Role of the androgen receptor in the central nervous system

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Abstract

The involvement of gonadal androgens in functions of the central nervous system was suggested for the first time about half a century ago. Since then, the number of functions attributed to androgens has steadily increased, ranging from regulation of the hypothalamic-pituitary-gonadal axis and reproductive behaviors to modulation of cognition, anxiety and other non-reproductive functions. This review focuses on the implication of the neural androgen receptor in these androgen-sensitive functions and behaviors.

1. Introduction

Androgens have been shown to regulate several neural functions ranging from reproduction to mood and cognitive abilities. This property starts as early as the perinatal period, which is characterized by prenatal and postnatal surges of gonadal testosterone, and continues through puberty and even adulthood. In the nervous system, the effects of testosterone are mediated either by androgen receptors (AR), or, after neural aromatization to 17β-estradiol, by estrogen receptors (ER) α and ERβ . Local synthesis of neuro-steroids has been reported in several cerebral regions such as the hippocampus, where they modulate hippocampal synaptic plasticity and healthy memory processes (Ooishi et al., 2012). AR and ER receptors are members of the nuclear receptor superfamily and act mainly through regulation of target genes at the transcriptional level, although several studies show rapid membrane and cytoplasmic changes. The present review focuses on studies in rodents that address the neural AR role in androgen-induced regulation of central nervous system (CNS) functions.

2. Pharmacological and genetic tools to assess neural AR function

2.1. Pharmacological tools

To discriminate between the androgenic and estrogenic pathways in testosterone-mediated regulation of CNS functions, gonadectomy and supplementation with 5-α-dihydrotestosterone (DHT) are used. However, this non-aromatizable metabolite of testosterone does not exclusively activate AR. Indeed, it can be metabolized to 5α-androstan-3β, which binds ERs and triggers neural responses (Handa et al., 2008). Systemic or local treatments in specific CNS regions with AR-antagonists, such as flutamide and cyproterone acetate, are also used to confirm an AR-dependent mechanism. These anti-androgens can act as partial agonists for neural AR in some situations (Nguyen et al., 2007). The use of selective AR modulators (SARM; Narayanan et al., 2017) has also been reported in a few studies.

2.2. Genetic models

Table 1 summarizes the different models used or generated in order to delineate the role of *AR* gene in CNS functions. The Testicular Feminization Mutation (Tfm) of the *AR* gene, which resides on the X chromosome, has been described in both rats and mice (Allison et al., 1971; Lyon & Hawkes, 1970). These spontaneous genetic models are widely used in studies addressing neural AR functions. Both rat and mouse mutations result in a similar, entirely feminine, external phenotype, but they inactivate *AR* to different extents. The rat mutation decreases AR function by 85–90% (Yarbrough et al., 1990). In the mouse model, a single nucleotide deletion introduces a reading frame shift and premature stop codon, thereby resulting in a shortened transcript and absence of AR protein (Charest et al., 1991; He et al., 1991). High testosterone levels are reported in Tfm rats while almost no secretion occurs in mutant mice (Yarbrough et al., 1990).

The global *AR* knock-out mouse models generated by Cre recombinase-mediated excision of Exon 1 or 2 display an androgen insensitivity phenotype comparable to that of Tfm mice (De Gendt et al., 2004; Sato et al., 2003; Yeh et al., 2002). Genetic (XY) males exhibit a typical female external appearance, small cryptorchidic azoospermic testes and very low levels of circulating testosterone. In both Tfm and global knock-out mice, it is difficult to distinguish between CNS and peripheral-mediated effects of testosterone through the AR, given the critical role of this hormone in several peripheral functions related, or not, to reproduction.

This observation motivated the generation of the first mouse line selectively invalidated for the neural *AR* by using mice with floxed *AR* Exon 2 and transgenic animals carrying Cre recombinase driven by the nestin promoter and nervous system-specific enhancer (Raskin et al., 2009; Figure 1). This conditional mutation, targeting *AR* in neuronal and glial precursor cells as early as embryonic day (ED) 10.5, does not interfere with the normal development of

the urogenital tract. The same mouse line was generated and reported by other groups (Chen et al., 2016; Juntti et al., 2010; Karlsson et al., 2016). Deletion of the *AR* gene in neurons was also performed using synapsin-I-Cre transgenic mice (Yu et al., 2013). Two mouse models overexpressing *AR* either globally or selectively at neural sites (neuronal and glial) were recently reported by Swift-Gallant et al. (2016a).

3. Neuroendocrine functions and behavior related to reproduction

Perinatal testosterone permanently potentiates male (masculinization) and inhibits female (defeminization) neuroanatomical and behavioral characteristics (Morris et al., 2004; Phoenix et al., 1959). These organizational effects of testosterone suppress the neuroanatomical characteristics required for sex steroids to induce the ovulatory surge of luteinizing hormone (LH) during adulthood (Corbier 1985; Homma et al., 2009). Sex steroids regulate gonadotropin release through an inhibitory or stimulatory feedback effect. Both male and female rodents respond to negative feedback, but only females respond to the positive feedback effect. The perinatal action of testosterone also triggers the adult expression of male-typical behavior (such as a preference for a receptive female, mounts and thrusts) and an inability to adopt the female posture (lordosis), which is triggered by male mounts (Phoenix et al., 1959; Baum et al., 1990). The pubertal testosterone increase also seems to be involved in the final maturation of neural structures underlying reproductive functions (Ahmed et al., 2008; De Lorme et al., 2012, 2016; Schulz et al., 2004). During adulthood, testosterone acts transiently on cerebral areas by exerting an activational role on neuroendocrine functions and behavior.

