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Sex Differences in the Effects of Gonadotropin Releasing Hormone Analogue Treatment on Adolescents' Limbic System

Essay

Gonadotropin Releasing Hormone Analogues (GnRHa) are used in conditions such as Gender Dysphoria and precocious puberty to suppress puberty in children or adolescents. This essay poses the question whether this blocking of sex hormones affects brain development of regions of the limbic system. It is hypothesized that the influence of GnRHAs on limbic system development shows differences between the sexes. While animal research has indeed indicated sex differences in the effect of puberty suppression on hippocampus and amygdala gene expression and amygdala volume, direct evidence in human subjects is lacking. It is suggested that well controlled studies in humans on the effects of GnRHAs on brain development could provide valuable insights into the origin of sex differences in the brain, as well as contribute to better psychological treatment of individuals who receive GnRHAs.

Keywords: GnRHa; gender; puberty; limbic system; animal model

INTRODUCTION

Puberty, the process in which a child's body sexually matures, is initiated when an individual's hypothalamus induces Gonadotropin Releasing Hormone (GnRH) secretion (Carlson, 2014). This neurohormone plays an important role in reproduction, but is involved in higher-order cognitive functions as well (Bryan et al., 2010; Casadesus et al., 2006). During puberty, its main function is to activate the pituitary to produce gonadotropic hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), that induce secretion of testosterone by the male testes or estradiol by the female ovaries (Carlson, 2014). This hormonal cascade causes changes in sex characteristics, such as breast development in females and lowering of the voice in males (Carlson, 2014), but puberty is also a period of marked changes in the brain (Giedd et al., 2006; Goddings et al., 2014). Research has shown that sex hormones affect brain structure and function (e.g. Hulshoff-Pol et al., 2006), and it has been suggested that adolescents undergo a sex-specific development of the limbic system (Koss & Frick, 2017), a system linked to long-term memory and emotions such as fear (Carlson, 2014). This partially sex-hormone-dependent development results in sex differences in structure, function, and connectivity of the limbic system (Koss & Frick, 2017; Lungu, Potvin, Tikász, & Mendrek, 2015; Peper, Hulshoff-Pol, Crone, & van Honk, 2011; Raper et al., 2013). For instance, women show larger grey matter volumes in the hippocampus (Filipek, Richelme, Kennedy, & Caviness, 1994; Lentini, Kasahara, Arver, & Savic, 2013; Murphy et al., 1996), while men show larger amygdala volumes (Gied et al., 2006; Lentini et al., 2013; Neufang et al., 2009). It has been suggested that such sex hormone dependent differences in limbic system development manifest on a

behavioural level; for instance, sex differences in amygdala connectivity during negative emotion processing may contribute to greater prevalence of depression and anxiety disorders in women (Lungu et al., 2015).

Sometimes the actions of GnRH are blocked in children or teenagers as part of a medical treatment for certain diseases, such as central precocious puberty, congenital adrenal hyperplasia and autism (Carel, Eugster, Rogol, Ghizzoni, & Palmert, 2009). This is done by administering GnRH analogues/agonists (GnRHa). This initial overstimulation of the pituitary initially results in an excess of FSH and LH. Through a feedback process, this further results in a down regulation of the hypothalamus, with subsequent 'puberty suppression' as a consequence. This treatment has been proven effective in the disorders mentioned above (Carel et al., 2009), as well as in teenagers with Gender Dysphoria, where it is used to suppress the development of undesired sex characteristics and where it makes the gender-transition less stressful (de Vries et al., 2014). One could hypothesize that this suppression of GnRH-action interferes with the sex-specific development of the limbic system described above. Knowing whether this is the case is of great importance for understanding the neuropsychological functioning of treated adolescents, and has theoretical implications for our current understanding of sex hormones and brain development. The aim of this article is to investigate indications for a sex-differential effect of GnRHa-therapy on the adolescent limbic system in the current scientific literature.

