Brain sexual development in Kallmann syndrome

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SUMMARY

Prenatal and one-two month postnatal testosterone influences human neural and behavioural development, since the prenatal and one-two month postnatal hormone environment clearly contributes to the development of sex-related variation in human behaviour, and plays a role in the development of the sexual brain and individual differences in behaviour within each sex, as well as differences between the sexes. Olfactory system development, brain sexual maturation and sexual behaviour in man and animals are closely related. Kallmann syndrome (KS) is a genetic disorder which combines hypogonadotropic hypogonadism and anosmia. Hypogonadism is characterized by the absence or reduced levels of gonadotropin-releasing hormone, and anosmia is due to aplasia of the olfactory bulb. The overlap between the formation of the olfactory system and the migration of neurons that synthesize the gonadotropin-releasing hormone (GnRH) is common knowledge. GnRH neurons migrate from the medial portion of the nasal epithelium through the olfactory nerves and the main olfactory bulb to the anterior hypothalamus. Furthermore, the clinical manifestations of KS are: anosmia, the absence of puberty, and modifications in sexual behaviour. The structures responsible for the maturation of the main and accessory olfactory systems, the sexual differentiation of the brain and its relationship with clinical manifestations and sexual behaviour in Kallmann syndrome are analyzed in this review. The importance of the treatment of KS at early ages is suggested in order to improve brain sexual development and its clinical and sexual behaviour manifestations.

Key words: Encephalic sexual maturation – Main and accessory olfactory systems – Kallmann syndrome – Sexual behaviour

INTRODUCTION

The foetal and perinatal hormonal environment plays an important role in the proper differentiation and development of the main olfactory bulb (MOB), the accessory olfactory bulb (AOB), the anterior hypothalamus (AH), specifically the organum vasculosum of the lamina terminalis (OVLT) and the medial preoptic area (MPA), and amygdaloidal complex (AC). These structures are involved in the sexual maturation of the brain, in the development of sexual behaviour and biological sex cell development during a critical period of postnatal development from the prenatal period until puberty, which is an important stage of life due to its impact on sexual maturation and sexual behaviour in adulthood (Hines 2011; Cao and Patisa 2013; Bai-

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leyand Silver, 2014; Hines et al., 2015).

MAIN OLFACTORY BULB AND ACCESSORY OLFACTORY BULB

The olfactory bulb (OB), in addition to participating in the perception of all odors, is connected to reproductive, aggressive and defensive behaviour. The OB is divided into two parts: the main olfactory bulb (MOB) and the accessory olfactory bulb (AOB). The main olfactory bulb comes from rhinencephalon, but the AOB is an independent unit located above the main olfactory bulb (MOB), its sensory innervation comes from the vomeronasal organ in rodents. Anatomically and functionally, the AOB appears to have two distinct parts: the anterior accessory olfactory bulb (aAOB) and the posterior accessory olfactory bulb (pABOA). The aAOB has the same origin as the MOB and is involved in reproductive behaviour, whereas the pAOB has a more caudal origin in the thalamic eminence in the diencephalic-telencephalic sulcus, and its role is to mediate defensive, aggressive and sexual behaviour (Blechschmidt, 1963; Huilgol el al., 2013; McGregor et al., 2004).