3.1. The hypothalamic-pituitary-gonadal (HPG) axis

The HPG axis plays a crucial role in steroid hormone production. In males, the testicular synthesis of testosterone is under the control of the hypothalamic gonadotropin-releasing hormone (GnRH) and pituitary LH. The liberated testosterone acts in turn to reduce GnRH release and thereby control gonadotropin secretion. In the hypothalamus, the negative feedback exerted by testosterone does not seem to directly target GnRH neurons, since these cells lack AR and ERα. Negative feedback seems to occur upstream of the GnRH neurons at the level of the kisspeptin system. This system acts as a CNS regulator of the HPG axis and is currently considered to be a key target of testosterone feedback regulation for GnRH and LH release (Navarro et al., 2011; Raskin et al., 2009; Ruka et al., 2016; Smith et al., 2005). Kisspeptin neurons are localized in two hypothalamic regions. The rostral periventricular area of the third ventricle involved in the ovulatory surge of LH in females contains fewer cells in males due to its perinatal masculinization by testosterone (Clarkson & Herbison 2006, 2009). The arcuate nucleus integrates the negative feedback exerted by sex steroid hormones in both sexes. In male mice, testosterone-induced regulation of *KiSS-1* expression has been shown to involve ERα and AR, which are both co-expressed in most *KiSS-1* neurons of the arcuate nucleus (Smith et al., 2005). In the arcuate nucleus, kisspeptin is co-expressed with neurokinin B, another major regulator of GnRH neurons (Navarro et al., 2009). Extracellular recording of brain slices from intact or castrated and treated males showed that agonists of both ERα and AR inhibit the neuronal response to activation of the neurokinin-3 receptor in adult mice (Ruka et al., 2016). However, data from global ERα knock-out mice suggested that the cerebral AR could play the primary physiological role in steroid feedback of LH secretion (Wersinger et al., 1999). Neural *AR* knock-out males exhibit elevated levels of circulating LH and testosterone (Raskin et al., 2009), indicating the involvement of hypothalamic AR in the negative regulation of the HPG axis. In contrast, neural AR does not seem to be involved in the perinatal organization of the rostral periventricular area of the third ventricle nucleus,

since the number of kisspeptin neurons was similar in control and mutant littermates (Table 2; Picot et al., 2014). This suggests that neural AR might be involved in the activational but not in organizational regulation of the HPG axis by testosterone.

3.2. Reproductive behavior

In rodents, reproductive behavior is induced by olfactory cues. Pheromone cues emitted by a sexually receptive female are detected by the main olfactory epithelium and vomeronasal organ of the male and are transmitted, respectively, to the main and accessory olfactory bulbs, then to chemosensory responsive nuclei in the medial amygdala, to the stria terminalis bed nucleus and the medial preoptic area, where they are processed into behavioral responses (Figure 2). Projections are sent from the hypothalamic paraventricular nucleus to spinal centers that promote penile erection and ejaculation like the spinal nucleus of the bulbo-cavernosus (SNB) and the gastrin-releasing peptide system. During the perinatal period, the organizational effects of testosterone result in sex differences at the structural, neurochemical and molecular levels along this circuitry (Morris et al., 2004). The widely-described sex differences in cell number and morphology or fiber density in these brain areas underlie the behavioral differences observed during adulthood.

3.2.1. AR expression in neural structures underlying reproductive behavior

In adult rats and mice, a high level of mRNA and protein expression occurs in the medial amygdala, lateral septum, stria terminalis bed nucleus, preoptic area and ventromedial hypothalamus (Pelletier, 2000; Raskin et al., 2009; Simerly et al., 1990). The AR is also present in the cerebellum, with a high expression level in superficial Purkinje neurons of the vermis (Perez-Pouchoulen et al., 2016; Simerly et al., 1990), where specific regions of the

cerebellar vermis become active during sexual behavior in rats (Garcia-Martinez et al., 2010; Manzo et al., 2008).

In the developing mouse hypothalamus, AR mRNA concentrations increase from ED 19 to postnatal day (PND) 7 and then decrease in both males and females, but with a higher expression level in males (Mogi et al., 2015). Mice carrying a knock-in *AR* allele, which permits co-expression of the nuclear β-galactosidase reporter gene, showed that at PND 4, AR expression is detected in the arcuate nucleus and ventral hypothalamus, but not in the medial amygdala, preoptic area and bed nucleus of stria terminalis, where only few AR-positive cells are present (Juntti et al., 2010). This number increases from PND 7 onwards in a sexually dimorphic manner. In line with these data, AR immunoreactivity was low in the early postnatal hypothalamus (anteroventral periventricular area, preoptic area, arcuate nucleus) of both males and females and strongly increased from PND 5 in male mice compared to females (Brock et al., 2015). In the SNB motoneurons, AR immunoreactivity is detected from PND 7 in both rats (Jordan 1997) and mice (Smith et al., 2012). These concordant data indicate that neural AR expression starts to increase after the perinatal period, meaning that the involvement of this receptor in the perinatal organizational effects of testosterone is unlikely.

