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The *Sturm und Drang* of anabolic steroid use: angst, anxiety, and aggression

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Abstract

Anabolic androgenic steroids (AAS) are illicitly administered to enhance athletic performance and body image. Although conferring positive actions on performance, steroid abuse is associated with changes in anxiety and aggression. AAS users are often keenly invested in understanding the biological actions of these drugs. Thus, mechanistic information on AAS actions is important not only for the biomedical community, but also for steroid users. Here we review findings from animal studies on the impact of AAS exposure on neural systems that are crucial for the production of anxiety and aggression, and compare the effects of the different classes of AAS and their potential signaling mechanisms, as well as context-, age- and sex-dependent aspects of their actions.

Keywords

anabolic steroid; anxiety; aggression; GABA_A receptor; corticotropin releasing factor; extended amygdala

Introduction

Anabolic androgenic steroids (AAS [1]; Box 1) comprise a large class of synthetic derivatives of testosterone that are predominately taken in an illicit manner for ergogenic purposes and image enhancement [2]. An estimated 10 million Americans, some starting as early as 13 years of age, have self-administered AAS [3,4], a pattern that is mirrored in other countries [5]. Although most users are male, an appreciable percentage is female [4]. Thus, both age and sex are crucial parameters to consider when assessing the impact of illicit steroid use. AAS are taken in intricate and complex patterns, typified by concurrent (stacking) and intermittent (cycling) coadministration of different AAS in which doses may be increased then decreased (pyramiding) and coupled with coadministration of masking agents (Box 1) [6,7]. The rationalization for these sophisticated regimens is the belief that activation of multiple signaling pathways will result in the synergistic augmentation of anabolic (muscle-building) effects with minimization of unwanted side-effects [7]. All illicit regimens result in suprathreshold levels of androgens and their metabolites: by an

estimated 10–100-fold in men and by >1000-fold in women and adolescents [7–9]. Thus, this rationalization aside, illicit steroid use results in highly nonphysiological levels of endogenous steroids and comparably high levels of synthetic steroids, all of which have the capacity to alter not only peripheral physiology, but also central nervous system processing and behavioral output [1,10,11].

Box 1

Classification of AAS

AAS are synthetic derivatives of testosterone, formed by chemical modification of the 4-ring 19-C androstane backbone into one of three classes (Figure I). The modifications of this fundamental chemical structure were made to endow new synthetic compounds with particular advantages as outlined below, and most modifications were derived to increase the anabolic (tissue-building) effects of the steroid while diminishing their androgenic (masculinizing) actions. Despite the explosion in the number of AAS that have been synthesized, all are active at the AR and thus have androgenic as well as anabolic properties [1]. The use-patterns of the AAS are typified by elaborate concurrent combinations of multiple AAS (stacking) with increasing (pyramiding) concentration believed by the users to limit the side effects of the compounds. A typical AAS schedule [7] is used to exemplify this (Figure II). Note the use of non-AAS compounds for energy replacement (insulin) and for fat loss (triiodothyronine; T₃) (Figure IIb), in addition to a large number of AAS of various classes (Figure IIa).

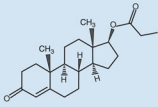
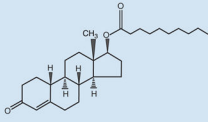
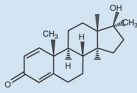
AAS Class	I	II	III
	C-17 Esterification	19-Nortestosterone	C-17 Alkylation
Structure			
Substitution	Long-chain ester	No methyl group at C-19; may also have C-17 esters	Alkyl moiety at C-17
Examples	Testosterone cypionate Testosterone propionate Methenolone	Nandrolone decanoate Trenbolone	17 α -Methyltestosterone Methandrostenolone Stanozolol Oxymetholone
Advantages	Injectable; modification retards liver metabolism and increases solubility in vehicle which prolongs release into circulation	Injectable; greater anabolic to androgenic ratio than dihydrotestosterone, the most biologically active androgen	Orally active; 17 α modification retards liver metabolism

Figure I.

The three major classes of anabolic androgenic steroids.