AMYGDALOID COMPLEX

The findings by Müller and O'Rahilly (2006) in humans describe the following: the future amygdaloid region is discernible at stage 14 and the amygdaloid primordium at stage 15; the anterior amygdaloid area and the corticomedial and basolateral complexes appear at stage 16, and these three major divisions arise initially from the medial ventricular eminence, which is diencephalic; individual nuclei begin to be detectable at stages 17-21, the central nucleus at stage 23 and the lateral nucleus shortly thereafter (Humphrey 1968; Müller and O'Rahilly, 2006). Müller and O'Rahilly (2006) also described that: the lateral eminence contributes to the cortical nucleus at stage 18; the primordial plexiform layer develops independently of the cortical nucleus; spatial changes of the nuclei within the amygdaloid complex and of the complex as a whole begin in the embryonic period and continue during the foetal period, during the early part of which the definitive amygdaloid topography in relation to the corpus striatum is attained. On the other hand, the influence of the olfactory bulb and tubercle on initial amygdaloid development, as postulated for rodents, is unlikely in the human (Müller and O'Rahilly, 2006). Therefore, this fact could explain that in human KS there is not hypoplasia or variations in amygdala (Yousem et al., 1993). Nevertheless, it is well recognized that neonatal exposure to estrogen, aromatized from testicular androgens, participates in the orchestration of structural sex differences within the amygdaloid complex that ultimately confer behavioural sex differences. Thus, the detailed profile of neonatal estrogen receptor (ER) mRNA levels provided by Cao and Patisa (2013) will help elucidate the relative roles each of the ERs play in the sex-specific, estrogen-dependent organization of the amygdaloid complex, and the sex-specific social and sexual behaviours elicited in response to the pheromonal, hormonal, social and other environmental cues mediated by this brain region (Cao and Patisa, 2013).

ANTERIOR HYPOTHALAMUS. ORGANUM VASCULOSUM OF THE LAMINA TERMINALIS (OVLT) AND PREOPTIC MEDIAL AREA (PMA)

