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Review

Behavioral and physiological responses to anabolic-androgenic steroids

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Abstract

Anabolic-androgenic steroids (AAS) are synthetic derivatives of testosterone originally designed for therapeutic uses to provide enhanced anabolic potency with negligible androgenic effects. Although AAS continue to be used clinically today, the medical benefits of low therapeutic doses of AAS stand in sharp contrast to the potential health risks associated with the excessive doses self-administered not only by elite athletes and body builders, but by a growing number of recreational users, including adolescent boys and girls. The deleterious effects of AAS on peripheral organs and the incidence of altered behaviors in AAS abusers have been well documented in a number of excellent current reviews for clinical populations. However, a comparable synthesis of nonclinical studies has not been made. Our purpose in this review is to summarize the literature for animal models of the effects of supraphysiological doses of AAS (e.g. those that mimic human abuse regimes) on behaviors and on the neural circuitry for these behaviors. In particular, we have focused on studies in rodents that have examined how AAS alter aggression, sexual behaviors, anxiety, reward, learning, and locomotion and how AAS alter the expression and function of neurotransmitter systems and other signaling molecules that underlie these behaviors.

Keywords: Anabolic steroids; Testosterone; Abuse; Rat, mouse; Hamster; Reproduction; Aggression, anxiety; Reward; Learning and memory; Locomotion; Neurotransmitter; Neurotransmitter receptors

Contents

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1. Introduction

Anabolic-androgenic steroids (AAS) are synthetic derivatives of testosterone originally designed to provide enhanced anabolic (tissue-building) potency with negligible androgenic (masculinizing) effects [79,93]. Approximately, 60 different AAS are available that vary in their chemical structure and thus in their metabolic fate and physiological effects [8,75,82,121,129,132,156]. All AAS are thought to have some androgenic activity, and the androgen receptor binding properties of several of these compounds have been characterized in brain tissue [124]. The AAS, like the endogenous androgens, are four-ringed structures with 19 carbon atoms (Fig. 1). Modifications of this androstane backbone have been introduced to prolong the metabolic half-life and thus the efficacy of synthetic AAS. Three main classes of AAS have been described. The first, used primarily as injectable compounds, are derived from esterification of the 17β-hydroxyl group of testosterone and includes testosterone propionate and testosterone cypionate. Esterification retards degradation and prolongs the duration of action after injection of the hormone by slowing its release into circulation [8,132]. Testosterone esters can be hydrolyzed into free testosterone, reduced to 5α-dihydrotestosterone, an androgen with higher biological activity at brain androgen receptors than testosterone [79,97,156], or aromatized to estrogens [79,156]. Molecules that have been 5α-reduced cannot be metabolized into estrogens but may be metabolized into other androgenic compounds such as 3α-androstanediol.

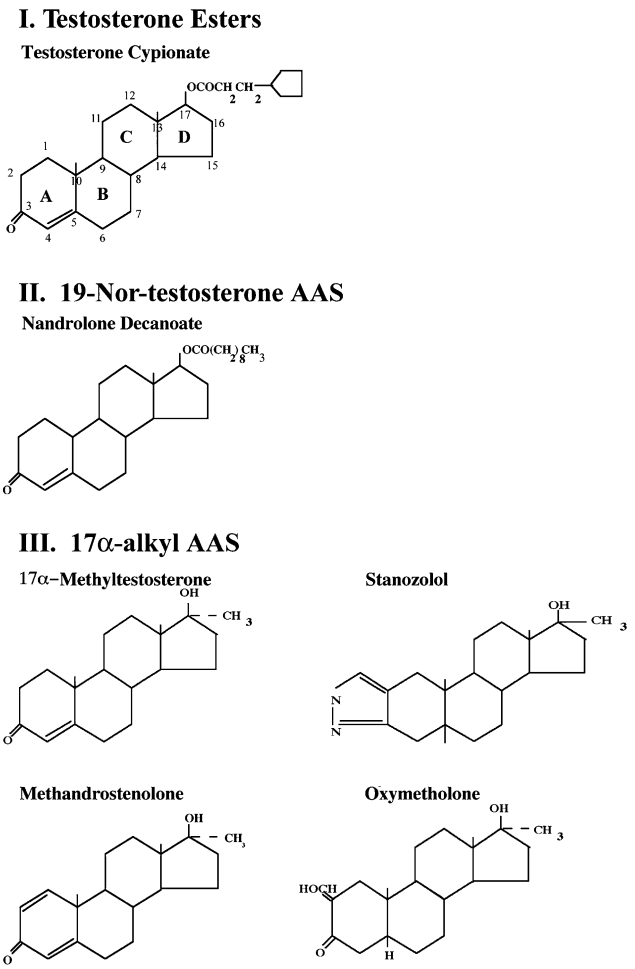

Fig. 1. Chemical structures of representative examples of the three major classes of the AAS.

The second class is also composed of injectable androgen esters called 19-nor-testosterone derivatives. These compounds have, in conjunction with the addition of long side chain moieties, a substitution of a hydrogen for the methyl group at C19 [8,132] (Fig. 1). AAS in this class include nandrolone decanoate. The substitution at C19 extends the half-life of this class of AAS beyond that contributed by esterification alone [132]. It should be noted that despite retention of the C4–C5 double bond in nandrolone decanoate, this compound has reduced androgenic activity at the androgen receptor compared to dihydrotestosterone [126,132,156]. Nandrolone decanoate, like the testosterone esters in class I, can be aromatized to 17β-estradiol, albeit only ∼20% as efficiently as testosterone [126,156]. Aromatizable AAS in both class I and class II may thus have additional and significant central nervous system effects not only at the androgen receptor, but also vis-à-vis the actions of their estrogenic metabolites at brain estrogen receptors [154].

The third class of AAS comprises those compounds that are alkylated at C17, including 17α-methyltestosterone, oxymetholone, methandrostenolone, and stanozolol (Fig. 1). Because alkylation retards metabolism by the liver, this group of AAS is orally active [8]. To the best of current knowledge, none of the 17α-alkylated steroids is converted into dihydrotestosterone or 17β-estradiol although other androgenic and estrogenic metabolites may be formed [156].

AAS were originally developed for the treatment of hypogonadal dysfunction in men, initiation of delayed puberty, and growth promotion [8,154]. They continue to be used today for these treatments, as well as for therapy in chronic conditions including HIV/AIDS, cancer, severe burns, anemia, hepatic and kidney failure, breast cancer, and

hereditary angioedema [8]. Originally synthesized to have maximal effects on protein synthesis and muscle growth with minimal androgenic or masculinizing effects, it is now clear from biochemical studies that there are no pure anabolic steroids that are devoid of androgenic actions [8,93,154]. Moreover, although originally developed for clinical use, AAS administration is now predominantly one of abuse, and the medical benefits of low doses of AAS stand in sharp contrast to the potential health risks associated with the excessive doses self-administered by elite athletes [48,49,92]. Assessments made a decade ago [161] indicate that, at that time, more than one million adult Americans had or were using AAS to increase physical strength, endurance, athletic ability or muscle mass. Over the past few decades, the misuse of AAS by college, Olympic, and professional athletes has become widely recognized [80]. However, recent reports highlight the fact that the more insidious growth in the abuse of these drugs is not among elite athletes, but among adolescent boys and girls [116]. Present estimations are that ~4% of high school students have used AAS [7,78,116,162], and the greatest increase in AAS use over the past decade has been reported in adolescent girls [7].

The potential behavioral effects of AAS abuse in human populations have received prominent coverage in a number of excellent recent reviews [6,29,48,56,82,93,162], as well as in the popular press [53,80,115,142]. However, less attention has been paid to the effects of AAS on neural circuits that underlie these behavioral effects, as determined in studies using animal models. Our purpose in this review is to summarize the literature with respect to studies employing animal models in conjunction with paradigms of AAS exposure that mimic human abuse regimes (i.e. supraphysiological doses). In particular, we have focused on studies that have examined how AAS alter aggression, reproductive behaviors, anxiety, learning and memory, reward and locomotion in rodents and how AAS alter the expression and function of neuronal signaling molecules that underlie these behaviors.

2. Experimental design

There are a number of experimental design considerations that will have an impact on the conclusions drawn by investigators assessing the behavioral and physiological effects of AAS. First, is the dose of AAS and whether it represents therapeutic androgen replacement levels or mimics human self-administration that results in supraphysiological androgen levels (abuse conditions). The present review focuses on studies that administer supraphysiological doses of AAS. Physiological doses of testosterone propionate, as assessed in gonadectomized male rats administered hormone replacement to maintain reproductive and aggressive behaviors, are ~1 mg/kg [32]. For the purposes of this review, we have focused on studies that administer doses of testosterone propionate and other AAS above 3 mg/kg/animal/day and define these doses as supraphysiological. We have limited our discussion of studies that administer lower doses to those that illustrate the contrasting effects of these low doses to those produced by AAS given at high doses. In addition, our review focuses on those AAS that humans report self-administering (in published studies) and does not address the effects of androgens that are not self-administered by humans.

Second, is the fact that AAS may be administered to laboratory animals following a variety of treatment regimens and that results will vary significantly depending on which specific regimen is employed. For example, some studies simultaneously administer combinations of two or three AAS, so-called AAS 'cocktails', to mimic the pattern of combining or 'stacking' of AAS that humans use [82]. Although the administration of combinations of AAS reflects human abuse paradigms, this method of exposure confounds interpretation of results and the understanding of the mechanism(s) because it is not known how AAS interactions affect behavior and physiological responses. In addition, AAS are heterogeneous in their effects on many endpoints, as illustrated by the fact that class I and II AAS (as defined above) may be aromatized and act at the estrogen receptor, whereas class III AAS are believed to have minimal estrogen receptor actions [156]. Conversely, while analyses of the actions of single AAS may provide better mechanistic insights, they do not as accurately mirror human patterns of self-administration.

Third, the timeframe of AAS exposure varies dramatically. In the studies described here, treatment times varied from 30 min to 6 months. The specific timeframe for AAS effects on behavior, minutes versus days, may provide valuable information regarding the underlying mechanisms. For example, behavioral responses to acute AAS administration would be consistent with allosteric modulation or post-translational actions of AAS at membrane delimited receptors, whereas behavioral responses that are evident only after long-term exposure may implicate changes in gene expression mediated by classical androgen receptor or estrogen receptor signaling pathways. However, such differences also make comparison across studies that employ widely divergent AAS exposures difficult. An overall conclusion that can be drawn from the studies presented in this review is that dramatically different results may be obtained with different experimental paradigms. Because of this, we believe that accurate interpretation of the studies presented in this review can best be made within the context of the experimental design and methodologies of these studies, and these details are provided herein.

Fourth, experiments may be performed on gonadally intact or gonadectomized animals. Numerous studies have shown that AAS have significant effects on the gonads, levels of gonadal steroids, and gonadotropins [154], however, we have concentrated on AAS effects on central nervous system

structures, and have not included a discussion of these studies in this review. Analysis of sexual behaviors in gonadally intact animals provides an excellent behavioral window on the impact of the AAS on the hypothalamic-pituitary-gonadal axis. Thus, studies in gonadally intact animals will reflect both the natural state and the intricate interactions of the exogenous AAS with the varying endogenous steroid milieu. However, the presence of circulating endogenous steroids can also confound results and make it difficult to interpret the critical neural substrates for AAS-modified behavior. For this reason, gonadectomized animals have also proved to be useful in delineating the mechanisms underlying the effects of AAS on behavior.

Finally, significant differences in AAS actions may arise not only from differences in hormonal state (e.g. intact or gonadectomized), but also with sex, age and species, and even the strain of species studied. Although there are only a few studies that have compared how sex, age or strain modify the actions of AAS, studies abound that categorically demonstrate that brain function varies significantly with sex, age, and genetic background [42,67,153]. Moreover, studies in animal models that directly assess how sex, age and strain modulate the impact of AAS on the brain and on behavior are particularly needed given reports from human subjects that indicate that some of the effects of AAS induced prior to puberty may not be reversible upon cessation of drug use [48,159] and that long-term consequences from AAS abuse may be greater in women than in men [43,48,66,68,138].

