), to accurately measure global and regional brain volumes. MRI has the advantage that it bypasses conventional dissection and photography methods, which carry higher risks of introducing errors in quantitative measurements (Pfefferbaum et al., 2004). In previous studies, we have shown different effects of GnRHa treatment in this animal model, specifically, sex specific effect on behavior and emotion regulation (Wojniusz et al., 2011) and gene expression in hippocampus without changing spatial orientation ability (Nuruddin et al., 2013). Although emotion regulation may be more closely linked to the amygdala and spatial navigation to the hippocampus; we anticipated that GnRHa treatment might have localized effects in both structures.

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 developmental effects of steroid transfer between siblings of different sexes, the study was conducted using forty six pairs of same-sex twin lambs (Scottish Mule Texel Cross, 22 female and 24 male). 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. Although sheep and humans have different timing of onset of puberty and periodicity of fertility, a common interaction between neurosecretory cells of the brain and the peripheral sexual organs is well documented in vertebrates (humans, primates and other mammalsl including sheep) (Wang et al., 2010). Intracerebral blockage of GnRH isoforms in both sheep and human will therefore suppress the hypothalamic-pituitary-gonadal axis, stop production of gonadal hormones in sheep as effectively as in humans, arresting sexual maturity in both species. In our study animals in the treatment group received subcutaneous implants of

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the GnRH agonist 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 difference in the timing of treatment initiation between males and females is necessary as onset of puberty in sheep, as in humans, is sexually dimorphic (Wood and Foster, 1998). Opposite to humans, ovine pubertal transition begins at about 10 weeks in males, and in females 20 weeks later (Claypool and Foster, 1990; Foster and Ryan, 1979). Our treatment protocol ensures that pharmacological inhibition of GnRH action begins approximately 2 weeks before the predicted time of onset of pubertal development in both rams and ewes. To confirm that GnRHa treatment blocked pubertal development, blood serum analyses were performed regularly during the animals' life. In addition, after death at 12 months of age, testes and ovaries were excised and weighed and histologically evaluated. The analyses confirmed that treatment prevented puberty by complete