The OVLT is a hypothalamic structure (Campos-Ortega and Ferres-Torre, 1965; Carmona-Calero et al., 2013; Wislocki and King, 1936) belonging to the so-called circumventricular organs (Höfer, 1959). The OVLT is located in the anterior ventral region of the third ventricle as a residual part of the forebrain (Castañeyra-Perdomo et al., 2013) and contains, angiotensin II, catecholamines and large amounts of GnRH (Herde et al., 2011; Teixeira et al., 2010). The OVLT is surrounded by the PMA (Fig. 1), both structures are the main brain areas for the synthesis of GnRH (Castañeyra-Ruiz et al., 2013; Schwanzel-Fukuda and Pfaff, 1989). The PMA makes its morphological appearance in the human species at eight to nine weeks of gestation and is located in the periventricular and middle regions of the anterior hypothalamus (Fig. 2). The PMA consists of small- and medium-sized neurons whose function is related to: the production of gonadotropin releasing hormone (GnRH), the regulation of body temperature and homeostatic control (Hasegawa et al., 2005; Kouttcherovet al., 2003). The innervation of PMA mainly comes from catecholaminergic pathways, since neurons and noradrenergic fibres from different parts of the brain are found in the PMA (Hasegawa et al., 2005; Kouttcherov et al., 2003; Castañeyra-Perdomo et al., 1992). GnRH positive cells and fibers are located in three parts of the PMA in the rat (Castañeyra-Ruiz et al., 2013; Herde et al., 2011) the dorsal medial preoptic area (APMD), the ventral medial preoptic area (APMV) and the ventrolateral medial preoptic area (APMVL) (Fig. 1). Immunohistochemistry study showed that the greatest amount of GnRH positive cells and fibres in the brain were found in the OVLT and the foremost part of the PMA, which then gradually decreased caudally down to the posterior parts of PMA (Fig. 1) and suprachiasmatic nucleus (SCN), where GnRH positivity was scarce (Castañeyra-Ruiz et al., 2013). On the other hand, structural differences in the medial preoptic area between the sexes in many species of animals and humans are described, which is known as sexual dimorphism of the medial preoptic area (Addison and Rissman, 2012; Orikasa and Sakuma, 2010; Perez-Delgado et al., 1987). At the same time, as described in previous
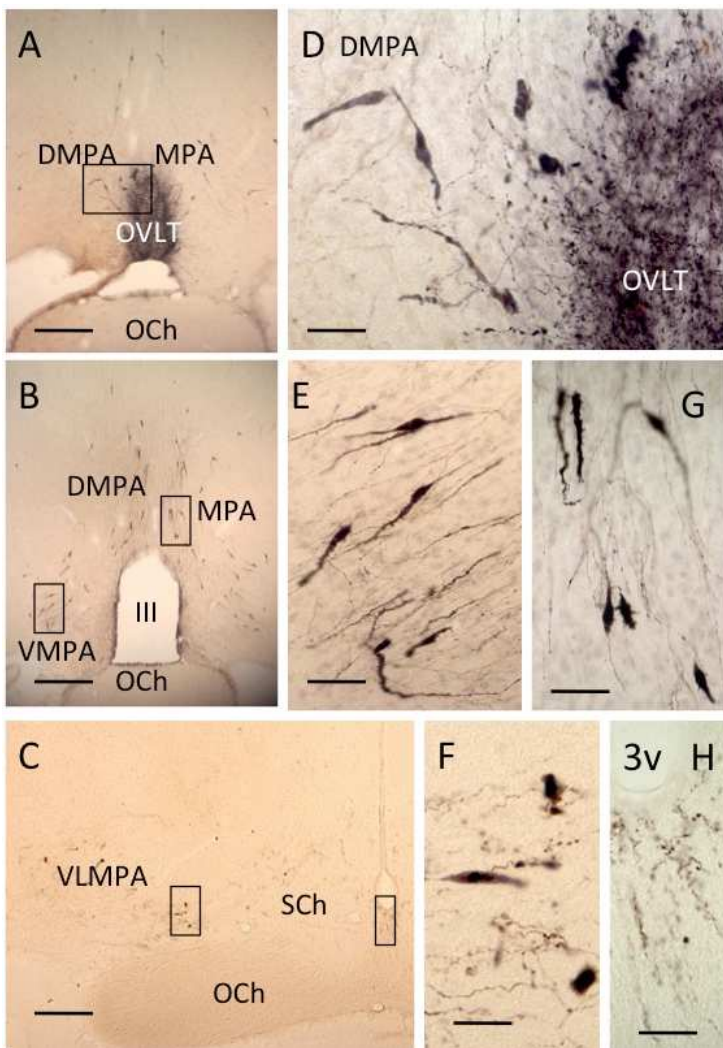
Fig. 1. Coronal sections of the rat brain at the different rostro-caudal levels, showing GnRH cells and fibers in DPMA, VPMA, VLMPA and OVLT. **(A)** panoramic view of the MPA and OVLT at rostral level; **(D)** magnification of DMPA and OVLT frame of A; **(B)** panoramic view of the MPA at the intermediate level; **(E)** magnification VMPA frame of B; **(G)** magnification of the VMPA frame of B; **(C)** panoramic view of the MPA at the caudal level; **(F)** magnification of VLMPA frame of C; **(H)** magnification of 3V floor frame of C. Scale bars: 200 μμm in A, B and C; 40 μμm in D,E,G,F and H. Images partially modified and reprinted from Castañeyra-Ruiz et al. (2013). 3v= third ventricle, DMPA= dorsomedial preoptic area, MPA= preoptic medial area, OCh= optic chiasm, OVLT= organum vasculosum of the lamina terminalis, SCh= suprachiasmatic nucleus, VMPA= ventromedial preoptic area, VLMPA= ventrolateral medial preoptic area.

studies (Castañeyra-Perdomo et al., 2013, 2014), it can be said that the preoptic area is neither a diencephalic nor telencephalic structure. Actually, during the early stages of its development the PMA comes from the primitive lamina terminalis and is located in the anterobasal forebrain (Fig.2), but in the following stages of its development, when from the prosencephalon develops and the diencephalon and the telencephalon differentiate, the preoptic area is anatomically located in the anterior hypothalamus (Castañeyra-Perdomo et al., 2013; Kouttcherov et al., 2003). Consequently, this zone has been called "residual forebrain", since this area, previously limited by the lamina terminalis would be part of the forebrain that would never have been differentiated in diencephalon or telencephalon (Blechschmidt, 1963; Castañeyra-Perdomo et al., 2013, 2014).

