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Role of Androgens and the Androgen Receptor in Remodeling of Spine Synapses in Limbic Brain Areas

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Abstract

Accumulating evidence indicates that structural synaptic plasticity in limbic areas plays a vital role not only in normal brain functions, such as cognition and mood, but also in the development of neurological and mental disorders. We have learned from studies investigating neuronal remodeling that estrogens have an exceptional synaptogenic potential that seems to be specific to limbic areas of the adult female brain. On the other hand, structural synaptic plasticity in the adult male brain and the synaptogenic effect of androgens received relatively little attention. During the last five years, the Leranth laboratory provided conclusive evidence that the hippocampus and prefrontal cortex of adult male rodents and non-human primates retain considerable structural synaptic plasticity similar to the female, and that androgens are capable of inducing spine synapse growth in both the hippocampus and prefrontal cortex similar to estrogens. Our recent work also demonstrates that androgen-induced remodeling of spine synapses in the prefrontal cortex of adult male rats is dependent, at least to some extent, on functional androgen receptors, while being entirely independent of the androgen receptor in the hippocampus. Based on these findings and on their many beneficial effects, we believe that androgens hold a great and undeservingly neglected therapeutic potential that could be employed to reverse synaptic pathology in various neurocognitive and neuropsychiatric disorders.

Keywords

Androgen; androgen receptor; testicular feminization mutant; hippocampus; prefrontal cortex; spine synapse; electron microscopic stereology

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Introduction

During recent years, the conventional view of the adult brain as an anatomically “fixed” structure has dramatically changed. As the pace of new developments has accelerated, there is now widespread acceptance of the idea that even the adult mammalian brain retains considerable structural plasticity. Neurogenesis continues in adulthood, while synapses are dynamically lost and formed, sometimes with extraordinary rapidity. More importantly, accumulating evidence indicates that structural synaptic plasticity in limbic areas plays a vital role not only in normal brain functions, such as cognition and mood, but also in the development of neurological and mental disorders. All of this has evolved from a most unexpected source. The initial observations and much of the ensuing evidence have come from studies on the effects of steroid hormones, the sex steroids playing a particularly important role. Much of the work on sex steroid-induced neuroplasticity has focused on the effects of estrogens, which participate in orchestrating sexual differentiation in development, as well as modulating adult hypothalamic and limbic structure (Parducz et al., 2006). Since 2003, when we reported that hippocampal spine synapse growth in castrated, adult male rats cannot be induced by estradiol but it is triggered by androgens (Leranth et al., 2003), it has become clear that androgens are capable of modulating structural synaptic plasticity in the adult brain via mechanisms considerably different from those utilized by estrogens. As a result, more studies and exciting findings have followed, both from our and other laboratories, most of which are summarized in this review.

Androgens and Higher Brain Functions

It is less known, surprisingly, that in *both* men and women, androgens comprise a substantial component of the total circulating pool of sex steroids in young adults, but then their production declines markedly with age. Circulating levels of the adrenal androgen, dehydroepiandrosterone (DHEA), in particular, undergo a precipitous drop over the course of middle age, falling more than 70% between the 3rd and 6th decades of life (Labrie et al., 2003). It has been hypothesized that such a change in androgen levels may contribute to variations in cognitive function, as well as to the incidence of certain types of neurological disorders, with cognitive dysfunction being their prominent symptom. Several studies provide support for this idea, as investigating androgen levels in patients with Alzheimer’s disease have demonstrated a reduction in the circulating concentrations of testosterone and DHEA-sulfate, in comparison to normal controls (Hogervorst et al., 2001; Sunderland et al., 1989; Yanase et al., 1996). A recent National Institute of Aging study has reported that older men with low levels of free circulating testosterone appear to be at higher risk of developing Alzheimer’s disease than men with higher serum levels of this hormone (Moffat et al., 2004). Subsequent research has also found androgens to have clear positive effects on cognitive performance in human beings, as well as in laboratory animals (Almeida et al., 2004; Bimonte-Nelson et al., 2003; Dohanich, 2002; Flood et al., 1995; Hirshman et al., 2004; Janowsky et al., 2000; Salminen et al., 2004). In addition, androgens appear to elicit a well-characterized positive influence on mood (Almeida et al., 2004; Barrett-Connor et al., 1999; Delhez et al., 2003; Seidman, 2003).