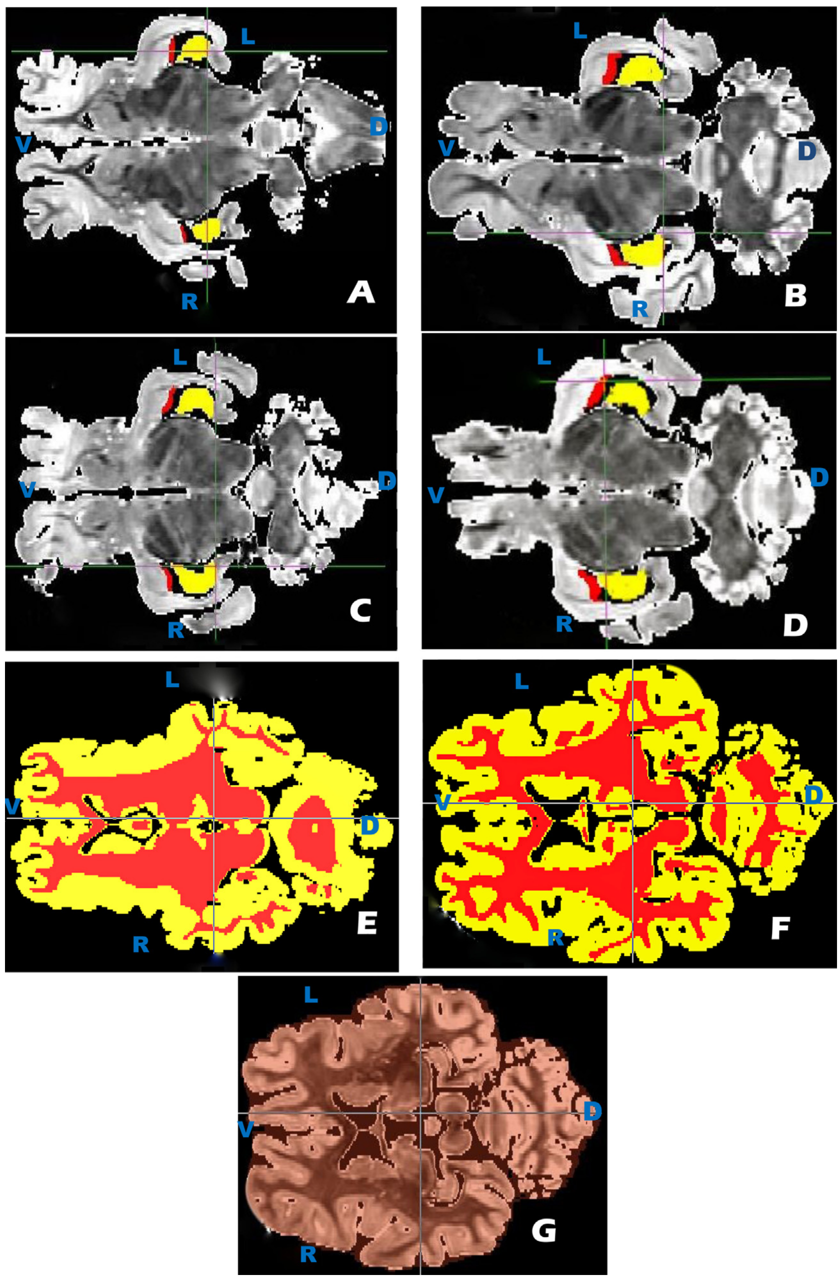

Figure 1 Magnetic resonance images including automatic and manual segmentations in axial view. Orientation of the picture are the following: V = ventral, D = dorsal, L = left, R = right. (A)—(D) represents magnetic resonance images of manual and automatic segmentation: (A) female untreated, (B) female treated, (C) male untreated, (D) male treated. Bilateral red colored areas indicate the amygdala and the yellow colored areas indicate the hippocampus. (E) and (F) represent automatic tissue segmentation images: (E) males, (F) females. The red colored area represents white matter, the yellow colored area represents gray matter; (G) represents an image of the manually drawn mask of the total brain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

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suppression of the hypothalamic-pituitary-gonadal axis (for more details see: Wojniusz et al., 2011).

2.2. Specimen collection

At approximately 50 weeks of age, animals were sacrificed with an overdose of barbiturate (Somulose 1 ml/kg body weight; Decra Veterinary Products, Shrewsbury UK), decapitated, and brains perfused, with 4% formaldehyde, through the carotid artery (bilaterally) using a peristaltic pump. In total 41 brains (17 treated; 10 females and 7 males, and 24 controls; 11 females and 13 males) were fixed in this way. These brains were stored in a 4% neutral buffered formalin solution and were the brains used for the MRI study.

2.3. Postmortem specimen preparation

To minimize movement during scanning, each postmortem specimen was suspended in agar, which was placed in a rectangular plastic container. The agar was semi-translucent, thus permitting gross land marking before scanning. Landmarks for scanning were drawn on each container and helped obtain the same alignment across scanning sessions.

2.4. MRI acquisition

Imaging was performed at the Interventional Centre (Oslo University Hospital, Oslo, Norway) using a 3T Philips Achieva Scanner (Release 2.5.3, Philips Healthcare, Netherlands) and a dedicated solenoid rat coil (signal to noise: 1064). Structural T1- and T2-weighted MRI data were obtained by acquiring a series of sagittal IR-spoiled GRE sequences with the following parameters: for the T1-weighted image; field of view (FOV): 90 mm $\times$ 90 mm $\times$ 72 mm, 144 slices of 0.5 mm with matrix size 180 $\times$ 180, bandwidth 95.8 Hz, inversion time = 450 ms, repetition time (TR)/echo time (TE) = 11/3.1 ms with segmented linear k-space filling (20 lines) and 20 repeated acquisitions to improve SNR with a total scan duration of 18 min. Voxel size was fixed to 0.5 mm $\times$ 0.5 mm $\times$ 0.5 mm. For the T2-weighted image; FOV: 90 mm $\times$ 90 mm $\times$ 72 mm, 144 slices of 0.5 mm with matrix size 180 $\times$ 179, bandwidth 137.0 Hz, inversion time = 450 ms, TR/TE = 2000/200 ms, voxel size was fixed to 0.5 mm $\times$ 0.5 mm $\times$ 0.5 mm and total scan duration was 34 min. Volumes covered the whole brain, cerebellum and brain stem. T1- and T2-weighted volumes were resampled to 0.23 mm $\times$ 0.23 mm $\times$ 0.5 mm on the scanner.