Studies in humans

Two types of studies could identify a potential influence of GnRHa-therapy on adolescents' limbic system. First, and ideally, one could investigate its effects through brain imaging research, by comparing grey and/or white matter structure and brain function between children/teenagers who are treated and untreated controls, and by associating these parameters with behavioural measures. So far, only one research group has performed such research (Staphorsius et al., 2015). They did not focus on the limbic system, however, but used a Tower of London-task, a task tapping into planning ability, to investigate the influence of long-term GnRHa-treatment on the adolescents' executive functions and associated functional brain activations. Participants were diagnosed with Gender Dysphoria, a condition in which one's biological sex differs from one's experienced gender, causing high levels of distress (American Psychiatric Association, 2013). Both transgirls (individuals with male sex assigned at birth and a female gender identity) and transboys (female sex assigned at birth, male gender identity) were investigated, and both groups included GnRHa-treated as well as untreated adolescents with Gender Dysphoria. No group differences were found on ToL-performance. The analysis of functional activity patterns revealed an effect of GnRHa-treatment on structures such as the precuneus and the dorsolateral prefrontal cortex. While this suggests that GnRHa-treatment affects the developing brain at least to some extent, it doesn't inform us about treatment effects on the limbic system since this brain area is not involved in the task at hand (Staphorsius et al., 2015).

A second way of answering the research question posed in this article, is by analysing the effects of GnRHAs on behaviours or functions that are associated with

the limbic system, such as emotion processing/regulation, learning, and memory (Carlson, 2014). While several single-sex studies on these functions have been conducted in adults (Craig, 2009; Craig et al., 2008a; Craig et al., 2008b; Henningson et al., 2015), no studies on sex differences in the effects of GnRHa-treatment or with adolescents have been performed so far. Some reports have been made, however, about the general emotional wellbeing and cognitive functions of adolescents under GnRHa-therapy. It has been shown that adolescents with Gender Dysphoria (Cohen-Kettenis, Schagen, Steensma, de Vries, & Delemarre-van de Waal, 2011; de Vries et al., 2014) as well as with precocious puberty (Neely & Crossen, 2014) generally fare well with the treatment and experience no adverse consequences in emotional or cognitive functions. Although these studies suggest that GnRHAs do not have any drastic effects on the limbic system of these children and teenagers, this remains rather anecdotic in nature and could hardly be considered as compelling evidence. No comparisons were made between the sexes, hence these studies cannot answer our question about sex differences in limbic development during GnRHa-treatment either.

Studies in animals

A set of valuable animal studies has been conducted by the Sex On Brain European Research Group (SOBER)(Evans et al., 2012; Nuruddin et al., 2013a; Nuruddin et al., 2013b; Nuruddin et al., 2013c; Wojniusz et al., 2011). The group created a model in which male and female sheep were compared during puberty while half of the animals received GnRHa-treatment (consisting of subcutaneous implants of goserelin acetate). The group conducted experiments with strong methodological designs, for instance by

using same-sex twins, of which one sheep was randomly allocated to the treatment group, while the twin-brother or -sister was allocated to the control group. Sex differences in onset of puberty (Wood & Foster, 1998) were taken into account and animal blood samples were collected to determine if GnRHa-treatment effectively blocked the hypothalamus-pituitary-gonadal axis, which it did.