3.2.2. Regulation of sexual behavior

In rodents, sexual behavior comprises pre-copulatory and copulatory phases. During the pre-copulatory (motivational) phase, the courting male displays a chemo-investigating behavior towards receptive females and produces ultrasonic vocalizations. The emission of these latter during courtship plays a role in attracting the female partner (Pomerantz et al., 1983). During the copulatory or consumatory phase, the male exhibits mounting, thrusting, and intromitting

behavior before reaching ejaculation. The studies addressing AR implication in each of these are summarized and discussed below.

3.2.2.1. Pre-copulatory behavior

Olfactory preference. Data from Tfm mice suggested a major role of AR in olfactory partner preference (Bodo & Rissman, 2007). However, further studies did not confirm these observations. Tfm male rats exhibit a normal preference for estrous females, suggesting that partner preference is normally masculinized (Hamson et al., 2009). Naïve and sexually experienced males lacking neural *AR* also display normal olfactory preference (Marie-Luce et al., 2013; Raskin et al., 2009). In line with these data, neural *AR* overexpression resulted in normal anogenital investigation and olfactory preference towards females. In contrast, global *AR* overexpression increased both behavioral patterns (Swift-Gallant et al., 2016a; 2016b). In neural conditional knock-out models, no differences were seen upon neuronal activation mapped by Fos-immunoreactivity, following exposure to estrus female-soiled bedding (Raskin et al., 2009; Swift-Gallant et al., 2016b). This discrepancy between global *AR* gene alteration (Tfm and AR overexpression) and neural *AR* mutations (deletion or overexpression) suggests that androgens may act through non-neural AR to trigger olfactory preference, as was recently proposed (Swift-Gallant et al., 2016a; 2016b). Alternatively, global *AR* gene alteration may alter other signaling pathways involved in testosterone-mediated regulation of olfactory preferences, such as ERα (Wersinger & Rissman, 2000). Complex regulation of odor preference by sex steroids was suggested by a previous rat study showing that AR blockade by flutamide in the posterodorsal medial amygdala seemed to induce a preference for male odors rather than reduce a preference for female odors (Hosokawa & Shiba, 2010).

Sexual arousal. Noncontact penile erection occurring in the male rat exposed to female odors or inaccessible sexually receptive females represent a useful model to assess sexual arousal. Previous studies pointed out a role for androgens, but not for estrogens, in this behavior (Cooke et al., 2003; Manzo et al., 1999). Further studies suggested that the medial amygdala is one of the neuronal structures that regulate male sexual arousal. Indeed, of males that received flutamide implants in the medial amygdala, fewer exhibited non-contact erections. Such males displayed longer latencies before their first sexual comportment and had less noncontact erections. Furthermore, they spent less total time in genital grooming in comparison to the control group (Bialy et al., 2011).

Sexual arousal can also be indexed by ultrasonic mating calls. In this context, it has been shown that castration and testosterone supplementation influence the production of courtship vocalization in male mice (Dizinno & Whitney, 1977). Treatment combining DHT and estradiol increased vocalizations in the same way as testosterone (Nunez et al., 1978). More recently, we have shown that neural *AR* knock-out males emit fewer and shorter vocalizations in the presence of sexually receptive females compared to their control littermates (Dombret et al., in press). The estrous state of the female must be important in eliciting this behavior, since neural AR mutants were shown to vocalize normally in the presence of intruder females (Juntti et al., 2010). It remains to be studied whether neural AR acts in the preoptic nucleus to increase the motivation to vocalize (Bean et al., 1982) or in the motor system (cortex, striatum, thalamus) to modulate vocalizations.

3.2.2.2. Copulatory behavior

Tfm rats show infrequent and incomplete copulatory responses to receptive females (Beach & Buehler, 1977), but exhibit normal mounting behavior and latency to mount (Hamson et al., 2009; Olsen 1979). More recent studies in Tfm mice reported a minor contribution of the

neural AR in the display of sexual behavior (Bodo & Rissman, 2007; Scordalakes & Rissman, 2004), while a major role was suggested from the analysis of global knock-out males (Sato et al., 2004). In these models, sexual behavior is limited to mounting and thrusting behavior due to the lack of external genitals (Ono et al., 1974; Sato et al., 2004). Therefore, conditional mouse models were analyzed to evaluate more precisely the neural contribution of androgens through AR in the display of full sexual behavior. Male mice lacking neural *AR* displayed increased latency to initiate mounting, thrusting and intromitting behavior and to reach ejaculation (Picot et al., 2014; Raskin et al., 2009; Raskin et al., 2012). The number of mounts with intromissions and total thrusts were also reduced. These behavioral alterations were seen in either intact or castrated mutants supplemented with testosterone to normalize their hormonal levels, and were maintained after sexual experience (Figure 3). Conversely, neural *AR* overexpression increased mounting and thrusting (Swift-Gallant et al., 2016a). The fact that neural *AR* invalidation did not affect olfactory preference strongly suggests that the sexual deficiency of neural *AR* knock-out males is caused by altered neural function in chemosensory responsive regions downstream from the olfactory bulb. Table 2 summarizes the analyses undertaken in the neural *AR* knock-out mouse model to determine whether the behavioral alterations were due to altered neuroanatomical organization. Interestingly, neural *AR* mutation did not affect neuronal populations such as the sexually dimorphic calbindin cluster, which is known to be organized by perinatal testosterone. Similarly, the perinatal organization of SNB motoneuron number was not altered by neural *AR* mutation. This confirms previous conclusions that testosterone-mediated sparing of SNB motor neurons from atrophy may depend upon peripheral AR action in the bulbo-cavernosus/levator ani muscles where this receptor is expressed from ED 15 onwards (Breedlove & Arnold, 1983; Smith et al., 2012). In contrast, SNB motoneuron soma size and dendritic length, which are influenced by testosterone until PND 12 and 28 (Breedlove & Arnold, 1983; Goldstein et al., 1990), were