2.5. MRI preprocessing

The raw DICOM files were converted to NIFTI and inspected for artifacts. Bias correction of the images was done using Freesurfer mri_nu_correct.mni to correct for intensity non-uniformity. Spurious noise outside the brain was removed, and Brain Extraction Tool (BET) (Smith, 2002), part of FMRIB's Software Library (FSL) (Smith et al., 2004) was used to strip residual gel artifacts outside the brain. A set of anatomical regions of interest (ROIs) were manually drawn on the best quality MRI brain of the sheep chosen to act as the template. These ROIs were then aligned to each individual's native space by first using FLIRT (Jenkinson and Smith, 2001) to calculate the affine transform of each individual brain to the template brain using 12 dof, and minimizing correlation ratio. Next, non-linear warp fields generated by FNIRT (Andersson et al., 2007a, 2007b) using a 6 mm warp resolution were calculated and inverted. The inverse warp was applied to the template-drawn ROI, followed by application of the inverse affine transform, bringing the template-drawn region of interest into the individual subject space. The ROI in subject space was then hand edited. Inter-rater reliability was calculated by comparing the percent overlap between two raters. Finally, the number of voxels of each ROI was extracted and multiplied with voxel size for use in statistical models.

2.6. Region of interest definition/selection and inter-rater reliability

Segmentation of the amygdala and the hippocampus was done by manual tracing using FSLView, part of FMRIB's Software Library (FSL) (Smith et al., 2004) by a single rater (M.B.) blinded to any subject characteristics. The hippocampal ROI included the cornu ammonis, dentate gyrus and the subiculum, as each of these components show different histological characteristics and topographically well-ordered afferents and efferents (Nolte, 1993). A combination of T1- and T2-weighted data was used to distinguish the anterior boundaries that separate the amygdala from the hippocampus. However, precise delineation of the boundaries is difficult even at a histological level (Bergin et al., 1994). Therefore, inter-rater reliabilities for each ROI were established by having two raters (M.B. and S.N.) independently segment five datasets. Percent inter-rater voxel overlap was 94% for hippocampus and 97% for amygdala, indicating high reliability.

2.7. Tissue segmentation

TBV was calculated using manually segmented brain masks covering both the gray and white matter, ventricular compartments and the cerebellum. FMRIB's Automated Segmentation Tool (FAST) (Zhang et al., 2001) was used to automatically segment the T2-weighted MRI volumes of the individual brains into gray matter (GM) and white matter (WM), while correcting for spatial intensity variations (bias field or RF in homogeneities). The underlying method of FAST is based on a hidden Markov random field model and an associated Expectation-Maximization algorithm. All segmented data were visually inspected for accuracy. Total GM and WM volumes were calculated by multiplying the number of voxels in each of the segmented classes by its voxel resolution. Four datasets (3 untreated males and 1 treated male) were excluded during the automatic tissue segmentation procedure due to imaging artifacts probably related to the formalin fixation of the brain (Tovi and Ericsson, 1992).

2.8. Statistical analysis

The mean and its standard deviation (SD) were calculated for the volume of the different ROIs. TBV, WM and GM volume, as well as the volume of the left and right amygdala and hippocampus, were compared between groups (control vs treated), while controlling for sex and treatment group using General Linear Models (GLMs). The sex by treatment interaction term was included in the models, allowing us to test for differential effects of treatment between male and female animals. The null hypothesis of no effects was rejected if $p < 0.05$, Bonferroni adjusted by a factor of 7 (corresponding to a nominal alpha of $p < 0.007$). The assumption of normally distributed residuals was evaluated using a normal quintile (Q—Q) plot and this assumption of normally distributed residuals was met for all parameters. Statistical analyses were performed using SPSS version 19.0 (IBM Corp., Armonk, NY, USA).