CELL DEVELOPMENT AND BIOLOGICAL SEX

It is important to note that virtually all eukaryotic cells have an endogenous circadian clock and biological sex. These cell-based clocks have been conceptualized as oscillators whose phases can be reset by internal signals, such as hormones, and external signals such as light. Therefore, one should consider the relationship between circadian clocks and gender differences. In mammals, the suprachiasmatic nucleus (SChN, Fig. 1), located on the optic chiasm surrounded by the caudal part of PMA, serves as a master clock for synchronizing the phases of all body clocks (Bailey and Silver, 2014; Vida et al., 2008). Gonadal steroid receptors are expressed in almost all structures that receive direct input from the SChN. So, sex differences in the circadian system in the hypothalamus-pituitary-gonadal axis, the hypothalamic-pituitary-adrenal axis, and sleep-wake system have been described in the literature (Bailey and Silver, 2014; Vida et al., 2008). It is also worth mentioning that, in the aforementioned systems, the forms of disruption of circadian rhythms differ by gender and are associated with dysfunction and disease. Better knowledge of the circadian timing systems, based on the sex, could lead to better treatment strategies in different pathologies (Bailey and Silver, 2014), as in the case of KS.

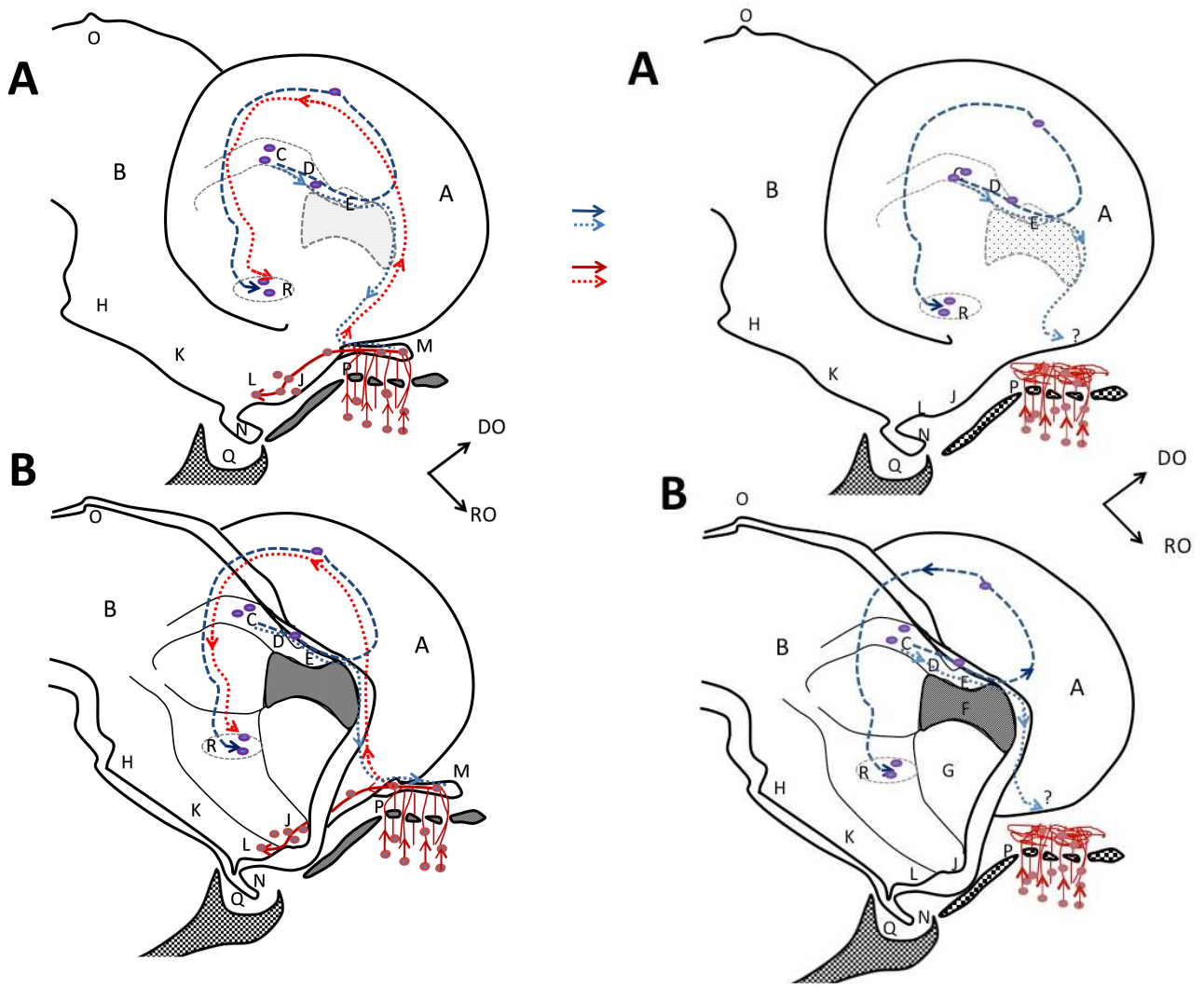## GONADAL HORMONES INFLUENCE HUMAN NEURAL AND BEHAVIOURAL DEVELOPMENT

Fig. 2 (left). Drawing of human diencephalon and telencephalon at 8th and 9th of gestational week of normal development. Lateral view **(A)**, Medio-sagittal view **(B)**. A = telencephalon, B = diencephalon, C = thalamic eminence, D = diencephalic-telencephalic border, E = choroid plexus, F = interventricular foramen Monro, G = striatum, H = mammillary hypothalamus, J = lamina terminalis, K = infundibular hypothalamus, L = Primordium of medial preoptic area and organum vasculosum of the lamina terminalis, M = Main olfactory bulb (MOB), N = neurohypophysis, O = epiphysis, P = Ethmoidal cribriform plate, Q= Sphenoid sella Turcica, R= Amygdala.　Caudal origin,　　Rostral origin,　　Only described in rodents. Modified from Blechschmidt (1963).