Given these experimental considerations, it should be noted that the majority of studies performed to date has used adult gonadally intact male rats. Although we discuss results from studies using other subjects, for clarity of presentation, we have not overtly designated each of these studies as having been performed on gonadally intact male rats, and the reader should assume that studies were performed in these animals unless otherwise designated.

3. Behavioral correlates of AAS administration

3.1. Aggression

Androgens have long been recognized as modulators of aggression in male rats [5,141]. Many studies of AAS effects on aggression have focused on intermale aggression, a pattern of aggressive behavior that is dependent on the presence of androgens [30]. Intermale aggression can be measured by systematically assessing the quality and quantity of aggressive acts displayed by a ‘resident’ male towards a strange or ‘intruder’ male. Lumia et al. [94] reported that long-term exposure (10 weeks) of gonadally intact Long-Evans rats to testosterone propionate (1 mg/rat 3 times per week) increased the display of dominance postures and threats, and reduced the number of submissive postures, relative to controls.

More recently, Breuer et al. [21] examined the effects of opponent status (intact or gonadectomized) and environment (home cage, opponent cage, neutral cage) on AAS-induced aggression. Testosterone propionate, nandrolone decanoate, or stanozolol was administered to Long-Evans rats (5 mg/kg 5 times per week for 12 weeks). Aggressive behavior was increased in testosterone propionate-treated rats relative to controls, whereas the nandrolone decanoate-treated and control groups exhibited similar levels of aggression. Surprisingly, stanozolol-treated rats exhibited significantly lower levels of aggression than rats receiving not only testosterone propionate or nandrolone decanoate, but also for those receiving vehicle. Rats receiving testosterone propionate continued to be responsive to the gonadal status of the opponent male, and thus were more likely to attack an intact versus a gonadectomized opponent, demonstrating that the testosterone propionate did not interfere with the ability to respond appropriately to these social cues. Regarding the environmental manipulation, whereas control rats displayed heightened aggression only in the home cage relative to the neutral cage, testosterone propionate-treated rats showed elevated aggression in both the opponent’s cage and in the home cage. The authors hypothesize that the administration of supraphysiological levels of testosterone propionate increased the propensity for aggression in male rats, while maintaining the ability to respond appropriately to social cues. Another noteworthy finding from this study was that stanozolol *suppressed* the display of aggressive behavior. The paradoxical effects of stanozolol highlight the disparity of action of different AAS and the need to understand the molecular mechanisms of each compound in the brain.

McGinnis et al. [106] tested whether a different type of provocation, mild physical provocation using a tail pinch, evokes an exaggerated aggressive response in rats receiving AAS and whether physical provocation impairs the ability of AAS-treated rats to discriminate between appropriate social and environmental cues. Rats were treated following the same three AAS conditions as above [21], 5 days a week for 12 weeks, and tested for intermale aggression beginning on week 12. Each rat underwent a brief (1 s) tail pinch and then was tested under home cage, opponent cage and neutral cage conditions with an intact or gonadectomized opponent male. Unlike controls, testosterone propionate-treated rats that received a tail pinch exhibited an increase in aggression in all social and environmental conditions; dominating their opponent whether the opponent was intact or gonadectomized and regardless of the testing condition (home, neutral or opponent’s cage). In contrast, controls that received a tail-pinch showed more aggression in their home cage than a neutral cage, and more aggression toward gonadally intact opponents. Thus, the social and environmental cues that normally modulate aggressive behavior were overshadowed by the chronic administration of high levels of testosterone propionate. In addition, testosterone propionate-treated rats also showed increased aggression when their opponents

were administered a tail pinch. The authors speculate that testosterone propionate sensitizes the rat to its surroundings and lowers the threshold to respond to provocation with aggression. The findings of McGinnis et al. [106] also confirmed those reported in Breuer et al. [21] demonstrating a lack of effect of nandrolone decanoate on aggressive behavior and a suppression of aggression in rats treated with stanozolol.

In one of the few reports to assess behavior after withdrawal from AAS, McGinnis et al. [107] recently examined whether AAS-enhanced aggression is reversible. Intermale aggression was assessed in rats following withdrawal from AAS (testosterone propionate, nandrolone decanoate or stanozolol; 5 mg/kg 5 days a week for 12 weeks). The experimental conditions (home cage, neutral cage, opponent cage) and opponent status (intact or gonadectomized) were varied on tests conducted beginning either 3 (acute condition; weeks 3–9) or 12 weeks (long-term condition; weeks 12–18) after withdrawal. Generally, on the acute tests (weeks 3–9), more rats in the testosterone propionate condition than in the control condition continued to display threats and mounts. Aggressive behavior in rats treated with nandrolone decanoate did not differ from controls whereas stanozolol-treated rats tended to display slightly lower levels of aggression than controls. By 18 weeks withdrawal from AAS, aggressive behavior in the testosterone propionate- and stanozolol-treated rats had returned to control levels. Thus, the effects of testosterone propionate and stanozolol on aggressive behavior appear to be dependent upon the continued presence of the AAS. In addition, the withdrawal from testosterone propionate did not itself induce aggressive behavior. To our knowledge no other studies have assessed aggressive behavior after long-term withdrawal from AAS.

In contrast to the aforementioned studies from McGinnis's group, Long et al. [90] reported that Sprague–Dawley rats receiving nandrolone decanoate (2 mg/day/rat or 20 mg/week/rat for 4 weeks) displayed heightened levels of aggression relative to controls. The bases for discrepancy between the findings of Long et al. [90] and more recent reports [21,106] are not clear, but may be related to differences in rat strain (Sprague–Dawley versus Long-Evans) and/or specific testing conditions; Long et al. [90] provided 2 weeks of experience with aggression before the AAS treatments began. Rats in McGinnis et al. [106] were not experienced in aggression before receiving AAS. The fact that the lack of effect of nandrolone decanoate on intermale aggression was replicated [21,106] demonstrates that under specific testing conditions nandrolone decanoate does not induce aggression in Long-Evans male rats.

In addition to studies in intact rats, the aggression-inducing properties of AAS have also been examined in gonadectomized Long-Evans rats [32]. 17α-Methyltestosterone (3 mg/day) or stanozolol (400 μg/day) was administered daily for 6 weeks to rats with tests for intermale aggression conducted on weeks 3–6. On week 6, the aggression scores for rats treated with 17α-methyltestosterone were equivalent to the levels displayed by rats treated with physiological doses of testosterone propionate (400 μg/rat) on most of the behavioral indices assessed. In contrast, stanozolol failed to elicit aggressive behavior in gonadectomized rats. These findings illustrate that AAS-induced aggression is compound-specific in gonadectomized, as well as in intact, male rats.

Bonson and Winter [18] tested the effects of testosterone propionate (30 mg/kg/day) on competitive aggression. In this task, pairs of Fischer rats were trained to compete for a food reward and a pattern of dominance is established. Once a stable dominance hierarchy was observed, testosterone propionate was administered to the non-dominant rat at the conclusion of each day's competition session. After 14 days of testosterone propionate, the previously non-dominant rats increased their level of competition compared to non-dominant rats receiving the vehicle. That is, administration of testosterone propionate reversed the dominance hierarchy. In agreement with these results, Mitchell and Wilson [114] reported that administration of unesterified testosterone (100 mg pellets) for 90 days, resulting in plasma testosterone levels of ~10 ng/ml significantly improved the success rate of rats tested on a competition task in which the incentive was copulation with a sexually receptive female. Lindqvist et al. [88] also examined competitive aggression in rats treated with nandrolone decanoate (15 mg/kg) for 14 days. Beginning one week after the last nandrolone decanoate injection, the Wistar rats were acclimated to new cages and cage mates for 3 days. On day 4, water intake was restricted and upon introduction of an active water-spout, the competitive aggressive behaviors displayed by AAS and vehicle-treated rats were recorded. At some point after the competition test, the rats then were placed in individual cages for the determination of baseline levels of water intake. The AAS-treated rats spent more time drinking during the competition task than controls, although baseline drinking did not differ between the groups. These findings are difficult to interpret because tests for competitive aggression took place 1 week after cessation of nandrolone decanoate treatment and the authors do not provide information on the clearance rate for nandrolone decanoate.

The effects of long-term treatment with stanozolol [99] and testosterone propionate [100] have also been tested in mice using an isolation-induced model of aggression. After 3 weeks of housing in isolation, the subject was paired with an anosmic stranger gonadally intact male mouse in a neutral cage and the display of aggressive behaviors was monitored. In the first set of studies [99], stanozolol was administered to Alderly Park mice at high (7 mg/kg), medium (0.7 mg/kg) or low (0.07 mg/kg) doses on alternate days for a three-week period, and tests for isolation-induced aggression were conducted 24 h after the last injection. Two cohorts of mice were tested; a peri-pubertal (29-day old) and an adult (56-day old) group. Stanozolol, at any dose tested,

had no effects on aggressive behavior in either age group, in agreement with the findings from intact [21,106] and gonadectomized [32] rats. In a later study, a range of doses of testosterone propionate was administered to OF-1 mice weekly for 10 weeks (3.75–30 mg/kg) [100] and isolation-induced aggression was measured during weeks 8–10, 24 h after the weekly testosterone propionate injection. Surprisingly, few behavioral effects of testosterone propionate were observed, with no effects on attack or threat frequency. On week 10, a significant reduction in latency to attack was observed in subjects receiving 3.75, 7.5 and 30 mg/kg testosterone propionate, but not in subjects receiving 15 mg/kg testosterone propionate. The authors speculate that the lack of responsiveness to androgens may reflect a reduced sensitivity of the OF-1 strain to the hormone-dependence of aggression. Although this hypothesis remains to be tested directly, it is known that different mouse strains vary significantly with respect to their sensitivity to endogenous gonadal steroids [137], strongly suggesting that genetic background will also influence sensitivity to AAS.

Additional studies in mice have assessed aggressive behavior following exposure to AAS combinations [22,23]. Bronson [22] administered silastic capsules containing testosterone, testosterone cypionate, 17α-methyltestosterone and norethandrolone to intact male and female CF-1 mice at low and high doses for 6 months. The latency to attack and the latency to accumulate 10 s of fighting were recorded. AAS (low or high dose) had no effect on aggression in the male mice, perhaps because of the high incidence (9/10) of fighting in the control group. In contrast, female mice receiving the AAS at low or high (5-fold the low dose) doses exhibited an increased number of attacks and were also more likely than control females to fight back when attacked. Similar results were observed in a follow-up study that focused exclusively on female mice [23]. To our knowledge, Bronson [22] and Bronson et al. [23] are the only studies to quantify AAS effects on aggression in female subjects.

Melloni and colleagues [109,110] have conducted a series of experiments examining the modulation of intermale aggression in gonadally intact adolescent male hamsters treated with a cocktail containing 2 mg/kg testosterone cypionate, 2 mg/kg nandrolone decanoate, and 1 mg/kg boldenone undecylenate (a 19-nor-testosterone ester). Each hamster received the AAS combination beginning on postnatal day 27 for 14–30 consecutive days, depending on the specific study. Aggression was measured using the resident-intruder paradigm test on the day after the final AAS injection. Hamsters receiving 14 days of AAS displayed more bites and attacks and a reduced latency to bite the intruder relative to controls [109,110]. In subsequent studies by this lab, a similar increase in the numbers of bites and attacks, and reduced bite latency, was observed in hamsters treated with the AAS combination for 30 days [41,55,62].

Harrison et al. [62] tested the hypothesis that arginine vasopressin system may mediate the increase in aggression accompanying AAS treatment. Specifically, an arginine vasopressin type 1_A antagonist (OH-Phaa-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-NH_2) was administered into the anterior hypothalamus of hamsters pre-treated with the AAS combination described above. AAS-treated hamsters receiving this antagonist exhibited a reduced number of bites and attacks, but continued to initiate biting with the same latency as hamsters receiving the vehicle. These results suggested a role for arginine vasopressin in the intensity and frequency, but not in the initiation, of aggressive behavior [62]. More recently, Grimes and Melloni [55] tested the effects of the selective serotonin reuptake inhibitor, fluoxetine, on the expression of AAS-induced aggression. Fluoxetine (20 mg/kg), administered 1 h before the aggression test, blocked AAS-induced aggression. Specifically, AAS-treated hamsters receiving fluoxetine displayed fewer attacks and took longer to initiate the first attack sequence relative to AAS-treated hamsters receiving the vehicle. These findings are consistent with the results of Bonson et al. [18,19] described earlier, and demonstrate that one mechanism by which AAS modulate aggression is via alterations in serotonin transmission.