reduced in mutant males lacking neural *AR*. Again, this suggests that neural *AR* mediates the postnatal, but not perinatal, effects of testosterone. Neural AR was also found to regulate gastrin-releasing peptide expression, as evidenced by the lower neuron number and intensity of axonal projections in neural *AR* knock-out male mice (Sakamoto et al., 2014). Whether this regulation involves developmental or adult AR effects is not known. Although the behavioral deficits observed in neural *AR* knock-out male mice indicate that this receptor is important, the precise period(s) of action (postnatal, pre-pubertal, pubertal or adult) still need to be identified using dedicated models. In a recent study, the subcutaneous treatment of male rats with flutamide during the first week postnatal had no effect on sexual behavior, whereas continuous treatment during the two first weeks altered intromission and ejaculation but not mounting behavior (Yamada et al., 2015).

3.2.3. Aggressive behavior

A comparison of wild-type and Tfm mice, which were gonadectomized and implanted with estradiol-containing implants, showed a minor difference in the display of aggressive behavior (Bodo & Rissman, 2007; Scordalakes & Rissman, 2004). In contrast, gonadally intact Tfm mice given a vehicle or anabolic androgenic steroids exhibited fewer aggressive events than wild-type males (Robinson et al., 2012). The major role of AR in the display of aggressive behavior was also shown in neural *AR* knock-out males (Raskin et al., 2009; Marie-Luce et al., 2013). In the resident-intruder paradigm, a low percentage of mutant males exhibited offensive attacks towards intruder mice compared to control littermates, despite normal anogenital chemo-investigation of intruders. The latency time to the first attack was increased, while the total aggression duration and the number of attacks were greatly reduced in mutant males. This less aggressive behavior was confirmed by further analyses of a similar neural *AR* knock-out model (Juntti et al., 2010; Studer et al., 2015). In social preference and

recognition tests, neural *AR* knock-out male mice showed normal social investigation but failed to recognize male conspecifics (Karlsson et al., 2016).

Arginine-vasopressin is a key neuromodulator of social behavior. The neuronal cell population expressing this peptide in the bed nucleus of stria terminalis is known to be sexually dimorphic and organized through the perinatal action of testosterone. Neural *AR* mutants showed comparable arginine-vasopressin cell numbers in the bed nucleus of stria terminalis and fiber density in the septum to those of their control littermates (Table 2). No modification was seen in the amount of galanin, a neuropeptide co-expressed with arginine-vasopressin. Neural AR does not seem to be involved in the perinatal organization of these brain areas that are essential for aggression, but may be implicated in adult activational effects of testosterone. In the territorial California mouse, winning fights increases the expression of AR in the bed nucleus of the stria terminalis, but also in brain regions that mediate motivation and reward such as the accumbens nucleus and the ventral tegmental area (Fuxjager et al., 2010). In Neotropical singing mice, the supplementation of castrated males with testosterone or DHT similarly restores both aggressive behavior and song effort in male-male aggression, suggesting an activational role of AR in both types of behavior (Pasch et al., 2011).

4. Cognition

Estrogen-mediated regulation of cognitive behavior and synaptic plasticity has been largely studied in rodents. In comparison, the neural function of AR has been investigated less thoroughly, although this receptor is expressed to a high level in brain structures that ensure underlying cognitive processes.

4.1. AR expression

AR is expressed in the adult cortex and hippocampus where it is predominantly expressed in Cornu Ammonis (CA) 1 and CA2/3 regions (Kerr et al., 1995; Kritzer 2004; Raskin et al., 2009; Simerly et al., 1990). Combined retrograde tracing and immuno-labeling of AR showed the prefrontal cortex to ventral tegmental area projection to be AR-enriched (Aubele & Kritzer, 2012). As a transcription factor, the AR protein is generally found in the nucleus, but by electron microscopy AR immunoreactivity was also revealed in axons and dendrites of the amygdala and cerebral cortex (DonCarlos et al., 2003; 2006). In these non-classical sites, AR may play roles in rapid behavioral effects of androgens.

In the developing male rat hippocampus, AR mRNA concentrations were shown to increase progressively from PND 1 to PND 14 and were then maintained until adulthood (Kimoto et al., 2010). Similar data were reported in mice where hippocampal mRNA concentrations significantly increased from basal levels at PND 5 in males and PND 1 in females through PND 7 (Mogi et al., 2015). In the same mouse study, cortical transcript concentrations increased significantly from ED 19 to PND 7 in both sexes. A more recent study confirmed the progressive increase in AR mRNA concentrations from PND 0 to PND 21 in the mouse cortex and hippocampus of both sexes (Tsai et al., 2015).