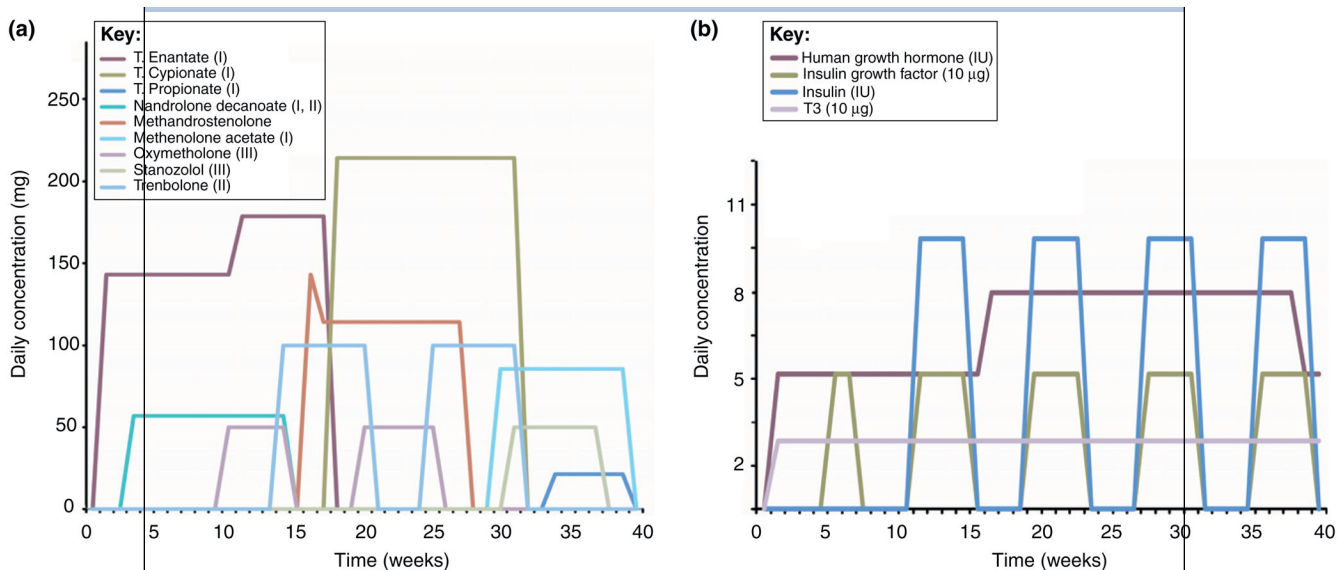


Figure II.

Representative AAS regime. (a) Individual lines represent the increases and decreases in concentrations of concurrent use of multiple AAS, as well as interruptions in steroid use that, together, represent pyramiding, cycling and stacking. Parentheses indicate class of AAS (I, II, III). (b) Individual lines represent the patterns of self-administration of non-steroidal compounds that AAS users would take in concert with the AAS shown above.

The matrix of interactions of these different steroid regimes with key variables such as age, sex, intrinsic genetic makeup, and social context promotes an array of effects that may be quite disparate from one individual to the next. With regard to behavior, data from case reports, large population surveys, and experimental studies in humans have shown that AAS use can result in a plethora of actions that range from euphoria and increased self-confidence to impulsivity, irritability, and aggression [6,9,12,13]. A growing body of literature demonstrates that behavioral outcomes of chronic AAS use are recapitulated in animal models. As such, these models provide crucial mechanistic insight into the underlying bases for AAS-induced effects. Here, we review these recent studies and highlight the advances in our understanding of the neuronal mechanisms that underlie AAS-induced changes in anxiety and aggression.

The behavioral effects of AAS on anxiety

As indicated above, case studies in humans demonstrate that AAS impose a wide range of changes in behaviors in individuals who use these steroids [8,9,12]. For example, adult men have reported transient euphoria and hypomania early in the course of AAS administration [14], but long-term users may manifest increased anxiety and marked irritability with generalized and undirected anger [6,12]. Men who administer the highest doses of AAS have been shown to have elevated scores on the Symptom Check List-90, a self-report system that includes several different dimensions of anxiety, and on the Hostility and Direction of Hostility Questionnaire, which reflects both the willingness to respond to social

situations with aggressive behavior and the tendency to evaluate people, including oneself, in negative terms [6]. Randomized clinical trials have been few owing to ethical constraints, and those that have been performed have been carried out using only moderate doses of a single AAS. However, men in these studies were also found to exhibit increased levels of hostility and anxiety [15,16]. Fewer studies have examined behavioral outcomes in women or in adolescents of either sex, but in such studies the data again suggest that chronic steroid use is associated with poor self-esteem, as well as eating and mood disorders, that may reflect a fundamental disruption in the neural circuitry that regulates anxiety [17,18].

Studies in laboratory rodents allow assessments of the behavioral effects of AAS exposure under conditions where the steroid regimen as well as the age and sex of the subjects can be controlled, and where direct observations of anxiety-like behaviors can be made by several independent means. Tests commonly used to assess AAS-induced anxiety in rodents include the acoustic startle response, the elevated plus-maze, the open-field test, the Vogel conflict test, shock-probe burying tests, and freezing behavior assays [19]. These studies indicate that the AAS have diverse effects on the expression of anxiety-like behaviors in rodents, exactly as they do in humans. For example, short-term exposure of adult male rats to low doses of the AAS, methenolone had anxiolytic actions as assessed by open-field behavior [20], a finding the authors suggest may be relevant to the reports of enhanced confidence and self-esteem that can be observed in human steroid users early in the course of self-administration. By contrast, chronic exposure to high doses of nandrolone decanoate increased anxiety-like behaviors in adult male rats on the elevated plus-maze [21] and in adult male mice, as assessed in both the open-field and elevated plus-maze tests [22].

Treatment of adult female mice with a high dose of 17 α -methyltestosterone was reported to have no effect on anxiety-like behaviors on the elevated plus-maze, light-dark transitions or defensive behavior tests [23], whereas treatment of female mice during adolescence with a mixture of AAS (methandrostenolone, nandrolone decanoate and testosterone cypionate) significantly increased anxiety-like behavior as determined by the acoustic startle response and the elevated plus-maze [24,25]. Interestingly exposure to a chronic low dose of testosterone propionate increased contextual fear responses in female mice, but only if these mice had been previously subjected to social isolation [26].

The diversity of AAS actions on anxiety-like behaviors in rodents is likely to reflect the interactions of age, sex, type(s) of AAS, the regimen employed to administer them, and environmental context. Beyond these variables, differences in experimental methodologies employed to measure anxiety may also add to the disparities. Studies that employ multiple behavioral tests in conjunction with a single paradigm of drug administration will be particularly valuable in discerning the impact of criteria such as sex or age on the expression of AAS-induced anxiety, and in parsing out how the AAS alter the different facets of the repertoire of anxious behaviors.