3. Results

MR images including example automatic and manual segmentations in difference data sets are shown in Fig. 1. The raw mean volumes of each ROI by group and sex and in total are summarized in Table 1. Significant effects of GnRHa treatment (Table 1) were observed on the volume of the left ($t = -13.76$, $p = 0.0001$) and right ($t = -16.56$, $p = 0.0001$) amygdala, indicating larger amygdalae in treated compared to untreated animals. Further, a significant ($t = -3.56$, $p = 0.001$) sex by treatment interaction effect on the volume of the left amygdala was observed, indicating that the effect of treatment was stronger in female (mean volume = 50 and SD = 1 in mm^3) compared to male (mean volume = 48 and SD = 1 in mm^3) animals. All treatment and treatment*sex effects on regional volumes remained practically unaltered when including TBV as an additional covariate (results shown in Supplementary Table 1). Table 1 also shows the effect sizes and the corresponding p-values for each of the estimated parameters from the GLMs. Briefly, significantly larger volumes in male compared to female animals were observed in total GM ($t = -3.05$, $p = 0.004$) and the right amygdala ($t = -5.12$, $p = 0.0001$). There was also a marginally significant sex difference in total WM volume ($t = -2.37$, $p = 0.024$), indicating larger volumes in males compared to females. Sex differences were still significant for total GM ($t = -2.91$, $p = 0.006$) and WM ($t = -3.12$, $p = 0.004$) when including TBV as an additional covariate.

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4. Discussion

In the present MRI study, we have reported sex differences in the volume of specific regions of the brain and effects of peripubertal GnRHa treatment on regional brain volumes in an ovine animal model. Specifically, total brain volume, total WM and GM, as well as hippocampal and amygdala volumes were compared between female and male animals that had been treated with GnRHa during the peripubertal period and their respective controls. The results demonstrated strong and specific GnRHa treatment effects on the volume of left and right amygdalae, indicating larger amygdalae in treated animals. Further, we found a significant sex by treatment interaction on the volume of the left amygdala, indicating stronger effects of treatment in female compared to male animals. Also, we have demonstrated sex differences in total GM and right amygdala volume, indicating larger volumes in male compared to female animals. As far as we know, this is the first study demonstrating effects on MRI derived regional brain volumes in GnRHa treated sheep. The interpretations and implications of these novel findings will be discussed below.

4.1. GnRHa treatment effects on amygdala volume

We observed a specific effect of pharmacological hypothalamus-pituitary-gonadal axis (HPG) blockage on amygdala volume, indicating larger volumes in GnRHa treated compared to untreated animals. This pattern of change indicates that GnRHa treatment did not affect the development of all areas within the limbic system but rather modulated the development of specific limbic structures. It should be noted that the experimental design does not allow differentiation between direct effects of GnRHa treatment or secondary effects such as the blockade of the reproductive axis with its consequent reduction in gonadal steroid secretion.

Previously published work has indicated that a number of factors could contribute to the observed morphological differences, for example, steroid hormones. Specifically, testosterone has been shown positively associated with increased regional volume of the medial amygdala (MeA) and posterodorsal subnucleus (MeApd) of the posterior MeA in males. In addition rising concentrations of androgen promote excitatory synaptic connectivity (Cooke and Woolley, 2009; Romeo and Sisk, 2001) in the amygdala.

It has previously been demonstrated, with this cohort of GnRHa treated sheep that GnRHa treatment had a sex specific effect on behavior and emotion regulation. Specifically, female animals in which the pubertal transition was blocked by GnRHa treatment displayed pronounced avoidance behavior, less emotional control capability or more anxiety behavior, whereas male animals exhibit risk-taking and exaggerated approach behavior (Wojniusz et al., 2011). The results of this study indicate that GnRHa treatment was also associated with increased amygdala volume in these animals. In line with these findings, amygdala volume in humans has been shown to be associated with both anxiety and depression during childhood and adolescence (Thomas et al., 2001), and children diagnosed with anxiety disorder show a larger and more reactive amygdala as well as greater processing bias for negative information (De Bellis et al., 2000; MacMillan et al., 2003; Barrós-Loscertales et al., 2006). Furthermore, an MRI study including children and adolescents (males age 6—16 years and females 4—11 years) has reported that changes in volume and functional activity of the amygdala might be due to excess androgens, estrogens or progestins; endogenous deficiency or exogenous excess of glucocorticoids; or some combination of these (Merke et al., 2003). This study focused on the examination of the effect of sex steroids on the growth and development of the amygdala, however possible involvement of GnRH has not been described elsewhere. Thus, volumetric effects of GnRHa treatment on the amygdala suggest that GnRH might also play role in the growth, development and functional activity of the amygdala.