The predominant cellular phenotype of AR-immuno-reactive cells in the developing and adult male rat forebrain is neuronal, but populations of AR-positive astrocytes also exist in discrete locations and at specific ages (Lorenz et al., 2005).

4.2. Behavioral, morphological and functional analyses

The neural role of AR in learning and memory processes was first suggested by intra-hippocampal infusions of DHT to gonadectomized male rats (Edinger & Frye, 2004). In the same way as for testosterone, DHT supplementation improved learning by treated males compared to vehicle controls in the inhibitory avoidance task. In the Morris water maze

(MWM), assessing spatial learning and memory, the performance of Tfm rats was intermediate between that of normal males and females, suggesting partial masculinization of this behavior through AR (Jones et al., 2005). In the Tfm mouse model, a greater role for AR in spatial learning and memory was supported by data showing that genetic XY animals underperform females in the MWM (Rizk et al., 2005). An AR blockade in the dorsal hippocampus interfered with cognitive performance in the inhibitory avoidance and MWM tasks by the male rat (Edinger & Frye, 2007). In particular, immediate administration of flutamide to the dorsal hippocampus at both pre- and post-training times induced a significantly poorer performance, while a simple injection 2 hours after training had no effect. Combined treatment with a SARM (ACP-105) and ERβ agonist improved cognitive performance in the MWM by gonadectomized male triple transgenic mice overexpressing both the human amyloid precursor protein presenilin 1 and tau and exhibiting the features of Alzheimer's disease (George et al., 2013). Finally, treatment with testosterone attenuated spatial memory defects in the MWM induced by castration (Moghadami et al., 2016) and increased the density of AR-immuno-reactive neurons in the hippocampus areas (Moghadami et al., 2016, Qiu et al., 2016). In a recent study, using conditional neural *AR* knock-out mice, we addressed temporal processing of information (Picot et al., 2016). This is the ability to remember the order in which items or events have been experienced. Neural *AR* invalidation impairs the processing of temporal information for visual objects and alters glutamatergic transmission in the hippocampus CA1 area. Indeed, this mutation affects basal glutamate synaptic transmission and decreases magnitude of N-methyl-D-aspartate receptor (NMDAR) activation and high-frequency stimulation-induced long-term potentiation.

At the cellular and molecular levels, DHT treatment increased Ca^{2+} stores in response to glutamate through a flutamide-sensitive pathway involving the up-regulation of sarco and endoplasmic reticulum calcium ATPase 2 expression in rat primary hippocampal neurons

(Foradori et al., 2007). Several studies have suggested that AR mediates the androgen-induced maintenance of normal spine synapse density in the CA1 area and the prefrontal cortex (Hajszan et al., 2007; Hatanaka et al., 2015; Leranth et al., 2003). It modulates the functional properties of pyramidal cells in both the CA1 (Pettorossi et al., 2013; Pouliot et al., 1996) and CA3 areas (Skucas et al., 2013) and increases CA3 dendritic thorns in hippocampal slices (Hatanaka et al., 2009). Hamson et al. (2013) showed that testosterone increases neurogenesis in the dentate gyrus of wild-type but not Tfm rats and that flutamide treatment blocks the DHT-induced increase of hippocampal neurogenesis.

5. The Hypothalamic-Pituitary-Adrenal (HPA) axis and anxiety-related behavior

Testosterone has been shown to modulate the HPA response to stress and attenuate anxiety-like behavior. In rats, supplementation or intra-hippocampal infusion of gonadectomized males with testosterone or DHT induced a lower anxiety-related behavior in the elevated-plus-maze, open field and defensive freezing tests (Edinger & Frye, 2004). Intra-hippocampal application of flutamide blocked DHT-induced effects, suggesting an implication of the hippocampal AR in an androgen-induced decrease of anxiety-like behavior (Edinger & Frye, 2006). In the Tfm rat model, Rizk et al. (2005) reported unchanged anxiety-state level assessed in the elevated-plus-maze and open field tests. In contrast, two other studies reported increased behavioral indices of anxiety in the open-field and elevated plus maze tests for Tfm rats compared to wild-type males and females (Hamson et al., 2014; Zuloaga et al., 2011). Mutant rats exhibited an increased corticosterone peak in response to mild stress, showing a chronic anxiety phenotype and dysregulated HPA axis (Zuloaga et al., 2011). An androgen-induced organization of the HPA axis through both AR and estrogen conversions was supported by data showing that postnatal treatment with flutamide or an aromatase inhibitor interfered with the adult HPA response to repeated stress (Bingham et al., 2011).

In mice, the Tfm mutation increased the anxiety-state assessed in the novel object and in light/dark box tests, whereas no changes were detected in the open field or elevated plus maze tasks (Zuloaga et al., 2008). As for Tfm rats, this seemed to be related to an increased HPA response to stress. The same group showed that global *AR* knock-out male mice exhibit similar behavioral deficiencies and HPA dysregulation (Chen et al., 2014). An elevated anxiety-state, in particular during the light phase, was seen in the open field, novel object and elevated plus maze tests, but not in the light/dark box and corticosterone levels remained high after mild stress. In contrast, neural *AR* invalidation did not affect androgenic regulation of anxiety or HPA activity (Chen et al., 2016; Raskin et al., 2009; Picot et al., 2016). It has been suggested that in neural *AR* mutant male mice, this lack of effect may be due to residual *AR* expression in the amygdala and hypothalamus, but not in the hippocampus or cortex (Chen et al., 2016). Alternatively, AR-expressing sites other than neurons and glial cells may be involved in such effects. Indeed, novel data suggest the involvement of the immune system including microglia cells in the development of anxiety in response to stress (reviewed in Reader et al., 2015).