Given that Melloni et al. [110] used a combination of AAS that includes nandrolone, and that nandrolone decanoate failed to induce aggression in male rats [21,106] it would be interesting to test whether nandrolone decanoate or testosterone propionate alone elicits aggressive behavior in hamsters. This point again raises the minimally explored issue of comparing the effects of AAS given individually versus in combination. In addition, a dose–response analysis to any AAS, individually or in combination, has yet to be conducted in hamsters. Finally, given the inhibitory effects of stanozolol on the expression of aggression in intact male rats [21] it would be interesting to test whether stanozolol antagonizes AAS-induced aggression in hamsters.

In summary, the effects of AAS on aggression are sex-, species- and compound-specific. The administration of testosterone propionate at supraphysiological doses for a long period of time consistently enhanced aggression in intact male rats [21,93,106]. Aggression in testosterone propionate-treated rats may be provoked more readily by physical stimuli (tail-pinch), and rats receiving testosterone propionate exhibited increases in aggression in social and environmental contexts that do not provoke aggression in controls [21,106]. Other AAS (stanozolol) either failed to stimulate aggression [32] or actually inhibited the display of aggression [21,106]. Estrogens, as well as testosterone, can stimulate aggressive behaviors in rats [30] and aggression is altered in mice with targeted deletions in the gene encoding the estrogen receptor alpha [117]. It is intriguing to speculate that the differential effects of testosterone propionate, 17α-methyltestosterone, nandrolone decanoate and stanozolol on aggression may reflect differences in the abilities of these compounds to act at androgen and estrogen

receptors and resulting differences in the balance of estrogen and androgen receptor-mediated signaling [106].

The studies done by Bronson and colleagues [22,23] suggest that AAS induce striking effects on aggression in female mice, however, surprisingly little research has been conducted on AAS effects on aggression in other female animal models. The neural systems underlying the AAS induction of aggression appear to overlap with the brain circuits underlying the regulation of aggression by endogenous androgens, that is neural systems utilizing arginine vasopressin, serotonin, and GABA [20,47,57] and AAS effects on these neural systems are discussed in detail below.

3.2. Sexual behaviors

3.2.1. Male sexual behavior

A number of studies have investigated the effects of AAS on the sexual behavior of intact male rodents. Clark et al. [37] assessed the effects of 12 weeks of treatment with three doses of each of six individual AAS on the expression of male sexual behavior in Long-Evans rats. The AAS and doses administered were as follows: 17α-methyltestosterone (0.075, 0.75 and 7.5 mg/kg); methandrostenolone (0.0375, 0.375, 3.75 mg/kg); nandrolone decanoate (0.056, 0.56, 5.6 mg/kg); stanozolol (0.05, 0.5, 5.0 mg/kg), oxymetholone (0.125, 1.25, 12 mg/kg); and testosterone cypionate (0.075, 0.75, 7.5 mg/kg). Twelve weeks of administration of the *high doses* of 17α-methyltestosterone, stanozolol, or oxymetholone eliminated the display of male sexual behavior; suppressing the expression of mounts, intromissions and ejaculations. In contrast, methandrostenolone, nandrolone decanoate, and testosterone cypionate had minimal effects on the display of sexual behaviors at any dose tested. Thus, in intact male rats, the six AAS examined in these studies evoked a range of behavioral responses that varied as a function of the specific compound and dose administered. In agreement with the lack of effects of low doses of AAS on sexual behavior in intact male rats, Lumia et al. [94] reported that long-term exposure of Long-Evans rats to testosterone propionate (1 mg/rat 3 times per week for 10 weeks) had no effects on the display of male sexual behaviors. In addition, Feinberg et al. [44] evaluated the sexual behavior of Long-Evans rats administered testosterone propionate for 16 weeks (1 mg/rat 3 times per week) beginning pre-pubertally (postnatal day 25) or peri-pubertally (postnatal day 40–45). Peri-pubertal exposure to testosterone propionate increased the proportion of rats that displayed ejaculation. Feinberg et al. [44] also examined the sexual behavior of rats that received testosterone propionate for 3 weeks and were tested at 16 weeks (i.e. following a 13-week withdrawal period) and determined that a smaller proportion of rats in the testosterone propionate-withdrawal group exhibited ejaculation compared with vehicle-treated rats. Finally, Bronson [22] observed no significant effects of 6 months of exposure to a combination of AAS administered at either low or high doses on the display of male sexual behavior in adult CF-1 mice.

It is noteworthy that in the study by Clark et al. [37] the AAS treatments that eliminated the expression of behavior in intact male rats (17α-methyltestosterone, stanozolol, oxymetholone) suppressed serum testosterone levels, whereas testosterone levels were not suppressed in rats receiving high doses of the AAS that did not affect sexual behavior (testosterone cypionate, nandrolone decanoate, methandrostenolone). Thus, the possibility cannot be excluded that AAS effects on sexual behavior in intact male rats reflected solely the suppression of endogenous testosterone secretion distinct from any direct action of AAS compounds in the brain areas modulating male reproductive behavior. To directly assess the ability and potency of AAS to elicit male sexual behavior in the absence of endogenous testosterone, Clark and colleagues [34,36] exposed gonadectomized subjects to AAS. Analyses of the ability of AAS to maintain sexual behavior following gonadectomy is a well-established paradigm for assessing the androgenic and estrogenic potency of compounds [69]. AAS treatments were begun on the day of gonadectomy and continued daily for six weeks with tests for sexual behavior conducted on weeks 1–6. The specific AAS and doses administered were as described above for intact male rats [37]. AAS were not equipotent in maintaining male sexual behavior patterns in gonadectomized male rats. Specifically, on week 6, 17α-methyltestosterone, stanozolol, and oxymetholone failed to maintain sexual behavior in gonadectomized rats at any of the doses tested. It is noteworthy that these are the AAS that interfered with sexual behavior in intact male rats. In contrast, gonadectomized rats receiving the high dose of methandrostenolone, nandrolone decanoate or testosterone cypionate continued to display male sexual behavior throughout the 6-week testing period suggesting that these AAS can substitute for endogenous testosterone and maintain the full expression of male sexual behaviors.

In summary, AAS have heterogeneous effects on the sexual behavior of male rats. AAS effects on sexual behavior in intact male subjects are largely absent except under periods of extended exposure and high doses [37], and low doses of AAS are generally without effect. Studies in gonadectomized male rats show that AAS, which at high doses disrupt sexual behavior in intact subjects, also fail to maintain sexual behavior in gonadectomized subjects suggesting that these specific AAS (17α-methyltestosterone, stanozolol, and oxymetholone) fail to stimulate androgen receptors in the brain to the level necessary to support the display of male sexual behaviors. It is noteworthy that no studies have been conducted in male rats assessing sexual behaviors in response to combinations of AAS. Given that human male users of AAS often report fertility complications such as decreased production of testosterone and sperm [4], it would be worthwhile to assess male reproductive function in animal subjects receiving combinations of low doses of AAS.

3.2.2. Female sexual behavior

The effects of AAS on estrous cyclicity in both rats and mice have been investigated. Clark and colleagues [12a,38] administered six individual AAS (17α-methyltestosterone, methandrostenolone, nandrolone decanoate, stanozolol, oxymetholone, testosterone cypionate) at low (0.037–0.075 mg/kg), medium (0.375–0.75 mg/kg) or high (3.75–7.5 mg/kg) doses to intact Long-Evans female rats for 2 weeks. The doses of the individual AAS were as described above in the studies of male rats [37]. Vaginal cytology and sexual behavior were monitored daily. Rats receiving the *high* doses of all AAS displayed significantly reduced periods of estrous cytology during the treatment period versus controls. Somewhat surprising was the finding that AAS affected sexual receptivity differently than vaginal cytology, suggesting a differential sensitivity to AAS of the neural substrates underlying the induction of sexual receptivity in ovariectomized rats and those regulating neuroendocrine function in the intact rat.

Whereas the high doses of 17α-methyltestosterone, methandrostenolone, and stanozolol all suppressed the display of sexual receptivity, rats receiving the high doses of nandrolone decanoate or oxymetholone continued to display regular cycles of sexually receptive behavior without interruption during the treatment period. In contrast, rats treated with testosterone cypionate showed a significant increase in the number of days of sexual receptivity displayed during the treatment period relative to controls. The disruptive effects of 2 weeks of exposure to AAS on female sexual behavior were also found to be reversible in most cases; regular cycles of vaginal cytology and sexual receptivity resumed during the 2-week follow-up (withdrawal) period from stanozolol, oxymetholone, 17α-methyltestosterone and methandrostenolone [12a,38]. In contrast, rats receiving nandrolone decanoate or testosterone cypionate displayed changes in vaginal cytology and sexual behavior that persisted through the 2-week withdrawal period. These results highlight the heterogeneity of AAS effects on both physiology and behavior and are one demonstration that the AAS that can be aromatized into estrogens (e.g. testosterone cypionate) may have distinct physiological and behavioral effects from AAS that are not subject to aromatization (e.g. stanozolol).

In addition to studies in gonadally intact female rats, combinations of four AAS (low and high doses) were administered via silastic capsules to CF-1 mice for either 9 weeks [23] or 6 months [22]. Not surprisingly, none of AAS-treated mice exhibited vaginal estrous cycles at the time of assessment (9 weeks or 6 months after initiation of AAS treatments). Sexual behaviors were not assessed in these experiments, and as noted above [12a,39], sexual behavior and vaginal cytology may be dissociated under some treatments.

AAS effects on the induction of sexual receptivity have also been assessed in gonadectomized Long-Evans female rats [12]. Gonadectomized rats were administered estradiol benzoate (2.0 μg/day on days 1–19) to induce receptivity and administered each of the six AAS mentioned above (at a dose of 7.5 mg/kg) on days 7–19. Tests for sexual receptivity were conducted on day 20 in the absence of progesterone replacement. Two weeks of administration of 17α-methyltestosterone, methandrostenolone, nandrolone decanoate, or stanozolol inhibited sexual receptivity in gonadectomized, estrogen-primed subjects. In contrast, estrogen-induced receptivity was not inhibited in gonadectomized rats receiving either oxymetholone or testosterone cypionate. The inhibition of sexual receptivity in gonadectomized, hormone-primed female rats by AAS has been shown to be dependent on the androgen receptor. The androgen receptor-specific antagonist, flutamide, completely reversed the inhibitory effects of 17α-methyltestosterone, stanozolol and nandrolone decanoate indicating that AAS actions at the androgen receptor are critical for the suppression of receptivity in gonadectomized subjects [13]. The respective roles of signaling mediated by the androgen receptor and other potential targets of the AAS on the disruption of vaginal cytology and on other sexual behaviors (e.g., proceptive behaviors, estrous termination) have not been determined.

In summary, a short- (2 week) or long- (9 months) term administration of AAS disrupts the rodent estrous cycle. In rats, there is evidence that sexual behavior, as well as peripheral cytology, is suppressed by some AAS. In mice, only vaginal cytology has been monitored. In addition, AAS that suppressed the display of sexual receptivity induced by priming with estrogen in gonadectomized rats did so via an androgen receptor-dependent process. Collectively, these results suggest that AAS act in the brain to interfere with events necessary for the estrogen-dependent induction of female sexual behavior and the regulation of the neuroendocrine events required for reproductive cyclicity.