Table 1 Mean volume (mm³) within each anatomical region of interest (ROIs) per group, sex and results from multiple linear regressions.

ROI	Female: Untreated (n = 11) Mean	SD	Treated (n = 10) Mean	SD	All (n = 21) Mean	SD	Male: Untreated (n = 13) Mean	SD	Treated (n = 7) Mean	SD	All (n = 20) Mean	SD	Total: Untreated (n = 24) Mean	SD	Treated (n = 17) Mean	SD	All (n = 41) Mean	SD	Multiple Linear Regression: Sex t	p	Treatment t	p	Sex* treatment t	p	Full model F	R^2
Hippocampus left	785	89	763	72	774	80	780	147	814	160	792	148	782	121	784	115	783	117	−0.86	0.394	−0.60	0.553	0.72	0.477	0.25	0.02
Hippocampus right	793	110	789	94	791	100	824	147	792	183	813	156	810	130	790	132	801	129	−0.05	0.959	0.50	0.617	−0.32	0.755	0.18	0.01
Amygdala left	29	3	50	1	39	11	32	3	48	1	38	8	31	3	49	1	38	10	2.02	0.051	**−13.76**	0.0001	**−3.56**	0.001	197.40	0.94
Amygdala right	23	2	43	2	32	11	30	3	49	1	37	10	27	5	45	4	34	10	**−5.12**	0.0001	**−16.56**	0.0001	−0.86	0.398	223.55	0.95
White matter (WM)	29,349	1755	27,706	4969	28,566	3655	32,887	1327	31,800	1663	32,615	1443	31,195	2355	28,876	4628	30,317	3525	−2.37	0.024	0.65	0.523	0.26	0.794	6.53	0.37
Gray matter (GM)	45,193	2332	41,009	10,531	43,201	7563	50,844	4052	52,212	3520	51,186	3859	48,142	4360	44,210	10,355	46,654	7353	**−3.05**	0.004	−0.38	0.705	1.24	0.225	5.86	0.35
TBV	103,974	7235	103,876	6489	103,928	6717	110,884	5998	109,169	9525	110,198	7416	107,579	7365	106,229	8184	106,986	7665	−1.54	0.131	0.52	0.606	−0.35	0.725	2.66	0.18

Mean volume (mm³) within each regions of interest (ROIs) per group and sex, and in total. White matter (WM) and gray matter (GM) were missing for one untreated male and three treated male animals. TBV: total brain volume as estimated using the manually segmented brain masks. In multiple linear regression analysis, the parameter estimates denote the effect size and the statistical significance of the parameter estimates from linear regressions with number of voxels in each region of interest (ROI) as independent variable, sex and treatment group as fixed factors. The sex*treatment interaction term was included in the models. *F* and R^2 reflect the full model fit. *Bold*: significant associations at $p < 0.05$, Bonferroni corrected. *Underlined*: significant associations at $p < 0.05$, uncorrected. White matter (WM) and gray matter (GM) volumes were missing for five datasets. For sex, negative *t* values indicate larger volumes in males compared to females. For treatment, negative values denote larger volumes in treated compared to non-treated animals. For the sex by treatment interaction term, negative values indicate stronger effects of treatment in female compared to male animal.

Animal research also suggests an association between hypertrophy and increased reactivity of the amygdala (Vyas et al., 2006). The GnRHa treatment effect on the volume of the amygdala observed in the present study, therefore, might be related to cognitive changes reported in our previous behavioral data (Wojniusz et al., 2011) and by extension, anxiety and mood parameter changes should be monitored in clinical human populations following GnRHa treatment.