Fig. 3 (right). Drawing of human diencephalon and telencephalon at 8th and 9th of gestational week of anatomical proposal for Kallmann Syndrome. **(A)** lateral view, **(B)** medio-sagittal view. A = telencephalon, B= diencephalon =, C = thalamic eminence, D = diencephalic-telencephalic border, E = choroid plexus, F = interventricular foramen Monro, G = striatum, H = mammillary hypothalamus, J = lamina terminalis, K = infundibular hypothalamus, L= Primordium of medial preoptic area and organum vasculosum of the lamina terminalis, N = neurohypophysis, O = epiphysis, P= Ethmoidal cribriform plate, Q= Sphenoid sella Turcica, R= Amygdala. .　Caudal origin,　　Rostral origin,　　Only described in rodents. Modified from Blechschmidt (1963).

The hypothesis that prenatal testosterone influences human neural and behavioural development has been revised by Hines et al. (2010, 2015), and the remarks and conclusion are that the prenatal hormone environment clearly contributes to the development of sex-related variation in human behaviour and plays a role in the development of individual differences in behaviour within each sex, as well as differences between the sexes. Thus, early hormone differences appear to be part of the answer to questions such as why some children are more sex-typical than

others, why some adults are more aggressive or better at targeting than others, and why some people are heterosexual while others are not. In other species, the early hormone environment exerts its enduring effects on behaviour by altering neural development. Similar neural changes are thought to underlie associations between the early hormone environment and human behaviour. The early postnatal surge in testosterone, sometimes called mini-puberty, may be more accessible than the prenatal surge. One study (Lamminmaki et al., 2012) measured testosterone repeatedly in urine samples from infants beginning at week 1 and continuing each month from weeks 4 to 26 of postnatal life (months 1 to 6 postnatal). Gender-typical play was then measured using a questionnaire, the Pre-School Activities Inventory (PSAI), and by observing toy choices in a playroom at age 14 months. The area under the curve for testosterone during the first 6 months of life was significantly larger in boys than in girls. In addition, the area under the curve for testosterone positively predicted PSAI scores for male-typical play, negatively predicted observed play with a baby doll in boys, and positively predicted observed play with a train in girls (Hines, 2011; Hines et al., 2015; Lamminmaki et al., 2012).