Androgen-Induced Spine Synapse Formation in the Hippocampus

Why is structural synaptic plasticity in limbic brain areas so important to study? Because based on mounting evidence, it appears that growth of dendritic spines and formation of their synapses represent a morphological substrate for learning and memory (Geinisman et al., 2001; Kasai et al., 2003; Lang et al., 2004; Silva, 2003). Moreover, in a recent review article, we have proposed that remodeling of hippocampal spine synapses may play a critical role in the mechanisms of depression and antidepressant therapy (Hajszan and MacLusky, 2006). Thus,

the synaptogenic activity of sex steroids may be an important component in the line of cellular and molecular responses that ultimately lead to beneficial effects on cognition and mood. It has been known for more than a decade that estradiol mediates the fluctuation of CA1 pyramidal cell spine synapse density during the estrus cycle of adult female rats (Woolley and McEwen, 1992). This finding has provided the first direct evidence that sex steroids indeed modulate the remodeling of spine synapses in the hippocampus, a limbic brain area with critical influence on mood, as well as on spatial, declarative, and contextual memory. At the same time, however, although a few studies have provided some positive indications (Harley et al., 2000; Lewis et al., 1995; Shors et al., 2001), no one knew whether the adult male retains hippocampal structural synaptic plasticity similar to the female, and whether androgens are capable of inducing hippocampal spine synapse growth similar to estrogens.

Male rat

In 2003, our laboratory provided definitive answers to both of the above questions. The initial work demonstrated that about half of the CA1 spine synapse pool is lost following castration in male rats, which was reversed by testosterone supplementation (Leranth et al., 2003). The issue became more exciting when we looked into potential mechanisms. The hippocampus is rich in androgen receptor-expressing cells (Kritzer M., 2004; Sar et al., 1990; Simerly et al., 1990), indicating that it is a target for testosterone action. In the CA1 area, androgen receptors appear to be located primarily in pyramidal neurons (Kerr et al., 1995). A reasonable interpretation of these findings may be that androgens regulate CA1 spine synapse density through androgen receptor-dependent mechanisms. However, given that the male hippocampus expresses the aromatase enzyme (Hojo et al., 2004; MacLusky et al., 1987), it also seems conceivable that testosterone works via intrahippocampal estrogen biosynthesis. To test these ideas, castrated rats were given additional replacement treatments with a non-aromatizable androgen, 5 α -dihydrotestosterone (DHT), or estradiol. Our expectation was that if the responses were due to intrahippocampal estrogen biosynthesis, then the negative impact of castration on synapses would be reversed by treatment with testosterone or estradiol, but not by the non-aromatizable DHT. In fact, the data revealed a completely different response. Estradiol had no significant effect, while DHT reproduced the actions of testosterone by returning CA1 spine synapse density to levels comparable to those in gonadally intact males (Leranth et al., 2003). These results suggest two important conclusions. First, because DHT cannot be converted to estradiol, and more importantly, because the male hippocampus did not respond to estradiol, the data indicate that androgens *per se* can induce spine synapse formation, without estrogen mediation, in the adult male hippocampus. Second, the hippocampal synaptogenic response to estradiol is sexually dimorphic. Thus, while previous data have repeatedly demonstrated how sensitive the female is to estradiol-mediated remodeling of CA1 spine synapses (Leranth et al., 2000b; Woolley and McEwen, 1992), the male apparently does not respond in the same way.

Female rat

A question raised by the above data was whether androgens also affect hippocampal spine synapse density in the female rat, or the synaptogenic response to androgens in the hippocampus is also sexually differentiated: strong in the male and absent in the female. Physiologically, this question is important because androgens are present in the circulation and play important roles not only in males, but also in females (Labrie et al., 2003; Rush and Blake, 1982). We treated ovariectomized female rats with testosterone or DHT, in combination with the non-steroid aromatase inhibitor, letrozole. Testosterone approximately doubled the density of CA1 spine synapses, compared to gonadectomized controls (Leranth et al., 2004a), a response comparable to that previously observed in males (Leranth et al., 2003). This change was almost completely inhibited in the presence of letrozole. DHT treatment increased CA1 spine synapse density to a level slightly but significantly less than that observed after

testosterone. In case of DHT, letrozole did not significantly diminish the synaptogenic effect (Leranth et al., 2004a).