6. Other brain functions

Recent reviews nicely summarize the role of expanded polyQ chain within the first exon of the AR in spinal and bulbar muscular atrophy and recent views on the development of this neurodegenerative disease (Giorgetti & Lieberman, 2016; Pennuto & Rinaldi, 2017). Otherwise, several studies documented the implication of androgens in neuroprotection. Pharmacological studies addressing the mechanisms underlying androgen-induced effects showed that pre-treatment of hippocampal neurons with testosterone or DHT, but not with estrogens, reduces cell death induced by different insults (Nguyen et al., 2010). In agreement with these data, the SARM RAD140 exerted a neuroprotective effect in both primary

hippocampal cells and the hippocampus of kainite lesioned males (Jayaraman et al., 2014). Flutamide counteracted testosterone-induced improvement of dendritic spine density in the hippocampal CA1 region of an Alzheimer's disease mouse animal model (Jia et al., 2016). The AR was also reported to mediate DHT-induced suppression of neuronal injury in rat organotypic hippocampal slice cultures as shown by flutamide treatment (Ishihara et al., 2016). However, in a male mouse model of cerebral ischemia, the AR was found to be involved in both neuroprotective and deleterious effects of androgens (Uchida et al., 2009).

In CNS lesions induced by chronic treatment with toxic agents, testosterone was effective in stimulating the formation of new myelin and in reversing myelin damage in the brain (Hussain et al., 2013). In the ventral spinal cord, it favored astrocyte recruitment and spontaneous oligodendrocyte-mediated re-myelination (Bielecki et al., 2016). Both types of glial cell express the AR. Interestingly, testosterone failed to induce these effects in Tfm mutants and neural *AR* knock-out mice, indicating a main role of this receptor (Bielecki et al., 2016; Hussain et al., 2013).

Although less documented than the other CNS effects of androgens, a regulatory role of circadian responses has been suggested. This hypothesis was based on high AR expression in the mouse suprachiasmatic nucleus, the brain clock controlling circadian rhythms in physiology and behavior (Iwahana et al., 2008). Hypothalamic implants of testosterone in this nucleus increases AR expression and restores locomotor activity in gonadectomized male mice (Model et al., 2015).

7. Conclusions and future directions

Information from in vitro and in vivo models is progressively allowing a more precise view of the neural role of AR in rodents. This helps to open and consider new perspectives in human studies since androgens are also suggested to modulate several brain functions related to

reproduction, cognition, anxiety or neuroprotection in man. However, much remains to be discovered and many questions still need to be addressed. For instance, although the effects of androgens are widely studied in the brain, the mechanisms underlying their effects in each brain area or nucleus still remain to be identified. In particular, AR targets and mediators remain largely unknown. A better knowledge of AR signaling pathways would be of particular relevance in studies of the physiological regulation of reproductive and non-reproductive functions as well as in physio-pathological situations such as exposure to anabolic androgenic steroids or endocrine disrupters with anti-androgenic activities. Furthermore, AR is expressed not only in neurons but also in glial cells including astrocytes, oligodendrocytes and microglia, which play a key role in brain physiology and behavior. Models targeting *AR* expression in each of these cell types would be of great help. Finally, more studies are needed in females. Although expressed to a lesser extent than in males, AR is present in the female brain, but its role in female brain physiology is not yet clear.

Acknowledgments

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Figure Legends

Figure 1. Neural *AR* knockout males were generated by crossing *AR* floxed mice (Exon 2 flanked by loxP sites; De Gendt et al., 2004) and transgenic animals carrying the Cre recombinase under the control of the promoter and neural enhancer of nestin (Tronche et al., 1999). The expression of the Cre recombinase in neural (neuronal and glial) cells triggers the excision of the *AR Exon 2* (Raskin et al., 2009).

Figure 2. Simplified scheme of the neural circuitry underlying male sexual behavior. **A-B**. Pheromonal cues emitted by receptive females are detected by the olfactory system and signals are transmitted to the medial amygdala (MeA), bed nucleus of stria terminalis (BNST) and to the hypothalamic medial preoptic area (POA). **C**. Signals are also transmitted from the hypothalamus (paraventricular nucleus) to spinal areas involved in erection such as the spinal nucleus of the bulbocavernosus (SBN). All these brain and spinal areas express the AR.