3.3. Anxiety

In contrast to the analysis of AAS effects on aggression and sexual behaviors, less is known about AAS effects on anxiety. AAS effects on anxiety were first reported by Bitran et al. [10] who assessed the effects of a high dose of testosterone propionate (silastic implants) on the performance of Long-Evans rats on the elevated plus maze. Animals were tested at either 6 or 14 days after implants were made. Serum levels of testosterone were elevated 7 to 10-fold in rats receiving implants relative to intact controls. Rats having 6 days of testosterone propionate exposure displayed an increase in the exploration of the open arms of the elevated plus maze relative to controls, a sign of reduced anxiety. However, the behavior of rats exposed to testosterone propionate for 14 days did not differ from controls. The differences in open arm exploration for rats treated with testosterone propionate for 6 days were exhibited in the absence of any changes in open-field activity, suggesting a specific effect of the testosterone

propionate treatment on anxiolytic behavior and not a generalized effect on activity levels.

Bitran et al. [10] suggest that the anxiolytic effects of AAS arise from metabolism of AAS to neurosteroid metabolites that induce allosteric modulation of the γ-aminobutyric acid type A ($GABA_A$ receptor) and that the bimodal effects of AAS on anxiety measures may reflect the development of tolerance at these receptors. The results suggesting development of tolerance to the anxiolytic effects of AAS are intriguing given that AAS effects on aggression or sexual behaviors do not appear to be transient. Perhaps this reflects the heterogeneity in AAS actions on different neuronal signaling systems (e.g. the GABAergic versus the serotonergic or androgen receptor-mediated signaling). Although this is an attractive hypothesis, no studies have been performed to directly test the hypothesis that exposure to testosterone propionate results in changes in $GABA_A$ receptor expression with a time course that parallels both the change in anxiolytic behavior and $GABA_A$ receptor function reported by these authors. Moreover, no studies to date have examined the dose–response relationship of testosterone propionate to the production of anxiolytic behaviors nor correlated the expression of these behaviors with levels of neurosteroid metabolites in the brain. Recent results demonstrate that the AAS themselves (in the absence of metabolism to neurosteroids) can allosterically modulate the $GABA_A$ receptor (Fig. 2) [74,102,103,160]. Therefore, the findings of Bitran et al. [10] need to be extended to establish whether the effects of AAS on anxiety behavior as measured in the elevated plus maze can be attributed to modulation of $GABA_A$ receptor function. Specifically, whether levels of either neurosteroid metabolites or the AAS themselves reach concentrations in the brain that will lead to allosteric modulation of these receptors has not been tested. Furthermore, it needs to be established that both the initial enhancement and the subsequent hypothesized tolerance to the behavioral and physiological effects of these steroids can be attributed to changes in $GABA_A$ receptor.

Further evidence that AAS may have anxiolytic effects is provided by the study of Bing et al. [9] in which Wistar rats received a single injection of testosterone (unesterified, 5 mg/kg) 24-h before testing on Vogel's conflict test. On this test, water-deprived rats were trained to lick from a spout in an operant chamber. Following a 24-h deprivation period, the rats were returned to the chamber and allowed to drink without punishment. Thereafter each lick was accompanied by an electric shock and the number of shocks received was recorded. Anti-anxiolytic agents increase punished drinking relative to controls [27], and Bing et al. [9] observed that testosterone-treated rats accepted significantly more shocks than control rats, consistent with an anti-anxiety action of testosterone.

It is noteworthy that Bing et al. [9] observe AAS-dependent changes in anxiety within 24 h after a single injection of testosterone. First, it is unlikely that anything beyond physiological levels of testosterone would be present at 24 h after a single injection, given the rapid clearance of unesterified testosterone [154]. However, if the anxiolytic effects were due to this testosterone treatment, then the narrow time frame, although not categorically excluding androgen receptor-dependent changes in gene expression, would be more consistent with a nongenomic mechanism of AAS action. Evidence that androgens do indeed elicit rapid anxiolytic effects has been provided by a recent study [2] on the effects of naturally occurring androgens in adult, C57BL/6J × AKR/J hybrid male mice. In this study, the authors found that a single dose of either testosterone (500 μg: an amount given to reflect the pulsatile, elevated release of testosterone that is observed during sexual encounters) or either of the testosterone-derived neurosteroids, androsterone or 3α-androstanediol (100 μg), reduced anxiety within 30 min. The anxiolytic effects of testosterone were blocked by co-injection of

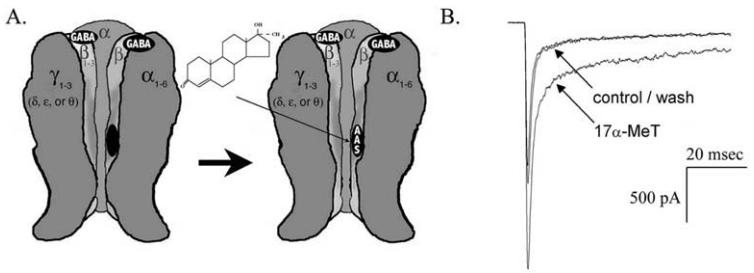

Fig. 2. Allosteric modulation of neurotransmitter receptor function by the AAS. (A) Schematic representation of the $GABA_A$ receptor. The $GABA_A$ receptor is a pentameric protein with a proposed stoichiometry of 2α, 2β and a γ subunit. Multiple α(1–6), β(1–3) and γ(1–3) subunit genes, as well as splice variants (not shown) have been identified. Less abundant subunits (δ, ε and Θ) may substitute for the γ subunit in native receptors. The cartoon illustrates the two binding sites for GABA at the interface of the α and β subunits and a putative binding site for the AAS hypothesized to reside in the transmembrane domain. Binding of the AAS with this site may induce a conformational change in the receptor (→) that alters transitions of the receptor between different kinetic states when either one or two of the GABA sites are bound, thus producing the allosteric modulation of the GABA-elicited chloride current. (B) Representative currents illustrating allosteric modulation of $GABA_A$ receptor function by the AAS, 17α-methyltestosterone (17α-MeT). Currents were elicited by ultrafast perfusion of 1 mM GABA (EC_{95}; 2 ms pulse application) (see Ref. [160] for methods) to recombinant $\alpha_2\beta_3\gamma_{2Long}$ $GABA_A$ receptors expressed in HEK293 cells (courtesy of Paul Yang; Dartmouth Medical School, Hanover, NH). The effects of 17α-MeT (increase in peak amplitude and prolongation of current decay) are immediate and reversible. Comparable positive modulation is observed for synaptic currents elicited from brain regions expressing predominantly $\alpha_2\beta_3\gamma_{2Long}$ $GABA_A$ receptors [74].

the $GABA_A$ receptor antagonists, bicuculline or picrotoxin, suggesting that the ability of testosterone to reduce anxiety arose from nongenomic allosteric modulation of this neurotransmitter receptor, although secondary effects on locomotor behavior observed with bicuculline or picrotoxin complicate interpretation of experiments with these antagonists. In contrast to the anxiolytic effects of AAS reported above, Minkin et al. [113] observed that Long-Evans rats (gonadally intact or gonadectomized) treated with nandrolone decanoate (10 or 50 mg/week for 8 weeks) spent more time than controls in the margins of the open-field (i.e. thigmotaxis), suggesting an increased level of anxiety. The opposing results of Minkin et al. [113] versus Bing et al. [9] and Aikey et al. [2] may reflect differences in AAS administered, dose and time course.

In summary, the number of studies that have tested the effects of AAS on anxiety behavior is limited. The findings of an anxiety-reducing action of testosterone reported by Bitran et al. [10] and Bing et al. [9] require follow-up with additional AAS, as well as time course and dose–response characterizations. As with aggressive behaviors, it would also be valuable to test the effects of AAS on anxiety behaviors in female subjects. Given the actions of AAS at the $GABA_A$ receptor as described in Section 4 that follows and the established role of GABA in the expression of anxiolytic behaviors, the analysis of AAS effects on the GABAergic system and anxiety behaviors provides an opportunity for further experimental study. In this regard, the experiments reported by Aikey et al. [2] are quite intriguing, but reflect endogenous changes in testosterone and need to be repeated with AAS given at high doses.

3.4. Reward

The potential reinforcing effects of AAS have been investigated using several paradigms. Clark et al. [35] tested whether AAS affect intracranial self-stimulation in Long-Evans rats. Rats received daily injections of methandrostenolone (~3 mg/kg) 1 h before daily testing for intracranial self-stimulation for 14 consecutive days and showed no changes in brain stimulation reward [35]. In a second experiment, the effects of D-amphetamine (0.5 mg/kg 15 min prior to the testing session) on brain stimulation reward were determined before and after the rats received a combination of AAS (2 mg/kg testosterone cypionate, 2 mg/kg nandrolone decanoate, and 1 mg/kg boldenone undecylenate) daily for 15 weeks [35]. Although brain stimulation reward remained stable in rats treated with this AAS combination for 15 weeks, AAS-treated rats exhibited a significantly larger response to amphetamine than vehicle-treated rats [35]. These findings suggested that although AAS did not have direct effects on brain reward, AAS potentiated the rewarding effects of amphetamine. No other studies have been conducted to determine whether AAS affect the rewarding properties of other drugs, although studies indicate that AAS alter neural systems important in reward.

The conditioned place preference task is used extensively to evaluate the rewarding properties of drugs. Several studies have reported that testosterone (~3 mg/kg) does not induce a conditioned place preference in male rats [4,26,51, 125 c.f.,3]. There are no published studies testing the effects of other AAS or doses on the conditioned place preference task, nor has the AAS-induction of conditioned place preference been tested in female subjects.

Given the frequent references in the media and scientific literature to the abuse of AAS [24], surprisingly little evidence has been forthcoming demonstrating that animals will self-administer AAS. Recently, Wood and colleagues [73,158], using a food-induced drinking model that makes testosterone available in an oral solution, demonstrated that gonadally intact adult male Siberian hamsters self-administer testosterone up to 4 mg/ml. Wood [158] reports considerable individual variability in the pattern and amount of AAS self-administration among male hamster subjects, and no evidence of a dose–response curve. Although the findings of Wood [158] are the clearest demonstration to date that AAS are reinforcing, additional studies assessing the self-administration of other AAS are needed.

In summary, the recent findings of AAS self-administration in hamsters provide an excellent model for the future analyses of the abuse potential of AAS, and will allow for testing of different compounds, doses and time courses [158]. In addition, experiments testing AAS effects on the rewarding properties of other drugs (cocaine, heroin) may reveal interactions between AAS and commonly abused drugs. Finally, tests of the ability of supraphysiological doses of testosterone propionate and other AAS to elicit a conditioned place preference in male or female subjects will extend our characterization of the abuse potential of AAS in animal models.

3.5. Learning and memory

As with reward, few studies have examined the effects of AAS on learning and memory. Two studies have assessed spatial memory in male rats treated with AAS. Clark et al. [33] administered an AAS combination (2 mg/kg testosterone cypionate, 2 mg/kg nandrolone decanoate, and 1 mg/kg boldenone undecylenate), methandrostenolone (0.375 mg/kg), or vehicle to Long-Evans rats for 10 weeks and tested rats in the water maze task with the platform hidden (place training) and removed (probe trials). AAS had no effects on performance on the water maze task. Rats receiving the combination AAS and methandrostenolone were able to find the platform as quickly, but no more quickly, than controls and spent comparable time swimming in the maze quadrant that previously housed the platform on the probe trials. Smith et al. [135] tested AAS effects on a spatial working memory task. Long-Evans rats received 17α-methyltestosterone (7.5 mg/kg), methandrostenolone (3.75 mg/kg) or

testosterone cypionate (7.5 mg/kg) for 30 days and were tested on the delayed non-match to sample radial arm maze task. AAS-treated rats and controls displayed comparable levels of motivation (rate of completion of the task) and spatial memory performance (number and types of errors). Based on these studies, spatial memory does not appear to be sensitive to AAS in Long-Evans male rats, nonetheless analysis of additional AAS (individually and in combination), dose–response characterizations, and tests in female rats and in other species are necessary to draw firm conclusions.