What are the implications of AAS-dependent effects on anxiety? First, there are many physiological and psychological correlates that suggest an intriguing reverse-parallelism between AAS users who manifest muscle dysmorphia and the disorders of body image that characterize individuals who suffer from anorexia nervosa. As with anorexia, AAS-induced

muscle dysmorphia is believed to reflect pathological anxiety, specifically a form of obsessive-compulsive disorder in which overly-muscled individuals perceive themselves as wimpy and weak [13]. Moreover, neuropeptides, such as corticotropin releasing factor (CRF), that are thought to play a key role in the animal models of anorexia [27], have also been shown to be dysregulated in animals exposed to suprathreshold levels of AAS [24]. Second, AAS-dependent increases in anxiety may be important contributors to the impulsivity and hostility often reported in steroid users, effects that are mirrored in enhanced levels of aggression in male rodents chronically exposed to these synthetic steroids.

The behavioral effects of AAS on aggression

Steroid abuse in humans is associated with poor impulse-control, extreme mood-swings, and abnormal levels of aggressive actions [6,9,12]. Although acts of violence (so-termed 'roid rage') and hostility have been in the spotlight of the popular press, the evidence of a link between AAS and overt acts of violence is complex and equivocal. By contrast, data on AAS and aggression in animal models are not ambiguous: chronic exposure of either adult or adolescent male rodents has consistently been shown to promote elevated levels of offensive aggression directed against not only male (Figure 1a–e), but also female conspecifics [28]. Offensive aggression is typically assessed by the resident-intruder test during which the proportion of animals fighting, the latency and duration of the attack, and the display of other aggressive behaviors such as leaps, lunges and tail rattles when an intruder is introduced to the home-cage of a male rodent, are tallied [19]. The level of offensive aggression depends upon the social history of the home-cage resident, the sex and hormonal status of the intruder, the age of exposure, and the environmental context in which behaviors are tested (Figure 1a–e).

AAS-induced aggression is also dependent upon the type of AAS administered. Adult male rats subjected to a mild physical provocation (e.g. tail pinch) demonstrate increased inter-male offensive aggression when chronically treated with testosterone propionate (Figure 1d), but nandrolone decanoate was without effect, and long-term exposure to stanozolol actually decreased aggressive behavior (Figure 1d) [29]. The suppressive effect of stanozolol on aggression in the absence of provocation (Figure 1c) has been observed in both adolescent and adult rats, as well as in adult mice [1,30,31], underscoring the importance of the identity and chemical nature of any individual AAS in its behavioral actions. Although a correlation exists between the relative binding affinities of several AAS to the androgen receptor (AR) and their ability to elicit inter-male aggression, there is no simple relationship between AR affinity, AR binding and behavioral effects [29,32]; the suppressive effects of stanozolol may reflect the actions of this AAS at the estrogen receptor (ER) [33], as much as its low affinity at the AR.

Intriguingly, treatment of adolescent male rats, as opposed to adults, with stanozolol does not suppress, but instead elevates aggressive behaviors when they are subjected to provocation, and this heightened level of aggression is maintained after AAS treatment is discontinued [34]. Moreover, although adolescent male rats chronically treated with testosterone show elevated aggressive behaviors, they nonetheless respond appropriately to social and environmental cues [31] (Figure 1b,c). Enhanced levels of aggressive behaviors

that last for weeks following AAS withdrawal have also been reported for adolescent hamsters [35,36]. These studies highlight not only the crucial importance of age in the behavioral effects of individual AAS, but also that enhanced levels of aggression in animals exposed during adolescence may persist for prolonged periods of time, even if steroid use is halted.

Such long-lasting effects of AAS, when coupled with the normal range of volatile behaviors that accompanies the transition through puberty, stand as a particularly important cautionary signal against AAS use in adolescence. The interactions of AAS with the hormonal milieu that characterizes adolescence may also contribute to the increases in risk-taking behavior that are observed in school-age children who use AAS [17,18]. Moreover, puberty is a developmental epoch during which endogenous hormones act through steroid receptors to organize the neural circuitries responsible for adult-like behaviors. Thus, neural circuits that have enhanced sensitivity to androgen exposure during adolescence may be permanently altered by AAS exposure during this time [37]. This concern is supported by studies showing that animals exposed to AAS during adolescence display higher levels of aggression than do animals who received AAS during adulthood [38,39]. It has also been shown that juvenile rats exposed to AAS had significantly smaller testes in adulthood following withdrawal from AAS than had rats exposed to AAS in adulthood, suggesting that early AAS exposure may impose a prolonged suppression of the hypothalamic/pituitary/gonadal (HPG) axis [38]. Such age-specific peripheral effects may, in turn, lead to long-term effects on aggression and anxiety through loss of the normal androgen-dependent regulation of cortisol/cortico-sterone synthesis and of stress-induced activation of the hypothalamic/pituitary/adrenal (HPA) axis [40,41].

Studies in rodents also provide insight into how AAS users may poorly process important social cues. Specifically, it is intriguing to note that AAS-treated adult male rats will respond more aggressively even when it is the intruder (rather than the AAS-treated animal himself) that is subjected to provocation [29] (Figure 1e). In addition, AAS treatment (nandrolone decanoate) of adult male rats can promote markedly-elevated levels of defensive aggression, even to an innocuous stimulus [42]. These studies in rodents may provide a window into understanding how persons who take AAS may misinterpret social interactions and respond with inappropriate levels of aggression when they themselves are not threatened.

AAS effects on the underlying neural architecture of anxiety

The evidence to date suggests that AAS, similarly to endogenous steroids, impart interwoven and complex effects on multiple nodes of the neural networks that are involved in the generation of anxiety and aggression (Figure 2; Box 2). The task of fitting together the AAS-dependent effects on these molecular jigsaw pieces into a coherent behavioral picture has only started. However, recent studies have demonstrated that signaling mediated by both classical neurotransmitters and neuromodulatory peptides in these circuits is significantly altered in AAS-treated animals.