KALLMANN SYNDROME

Kallmann syndrome (KS) is a developmental disorder that combines hypogonadotropic hypogonadism with anosmia and / or hyposmia, associated with aplasia or hypoplasia of the olfactory bulbs and tracts (Vidal et al., 2007; Delezoide et al., 1991, Dodé and .Hardelin, 2009; Hardelin et al., 1992; Legouis et al., 1991; Schwanzel-Fukuda et al., 1989, 1996). The interruption of GnRH cell migration is responsible for hypogonadism in KS (Herde et al., 2011; Franco et al., 1991). Several studies (Schwanzel-Fukuda et al., 1989, 1996; Schwanzel-Fukuda and Pfaff, 1989) describe how the GnRH cells migrate from the medial part of the high nasal epithelium to forebrain (Fig. 2), and this migration in humans begins during the sixth week of embryonic development (Delezoide et al., 1991; Schwanzel-Fukuda et al., 1989). When telencephalon first appears (Blechschmidt, 1963), GnRH cells reach the telencephalon anteroventral region and penetrate along with the central processes of nerve terminals into olfactory bulb (Fig. 2). GnRH cells caudally then move and reach the anterior hypothalamus, specifically to the early primordium of PMA and OVLT. In a study by Schwanzel-Fukuda et al. (1989) in a fetus that had no olfactory bulbs, a total lack of GnRH cells was found in the brain, however certain groups of such cells were found in the nasal region and the dorsal surface of the cribriform plate, adjacent to the entangled fibers of the olfactory terminals nerves, which do not come into contact with the forebrain (Fig.

3). This case corresponded to a KS fetus that presented a chromosomal deletion that included the Xp22 KAL1 X chromosome gene responsible for the type KAL1 of Kallmann syndrome (Vidal et al., 2007; Ayari et al., 2012).

DISCUSSION

There is probably virtually no difference between the normal brain and the Kallmann syndrome brain in normal human brain development during the first 4 or 5 gestational weeks (Castañeyra-Perdomo et al., 2013). However, at the beginning of the sixth week of gestation the telencephalon primordium first make their appearance in the anterolateral parts of the forebrain, with a part of anteromedial forebrain remaining undifferentiated called "primitive lamina terminalis" (PLT) or "residual forebrain". The PLT will later lead the development of the lamina terminalis, PMA, OVLT and rhynencephalon (RPh). The RPh will later induce the formation of the olfactory bulbs, stimulating the olfactory nerves from the nasal epithelium connected to it. This will allow the migration and / or maturation of olfactory and GnRH-producing cells which move to and settle in the olfactory bulbs, olfactory tubercles (OT) and the OVLT and PMA (Castañeyra-Perdomo et al., 2013; Schwanzel-Fukuda et al., 1989, 1996; Ayari et al., 2012; Krisch, 1978).

What probably occurs in Kallmann syndrome (Castañeyra-Perdomo et al., 2013, 2014), is that during the sixth week of gestation the telencephalic primordium could originate very close to one another in the anterior part of the forebrain, thereby preventing the formation of the residual primitive forebrain or lamina terminalis between them (Schmidt et al., 2001), therefore, there is no rhinencephalon differentiation and the MOB does not appear or develop which would explain the anosmia in KS. Furthermore, no anterior hypothalamic structures are properly formed, such as the PMA and OVLT and therefore, the olfactory nerves from the nasal epithelium cannot connect MOB, structure, which do not exist in KS, and GnRH producing cells cannot migrate and reach their destination in PMA and OVLT, and consequently sexual differentiation cannot occur as is described in KS.