The preceding data suggest that, in both sexes of rats, androgens are capable of inducing formation of CA1 spine synapses. In the male, while DHT reproduces the effect of testosterone, there is no response to estradiol. In case of the female, the synaptogenic activity of testosterone reflects a large contribution from intrahippocampal estrogen biosynthesis. However, aromatization is clearly not essential, even in the female, as DHT induces spine synapse formation in both sexes. Finally, it has to be noted that one might argue with the interpretation of the above findings based on the fact that these initial studies have investigated only spine synapse densities. However, most of these experiments were repeated later, as parts of follow-up studies, using more sophisticated stereology techniques that take into account potential confounding variables, such as changes in tissue volume. The results of these repeated experiments (we discuss some of them later in this review) fully validated the data presented above.

Male non-human primate

A vitally important question is, in terms of relevance of the above-discussed findings to human beings, whether similar changes do occur in the primate brain. Major differences have been shown between primates and rats in the morphology and functional interconnections of the hippocampus. Rats have a 4-day estrous cycle, while humans have a 28-day menstrual cycle, indicating further substantial disparities in their endocrine systems, particularly in the hypothalamic - pituitary - gonadal axis. On the other hand, the monkey and human hippocampus are structurally similar (Rosene and Van Hoesen, 1987), and monkeys and humans both have a menstrual cycle with a true luteal phase, as opposed to the much shorter estrous cycle of rats. Therefore, determining whether sex steroids influence spine synapse growth in the brain of non-human primates could provide a good indication of whether similar changes are likely in humans.

To investigate whether androgens regulate the remodeling of CA1 spine synapses in the primate, we utilized male St. Kitts vervet monkeys (*Chlorocebus aethiops sabaues*). Animals either gonadally intact or castrated for one month were examined. In the castrated males, there was a significant 40% decrease in CA1 spine synapse density compared to gonadally intact monkeys. This observation indicates that similar to male rats, normal levels of circulating testicular hormones are required for the maintenance of hippocampal spine synapses (Leranth et al., 2004b).

Female non-human primate

To date, there is no information as to whether androgens are capable of inducing hippocampal spine synapse growth in female primates, but direct evidence for regulation of CA1 spine synapse density by estrogens in female primates was first obtained in our laboratory. Female St. Kitts vervet monkeys (*Chlorocebus aethiops sabaues*) of reproductive age were ovariectomized, and they received 4-cm silastic capsules either empty or filled with estradiol-benzoate, implanted below the skin of the back at the time of ovariectomy. One month later, there was a striking difference in the density of spine synapses between the two groups, with estrogen-replaced animals having approximately 40% more CA1 spine synapses than control monkeys (Leranth et al., 2002). This finding indicates that estrogens are essential in maintaining the morphological integrity of the female primate hippocampus. These data, together with additional observations on the beneficial effects of estrogens on other brain structures, e.g., (Kritzer M.F. and Kohama, 1999; Leranth et al., 2000a), further support the view that despite the differences that exist between primates and rodents, the influence of estrogens on cortical anatomy may well be evolutionarily conserved.