Box 2**Outstanding questions**

- How does the extensive overlap of neural circuits involved in anxiety and aggression influence AAS actions? Does AAS-induced aggression arise from steroid-induced anxiety (Figure 2)? Are rodents that display inherently lower or higher levels of anxiety [53,118] more likely to show AAS-related aggression? Are there differential effects on anxiety in animals that have inherent differences in aggression [99]? Experimentally addressing such questions may point to important genetic determinants that could increase or decrease individual susceptibility to the negative behavioral effects associated with AAS use.
- What role do anxiety-related circuits play in substance abuse among AAS users? Do AAS effects on the neural circuits that underlie the expression of anxiety play a role in the observed higher levels of administration of other drugs of abuse in AAS users versus non-users [4]? This is especially intriguing with regard to the actions of AAS and ethanol because the behavioral effects of both drugs depend on CRF-mediated changes in GABAergic transmission in the extended amygdala. Does an AAS-induced increase in anxiety lead to increased ethanol consumption in efforts to quell this behavioral effect (i.e. 'self-medication')? Could chronic AAS use lead to increased physiological tolerance to ethanol because of the convergence of effects on CRF signaling (Figure 3)?
- What are the long-term effects of prior AAS use? With a large population of men who used AAS as teenagers and young adults now entering middle age, some data have suggested that these individuals are at higher risk of negative psychological outcomes [8]. Animal studies that model such long-term effects in former AAS users are warranted.
- How do AAS-related actions in one brain region affect those in another? How do these changes result in behavioral modifications in both rodents and humans? There is ample evidence that AAS can alter the cellular biology of specific brain regions [11]. However, techniques employed by studies to date have not allowed assessments beyond isolated regions to determine how activity in whole circuits in a behaving animal is altered with AAS use. New advances in functional imaging of freely moving animals, as well as current optogenetic approaches [95,98], may provide answers to these questions.
- How general are the underlying mechanisms of AAS-induced anxiety and aggression? Anxiety may be both a symptom and a cause of other psychiatric disorders. The experimental generalization of the underlying mechanisms of AAS-induced anxiety and aggression in rodents may yield significant clues into the etiology of these conditions within the human population.
- What is the impact of exercise on AAS-induced aggression and anxiety? The effects of AAS on peripheral tissue clearly depend on exercise. Behaviorally, exercise is known to diminish anxiety and impulsivity in both humans [119] and

in rodents [120]. No study to date has assessed the effects of chronic AAS on the expression of anxiety or aggression in animals that exercise. In particular, although moderate exercise might be expected to diminish AAS-induced anxiety, it will be important to examine the effects of excessive exercise, which may better reflect the conditions of professional athletes, to determine if it exacerbates AAS-induced anxiety and aggression.

A corridor of interrelated forebrain structures termed the extended amygdala, which includes the central amygdala (CeA), the shell of the nucleus accumbens [NAc(s)], and the adjoining bed nucleus of the stria terminalis (BnST), comprise the neural circuitry fundamental to the expression of anxious states [43,44] (Figure 3). The CeA is paramount in the acquisition and expression of phasic fear to specific threats whereas the BnST is crucial for sustained fear/anxiety [44], and the connection between these two regions is pivotal to the normal balance between adaptive, specific fear, and maladaptive, diffuse fear (i.e. anxiety). Changes to both specific threats and generalized anxiety are noted in steroid abusers, but the latter may be particularly important with respect to the expression of inappropriate reactions and increased negative perception of self and others [6].

The extended amygdala, as a component of the limbic system, integrates higher-order cognitive processes and homeostatic information, and neurons within these structures express a host of neuromodulatory peptides, including CRF [24,45], brain-derived neurotrophic factor [41], neuropeptide Y [46], pituitary adenylate cyclase activating peptide (PACAP) [47], nociceptin [48], and calcitonin gene-related peptide [49], as well as classical neurotransmitters including GABA, glutamate, serotonin (5-hydroxytryptamine; 5-HT) and dopamine [22,25,50–53] that regulate the expression of anxiety. Chronic AAS exposure has been shown to alter the expression of a wide range of these molecules and their cognate receptors within these regions (Figure 3). Knitting together AAS-dependent changes along the continuum from expression of signaling molecules to alterations in neurophysiology to behavior has not often been done, however recent work suggests an important functional mechanism by which AAS exposure gives rise to anxiety. Specifically, AAS treatment of female mice throughout adolescence resulted in an increase in anxiety-like behaviors (but not fear), as assessed by the acoustic startle response (Figure 4a) and the elevated plus-maze, and that this action of AAS could be blocked by intracerebroventricular infusion of the CRF receptor type 1 (CRF-R1) antagonist, antalarmin (Figure 4b) [25]. Although effects on other important signaling pathways cannot be ruled out, the increase in anxiety elicited by chronic AAS exposure was accompanied by increased activity in GABAergic neurons that project from the lateral CeA to the dorsolateral BnST, an increase in the frequency of GABA_A receptor (GABA_AR)-mediated spontaneous inhibitory synaptic currents (sIPSCs) in downstream BnST neurons (Figure 4c) and a concomitant decrease in their activity. The effects of chronic AAS treatment on inhibitory transmission to the BnST neurons could be blocked by the CRF-R1 antagonist, antalarmin (Figure 4d) or by the GABA_AR antagonist, picrotoxin, and could be mimicked by direct application of CRF [25]. Furthermore, chronic treatment with AAS resulted in an increase of CRF mRNA in the CeA and of CRF peptide in processes in the BnST. Taken together, these data are consistent with a mechanism by which AAS promote an anxiety-like state by enhancing GABAergic inhibition in the CeA to

BnST pathway via CRF-R1-dependent increases in presynaptic release of GABA – where the increased GABAergic tone could arise from the CeA afferents themselves or from nearby terminals of interneurons within the BnST [24,25].