Figure 3. Behavioral analysis of neural *AR* knockout males backcrossed for at least nine generations into strain C57BL/6. Both control and mutant littermates were castrated and supplemented with testosterone implants in order to normalize their hormonal levels as previously described (Raskin et al., 2012). Males (n = 12-15 per genotype) were tested twice (Test 1: naïve, Test 2: sexually experienced) with a week-interval in the presence of receptive females (ovariectomized and primed with estradiol and progesterone). Two-way ANOVA showed a significant effect of genotype on the latencies to the first mount (Mo: $F_{(1, 25)} = 20.84$, p = 0.0001), thrust (Th: $F_{(1, 25)} = 26.45$, p < 0.0001), intromission (In: $F_{(1, 25)} = 26.28$, p < 0.0001) and to ejaculation (Ej: $F_{(1, 25)} = 26.88$, p < 0.0001). There was also a significant effect of experience on Th ($F_{(1, 25)} = 7.488$, p = 0.0113), In ($F_{(1, 25)} = 6.164$, p = 0.0201) and Ej ($F_{(1, 25)} = 6.307$, p = 0.0189). There was no amelioration of behavior induced by sexual experience in

mutants by contrast to their control littermates, which exhibited reduced latency to ejaculation

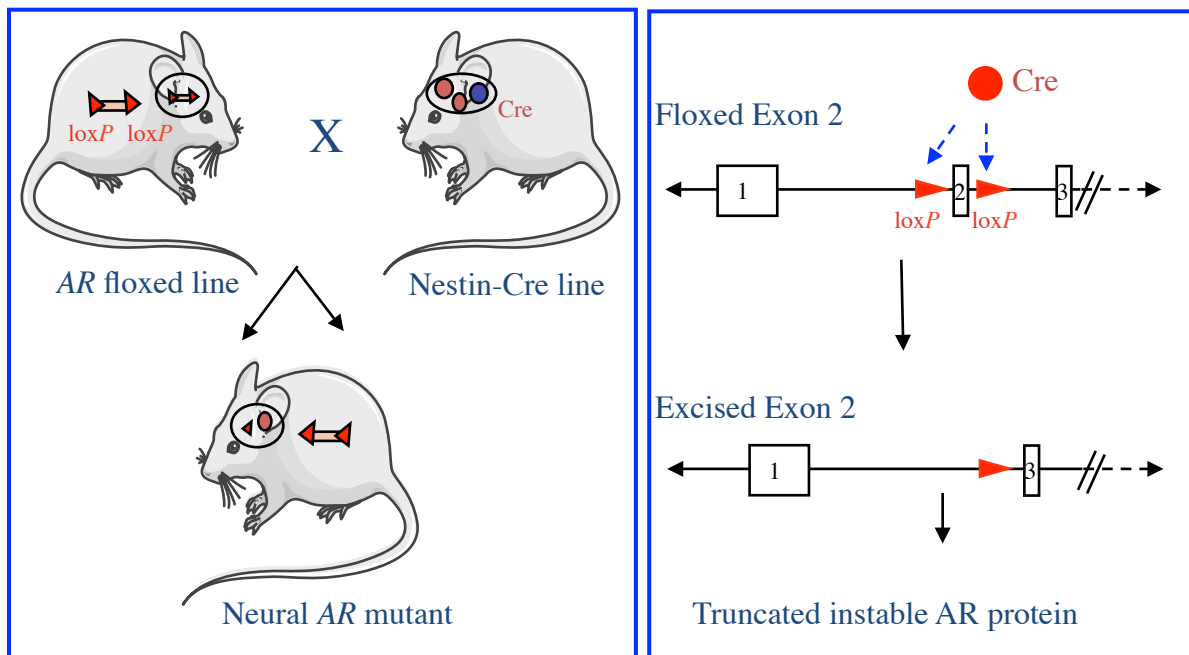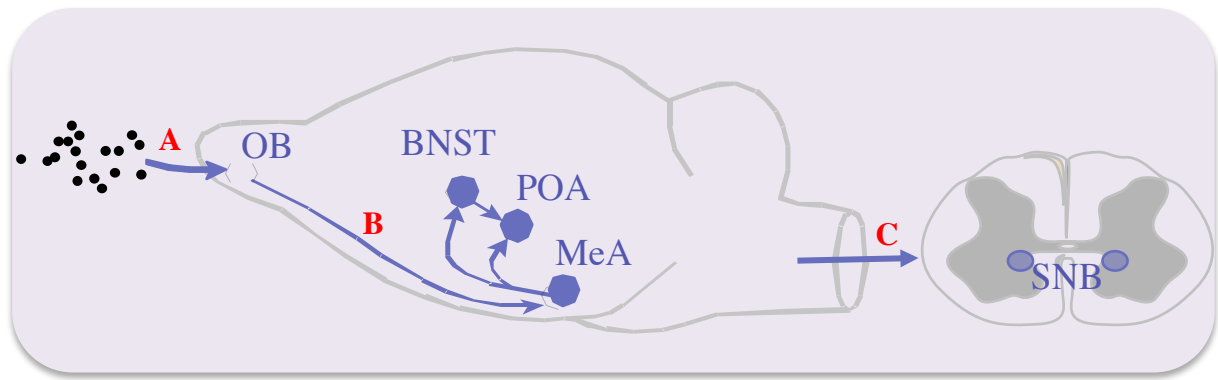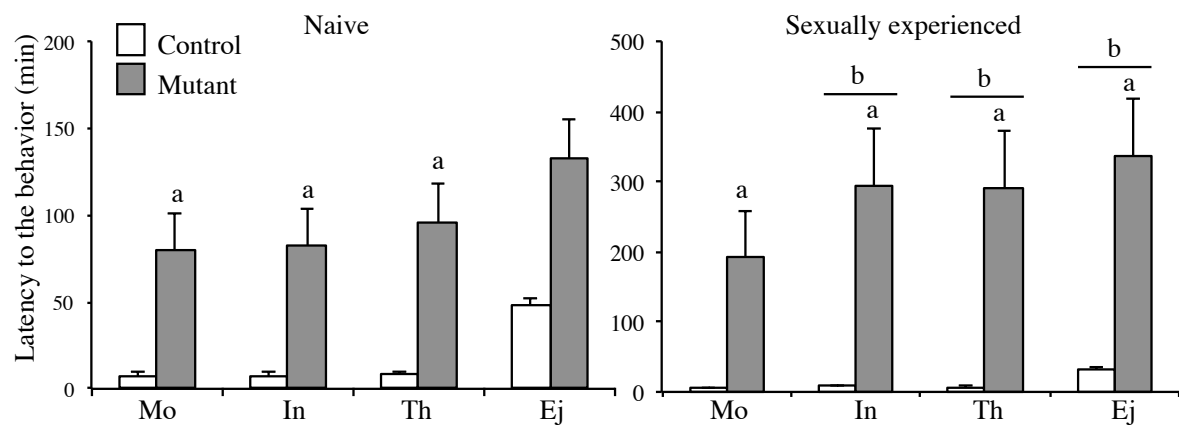when sexually experienced. a, $p < 0.05$ vs control males; b, $p < 0.05$ vs naïve males.