Androgen-Induced Spine Synapse Formation in the Prefrontal Cortex

While much of the work on the synaptogenic effects of sex steroids so far has focused on the CA1 area, it is important to recognize that this phenomenon is clearly not confined to the hippocampus. In humans, non-human primates, and rats, the prefrontal cortex is also involved in cognitive, emotional, and locomotor functions, including spatial orientation, habituation, temporal ordering, and most importantly, working memory (Kolb, 1984). Similar to the hippocampus, there is growing evidence that the prefrontal cortex retains considerable potential for structural synaptic plasticity in adulthood. For example, it is well established that in rat and monkey models of schizophrenia, subchronic administration of the non-competitive NMDA-receptor antagonist, phencyclidine, impairs attention and performance in prefrontal cortex-dependent cognitive tasks (Jentsch et al., 1997). We have shown that the number of asymmetric spine synapses in the prefrontal cortex of phencyclidine-treated male rats is significantly decreased when compared to controls (Hajszan et al., 2006). Recent human clinical and animal behavioral studies suggest that treatment with estrogens enhances performance not only in hippocampus-associated memory tests, but also in working memory tasks that are reliant on the prefrontal cortex (Duff and Hampson, 2000; Keenan et al., 2001; Rapp et al., 2003). Whether this estrogen-induced improvement in prefrontal cortex-dependent cognitive function is associated with remodeling of prefrontal spine synapses, not to mention androgen effects, has remained a question for a long time.

Male and female rat

Until very recently, there was no direct, electron microscopic evidence available about the existence of structural synaptic plasticity in the prefrontal cortex of *any* species. We have just reported that both androgens and estradiol are effective in inducing spine synapse formation in the medial prefrontal cortex of castrated male rats, although estradiol was significantly less potent (Hajszan et al., 2007). The most interesting aspect of these findings is the response to estradiol. A few studies indicate that, similar to androgens (Janowsky et al., 2000), estrogens are also effective in enhancing cognitive performance in males (Friedman, 2000; Kampen and Sherwin, 1996), as well as in females. In contrast to the beneficial cognitive effect of estrogens in males, our previous study indicates that estradiol does not increase the density of CA1 spine synapses in castrated male rats (Leranth et al., 2003). This finding seems to contradict the hypothesis that spine synapse remodeling contributes to changes in cognitive performance. Although estradiol was not as potent as androgens, our findings show that estradiol is capable of enhancing spine synapse formation in the medial prefrontal cortex of castrated male rats (Hajszan et al., 2007). Thus, spine synapse remodeling in the prefrontal cortex, rather than in the hippocampus, may contribute to the cognitive benefits of estrogen treatment in males.

On the other hand, the existence of structural synaptic plasticity in the prefrontal cortex of adult female rodents still remains to be proven.

Non-human primate

The majority of what we know about dendritic spine plasticity in the prefrontal cortex has come from the monkey studies performed in the Morrison and Rapp laboratories (Hao et al., 2007). Estrogens improve prefrontal cortex-related cognitive performance of ovariectomized non-human primates (Rapp et al., 2003) and rats (Wallace et al., 2006). In line with this, a recent study using spinophilin as a marker for dendritic spines shows that in layer I of the prefrontal cortex, estrogen-replacement increases the number of spines by 55% when compared to ovariectomized, vehicle-treated monkeys. Other layers of the prefrontal cortex were not affected. Furthermore, no differences have been observed in the number of spines in layer I of the visual cortex (Tang et al., 2004). In another elegant study performed by the same group (Hao et al., 2006), the authors have analyzed the potential beneficial effects of estrogens

(administered every 3 weeks for 2–3 years) in the prefrontal cortex of aged (~22 years old), ovariectomized, non-human primates. Although administration of estrogen did not affect several dendritic parameters, it increased apical and basal dendritic spine density and induced a shift toward smaller spines, a response linked to spine motility, NMDA-mediated activity, and learning. These observations clearly demonstrate that the aged prefrontal cortex, at least in the primate, remains responsive to long-term, cyclic estrogen treatment. It has to be noted, however, that in the female rat, aging seems to impair recognition memory and decreases the density of dendritic spines, which may be due to reduced sensitivity to estrogen (Wallace et al., 2007).