AAS effects on the underlying neural architecture of aggression

Although the neurophysiological interface has not been fully assessed in relating molecular changes to behavior in studies of aggression, a substantive body of literature demonstrates that chronic exposure to AAS alters the expression of molecules known to regulate the expression of aggression in those brain regions that subservise this behavior. For example, in many species the lateral hypothalamic area has been demonstrated to be important in attack behaviors [54] (Figure 2). Chronic AAS treatment of male hamsters during adolescence elicits significant changes in serotonergic, dopaminergic and glutamatergic signaling [10,55–57] in a network of regions that includes this part of the mediobasal hypothalamus, as well as its connections to other limbic structures termed the latero-anterior hypothalamus [10,56]. The importance of 5-HT in contributing to AAS-induced aggression is supported by work demonstrating decreased 5-HT_{1A} receptor (5-HT_{1A} R) and 5-HT_{1B} Rs immunoreactivity in the latero-anterior hypothalamus of AAS-treated hamsters [58,59], but increased 5-HT_{2A} R immunoreactivity in these same regions [60] (Figure 2). Interestingly, a shift in the balance of serotonergic actions from inhibition (5-HT_{1A} Rs) to excitation (5-HT_{2A} R) is hypothesized to be a major contributor to pathological anxiety states [61]. Similar AAS-induced shifts in the hypothalamus may also contribute to increased aggression. As has been shown with anxiety [61], the relationship between 5-HT and aggression, as it relates to AAS-initiated actions, is complex and may be highly state-dependent. For example, whereas depletion of 5-HT led to increased levels of aggression in AAS-treated rats [62,63], AAS treatment increased 5-HT levels in the frontal cortex in rats [63]. Moreover, the timing of changes in aggressive behaviors did not correlate with changes in expression of 5-HT, but did with another key regulator of aggression, arginine vasopressin (AVP) [64].

The fact that AAS treatment can alter the levels of AVP, as well as of substance P [65], another peptide known to play an important role in the expression of aggression, begs caution with respect to attributing behavioral actions based on histochemical correlations alone. The interplay between neurons in the hypothalamus expressing neuromodulators such as AVP and 5-HT neurons in the dorsal raphe have been postulated to converge on a single population of neurons that facilitates aggression, although the presumptive identity of these neurons is not known [10], and no functional studies have been performed to test this mechanism. Furthermore, the ability of the AAS to alter the biosynthesis of endogenous neurosteroids, such as allopregnanolone [66–68], must not be overlooked when searching for molecular correlates of AAS-induced changes in anxiety and aggression. In fact, for both these behaviors, the changes imposed by AAS are likely to reflect an integration of a myriad of effects on classical neurotransmitter systems and their modulators.

Mechanisms of AAS signaling

Steroid receptor signaling

The mechanisms by which chronic AAS exposure modifies the expression of anxiety and aggressive behaviors are only beginning to be uncovered, but any discussion of AAS action in the brain must begin with steroid receptors. All AAS bind to ARs, albeit with significantly different activities. AAS can also be aromatized to estrogens with the capability to interact with both ER α and ER β . AAS can also signal through ER α and ER β and the progesterone receptor without metabolic conversion [11]. The regions of the mammalian forebrain that regulate anxiety and aggression are rich in the expression of these steroid receptors and the enzymes that both synthesize and metabolize steroids [68–72]. It is likely that AAS impart their effects through interactions with all of these receptors, as well as by altering the activity of steroid-metabolizing enzymes.

The importance of AR and ER signaling on the expression of aggression has been demonstrated through several elegant experiments using knockout mice [73–75] and naturally occurring AR-deficient (*tfm*) mice [76]. These studies suggest that both AR-dependent and AR-independent signaling are important in the ability of AAS to increase aggression in mice. AAS can also have important effects by indirectly altering signaling via ER by their ability to allosterically inhibit aromatase, thus limiting the availability of endogenous estrogens [77]. Furthermore, they may have key non-receptor-mediated effects on both aggression and anxiety through their ability to decrease the biosynthesis of endogenous neurosteroids [66,68,77].

Allosteric modulation of GABA_A receptors (GABA_ARs)

Although the chronic use of supratherapeutic levels of AAS is most commonly associated with negative behavioral outcomes, including decreased libido, increased aggression, irritability, anxiety, and impulsivity [2,6,8,12,15,16,78], humans who use AAS, both therapeutically and illicitly, also report positive mood symptoms, including increased libido, increased self-confidence, diminished anxiety, a sense of euphoria, and hypomania [15,16,78]. Increases in anxiety and aggression are evident after chronic exposure to AAS, and these effects are likely to arise from genomic mechanisms. However, the positive effects of AAS in diminishing anxiety may arise from more rapid, non-genomic mechanisms. Indeed, significant, rapid anxiolytic effects (within 30 min) have been reported for adult male rats and mice given acute doses of testosterone or androgenic neurosteroids [79,80], and these actions could be antagonized by concomitant administration of bicuculline or picrotoxin [80], implicating an allosteric action of these androgens at the GABA_AR. As recently summarized [81], GABA_AR activation is the key mediator in regulating the balance of anxiolytic and anxiogenic tone.