4.2. Sex differences in brain volumes

Sex specific volumetric differences in global and regional brain structures can be important indicators for the development of specific behaviors and diseases (Filipek et al., 1994; Caviness et al., 1996; Allen et al., 2002; Giedd, 2004; Sowell et al., 2007). This is of interest as sexual maturation is linked with changes in brain morphometry in normal developing children and adolescents (Giedd et al., 1997, 2006; Wilke et al., 2007; Neufang et al., 2009). For example, the hippocampi in young females have been shown to be larger compared to males (8—18 years of age) (Filipek et al., 1994; Giedd et al., 1996), and larger amygdalae have been shown in young male compared to female individuals (7—11 years of age) (Caviness et al., 1996; Giedd et al., 1997). Furthermore, extensive evidence has demonstrated that the hippocampi in males and females differ significantly in their anatomical structure, their neuroanatomical makeup and their reactivity to stress (Juraska, 1991; Madeira and Lieberman, 1995). In contrast to human studies, our results show no significant sex difference in hippocampal volume in sheep.

Human studies have also shown sex differences for GM and WM volume during pubertal development (Peper et al., 2011) where GM and WM volumes were larger in males than females. The observed significant sex differences for global GM and the trend differences for WM in the present study are, thus, in concordance with human studies. The sex difference in right amygdala volume, seen in this study, could add further weight to the proposals for sex dependent lateralization of the amygdala (Peper et al., 2011; Schneider et al., 2011). However, it is worth issuing a note of caution relative to this possibility, as while the male and female subjects were age-matched, the interpretation of sex differences is complex because male sheep reach puberty some 20 weeks earlier than female sheep and, thus, the male animals had received GnRHa treatment for a longer duration than females. The results, nevertheless, showed a significant treatment by sex interaction on the volume the left amygdala, which would indicate a stronger effect of treatment in females compared to males.

Sex related hemispheric lateralization of amygdala function in relation to emotion regulation has been reported in imaging studies where the left amygdala has been implicated as dominant in females while the right amygdala was more dominant in males (Cahill et al., 2004; Koscik et al., 2010). The present results indicate effects of GnRHa treatment in both male and female animals. Interestingly, this corresponds with the fact that behavior was also affected in both sexes of the treatment group (Wojniusz et al., 2011). Final interpretations regarding the observed sex by treatment interaction should be performed with caution and warrant replication in future studies.

In a recent study, we found that GnRHa treatment affects hippocampal synaptic plasticity and endocrine signaling gene expression without changing spatial orientation ability which is a hippocampus-related function (Nuruddin et al., 2013), thus we could expect that this treatment could affect hippocampal volumes in the same animals. Our data show no effects of treatment on hippocampal volume. The reason for this is unknown but it could be that treatment affects hippocampal processes at the molecular level without affecting microstructural processes in this region. An important limitation, using this technique/approach to assess brain morphology is that the exact neurobiological substrates of the volumetric effects of the GnRHa treatment are still unknown.

It could be speculated that increase in pubertal GnRH, androgen and estrogen release might affect the growth and development of the amygdala during puberty; or possibly that GnRH along with sex hormones alter the neural substrates of the circuitry involved in growth and development of amygdala and other brain structures. These speculations can be supported by the fact that GnRH and sex hormones receptors are abundant in limbic regions particularly in the amygdala and hippocampus (Simerly et al., 1990; Beyenburg et al., 2000; Skinner et al., 2009). Further studies will therefore be required in which histological, cellular, and genetic analysis such as gene expressions and proteomics are combined in order to identify the molecular biological mechanisms that underlie these GnRHa-induced morphological changes in the amygdala.

4.3. Conclusion

Our results demonstrate that GnRHa treatment modulates specific facets of the development of the amygdala, as indicated by increased amygdala volume in treated compared to untreated animals. These novel findings demonstrate that gonadotropin releasing hormone concentrations during puberty are important for normal brain development. Pharmacological manipulation of gonadotropin releasing hormone action in clinical settings using young patients should therefore be accompanied by carefully monitoring for neurobiological side effects.

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Conflict of interest

The authors have nothing to disclose.

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