Unfortunately, we still have no published electron microscopic evidence for the existence of structural synaptic plasticity in the prefrontal cortex of adult primates. In a preliminary study (Hajszan, T., MacLusky, N.J., Leranath, C., unpublished observation), we calculated the density of spine synapses in layer I of the prefrontal cortex (Walker's area 46) in gonadally intact and castrated adult male St. Kitts vervet monkeys (*Chlorocebus aethiops sabaeus*), as well as in ovariectomized+vehicle-treated and ovariectomized+testosterone-treated adult female monkeys. For the latest updates on our electron microscopic stereology methods, see (MacLusky et al., 2006) and (Hajszan et al., 2006). Density of spine synapses in gonadally intact male animals was ~70% higher than that in castrated monkeys, while the density of spine synapses was ~80% higher in ovariectomized+testosterone-treated females when compared to ovariectomized+vehicle-treated animals (Fig. 1). These preliminary findings support the expectations that structural synaptic plasticity is retained in the prefrontal cortex of adult primates, and that androgens may mediate the growth of prefrontal spine synapses in both sexes of the primate.

Is the androgen receptor involved - or not?

We have concluded above that in certain cases, androgens induce the formation of CA1 spine synapses via androgen specific mechanisms (Leranath et al., 2004a; Leranath et al., 2003). A reasonable interpretation of this finding may be that androgens regulate CA1 spine synapse density through androgen receptor-dependent mechanisms. In the hippocampus, particularly in the CA1 subfield, there is a high level of androgen receptor expression both at nuclear (Kritzer M., 2004; Sar et al., 1990; Simerly et al., 1990) and extranuclear locations (DonCarlos et al., 2003; Tabori et al., 2005). Therefore, a plausible hypothesis for the effects of androgens in both males and females may be that these steroids activate CA1 synaptogenesis via intrahippocampal androgen receptors. There are reasons, however, to question the involvement of the androgen receptor.

Probably the most challenging question is the pharmacological specificity of the hippocampal synaptogenic response to androgens, as it is not what one might expect for an androgen receptor-mediated effect. Different androgens have radically different potencies in the reproductive tract, based largely on the way that they interact with the ligand-binding domain of the androgen receptor. Thus, DHT has a rather high affinity for the androgen receptor and is a very potent androgen, in organs such as the ventral prostate gland. Testosterone is a somewhat less potent androgen (Bardin and Catterall, 1981; Naess, 1976), and other androgens, such as DHEA, have relatively low androgen receptor affinity, and exert weak effects on non-neural androgen target tissues (Labrie et al., 2003). These effects are all blocked by androgen receptor antagonists, such as flutamide (Richie, 1999). Studies designed to examine the specificity of the CA1 synaptic response to androgens, with and without flutamide pretreatment, have yielded results that were discordant with predictions based on systemic androgenic potency. Thus, in castrated male rats, treatment with comparable doses of DHEA, DHT, or testosterone induced almost identical CA1 spine synapse levels (Leranath et al., 2003; MacLusky et al., 2004), despite the fact that these treatments had different effects on

ventral prostate weight in the same animals (MacLusky et al., 2004). Moreover, flutamide appeared to be an androgen *agonist* as far as remodeling of CA1 spine synapses was concerned. Treatment with flutamide alone, in either males or females, induced an increase in CA1 spine synapse density, with the synaptogenic potency of flutamide being about half of those of the androgens. Co-administration of flutamide with the androgens demonstrated that the effects are additive, rather than competitive. In both sexes, co-administration of flutamide further increased CA1 spine synapse densities, to levels we had never previously observed in *any* animals following androgen administration alone (MacLusky et al., 2004).

Although these data with flutamide are intriguing, they do not yet lead to a definitive conclusion regarding the involvement of the androgen receptor in hippocampal synaptogenesis. Thus, we brought our studies a step further by utilizing testicular feminization mutant (Tfm) male rats. In the rat, the *Tfm* mutation results in the transcription of a defective androgen receptor, with a glutamine for arginine substitution at position 734 in the ligand-binding domain (Yarbrough et al., 1990). This reduces the binding capacity of the receptor to ~10–15% of normal.