The native GABA_AR is a pentameric ionotropic transmembrane protein for which sixteen different receptor subunit genes (α_1 – α_6 , β_1 – β_3 , γ_1 – γ_3 , δ , ϵ , π , and θ) have been identified in mammals [82]. Receptors containing the α_2 subunit are highly expressed in the extended amygdala and are implicated as being the key regulators of the expression of anxiety [81]. The AAS, 17 α -methyltestosterone, was shown to augment currents through α_2 -containing

receptors elicited by GABA under synapse-like conditions, suggesting direct allosteric modulation of α_2 -containing receptors in the extended amygdala may promote acute anxiolytic actions [82]. Although these studies are consistent with a putative mechanism by which acute AAS administration promotes transient positive mood symptoms, perhaps through enhancement of GABA_AR function, behavioral studies have failed to observe decreases in anxiety-like behaviors on the acoustic startle response in adult female mice given a single injection of a mixture of testosterone cypionate, methandrostenolone and nandrolone decanoate [25]. The disparity in these studies may reflect a sex-specific difference, or an inherent difference in allosteric modulation induced solely by naturally occurring neurosteroids, such as 5 α -androstane-3 α ,17 β -diol (3 α -diol) [79,80] and the chemically modified, synthetic AAS [82].

Concluding remarks

Illicit use of AAS is associated with behavioral effects that promote social dysfunction, depression, and an increased incidence of abuse of other addictive substances. Despite these reports from human subjects, steroid users dismiss behavioral effects of these drugs ('Anabolic androgenic steroids can increase aggression, but they cannot alter your mind'; <http://www.steroid.com>), in part because mechanistic results from animal studies have not been sufficiently disseminated. Work summarized here demonstrates that the AAS have significant effects in rodents on the neural circuits that underlie the expression of anxiety and aggression. It is interesting to speculate that AAS effects on aggression and anxiety are intertwined because they involve overlapping neural systems (Figure 2; Box 2). Thus, enhanced generalized anxiety and irritability may contribute to increased levels of aggression and the expression of poor social behaviors observed in steroid users.

Intriguingly, data from animal models also demonstrate a convergence in behavioral and neurophysiological effects of AAS and other illicit drugs whose use is known to covary with AAS [83]. For example, both AAS [25] and ethanol [84,85] impose significant effects on CRF-dependent modulation of GABAergic transmission in the extended amygdala, raising the possibility that AAS use may predispose individuals to alcohol abuse by altering the sensitivity of these circuits to the effects of alcohol. Similarly, AAS effects on dopaminergic transmission have been shown to alter the rewarding properties of cocaine [86]. These data highlight that the neuropsychological complications associated with illicit AAS use should not be considered solely in isolation, but also as a crucial factor in the treatment and counseling for other abused drugs.

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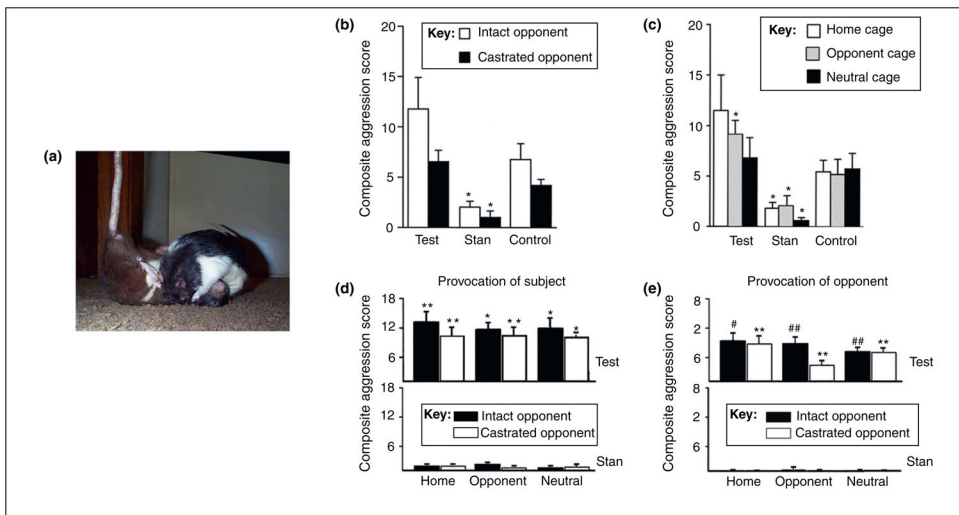


Figure 1.

AAS effects on aggression are compound- and context-specific. **(a)** Offensive inter-male aggression in rats. Reproduced, with permission, from <http://www.ratbehavior.org/>. **(b,c)** Social discrimination in AAS-induced aggression. Rats ($n = 10$ each group) were treated during adolescence with testosterone (Test; class I), stanozolol (Stan; class III) or vehicle (control). **(b)** Although aggressive behavior was greater in testosterone-treated than control rats, each group showed comparable social discrimination in aggression towards a gonadally-intact versus a castrated intruder. These experiments also demonstrate the importance of the chemical signature of the AAS because stanozolol inhibited offensive aggression compared to controls ($*P < 0.05$) [31]. **(c)** Rats treated with testosterone were more aggressive than controls in the opponent's cage, but rats treated with stanozolol showed decreased aggression relative to controls ($P < 0.05$) irrespective of environment [31]. Such findings demonstrate the importance of environmental cues, as well as inherent differences in behavioral responses to distinct AAS compounds. **(d)** Provocation (tail pinch) enhanced aggression in rats treated with testosterone propionate (but not stanozolol) in each environmental (home, opponent or neutral cage) and social (gonadally-intact or castrated) condition compared to controls ($*P = 0.0001$; $**P = 0.001$) [29]. Controls showed more aggression to an intact than a castrated male, even when provoked (data not shown). **(e)** Moreover, enhanced aggression was elicited in rats treated with testosterone propionate even when the provocation was given to the opponent male ($**P = 0.001$; $\#P = 0.01$; $###P = 0.05$). Modified, with permission, from [31] **(b,c)** and [29] **(d,e)**.