Compound-specific effects of AAS on passive avoidance have been reported by Rivas-Arancibia and colleagues [123,149]. In one study, Wistar rats received 10 weekly injections of nandrolone decanoate (4 mg/rat) or testosterone enanthate (20 mg/rat) 24 h before a training session and 24 h before the memory test. On the test, rats receiving testosterone enanthate performed better than controls whereas rats treated with nandrolone decanoate performed more poorly than controls [123]. In a second study, Wistar rats received testosterone enanthate (5–30 mg/rat) or nandrolone decanoate (1–6 mg/rat) 45 min before training on a one-trial passive avoidance task [149]. Rats receiving testosterone enanthate (30 mg) or nandrolone decanoate (4 mg) performed better than controls on tests conducted both at the short (10 min) and long (24 h) interval, whereas rats receiving the 20 mg dose of testosterone enanthate performed better than controls only on the 10 min test. The authors hypothesize, albeit in the absence of any direct data, that the effects of testosterone enanthate are due to aromatization, and that estrogen receptor-mediated signaling subsequently affects functions of the cholinergic system. In contrast, the authors attribute memory-enhancing effects of nandrolone decanoate to the 'anabolic' actions of this compound on protein synthesis. These hypotheses have yet to be experimentally tested.

Finally, Minkin et al. [113] investigated the effects of nandrolone decanoate (10 or 50 mg/rat, weekly for 8 weeks) on the acquisition of lever-pressing behavior and extinction in gonadally intact and gonadectomized male Wistar rats. Behavioral measures were collected beginning following the sixth nandrolone decanoate injection. There were no group differences in the acquisition of lever-pressing behavior nor did nandrolone decanoate affect the extinction of lever pressing.

In summary, the two studies of AAS effects on spatial memory found no deficits in the AAS-treated rats [33,135]. Moreover, no consistent results were found for the three studies assessing AAS effects on tests of short- and long-term memory [113,123,149].

3.6. Locomotion

Results of all the studies of AAS effects on locomotion in male rats and mice, tested either in an activity chamber or an open-field, have shown no significant changes in activity in AAS-treated subjects versus controls [9,10,22,32,34,36,37,88,99,101,113]. In contrast, Bronson and colleagues [22,23] have reported that female mice treated with a combination of AAS at low and high doses for either 9 weeks or 6 months (high dose only) exhibited significantly reduced spontaneous activity in a running wheel relative to controls. Bronson [22] hypothesizes that female-specific effects of AAS on locomotion may reflect AAS antagonism of estrogen-induced spontaneous activity. Given the marked effects of AAS on the activity of female mice, it would be interesting to determine whether these findings extend to gonadectomized females and to females of other species.

Although AAS did not affect locomotor activity in male rodents, two studies have measured locomotor activity in rats treated with both AAS and cocaine to assess the effects of interactions between these two agents on activity. Martínez-Sanchis et al. [101], administered a range of doses of testosterone (2, 5, 10, and 14 mg/kg) to Swiss-Webster mice in tandem with a range of doses of cocaine (2, 4, 8, 10, and 12 mg/kg). AAS were administered 45 min before, and cocaine 15 min before, the 20-min test for locomotion in an open-field. Testosterone alone had no effect on locomotor activity, however, the activity-promoting effects of cocaine (10 mg/kg) were enhanced by testosterone (2, 6, 10 and 14 mg/kg). In contrast to these results, Long et al. [89] reported a suppressive effect of testosterone (100 mg pellet), administered for 30 days to gonadectomized and intact adult male Wistar rats on locomotor activity stimulated by orally administered cocaine (20, 40 and 80 mg). Intact rats receiving testosterone and cocaine (40 and 80 mg/kg) did not show the same elevation in activity as rats receiving the vehicle plus cocaine. The reasons for the discrepancies between these two studies are not clear, but may relate to the difference in the species, route of cocaine (i.p. versus oral), and timecourse of AAS administration (single exposure versus 30 days). Thus, it would appear that additional studies are needed to determine the effects of AAS on the locomotor-stimulating effects of psychostimulants.

In summary, AAS appear to have no effects on generalized locomotor activity as measured in an open-field. In female mice however, tests of running wheel activity revealed a dramatic suppression of spontaneous activity by AAS. Further studies are necessary to test whether the AAS suppression of wheel running is due to disruption of the hypothalamic-pituitary-gonadal axis, as suggested by Bronson et al. [23]. Finally, given the conflicting results of AAS enhancement [101] and suppression [89] of cocaine-induced locomotor activity, further studies are necessary to clarify whether and how AAS affect activity induced by psychostimulants.

4. Physiological correlates of AAS administration

The breadth of behaviors altered in animals subjected to supraphysiological levels of AAS suggests widespread

involvement of numerous brain regions and different signaling systems. Perhaps, the most obvious target for AAS action are the steroid-sensitive regions of the forebrain, hypothalamus and pituitary that express high levels of steroid receptors (androgen receptors, estrogen receptors, and progestin receptors), are exquisitely sensitive to changes in endogenous levels of circulating gonadal steroids, and regulate reproductive and aggressive behaviors. However, the reported effects of AAS on anxiety and reward implicate changes in neural systems beyond these classic neuroendocrine control regions. Although studies delineating the molecular and cellular mechanisms that underlie the changes in behavior elicited by AAS exposure are by no means exhaustive, data from a number of laboratories indicate that the AAS alter disparate signaling pathways mediated by classical neurotransmitter systems and by neuromodulators. Based on the evidence to date, the AAS also appear to be able to modulate neural transmission both by classical androgen receptor-dependent changes in gene transcription and by nongenomic, allosteric modulation of specific receptors. However, the roles of AAS-dependent genomic and nongenomic changes in these signaling systems on behavioral outputs remain to be determined. Moreover, even the limited numbers of studies that have been performed to assess the actions of AAS in the brain have focused predominantly on biochemical and physiological effects. Although Clark et al. [33] demonstrated that AAS did not alter neuronal number in the hippocampus, to our knowledge, no study to date has examined whether AAS exposure induces changes in neuronal morphology or connectivity in the brain. With these limitations in mind, in the sections below, we review the current knowledge of the effects of high levels of AAS on neural signaling systems in the brain.

4.1. Androgen receptors

Signaling mediated by androgens, estrogens, and progestins is critical not only for the expression of reproductive and sexual behaviors [15,69], but also plays an important role in expression of a wide range of nonreproductive behaviors, including aggression, anxiety and cognition [6, 64,157], that may be altered during AAS abuse. To our knowledge, studies have not been performed to determine AAS effects on the expression of estrogen or progestin receptors in the mammalian brain, although treatment of adult, gonadectomized, estrogen-primed female Sprague–Dawley rats with 10 mg/kg of the non-aromatizable androgen, 5α-dihydrotestosterone, every 12 h for 4 days decreased estrogen receptor levels in the ventrolateral portion of the ventromedial nucleus of the hypothalamus [25]. These data suggest AAS may have similar effects on estrogen receptors levels, but this remains to be tested directly.

Although direct assessments of AAS effects on estrogen and progestin receptor expression have not been made, AAS have been shown to alter the expression of androgen receptors in the central nervous system. Treatment of Long-Evans or Sprague–Dawley rats with an AAS cocktail consisting of 2 mg/kg testosterone propionate, 2 mg/kg nandrolone decanoate, and 1 mg/kg boldenone undecylenate for 2 weeks increased androgen receptor immunoreactivity in classical androgen target sites including the medial preoptic area, the dorsal division of the lateral septal nucleus, a subregion of the medial division of the bed nucleus of the stria terminalis, the ventrolateral and dorsomedial divisions of the ventromedial nucleus of the hypothalamus, and the posteroventral division of the medial amygdala [95,111]. Upregulation of androgen receptors in brain regions that express high levels of androgen receptors recapitulates testosterone-mediated increases of androgen receptors in steroid-sensitive peripheral tissues [128,143] and supports the assertion that AAS enhance androgen receptor expression via androgen receptor-mediated signaling pathways. Although AAS-mediated increases in androgen receptor-immunoreactivity were observed in all central nervous system classical androgen target sites in these studies, region-specific differences were encountered in terms of (a) whether increases were due to increases in the numbers of androgen receptor-immunopositive cells, in the intensity of androgen receptor staining in immunopositive cells, or both, (b) whether the differences were due to actions of androgens at the androgen receptor or due to activation of estrogen receptors following aromatization of the AAS and (c) whether exogenous AAS interacted with endogenous steroids to yield differential regulation of androgen receptor-immunoreactivity.

Menard and Harlan [111] also report that high levels of AAS increased androgen receptor immunoreactivity in several ‘nonclassical’ brain regions that normally express few steroid hormone receptors (e.g. frontal cortex, hippocampus, substantia nigra, midbrain central gray). The ability of AAS to induce significant changes in both ‘steroid-sensitive’ and ‘steroid-insensitive’ regions of the central nervous system has also been noted by Blanco et al. [14]. These investigators report that a 4 week treatment of Long-Evans rats with silastic capsules of testosterone propionate that elevated serum testosterone levels 5- to 10-fold significantly increased expression of the mRNA encoding the synthetic enzyme choline acetyltransferase by 150–250% not only in spinal motoneurons that innervate sexually dimorphic muscles and express high levels of androgen receptors, but also throughout lateral column motoneurons that express few steroid hormone receptors and supply nondimorphic muscle. These data support the hypothesis that AAS may alter circuitry not only in neuroendocrine control or sexually dimorphic regions, but also throughout the central nervous system, and that the effects of AAS in these regions may arise from signaling pathways that are separate from the classical nuclear hormone receptor pathways.

4.2. γ-Aminobutyric acid type A ($GABA_A$) receptors

To date, the best-characterized example of AAS effects in the central nervous system that are independent of nuclear hormone receptor signaling is the ability of these steroids to allosterically modulate the function of the $GABA_A$ receptor. GABAergic transmission in the mammalian forebrain has been implicated as playing a pivotal role in the expression of reproductive behaviors [45,104,118], anxiety and stress [155], and aggression [133]. AAS have been shown to alter $GABA_A$ receptor expression when given chronically [105], but have also been shown to allosterically modulate $GABA_A$ receptor function when given acutely [74,102, 103,160].

The $GABA_A$ receptor is a heteropentameric ligand-gated ion channel. The ion channel is predominantly permeable to chloride ions, and inwardly directed chloride flux through this receptor provides the major mechanism for fast acting inhibition in the adult mammalian nervous system [108, 134]. In addition to their pivotal role in transducing inhibitory chemical transmission, $GABA_A$ receptors are the molecular targets of an extraordinarily diverse range of therapeutic drugs, toxins, and endogenous hormones that includes anxiolytic benzodiazepines, sedative/hypnotic barbiturates and neurosteroids, anticonvulsants, convulsants (including a number of insecticides), general anesthetics, ethanol, and zinc [108,134]. These drugs act by binding to specific sites on the receptor that are separate from the agonist binding sites for GABA. When bound, these allosteric modulators are believed to alter the way the receptor responds to GABA, thus changing the total amount of chloride that flows across the cell membrane and the overall amount of inhibition [134] (Fig. 2).

4.2.1. Allosteric modulation of $GABA_A$ receptors

The first studies to suggest that AAS have modulatory actions at the $GABA_A$ receptor were those provided by Bitran and colleagues [10]. These investigators showed that a 6 day exposure to moderate to high levels of testosterone propionate (~3.5–5 mg/kg/day from silastic capsules resulting in ~40 ng/ml serum testosterone levels) not only produced anxiolytic behavior in Long-Evans rats as described earlier, but also left-shifted the EC_{50} for $^{36}Cl^-$ flux induced by GABA in cortical synaptosomes, suggesting an increased affinity of the $GABA_A$ receptor for GABA-induced chloride flux. A number of reports published prior to this study had demonstrated that endogenous testosterone metabolites could act as allosteric, neurosteroid modulators of $GABA_A$ receptor function [52,120,147,148]. Bitran et al. [10,11] hypothesized that AAS effects on both $GABA_A$ receptor function and the anxiolytic behavior elicited with AAS exposure were consistent with allosteric modulation of the $GABA_A$ receptor. However, because the AAS do not have the structural entities believed to confer efficacious binding to the neurosteroid site and subsequent modulation of the receptor [83], Bitran and colleagues hypothesized that metabolism of the AAS to neurosteroid derivatives, in particular androsterone and 3α-androstanediol, was required for the observed anxiolytic action. In particular, the ability of the androstane steroids to induce allosteric modulation of the $GABA_A$ receptor is strongly dependent upon the presence of a 3α-hydroyx group. Furthermore, a keto group at C_{17} is believed to enhance neurosteroid modulation of the receptor (although it was deemed nonessential as long as there was an oxygen on the D ring) [52,120,148]. Neither of these structural entities is present on testosterone propionate or other commonly abused AAS.