Adult synaptogenesis in the hippocampus of Tfm rats

We have investigated the effects of DHT, the antiandrogen hydroxyflutamide, and estradiol on CA1 dendritic spine synapses in adult male Tfm and littermate wild-type rats (MacLusky et al., 2006). In wild-type males, DHT and hydroxyflutamide both induced CA1 spine synapse growth. DHT almost doubled the number of spine synapses observed, whereas hydroxyflutamide increased spine synapse levels by approximately 50%, compared to vehicle-injected controls. Surprisingly, in Tfm males, the effects of DHT and hydroxyflutamide were all indistinguishable from those observed in wild-type animals. In the case of estradiol, the hormone remained ineffective both in Tfm and wild-type rats, in line with our initial observations (Leranth et al., 2003). Despite the reduction in androgen sensitivity because of the Tfm mutation, hippocampal synaptic responses to both DHT and the androgen antagonist, hydroxyflutamide, remain intact in Tfm males. These data are consistent with our previous results and expectations that androgen effects on hippocampal spine synapses may involve response mechanisms other than the androgen receptor (MacLusky et al., 2006).

Adult synaptogenesis in the prefrontal cortex of Tfm rats

Analyzing the prefrontal cortex of the same animals used in the previous study, we have observed a considerable decrease in the number of prefrontal spine synapses both in gonadally intact and in DHT-treated, castrated Tfm males, when compared to their wild-type counterparts (Fig. 2). Thus, the potency of DHT to enhance spine synapse formation in the prefrontal cortex of castrated Tfm males is markedly reduced, but not completely lost (Hajszan et al., 2007). The most logical conclusion is that androgen-induced prefrontal spine synapse remodeling in male rats is dependent, at least to some extent, on functional androgen receptors. In this respect, the prefrontal cortex is considerably different from the hippocampus, where the effect of androgens on spine synapse remodeling seems to be entirely independent of the androgen receptor (MacLusky et al., 2006). Nevertheless, the mechanisms mediating the *residual* synaptogenic activity of androgens in the prefrontal cortex are still unknown; and the important question remains...

If not the androgen receptor, then what?

There are potential mechanisms of androgen action in the brain that do not require androgen receptor participation. Here we draft our theory that seems to be the most supported by available data. In the original studies from Bruce McEwen's laboratory on CA1 spine density during the estrous cycle (Gould et al., 1990; McEwen and Woolley, 1994; Woolley and McEwen, 1993), two distinct hormonal contributions have been identified. Estradiol shows an inductive influence leading up to the time of ovulation, followed by a biphasic effect of progesterone,

initially potentiating the dendritic response to estradiol (within the first 2–6 h), and then later inhibiting it. Woolley has concluded, in part based on studies of GABA-mediated responses in cultured hippocampal neurons (Murphy et al., 1998), that estradiol effects on dendritic spine density involve GABA-mediated disinhibition of pyramidal neurons. A potential anatomical substrate for GABA-mediated “feed-forward” disinhibition of the hippocampus was described almost 20 years ago. GABAergic afferents originating from the septum innervate inhibitory interneurons in the hippocampus (Freund and Antal, 1988), consistent with the idea that hormonal activation of sub-cortical afferent inhibitory pathways could lead to disinhibition of pyramidal neurons (Leranth et al., 2000b). The biphasic effects of progesterone are also consistent with a critical role for GABA-mediated responses. Progesterone, like DHT, is rapidly converted in the brain to 5 α -reduced metabolites, some of which potentiate GABA action on the GABA-benzodiazepine-chloride channel complex (Purdy et al., 1990). Our hypothesis is that the immediate effect of introducing progesterone may be to further enhance the activity of afferent GABAergic input to the hippocampus. Over time, however, progesterone acting via progesterone receptors may antagonize the effects of estradiol, as it has been shown in other tissues (Okulicz et al., 1981), thereby terminating the dendritic response.

Hypothetically, the effects of both androgens and flutamide could be mediated via similar mechanisms. DHT and other natural androgens are converted in the brain to 5 α -androstane-3 α ,17 β -diol, which like the analogous metabolite of progesterone, allopregnanolone, potentiates the activity of GABA on the GABA-benzodiazepine-chloride channel complex (Frye et al., 1996; Reddy, 2004). Therefore, the effects of the androgens could be mediated in much the same way as the rapid responses to progesterone, via enhancement of GABAergic neurotransmission. Flutamide at high doses could partially activate the same response mechanism, because flutamide has been reported to have weak benzodiazepine-like activity (Ahmadiani et al., 2003). The idea that androgens could act via the GABA receptor system is attractive, because it could potentially provide a common cellular mechanism for regulation of adult synaptogenesis by both the male and female sex steroids.