First of all, behavioural measures that might identify possible treatment effects on the limbic system were investigated (Wojniusz et al., 2011). A Food Acquisition Task was designed, in which the animals individually could acquire food. The most attractive food was placed furthest away from the group of sheep, which was thought to instigate fear in the animals. Hence, in order to get the most attractive food, animals had to rely on emotion regulation, a function linked to the amygdala (Carlson, 2014). Heart rate variability (HRV) was measured in parallel as a physiological parameter of emotion regulation. The results indicated clear sex differences in both measurements, with male pubertal sheep showing better emotional regulation than female sheep, indicated by higher HRV. GnRHa-treatment even seemed to exaggerate these sex differences: the GnRHa-treated rams showed the highest HRV and engaged in risk-taking behaviour, while the GnRHa-treated ewes had the lowest values of HRV and expressed avoidance behaviour. It seemed that the female sheep couldn't properly regulate their feelings of anxiety in order to reach the food, contrary to male sheep, which was intensified by GnRHAs. These findings point to a possible sex-specific treatment effect and were extended by an analysis of emotional reactivity (Evans et al., 2012). This study compared four groups of pubertal sheep (control males, control females, GnRHa-treated males, GnRHa-treated females) as well. The animals were

exposed to stress while measurements of cortisol secretion and psychophysiological motoric reactivity (PMR) were taken as proxies for emotional reactivity. This time, GnRHa-treatment did not affect parameters of female sheep, while it did affect male sheep's PMR by first increasing it, then decreasing it again as the sheep aged. This again points to a sex-specific action of GnRHAs in pubertal sheep.

Hypothesizing that GnRHa-treatment might affect the structure/function of the hippocampus, performance on a spatial orientation task, which is known to depend on this structure (Carlson, 2014; Levita & Muzzio, 2010), was investigated as well (Nuruddin et al., 2013c). Long-term treatment did not show to have an effect on task-performance. GnRHa-treatment did have an effect, however, on hippocampus gene expression. Sex- and hemispheric-specific changes were found in hippocampal genes involved in neuroplasticity and endocrine signalling. For instance, brain derived neurotrophic factor (BDNF) mRNA expression was elevated in the right hippocampus of female sheep only, and while neural cell adhesion molecule 1 (NCAM1) mRNA expression was reduced in the right hippocampus of male sheep, it was reduced in the left hippocampus of female sheep. Expression of amygdala genes was investigated as well (Nuruddin et al., 2013b). Treatment affected the expression of multiple genes, especially in the left amygdala of female sheep. Treatment did not affect amygdala gene expression in male animals.

Most importantly for the current topic, the structure of the animals' brains was analysed (Nuruddin et al., 2013a). Upon comparing the limbic structures of both treated and control, both male and female sheep, no differences in grey matter volume were found in the hippocampi. The opposite was the case for the amygdala: the

researchers found main effects of treatment and sex as well as an interaction of the two on grey matter volume. Rams had bigger volumes of grey matter in the right amygdala than ewes. The effect of treatment was manifested as grey matter volume increases in both left and right amygdala in the treated animals versus controls, an effect especially strong in the left amygdala of female sheep, which explains the interaction. The authors conclude that GnRHa-treatment has sex-differential effects on amygdala volumes in sheep.

DISCUSSION AND FUTURE DIRECTIONS

As the above section on human research indicates, there is no direct evidence for a sex-specific effect of GnRHa-treatment during adolescence on the human limbic system – at least not yet. The ovine model by the SOBER-group substantiates the need for similar studies on humans in order to investigate whether the findings in sheep (i.e. sex-differential effects of GnRHa-treatment on hippocampus and amygdala gene expression and amygdala volume) translate to humans. Experiments should be conducted in which four groups of adolescents (control males, control females, GnRHa-treated males, GnRHa-treated females) are compared with respect to brain structure and function as well as relevant behavioural and psychological parameters. Ideally, these designs should be longitudinal in nature, and ultimately investigate long-term outcomes. It is of great importance to investigate potential neurobiological consequences of puberty suppression, given its widespread application (Carel et al., 2009). Until we have explicitly investigated the effects of GnRHa-treatment on brain

structures involved in emotion regulation and on neuropsychological performance, we cannot know to what extent we need to further support treated adolescents with extra psychotherapy. From a more theoretical viewpoint, this research might be interesting to further clarify the relation of sex hormones to sex differences in brain structure/function and ultimately behaviour.

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