In a second study, Bitran et al. [11] show that treatment of Long-Evans rats with 10 mg/kg/day of methandrostenolone induced a significant increase in Cl^- flux through $GABA_A$ receptors in cortical synaptoneurosomes from animals treated for 2 weeks and a further increase in animals treated for 4 weeks. In contrast to the prior study with testosterone propionate [10], no significant effect was apparent in animals treated for 1 week. While Cl^- flux was elevated in animals receiving long-term methandrostenolone treatment, the increases in $GABA_A$ receptor function did not correlate with comparable changes in serum levels of 3α-androstanediol, which were elevated relative to control at 2 weeks, but depressed relative to control at 4 weeks. It is possible, but has not been tested, that androsterone and/or 3α-androstanediol may contribute to the anxiolytic effects of testosterone propionate since testosterone is metabolized to these compounds [156]. However, to our knowledge, no studies to date have demonstrated that methandrostenolone is metabolized to neuroactive metabolites, and the lack of correlation between serum levels 3α-androstanediol and enhanced Cl^- flux through the $GABA_A$ receptor does not support the hypothesis that allosteric modulation of the receptor by neurosteroids is mediating the anxiolytic effects observed in animals treated chronically with methandrostenolone.

Studies have now shown that the AAS, in the absence of metabolism, can directly modulate the $GABA_A$ receptor. Masonis and McCarthy [102] showed that acute application of micromolar concentrations of either stanozolol or 17α-methyltestosterone significantly inhibited the binding of the benzodiazepine site ligand, flunitrazepam, to rat brain synaptoneurosomal membrane preparations from Sprague–Dawley rats. In a subsequent study [103], Masonis and McCarthy went on to show that stanozolol (5 μM) both potentiated GABA-mediated Cl^- flux in cortical synaptoneurosomes and inhibited the flunitrazepam-mediated enhancement of this GABA-elicited Cl^- flux. These investigators concluded that the AAS act as direct allosteric modulators of the $GABA_A$ receptor. Masonis and McCarthy [102] also showed that while stanozolol or 17α-methyltestosterone inhibited benzodiazepine binding, the neurosteroid, tetrahydroxycorticosterone, enhanced benzodiazepine binding, suggesting distinct functional mechanisms and

perhaps separate binding sites for these two classes of steroid modulators.

Results using these biochemical approaches to demonstrate that the AAS are indeed allosteric modulators of $GABA_A$ receptors have been recently confirmed and extended by direct electrophysiological assessments of primary neurons and of recombinant $GABA_A$ receptors of known subunit composition expressed in heterologous cell lines. Jorge-Rivera et al. [74] demonstrated that acute and direct application of 1 μM stanozolol, nandrolone, or 17α-methyltestosterone induced significant modulation of synaptic currents for neurons of both the ventromedial nucleus of the hypothalamus and the medial preoptic area in slices from pre-pubertal (postnatal day 3 to postnatal day 14) female Sprague–Dawley rats. Moreover, 17α-methyltestosterone was shown to modulate currents elicited by ultrafast (10–90% rise times of on/off application ~100 μs, duration 2–4 ms) perfusion of GABA for both primary neurons [74] and recombinant $GABA_A$ receptors expressed in the human embryonic kidney (HEK293) cell line [160] (Fig. 2). These data unambiguously demonstrate that this AAS can itself induce allosteric modulation of the $GABA_A$ receptor, and that metabolism to neurosteroids, androsterone and 3α-androstanediol, is not required for AAS activity at the $GABA_A$ receptor. It should also be noted that while it is not yet possible to directly measure AAS concentrations in specific brain regions, serum levels of AAS have been estimated to reach micromolar concentrations in human subjects [102,159].

Experiments using ultrafast perfusion techniques also confirmed and extended the flux studies of Masonis and McCarthy [102] to demonstrate that the AAS, 17α-methyltestosterone, acts via a different allosteric mechanism than do the neurosteroids. Specifically the neurosteroids are believed to act by altering desensitization of the receptor in the presence of GABA [164] and, at high (>1 μM) concentrations, can also directly activate the receptor in the absence of GABA [84]. In contrast, 17α-methyltestosterone was found to have no effect on either desensitization or recovery from desensitization for recombinant receptors (composed of the α_1, β_3, and γ_2 subunits; [160]), and concentrations of this AAS as high as 10 μM were unable to activate either recombinant or native $GABA_A$ receptors in the absence of GABA [74,160]. In addition, kinetic modeling studies indicated that for $\alpha_1\beta_3\gamma_2$ receptors, 17α-methyltestosterone acted by enhancing entry of singly liganded receptors into the open state, a mechanism distinct from those for either the neurosteroids or the benzodiazepines [160]. Finally, the benzodiazepine site antagonist, flumazenil, did not block modulation induced by 17α-methyltestosterone, indicating that this AAS does not act at the high affinity benzodiazepine site [160], see [103] for discussion.

As with a wide range of other allosteric modulators [108,134], published studies have suggested that the ability of the AAS to alter the activity of the $GABA_A$ receptor varies with subunit composition of this pentameric receptor. The first report of subunit specificity was provided by Jorge-Rivera et al. [74] in which it was concluded that the polarity of AAS modulation could be reversed from positive to negative with substitution of the γ_1 for the γ_2 subunit in $\alpha_2\beta_3\gamma_x$ recombinant receptors. However, subsequent experiments from the Henderson laboratory have failed to confirm these original findings, and current data indicate that 17α-methyltestosterone potentiates currents elicited by brief (2–4 ms) pulses of high (1 mM) GABA and subsaturating (20 μM) concentrations of GABA, for both $\alpha_2\beta_3\gamma_2$ and $\alpha_2\beta_3\gamma_1$ recombinant receptors [39]. While it appears that γ subunit composition does not confer distinctive modulation by AAS, α subunit composition has been shown to confer significant differences in the sensitivity of the $GABA_A$ receptor to the AAS. 17α-Methyltestosterone does not modulate currents elicited by brief pulses of millimolar concentrations of GABA (i.e., those that reflect GABA concentrations and kinetics at the synapse) from $\alpha_1\beta_3\gamma_2$ receptors [160], the predominant receptor isoform expressed in the adult cortex and cerebellum [50]. However, 17α-methyltestosterone does significantly potentiate currents elicited by these 'synaptic' conditions at $\alpha_2\beta_3\gamma_2$ receptors [74], the predominant receptor isoform expressed in the forebrain [50]. Yang et al. [160] hypothesize that the α subunit composition-dependent sensitivity to AAS modulation underlies the fact that AAS potentiate synaptic currents in forebrain regions (α_2-containing receptors), but have no effect on synaptic currents recorded from Purkinje cells (α_1-containing receptors) in the cerebellum. While AAS do not have a significant effect on currents mediated by $\alpha_1\beta_3\gamma_2$ receptors under synaptic conditions, Yang et al. [160] go on to report that 17α-methyltestosterone does potentiate $\alpha_1\beta_3\gamma_2$ receptors under conditions of subsaturating concentrations of GABA. These authors hypothesize that the AAS may influence processing in neurons that express this receptor isoform under conditions when GABA levels are low (e.g., tonic release or spillover from the synaptic cleft that occurs with high levels of presynaptic activity). Tonic $GABA_A$ receptor-mediated conductances are known to significantly modulate activity in neurons in regions such as the cerebellum [63]. However, the contribution of $\alpha_1\beta_3\gamma_2$ receptors to tonic conductances in different brain regions and the effects of the AAS on these conductances in primary neurons remain to be tested.

4.2.2. Changes in $GABA_A$ receptor expression

AAS-dependent changes in $GABA_A$ receptor function (Cl^- flux) as reported by Bitran et al. [11] were not noted until after 2 weeks of treatment, a time frame suggesting that chronic AAS exposure induces changes in $GABA_A$ receptor expression. A recent study by McIntyre et al. [105] determined steady-state levels of α_1, α_2, α_5, γ_1, γ_2, and ε $GABA_A$ receptor subunit mRNAs in adult and peri-pubertal male and female C57Bl/6J mice treated with AAS. Subjects were given a 4-week exposure to moderate (0.75 mg/kg) or

high (7.5 mg/kg) doses of 17α-methyltestosterone (beginning with postnatal day 24 for peri-pubertal animals and between postnatal days 56 and 63 for adult animals). This AAS regime significantly decreased the levels of individual $GABA_A$ receptor subunit mRNAs in the ventromedial nucleus of the hypothalamus, the medial preoptic area and the medial amygdala [105]. However, the ability of this AAS to induce significant changes in $GABA_A$ receptor mRNA levels depended upon the dose of AAS used, and the age and the sex of the animals. In particular, peri-pubertal females were markedly more sensitive to the effects of AAS exposure than were adults of either sex or peri-pubertal males. These results may be of particular relevance with respect to AAS abuse in human populations since the greatest increase in AAS use over the past decade has been reported for adolescent girls [7]. However, the results presented in McIntyre et al. [105] were limited to mRNA expression, and experiments need to be done to determine whether these AAS-dependent changes are reflected in changes in $GABA_A$ receptor protein and in receptor function.

The mechanisms by which AAS regulate $GABA_A$ receptor subunit mRNA expression have not been determined. $GABA_A$ receptor subunit gene expression is regulated by both 17β-estradiol [65] and testosterone [163], presumably by signaling mediated by nuclear estrogen and androgen receptors, and AAS may mimic these gonadal steroids by actions at these nuclear hormone receptors. In addition, allosteric modulators of the $GABA_A$ receptors can induce changes in $GABA_A$ receptor subunit expression by altering neuronal activity [64,108], thus providing an additional mechanism by which AAS may alter expression not only of $GABA_A$ receptors, but also of other neuronal proteins whose expression is activity-dependent. Experiments using pharmacological agents and genetically altered mice may be useful in determining the respective importance of signaling through androgen receptors, estrogen receptors, or the $GABA_A$ receptors in AAS-dependent changes in $GABA_A$ receptor expression.

Highlighting the complexity of AAS effects on the GABAergic system, it has also been shown that AAS alter the synthesis of endogenous neurosteroids and of GABA synthesizing enzymes. Stürenburg et al. [139] report that stanozolol, metenolone, and nandrolone act in an in vitro assay of cortical homogenates prepared from Wistar rats to inhibit production of 5α-pregnane-3,20-dione (5α-DHP), as well as 3α,5α-THP, via actions on the synthetic enzymes, 3α-hydroxysteroid dehydrogenase (3α-HSDH) and 5α-reductase. Such changes in endogenous neurosteroid levels may have actions throughout the brain and on all of the behaviors implicated in AAS abuse.

In summary, AAS directly alter $GABA_A$ receptor function via allosteric modulation, as well as the expression of enzymes responsible for the synthesis of endogenous allosteric modulators. The allosteric actions of the AAS depends upon the subunit composition of the $GABA_A$ receptor, and varies among different brain regions and under conditions of saturating versus nonsaturating neurotransmitter release. Chronic exposure to AAS also changes $GABA_A$ receptor subunit gene expression. That AAS have both allosteric and chronic effects at the $GABA_A$ receptor may promote quite different behavioral effects with respect to acute changes upon steroid administration versus long-term changes associated with continued steroid use. Experiments designed to test this hypothesis need to be performed.

4.3. 5-Hydroxytryptamine (5-HT) and 5-HT receptors

Neurotransmission mediated by 5-HT (serotonin) has widespread modulatory effects, and serotonergic transmission is known to regulate sexual behaviors, aggression, fear, anxiety, and reward [17,69,87]. In particular, it has been hypothesized that increases in aggressive behaviors are correlated with decreases in neural activity mediated by 5-HT [150]. Consistent with this hypothesis, an early report by Martinez-Conde et al. [98] demonstrated that testosterone propionate (10 mg/kg) administered every 7 days to Sprague–Dawley rats (beginning at postnatal day 23) resulted in significant decreases in the levels of 5-HT in the diencephalon by postnatal day 45. Similar results were obtained by Bonson et al. [19], who report that treatment of Fischer rats with 30 mg/kg/day testosterone propionate significantly decreased both 5-HT and the 5-HT metabolite, 5-hydroxyindoleacetic acid, in the hippocampus, although no significant changes were induced by testosterone treatment for either 5-HT or 5-hydroxyindoleacetic acid in the striatum or frontal cortex.