Another metabolite of DHT, 5 α -androstane-3 β ,17 β -diol is a potent agonist at estrogen receptor- β (Pak et al., 2005). Handa and colleagues in this issue review this mediator of androgen effects and its role in the brain in detail. For more information, see... Regarding remodeling of spine synapses, this estrogenic metabolite may also play a significant role in mediating the synaptogenic action of androgens primarily in females, as the responsiveness of adult male synaptogenesis to estrogens is low (Hajszan et al., 2007; Leranth et al., 2003). A considerable amount of additional work remains to be done, however, to determine the extent of contributions from androgen receptor-mediated vs. androgen receptor-independent mechanisms of androgen action on the remodeling of spine synapses. Critical to these studies will be the further exploration of effects of androgens in genetic models of androgen insensitivity, in which androgen receptor expression is completely abolished (French et al., 1990). In addition, as the summary of our most important findings indicates (Table 1), more basic information is still missing. The area of our current interest is the prefrontal cortex, as we lack fundamental electron microscopic evidence in this cortical region about the existence of adult synaptogenesis in female rodents, as well as in primates. Furthermore, we don't know whether sex steroid-induced remodeling of spine synapses is specific to the prefrontal cortex, whether it is confined to certain layers here, or other cortical areas and several layers are also affected. Finally, the most important among the many questions is how we could exploit this knowledge in clinical practice? The following section provides a brief summary of the most reasonable points of clinical significance.

Concluding Remarks on Potential Clinical Implications

We have just begun to recognize that neuroplasticity, particularly hippocampal synaptic remodeling, is involved in the neuropathology of a set of disorders that currently represent the highest burden for society, such as neurodegenerative diseases, schizophrenia, depression, and stress. Because the hippocampus is commonly involved, it is not surprising that the vast majority of these patients all suffer from cognitive impairment, memory dysfunction, affective problems, as well as an abnormal stress response. Another striking feature of these hippocampus-related disorders is that sex steroids are known to influence their incidence and pathology. We mentioned above the association of testosterone levels and the risk for Alzheimer's disease in men (Moffat et al., 2004). In addition, a number of other studies have reported that the incidence of Alzheimer's disease may be reduced in postmenopausal women who have taken estrogen-based hormone replacement therapy (Sherwin, 2003). In case of schizophrenia, psychotic symptoms are exacerbated when estrogen levels are low, while estradiol treatment produces beneficial effects (Seeman, 1997). Furthermore, reproductive changes in women accompanied by abrupt declines in sex steroid levels are frequently associated with mood disorders, such as postpartum and postmenopausal depression (Steiner et al., 2003).

In light of the role of dendritic spine synapses in hippocampal function, it is not surprising that various factors causing a global reduction in the number of these synapses all lead to disturbed hippocampal function, which may commonly contribute to the above-mentioned brain disorders. A growing body of evidence indicates that Alzheimer's disease (Scheff and Price, 2003), schizophrenia (Harrison, 2004), and stress (Xu et al., 2004) are all associated with hippocampal synaptic pathology that is mainly manifested as a substantial loss of dendritic spine synapses. Furthermore, we have recently demonstrated that fluoxetine-treatment increases spine synapse density in several hippocampal areas (Hajszan et al., 2005). Synaptic pathology may play a particularly important role in rapidly evolving symptoms of both schizophrenia and depression, such as the fast onset of psychotic and depressive episodes, which are difficult to reconcile with current theories for these diseases (Henn and Vollmayr, 2004). Although we have no conclusive evidence yet that synaptic pathology is causally related to these neurological disorders, according to the potential rapidity of synaptic changes (MacLusky et al., 2005), it is reasonable to hypothesize that abrupt deterioration of hippocampal synaptic connectivity, i.e., loss of spines and their synapses, may substantially contribute to these rapidly evolving symptoms.