Furthermore, a temporary increase of testosterone (Jean-Faucher et al., 1978, 1985) inducing sexual maturation of the brain (Hines, 2011; Hines et al., 2015) is produced at postnatal age (mini-puberty). This increase in testosterone does not appear to occur in KS, so maturation of the nonexistent OVLT, PMA and OT structures in KS does not occur and this structures are important neuroanatomical parties for the sexual maturation of the brain. However, it should be taken into account that several structures forming part of the olfactory and sexual brain systems are not affected in KS such as: pABO and amygdala, because they have

an origin at the thalamic eminence level in the di-encephalic-telencephalic sulcus and these structures could develop if a proper hormonal environment was generated at postnatal age (Hines, 2011; Hines et al., 2015; Castañeyra-Perdomo et al., 2014).

At present, the treatment of hypogonadism in KS is prepuberal (Dodé and Hardelin, 2009; Hardelin et al., 1992; Grumbach, 2005), and aims to: first start virilization or breast development, and secondly trigger the development of fertility. Hormone replacement therapy usually uses testosterone for men and a combination of estrogen and progesterone for women, which is a treatment aimed almost exclusively at stimulating the development of secondary sex characteristics. For those wishing fertility treatments, GnRH pulsatile gonadotropin can be used for both testicular growth and sperm production in males or ovulation in women (Dodé and Hardelin, 2009).

A series of questions were raised in a previous work (Castañeyra-Perdomo et al., 2014): What happens to the accessory olfactory system (AOS) in patients with Kallmann syndrome where the main olfactory system does not develop properly? Could the AOS develop completely or at least partially (posterior accessory olfactory bulb and medial amygdala) in humans with KS despite the fact that humans are reported to not have an accessory olfactory bulb but they have amygdale (Huilgol et al., 2013; Korzan et al., 2013)?. And what would happen in animal models of KS, such as the mouse, where the accessory olfactory bulb exists? Besides, if one takes into account the importance of sex in cellular circadian sync rhythms (Bailey and Silver, 2011; Simerly, 2005), one could add another question: what happens to the timing of circadian rhythms in KS during the period from the prenatal period until puberty, which is so important to the future brain sexual development and maturation when no treatment has been started?

On the other hand, testosterone levels are high in human males from about weeks 8 to 24 of gestation, and then again during early postnatal development. The postnatal surge in testosterone in male infants, sometimes called mini-puberty, may provide a more accessible opportunity for measuring early androgen exposure during typical development (Lamminmäki et al., 2012). This approach has recently begun to be used, with some promising results relating testosterone during the first few months of postnatal life to later gender-typical play behaviour. In replicating and extending these findings, it may be important to assess testosterone when it is maximal – months 1 to 2 postnatal and to take advantage of the increased reliability afforded by repeated sampling (Hines et al., 2015; Lamminmäki et al., 2012). But individuals exposed to atypical concentrations of testosterone or other androgenic hormones prenatally, for example because of genetic conditions or because their moth-ers were prescribed hormones during pregnancy, have been consistently found to show increased male-typical juvenile play behaviour, alterations in sexual orientation and gender identity (the sense of self as male or female), and increased tendencies to engage in physically aggressive behaviour (Hines et al., 2015). Therefore, if one bears in mind the described above, and the fact that in KS some brain sexual structures, pAOB and amygdala, could develop, the question would be: could the postnatal surge in testosterone (mini-puberty) be used in KS infants in order to obtain a proper development of sexual behaviour and identity during the critical period which goes from the early postnatal age to puberty and even at later ages?

Regardless of the fact that human sexual behavior and identity with respect to emotions and instincts is different for biological reasons, and that sophisticated sexual behaviour is determined by the culture of the environment, one could say that the clinical manifestations and sexual behaviour in KS are not only the direct expression of the lack of development of gonadal and main olfactory system, but the absence of the accessory olfactory system development and properly amygdala development, and furthermore, the altered circadian rhythms in different cells are also involved in clinical and sexual behaviour and identity. Early diagnosis of KS would allow the start of early treatment (1 to 2 months postnatal) and prevent the absence of virilization, the appearance of a eunucoide body, a deficiency in the development of the accessory olfactory system and amygdala, and a disruption of sexual maturation and the consequential disturbances of sexual behaviour and identity.

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