Bonson and colleagues have investigated the role of 5-HT specifically in androgen-induced aggression. As described earlier, dominant behavior was elicited by administration of 30 mg/kg/day of testosterone propionate to Fischer rats. Acute administration of the serotonergic agonist, quipazine, reduced this testosterone-induced dominance in a dose-dependent fashion [18]. Moreover, the effects of quizapine were themselves antagonized by the $5\text{-}HT_{1A}$ and $5\text{-}HT_{1B}$ receptor antagonist, pindolol, demonstrating that the actions of quipazine in reducing androgen-induced aggression were specific for 5-HT-mediated transmission [18]. In a subsequent study [19], this laboratory went on to test the role of specific 5-HT receptor subtypes in the regulation of androgen-induced dominance. The $5\text{-}HT_{1A}$ specific agonists, 8-OH-DPAT, buspirone, and gepirone, all elicited dose-dependent decreases in dominance. Qualitatively similar results were obtained with the $5\text{-}HT_{1A}/5\text{-}HT_{1B}$ agonist, eltoprazine, the $5\text{-}HT_{1B}/5\text{-}HT_{2C}$ agonist, TFMPP, and the $5\text{-}HT_{2A}/5\text{-}HT_{2C}$ agonist, DOM. The benzodiazepine, chlordiazepoxide, a nonserotonergic positive allosteric modulator of the $GABA_A$ receptor, had no significant effect on dominance, except at high doses where motor ability was impaired. In addition to suppression of androgen-induced dominance by various 5-HT

agonists, these investigators tested the ability of specific 5-HT receptor antagonists to interfere with the agonist-dependent reduction in androgen-induced aggression [19]. While the authors note that effects of some specific antagonists do not fit easily in a single unifying hypothesis with respect to categorically identifying 5-HT receptor isoforms that mediate androgen-induced dominance, these pharmacological studies support a role for 5-HT_{1A} receptors in this androgen-induced behavior. The conclusion that 5-HT_{1A} receptors are involved is also supported by results demonstrating that chronic treatment with testosterone propionate caused a significant decrease in the affinity of 5-HT_{1A} receptors in the hippocampus, but had no effect on either the density or affinity of 5-HT_2 sites in frontal cortex [19].

Work in hamsters, as well as rats, indicates that changes in serotonergic signaling underlie AAS-induced aggression. Specifically, Grimes and Melloni [55] have proposed that AAS exposure during adolescence leads to enhanced aggressive behaviors by diminishing the development of serotonergic innervation in the hypothalamus and forebrain. In support of this hypothesis, they demonstrated that treatment of preadolescent (postnatal day 27–56) male hamsters for 30 days with a AAS cocktail (2 mg/kg testosterone cypionate, 2 mg/kg nortestosterone, 1 mg/kg dihydroxytestosterone undecylenate) led to significant decreases in the numbers of terminals that are immunoreactive for 5-HT in the anterior hypothalamus, the ventrolateral hypothalamus and the medial amygdala; brain regions known to be critical for the display of aggression. No differences in 5-HT immunoreactivity however, were noted in other areas important in aggression, including the bed nucleus of the stria terminalis and the lateral septum, nor were differences noted in neocortex or ventral palladium (areas not implicated in the control of aggression). Moreover, Grimes and Melloni [55] demonstrated that AAS-induced aggression was inhibited by concomitant administration of the selective serotonin reuptake inhibitor, fluoxetine. These authors hypothesized that the decreased serotonergic tone in AAS-treated animals may lead to de-repression of arginine vasopressin pathways in the hypothalamus and disinhibition of neural pathways in the amygdala that result in increased aggression (see below).

In contrast to these studies, Thiblin et al. [144] treated Sprague–Dawley rats with 5 mg/kg given once per week for 6 weeks of testosterone propionate, nandrolone propionate, methandrostenolone, or oxymetholone. The authors found that 5-HT metabolism (as measured by the ratio of 5-HT to 5-hydroxyindoleacetic acid) was increased in the hippocampus by any of the four AAS, increased by methandrostenolone in the hypothalamus, and increased by oxymetholone or testosterone propionate in the frontal cortex. However, in contrast to previous studies discussed above which did not examine the effects of oxymetholone, Thiblin et al. [144] report that oxymetholone increased the levels of 5-HT levels in the hippocampus and levels of 5-hydroxyindoleacetic acid in frontal cortex, as well as in the hippocampus. Increased monoamine oxidase activity in the hypothalamus was also observed in these oxymetholone-treated animals. The authors suggest that enhanced activity of serotonergic neurons may give rise to a compensatory increase in the monoamine oxidase activity of these projection neurons, but this hypothesis was not tested. The authors also suggest that paradoxical effects of AAS on 5-HT metabolism reported in their study versus those studies summarized above may reflect the different actions of AAS when given at a moderate versus a high dose, but their results are inconsistent with the hypothesis that androgen-induced aggression is reflected in diminished serotonergic tone.

In summary, there is convincing evidence that AAS, in a manner similar to endogenous testosterone, induce aggression in rodents and that decreased serotonergic tone is pivotal to the ability of the AAS to increase aggression. The roles of specific 5-HT receptor isoforms, as well as pathways mediating AAS-dependent changes in 5-HT receptor expression and in 5-HT metabolism remain to be determined. Moreover, experiments need to be performed on females and with adolescent animals to determine if there are sex- and age-specific differences in the effects of AAS on the 5-HT system.

4.4. Dopamine and dopamine receptors

As with serotonin, neural transmission mediated by dopamine receptors has widespread effects on a number of neural systems implicated in regulating behaviors altered with high doses of AAS. In particular, a number of studies has assessed whether AAS alter signaling in dopaminergic (and peptidergic) systems of the mesolimbic region and subregions of basal forebrain that comprise the brain's reward circuitry [81,87]. Early studies by Vermes et al. [151] provided the first evidence that acute exposure to high doses of AAS (5 mg/kg of testosterone propionate, norandrostenolone propionate, dihydrotestosterone or androstenedione) altered dopamine, but not 5-HT or norepinephrine levels in the brains of CFY rats. More recent studies have extended these results to show that chronic AAS exposure alters dopaminergic-signaling components. Kindlundh et al. [76] report that a 2-week treatment with 15 mg/kg of nandrolone decanoate altered the levels of both D_1 and D_2 dopamine receptors in the mesocorticolimbic system of Sprague–Dawley rats. Specifically, they report that this AAS treatment regime decreased the numbers of binding sites attributed to D_1 receptors in the caudate/putamen and in the core and the shell of the nucleus accumbens. D_2 receptor binding sites were also decreased in the shell of the nucleus accumbens, but increased in the core of the nucleus accumbens and in the caudate/putamen. In a later report [77], these authors employ the same AAS paradigm in conjunction with positron emission tomography (PET) to

assess the numbers of D_1 and D_2 receptors, as well as the binding of ligands to the dopamine transporter in the mesolimbic system of Sprague–Dawley rats. In this later report, the authors detect no significant change in the level of D_1 receptors in the striatum. Moreover, while experiments assessing binding to D_2 receptors, as analyzed by PET, were performed in this study, no data is presented because the authors state that they had too few animals ($n = 5$) to perform a statistical analysis. Thus, with respect to D_1 receptors, results from this latter study [77] do not support their previous findings using conventional autoradiography [76], and with respect to D_2 receptors, results from this latter report are not conclusive [77].

Thiblin et al. [144] provide biochemical data that AAS exposure alters the activity of dopaminergic neurons in the mesocorticolimbic system. In this study, treatment of Sprague–Dawley rats with a moderate dose of AAS (one injection per week for 6 weeks of 5 mg/kg testosterone propionate, nandrolone propionate, methandrostenolone, or oxymetholone) increased the concentration of dopamine in the striatum, as well as the levels of the dopamine metabolites, 3,4 dihyroxyphenylacetic acid and homovanillic acid. All AAS tested were found to increase dopamine metabolism in the striatum, however, the increase was significant only with oxymetholone. The authors conclude that the AAS-dependent increases in DA metabolism reflect increased activity of dopaminergic neurons and stimulation of the mesolimbic reward pathways and thus may contribute to reinforcement behaviors [144].

In summary, studies to date suggest that chronic exposure to high doses of AAS alters both dopamine and dopamine receptor expression in regions of the brain important for mediating reward. Although enhanced activity of the mesocorticolimbic dopaminergic system is critical for the acute rewarding effects of cocaine and amphetamines [81], it remains to be established if the AAS-dependent changes in dopamine signaling are comparable to those produced by psychomotor stimulants. Conversely, it may be that the AAS, while not themselves rewarding, alter neural circuits involved in reward so as to make the brain more sensitive to the effects of other drugs [31,60]. A mechanism such as this that resets the sensitivity of the brain reward circuitry is consistent with behavioral data of Clark et al. [35] that demonstrated that AAS potentiate the effects of amphetamines in reward behaviors and with human studies indicating that AAS abusers are more likely to abuse other drugs [6,16,93,140].

4.5. Opioids and opioid receptors

Opioids and opioid receptors are highly expressed in brain regions that mediate both reward and reproductive behaviors [69,96]. Moreover, opioids acting on the hypothalamus regulate the release of a number of hormones, including corticosteroids and arginine vasopressin [96] that, in turn, may alter the expression of aggression, fear and anxiety. Menard et al. [112] report that a 2 week exposure to an AAS cocktail (2 mg/kg testosterone propionate, 2 mg/kg nandrolone decanoate, and 1 mg/kg boldenone undecylenate) of Sprague–Dawley rats significantly decreased the number of β-endorphin immunoreactive neurons in the rostral part of the arcuate nucleus and diminished the number of lightly to moderately (but not intensely) stained neurons in this region (β-endorphin immunoreactivity was not altered in caudal and medial aspects of the arcuate nucleus). Harlan et al. [61] treated Sprague–Dawley rats for 15 days with one of three different AAS cocktail regimes: (1) 4 mg/kg nandrolone decanoate and 4 mg/kg 17α-methyltestosterone, (2) a combination of testosterone cypionate and nandrolone decanoate given in an ascending series of doses from 0.5 mg/kg testosterone cypionate and 0.25 mg/kg nandrolone decanoate to 1 mg/kg testosterone cypionate and 0.5 mg/kg nandrolone decanoate, (3) 2 mg/kg testosterone cypionate, 2 mg/kg nandrolone decanoate and 1 mg/kg boldenone undecylenate. In contrast to Menard et al. [112], Harlan et al. [61] report that β-endorphin levels were significantly increased, albeit in a different brain region, the paraventricular thalamic nucleus of pairs of rats subjected to one of the four AAS cocktails. Because the paraventricular thalamic nucleus sends a glutamatergic projection to the striatum, the authors speculate that AAS modulation of β-endorphin levels in this brain region may alter brain reward circuitry.

A number of studies have also been carried out by Nyberg and colleagues to determine whether AAS exposure alters opioid signaling in brain areas involved in reward. Johansson et al. [70] treated Sprague–Dawley rats daily with 15 mg/kg nandrolone decanoate for 2 weeks, and levels of opioid peptides were assessed using radioimmunoassay. In this initial study, these authors report that this AAS treatment regime did not significantly alter levels of Met-enkephalin-Arg-Phe, a μ and δ opioid receptor agonist, in any of seven brain areas examined, including the hypothalamus. In a subsequent study, Johansson et al. [71] report that this regime of AAS induced significant increases in Met-enkephalin-Arg-Phe in the hypothalamus, as well as in the striatum and the periaqueductal gray. A concurrent report from this same group [72] again indicated that this treatment regime increased levels of Met-enkephalin-Arg-Phe in the hypothalamus, however, in this study, no significant effect was seen on Met-enkephalin-Arg-Phe levels in the striatum and AAS treatment was found to significantly decrease Met-enkephalin-Arg-Phe levels in the periaqueductal gray.