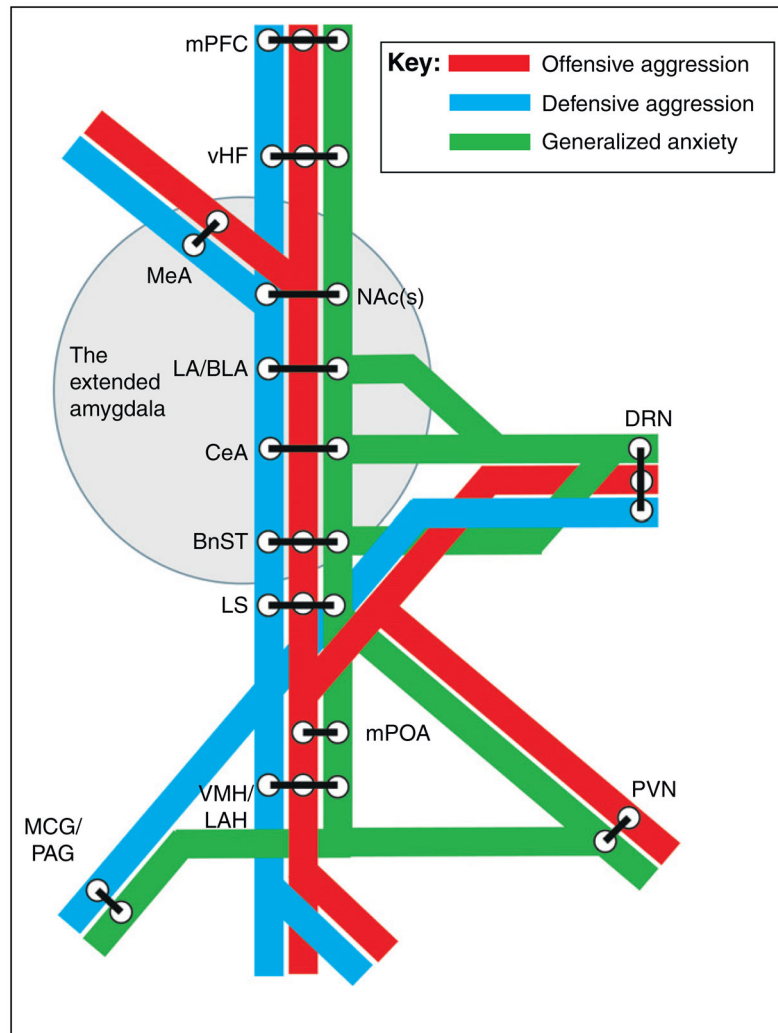


Figure 2.

The mind's metro of anxiety and aggression. The neural circuitry implicated in the expression of both aggression and anxiety-like behaviors exhibits striking overlap. These neural areas are presented as train stations on different train lines of a subway or metro map, to illustrate the overlap and complexity of affective and aggressive circuitry. The medial pre-frontal cortex (mPFC) [87–89], the extended amygdala, and the dorsal raphe nuclei (DRN) [89,90] have been implicated independently and together by numerous studies in the modulation of both anxiety and aggressive behaviors. Sub-divisions of the extended amygdala [43], including the bed nucleus of the stria terminalis (BnST) [43,47,54,87,91] the shell of the nucleus accumbens [NAc(s)] [92–94], and the three parts of the amygdala proper {the lateral and basolateral (LA/BLA) nuclei [53,90,95], the medial nucleus (MeA) [73,87,91,96], and the central nucleus (CeA) [43,44,52,95,97]}, have been shown to be involved in aggression and anxiety, as have the ventral hippocampus (vHF) [87,88], the lateral septum (LS) [73,87,91,97], and parts of the hypothalamus [88,98] {the ventromedial nucleus and lateral anterior hypothalamic areas (VMH/LAH) [54,55,96,99], the paraventricular nucleus (PVN) [40,97,99] and the medial preoptic area (mPOA) [88,96]}.

The lower brainstem {midbrain central grey (MCG) [88] and periaqueductal grey area (PAG) [43,88]} is also an integral part of the circuitry.

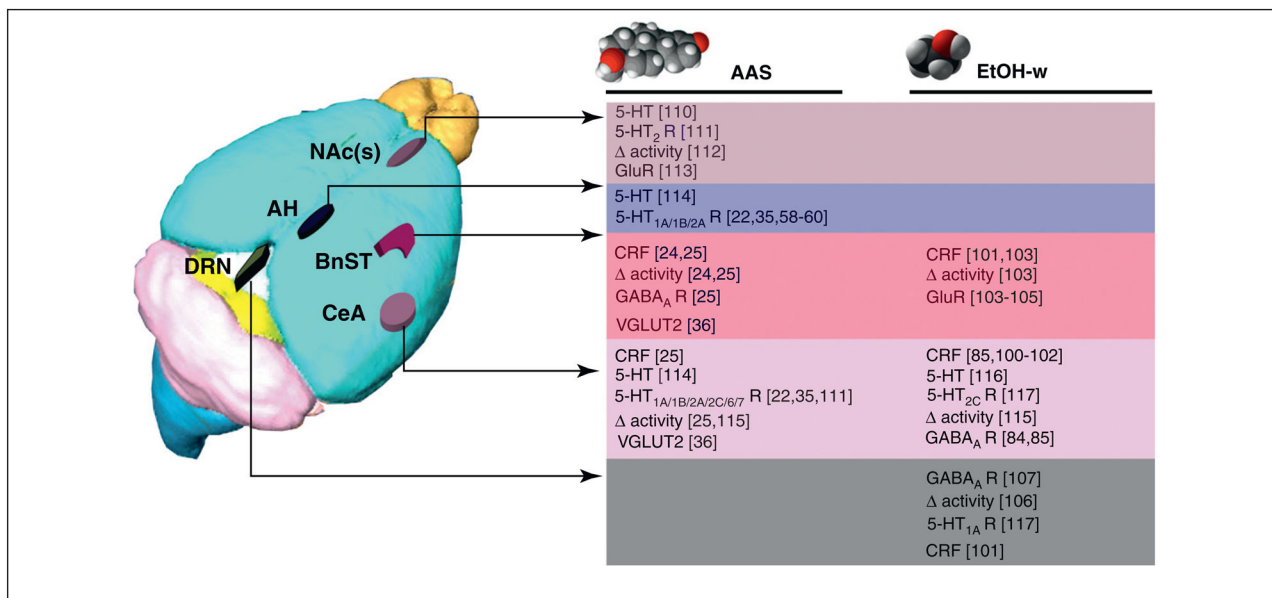


Figure 3.