A critical question is whether the synaptogenic power of androgens could be employed to reverse synaptic pathology in the clinical management of the above-mentioned disorders. In light of what we have just discussed above in this review, we strongly believe that androgens hold a great and undeservingly neglected therapeutic potential. Sex steroid hormones proved to be the most potent synaptogenic agents in our hands, in terms of both rapid onset of action (by 30 min) and magnitude of effects (increase in spine synapse levels by up to 130%). They are paralleled by antidepressants (fluoxetine and desipramine) only in the magnitude of effects, as antidepressants need significantly more time (1–2 weeks in rodents) to achieve the same increase in the number of spine synapses. We don't know, of course, whether this synaptogenic potential of androgens is preserved in the severely diseased brain; the good news is, however, that the above-mentioned disorders appear to be significantly influenced by sex steroids.

Two specific properties of androgens make them attractive for clinical use, particularly when endogenous sex steroid secretion falls below normal. The first property, which has been extensively commented on by others (Labrie et al., 2003), is the ability of these steroids to act as precursors for local metabolism. This raises the possibility of "targeted" androgen treatment, designed to achieve a selective biological response. For example, aromatizable androgens with

little intrinsic androgenic bioactivity, such as DHEA, could theoretically be used to deliver estrogen precisely where it is needed in the brain, through local metabolism, without exposing other tissues in the body to either high circulating estrogen levels, or potentially unacceptable anabolic effects. Because responses to DHEA are observed in both sexes (Hajszan et al., 2004; MacLusky et al., 2004), there is no theoretical impediment to the use of DHEA for treatment of synaptic pathology as well as hormonal deficiency syndromes, associated with aging in men, as well as women (Almeida, 1999; Delhez et al., 2003). Second, the unusual specificity of the hippocampal synaptoplastic response to androgens raises the possibility of pharmacological distinction between the effects of androgens on the brain, as opposed to the masculinizing influence of these steroids elsewhere in the body. Obviously, a great deal remains to be done to determine whether such distinctions may be feasible, let alone therapeutically useful. Nevertheless, it seems to be clear that induction of CA1 spine synapse growth by DHEA is possible to achieve without systemic hormone effects (Hajszan et al., 2004; MacLusky et al., 2004), at least in rodents. Of course, the ultimate question that largely determines the relevance of our work to human health is whether the increasing number of spine synapses observed after sex steroid administration results in a concomitant enhancement of brain function, in terms of improvements in cognition and/or mood state. Fortunately, our ongoing experiments seem to reveal a strong positive relationship between spine synapse levels and performance in various behavioral tasks.

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Figure 1.

The density of spine synapses in layer I of the prefrontal cortex (Walker's area 46) in gonadally intact and castrated (GDX) adult male St. Kitts vervet monkeys (*Chlorocebus aethiops sabaeus*), as well as in ovariectomized+vehicle-treated (OVX+Oil) and ovariectomized +testosterone-treated (OVX+T) adult female monkeys.

Figure 2. Total number of prefrontal (mPFC) spine synapses in gonadally intact, castrated oil-treated (GDX+Oil), and castrated DHT-treated (GDX+DHT) Tfm (TFM, solid columns) and littermate wild-type (WT, open columns) adult male rats. Data obtained from (Hajszan et al., 2007).

Table 1
Summary of our findings in the prefrontal cortex and hippocampus of both male and female rats.

Male PFC ¹	Male HIP	Receptor manipulation	Hormone	Receptor Manipulation	Female HIP
+2	-	None	Estradiol	None	+++
+++	-	Tfm	Estradiol	Tfm	N/A
+++	+++	None	Testosterone	None	+++
+	+++	Tfm	Testosterone	Tfm	N/A
+++	+++	None	DHT	None	+++
N/A	++++	Flutamide	DHT	Flutamide	++++
+	+++	Tfm	DHT	Tfm	N/A

¹ Abbreviations: DHT, 5 α -dihydrotestosterone; HIP, hippocampus; N/A, not available; PFC, prefrontal cortex; Tfm, testicular feminization mutant.

² Magnitude of synaptogenic responses:

- , no response;
- + , moderate response;
- +++ , strong response;
- ++++ very strong response.