Similar conflicting findings on the effects of 15 mg/kg nandrolone decanoate for 2 weeks on the levels of dynorphin-B, a κ opioid receptor agonist, have been provided by this group. Johansson et al. [70] report that this AAS treatment induced a significant increase in β-endorphin levels (as measured by radioimmunoassay) in the ventral tegmental area. Johansson et al. [71] reported that nandrolone decanoate treatment also led to an increase

dynorphin-B levels in the hypothalamus, striatum and periaqueductal gray (the ventral tegmental area was not examined in this latter study). However, results published concurrently in Johansson et al. [72] do not replicate these findings, and in this study the authors report no significant changes in dynorphin-B levels in the hypothalamus, striatum or periaqueductal gray. The variability in the effects of AAS on opioid peptide levels elicited by the same AAS regime may arise from the fact that the biochemical approaches may not be able to consistently detect significant changes if those changes are limited to a subpopulation of neurons within a given brain region. For example, Menard et al. [112] reported that significant changes in β-endorphin expression, as assessed using immunocytochemistry, were evident only in the rostral aspect of the arcuate nucleus. Such changes may have gone undetected in a radioimmunoassay of the arcuate nucleus as a whole. In addition, the small number of subjects assayed in the study by Harlan et al. [61] (two animals per AAS regime) may have precluded an accurate assessment of AAS-dependent changes in opioid expression.

Although the focus of this review is on studies performed with mammals or mammalian tissues, it is worth noting that AAS have been shown to alter molecules involved in opioid signaling in a simplified in vitro system that obviates unknown variables associated with AAS metabolism and the complex indirect interactions among different neural populations that confound interpretation of results from in vivo studies. Pasquariello et al. [119] report that exposure of the androgen-sensitive GT1-1 hypothalamic cell line to nandrolone significantly decreased levels of both δ opioid receptor mRNA and the number of δ opioid receptor binding sites. Interestingly, these changes in δ opioid receptor levels of mRNA and protein were not blocked by coincubation with the androgen receptor-specific antagonist, flutamide, indicating that this effect of nandrolone is *independent* of androgen receptor activation. Aromatization of nandrolone is unlikely in this in vitro system, suggesting that the effects of nandrolone may be mediated via alternative membrane-delimited signaling mechanisms. Treatment of this same cell line with flutamide did block the nandrolone-dependent decrease in androgen receptor mRNA in this cell line, consistent with previous reports in peripheral tissues demonstrating that androgens down regulate the expression androgen receptor mRNA via the classical androgen receptor signaling pathway [143].

In summary, results are equivocal with respect to the in vivo studies and the ability of AAS to alter opioid or opioid receptor expression in brain regions important for reward. Further studies need to be done to determine if there is (or is not) a clear pattern of changes in opioid signaling components elicited by high doses of AAS and if changes in these molecules are reflected in changes in the physiology of brain reward circuits. It should be noted that AAS actions via separate signaling pathways may converge in brain reward areas. For example, Menard and Harlan [111] note that AAS treatment increased androgen receptor immunoreactivity in regions containing dopaminergic and opioid neurons. These authors hypothesize that changes in androgen-sensitivity induced by the AAS-dependent upregulation of androgen receptors in these regions may induce a feed-forward mechanism that could amplify androgen-mediated modulation of brain circuits involved in reward. Similarly, Johansson et al. [70] suggest that AAS may modulate the activity of inhibitory GABAergic synapses from the ventral tegmental area that synapse upon dopaminergic neurons in the nucleus accumbens. While such complex interactions among different signaling pathways are quite probable, no experiments to date have directly tested these hypotheses.

4.6. Other molecules in reward

Induction of immediate early genes in brain reward systems is a hallmark of all abused drugs [59]. Harlan et al. [61] demonstrated that, in contrast to morphine, acute AAS administration of a number of different AAS cocktails had no effect on immediate early gene induction in the striatum of Sprague–Dawley rats. However, in this study, AAS significantly inhibited the ability of morphine to induce immediate early gene expression. Similarly, Le Greves et al. [86] report that a 2 week treatment of Sprague–Dawley rats with 15 mg/kg/day nandrolone decanoate did not significantly alter the levels of the NMDA receptor, NR1 subunit mRNA within the nucleus accumbens or the periaqueductal gray (although see Ref. [85] for different results). This AAS regime, however, was reported to augment the ability of cocaine hydrochloride (5 mg/kg/day) to decrease the levels of NR1 mRNA in these brain regions. Finally, Long et al. [91] report that high intermittent doses of nandrolone decanoate (20 mg twice weekly for ∼4 weeks) had no ability to induce seizures in Sprague–Dawley rats. However, nandrolone decanoate increased the ability of cocaine to induce seizures in these animals.

In summary, the number of studies examining AAS effects on signaling systems implicated in reward, other than dopamine and opioid pathways, is quite limited. Taken together, however, these studies are again consistent with the hypothesis that the AAS may alter the sensitivity of reward circuitry and thus the sensitivity of the brain to other drugs of abuse.

4.7. Other neuropeptides and neurotrophins

Arginine vasopressin has been postulated to regulate the expression of aggressive (for discussion, see Ref. [62]) and sexual behaviors [69]. Harrison et al. [62] have shown that exposure of preadolescent male hamsters (postnatal day 27–56) to a cocktail of AAS (2 mg/kg testosterone cypionate, 2 mg/kg nandrolone decanoate, and 1 mg/kg boldenone undecylenate) for 30 days led to enhanced aggressive behavior relative to control animals, a 3-fold

increase in the average area innervated by arginine vasopressin-immunoreactive fibers in the anterior hypothalamus, and a 2.5-fold increase in arginine vasopressin content. This increase in peptide expression was not accompanied by a change in arginine vasopressin mRNA levels. The increase in aggression could be blocked in AAS-treated hamsters by intracranial infusion of a specific arginine vasopressin V1A receptor antagonist.

Substance P has also been implicated in modulating neural circuits that underlie aggression, fear, and stress, including the amygdala, hypothalamus and the periaqueductal gray (for discussion, see Ref. [58]) as well playing a role in brain reward [81,87]. Hallberg et al. [58] have demonstrated that 15 mg/kg/day nandrolone decanoate increased the expression of substance P (as indicated by radioimmunoassay) in the amygdala, hypothalamus, striatum and periaqueductal gray of Sprague–Dawley rats. No changes in substance P immunoreactivity were seen in either the nucleus accumbens or in the hippocampus.

Neurotrophins, including nerve growth factor and related family members, brain-derived neurotrophin factor and neurotrophin-3 play important roles not only in neuronal survival, but also in neuronal differentiation and synaptic plasticity [28]. Levels of neurotrophic factors and their respective receptors are regulated by androgens and estrogens [122,136,146]. Tirassa et al. [145] demonstrated that treatment of Sprague–Dawley rats with 5 mg/kg nandrolone once per week for 6 weeks resulted in a significant increase in the levels of nerve growth factor in the septum and hippocampus and a significant decrease in nerve growth factor levels in the hypothalamus. This treatment also significantly decreased immunoreactivity for the low affinity nerve growth factor receptor (p75NGFR) in the septum and in the vertical and horizontal bands of Broca. Levels of the high affinity receptor selective for nerve growth factor (TrkA) were not assayed.

In summary, high doses of AAS may affect the expression of a broad range of neuropeptides and their receptors in the brain. However, the numbers of studies examining AAS effects on neuropeptides is too limited to draw definitive conclusions with respect to how changes in these neuropeptides may contribute to AAS-dependent changes in specific behaviors. The exception is that the studies demonstrating AAS-dependent increases in arginine vasopressin [62] and substance P [58] are consistent with the hypothesized roles of these peptides in the expression of aggression in rodents [40,46].

4.8. Stress hormones

In the central nervous system, glucocorticoids, and therefore, the peptides that regulate their synthesis and release, impose significant modulation upon the hypothalamic-pituitary-gonadal axis. Gonadal steroids, in turn, regulate the hypothalamic-pituitary-adrenal axis, thus establishing complex and reciprocal interactions between the two steroid systems [152] and a myriad of potential cellular targets by which AAS may influence brain function by altering glucocorticoid synthesis, release and signaling. In addition to their effects on neuroendocrine control regions, glucocorticoids have been shown to modulate synaptic plasticity [127] and neurogenesis [54] in the hippocampus. With respect to steroids and peptides involved in stress, Ahima and Harlan [1] demonstrate that a 1 week treatment of adrenalectomized Sprague–Dawley rats with an AAS cocktail (2 mg/kg testosterone cypionate, 2 mg/kg nandrolone decanoate, and 1 mg/kg boldenone undecylenate) partially restored glucocorticoid receptor immunoreactivity in the pyramidal cell layer of CA1 and granule cell layer of the dentate gyrus to levels to that observed in control animals, however, effects of the AAS cocktail on glucocorticoid receptor immunoreactivity in intact animals was not assessed. Schlussman et al. [130] report that a 3 day treatment with 15 mg/kg/day of nandrolone decanoate to Sprague–Dawley rats increased circulating levels of corticosterone and adrenocorticotropic hormone immediately following the final injection. No changes were evident in levels of mRNA encoding corticotropin releasing factor in the amygdala or proopiomelanocortin mRNA in the hypothalamus immediately following the final injection, but the authors state that the levels of these mRNAs were found to be decreased 24 h later. No changes were noted in corticotropin releasing factor mRNA in the hypothalamus or the anterior pituitary or in proopiomelanocortin mRNA in the amygdala. In a subsequent report, Schlussman et al. [131] again report that a 3 day treatment with nandrolone decanoate elevated circulating levels of adrenocorticotropic hormone and corticosterone, but did not alter levels of hypothalamic corticotropin releasing factor or proopiomelanocortin mRNA in the anterior pituitary. However, in this same report [131], the authors find that treatment with 15 mg/kg/day nandrolone decanoate for 2 weeks did not induce significant changes in the circulating levels of corticosterone or on hypothalamic corticotropin releasing factor mRNA or corticotropin releasing factor receptor R1 and proopiomelanocortin mRNAs in the anterior pituitary (unpublished data).

In summary, AAS may influence the expression of stress hormones and releasing factors. However, conclusive data regarding how the AAS alter the expression or activity of these factors and the potential effects of these alterations on behavior remain to be determined.

5. Conclusions and future directions

Studies in rodents have shown that exposure to AAS at doses that mimic levels observed with human abuse elicit significant changes in aggression, anxiety, and sexual behaviors: all behaviors implicated as being altered in human steroid abusers. Studies examining AAS effects on

reward behaviors, locomotion, and learning and memory are fewer in number and less conclusive. Further work is needed to establish whether high doses of AAS have consistent and significant effects on these behaviors. Studies in rodents also provide convincing evidence that exposure to AAS at doses that mimic levels observed with human abuse elicit significant changes in the expression and/or function of molecules involved in serotonergic and GABAergic transmission in a manner consistent with the observed behavioral effects of AAS on aggression and anxiety. Chronic exposure to AAS clearly also alters the expression of a broad range of other signaling molecules in the brain, but studies are equivocal with respect to consistency observed in those changes and speculative with respect to the relation to behavioral changes observed with AAS. As more data accrues determining what AAS do to the brain and to behaviors, more studies are needed that mechanistically tie the former to the latter. Furthermore, as mentioned often throughout this review, most studies of AAS effects have been performed on adult male subjects. It is clear that the effects of these steroids differ with sex and age, and more studies need to be performed using female subjects and with adolescent subjects. It is also clear that the effects of AAS vary with the identity of the AAS given and whether the AAS are given singularly or in combination. Studies are needed both to define further the actions of individual AAS and the interactions of AAS that are commonly taken in combination as part of stacking regimes. Finally, few data exist on either the protracted effects of multiple cycles of AAS or the effects of withdrawal. While the data in animal models, as presented in this review, has provided a solid basis delineating the behavioral and physiological actions of AAS in the nervous system, many questions remain to be explored.

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