Comparisons and similarities between AAS and ethanol actions. Chronic AAS exposure [24,25] and withdrawal from ethanol [85,100–102] both result in increased anxiety-like behavior. Molecular and cellular studies suggest that both of these drugs of abuse impose similar changes in neural signaling along a shared template that includes reciprocal connections between structures in the extended amygdala [43] {including the nucleus accumbens shell [NAc(s)], the bed nucleus of the stria terminalis (BnST), and the central nucleus of the amygdala (CeA) and the dorsal raphe (DRN)}, as well as their connections with structures, such as the anterior hypothalamus (AH), ventrolateral nucleus of the hypothalamus, hippocampus and medial prefrontal cortex, that are pivotal for the expression of both anxiety and aggression (Figure 2). Although some molecular targets have not been assessed in all of these key regions for both classes of drugs, the available data converge to suggest potential mechanisms by which both AAS exposure and ethanol withdrawal (EtOH-w) augment the expression of corticotropin releasing factor (CRF) in key regions of the anxiety circuitry, including the BnST [24,103], the CeA [25,84,85,100–102], and the DRN [102]. AAS and/or alcohol-dependent changes in CRF may, in turn, alter classical neurotransmission mediated by both GABA_ARs [25,84,85] and glutamate (as assessed by vesicular glutamate transporter 2, VGLUT2) [36] and glutamate receptors (GluRs) [56,103–105]. Finally, AAS and alcohol may significantly alter the activity of 5-HT neurons in the DRN [106] through GABAergic afferents to this region [90,107]. These DRN neurons may reflect a crucial focal point in the control of anxiety because they provide greater than 50% of the serotonergic innervation to the forebrain [108], including regions such as the BnST [61,109] and the CeA [52], implicated in the expression of anxious states, and regions such as the AH that are crucial for the expression of aggression [10]. Mouse brain adapted from an image in the Magnetic Resonance Microimaging Neurological Atlas (MRM NeAt) Mouse Brain Database (<http://brainatlas.mbi.ufl.edu/ImageGallery.php>).

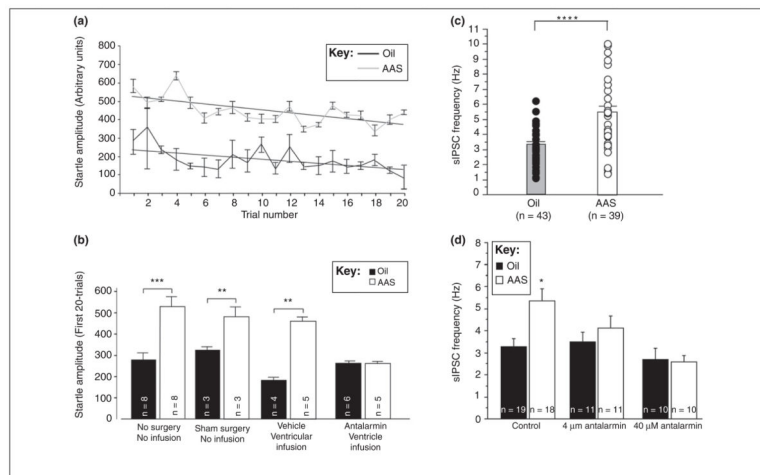


Figure 4.

AAS enhances anxiety and GABAergic transmission in the BnST by a CRF-dependent mechanism. **(a)** Data demonstrating that the amplitudes of the acoustic startle response were significantly greater on all trials in animals receiving a chronic mixture of three commonly used AAS (testosterone cypionate, nandrolone decanoate and methandrostenolone; 7.5 mg/kg/day) than in control animals receiving a comparable volume of sesame oil (vehicle) ($n = 8$ in each group) [25]. **(b)** Mean startle amplitudes over the first 20 trails for oil- and AAS-treated animals that received no infusions or surgery, sham surgery, ventricular infusion of saline, or ventricular infusion of the CRF-R1 antagonist, antalarmin. n values represent the number of animals per group/condition (** $P < 0.01$; *** $P < 0.001$) [25]. **(c)** sIPSC frequencies from individual dorsolateral BnST neurons from oil-injected (black circles) and AAS-treated (white circles) mice. Histograms display the same data as means with error bars (SEM); n values represent the number of cells (**** $P < 0.0001$). **(d)** Average sIPSC frequency recorded from neurons in the dorsolateral BnST in saline alone (control) or in the presence of 4 or 40 μ M of antalarmin. *The frequency of sIPSCs in these neurons of the dorsolateral BnST from AAS-treated animals under control (saline) recording conditions was significantly ($P < 0.05$) different than the frequency recorded from neurons of oil-injected mice; n values represent the number of cells. Acute application of the CRF-R1 antagonist, antalarmin, abrogated the difference in sIPSC frequency in dorsolateral BnST neurons between oil- and AAS-treated mice [25]. Reproduced, with permission, from [25].