Figure 1

Figure 2

Figure 3

Table 1

AR mutation	Spontaneous		Transgenic mouse lines crossed		
	Tfm	*Reference*	*Floxed AR*	*Cre recombinase*	*Reference*
Global	Rat	Allison et al. 1971	Exon 2	ACTB-Cre	Yeh et al. 2002
	Mouse	Lyon & Hawks, 1970	Exon 1	CMV-Cre	Sato et al. 2003; Sato et al. 2004
			Exon 2	PGK-Cre^m	De Gendt et al. 2004
Neural knockout: neuronal and glial			Exon 2 (De Gendt et al. 2004)	Nestin-Cre (Tronche et al. 1999)	Raskin et al. 2009 (C57BL/6 x 129SvEv) Juntti et al. 2010 (C57BL/6 x 129SvEv) Raskin et al. 2012 (C57BL/6) Marie-Luce et al. 2013 (C57BL/6) Picot et al. 2014 (C57BL/6) Picot et al. 2016 (C57BL/6) Karlsson et al. 2016 (C57BL/6) Chen et al. 2016 Dombret et al. in press (C57BL/6)
Neuronal knockout:			Exon 2 (Yeh et al. 2002)	Synapsin-I-Cre (Cohen et al. 2001)	Yu et al. 2013

AR overexpression			*Transgenic mouse lines*		*Reference*
Global			CMV-Stop-AR	CMV-Cre	Swift-Gallant et al. 2016a (C57BL/6)
Neural:neuronal and glial			CMV-Stop-AR	Nestin-Cre (Tronche et al. 1999)	Swift-Gallant et al. 2016b (C57BL/6)

Genetic models used to delineate the role of AR in CNS functions. Models with global or selective (neural, neuronal) *AR* mutation or overexpression are presented. For each genetically engineered model, the two mouse lines crossed to induce Cre-loxP-mediated invalidation or overexpression are shown. The genetic background of mouse lines is indicated when available. ACTB: actin beta; AR: androgen receptor; CMV: cytomegalovirus, PKG: phosphoglycerate kinase-1; Tfm: Testicular feminization mutation.

Table 2

Region	Neuronal population	Dimorphism	Technique	Effect of neural *AR* deletion	Reference
Preoptic nucleus	Kisspeptin neurons Calbindin neurons	Cell number (M < F) Cell number (M > F)	IHC	Unchanged Unchanged	Picot et al., 2014
BNST	AVP neurons Galanin neurons	Cell number (M > F) Cell number (M > F)	ISH	Unchanged Unchanged	Marie-Luce et al., 2013
Septum	AVP Fibers Galanin Fibers	Density (M > F) Density (M > F)	IHC	Unchanged Unchanged	Marie-Luce et al., 2013
Spinal cord	SNB motoneurons	Cell number (M > F) Soma size (M > F) Dendritic arborization (M > F)	Retrograde labeling	Unchanged Reduced Reduced	Raskin et al., 2012
Spinal cord	GRP neurons	Cell number (M > F) Fiber density	IHC	Reduced Reduced	Sakamoto et al., 2014

Analysis of sexually dimorphic populations in brain (preoptic nucleus, BNST, septum) and spinal regions of male mice lacking the neural androgen receptor gene (*AR;* Raskin et al., 2009) and backcrossed onto the C57BL/6 strain. M > F and M < F designate cell number, soma size, fiber density or dendritic arborization, which are higher or lower, respectively, in males (M) compared to females (F) as previously shown (Breedlove et al., 1980; De Vries et al., 1990; Goldstein et al., 1990; Kauffman et al., 2007; Orikasa & Sakuma, 2010; Sakamoto et al., 2008; van Leeuwen et al., 1985). AVP: arginine-vasopressin; BNST: bed nucleus of stria terminalis; GRP: gastrin-releasing peptide; IHC: immunohistochemistry; ISH: in situ hybridization; SNB: spinal nucleus of the bulbocavernosus.