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Serotonin modulates anxiety-like behaviors during withdrawal from adolescent anabolic–androgenic steroid exposure in Syrian hamsters

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ABSTRACT

From the U.S. to Europe and Australia anabolic steroid abuse remains high in the adolescent population. This is concerning given that anabolic steroid use is associated with a higher incidence of pathological anxiety that often appears during withdrawal from use. This study uses pubertal Syrian hamsters (*Mesocricetus auratus*) to investigate the hypothesis that adolescent anabolic/androgenic steroid (AAS) exposure predisposes hamsters to heightened levels of anxiety during AAS withdrawal that is modulated by serotonin (5HT) neural signaling. In the first two sets of experiments, adolescent AAS-treated hamsters were tested for anxiety 21 days after the cessation of AAS administration (i.e., during AAS withdrawal) using the elevated plus maze (EPM), dark/light (DL), and seed finding (SF) tests and then examined for differences in 5HT afferent innervation to select areas of the brain important for anxiety. In the EPM and DL tests, adolescent AAS exposure leads to significant increases in anxiety-like response during AAS withdrawal. AAS-treated hamsters showed long-term reductions in 5HT innervation within several areas of the hamster brain implicated in anxiety, most notably the anterior hypothalamus and the central and medial amygdala. However, no differences in 5HT were found in other anxiety areas, e.g., frontal cortex and lateral septum. In the last experiment, adolescent AAS-treated hamsters were scored for anxiety on the 21st day of AAS withdrawal following the systemic administration of saline or one of three doses of fluoxetine, a selective serotonin reuptake inhibitor. Saline-treated hamsters showed high levels of AAS withdrawal-induced anxiety, while treatment with fluoxetine reduced AAS withdrawal-induced anxiety. These findings indicate that early AAS exposure has potent anxiogenic effects during AAS withdrawal that are modulated, in part, by 5HT signaling.

Introduction

Despite educational programs and legal prohibitions, the illicit use of androgenic–anabolic steroids (AAS) has remained constant in the adolescent population over the past decade in the U.S., with more than a half million 8th–10th graders reporting use each year and a lifetime use of nearly 2% in males (NIDACapsules, 2007). This pattern is eclipsed however by use in European countries and Australia where AAS use among adolescents can well exceed 6% (Palleson et al., 2006; Williamson, 1993a; Williamson, 1993b). This trend is alarming given that AAS use has been shown to be associated with an increased frequency of an array of adverse psychiatric side effects, including impulsivity, irritability, aggression and mood disorders (Pope and Katz, 1994; Pope et al., 1988). This is significant as much of the clinical data on the psychiatric side effects of AAS stem from the study of users in the 1960s–70s, i.e., a time when few adolescents had access to AAS and the doses used were generally much lower (Kanayama et al., 2008), therefore young AAS users of today may be more vulnerable to a wider range of more severe psychiatric side effects that may persist long after cessation of AAS use.

A common psychological side effect of AAS abuse in adult users is pathological anxiety (Bahrke et al., 1990; Daly et al., 2003; Johnson, 1990; Pagonis et al., 2006a, 2006b; Pope and Katz, 1994; Pope et al., 1988) that often appears during withdrawal from AAS use, either at the end of a use cycle or long after discontinuing AAS use (Bahrke et al., 1990; Brower, 1992; Brower, 2002; Corrigan, 1996; Kashkin and Kleber, 1989; Lindqvist et al., 2007; Malone and Dimeff, 1992; Malone et al., 1995; Perry and Hughes, 1992; Perry et al., 1990; Pope et al., 1996; Su et al., 1993). Often, the range and extent of the anxiogenic effects of AAS correlate with the severity of abuse (Pagonis et al., 2006a, 2006b). In contrast, preclinical studies investigating the relationship between adult AAS exposure and anxiety have produced mixed results indicating that AAS administration has both *anxiolytic* and *anxiogenic* effects (Agis-Balboa et al., 2009; Aikey et al., 2002; Ambar and Chiavegatto, 2009; Bing et al., 1998; Bitran et al., 1993; Fernandez-Guasti and Martinez-Mota, 2005; Koukoulas et al., 1999; Minkin et al., 1993; Ovsiukova et al., 2003; Parrilla-Carrero et al., 2009; Rocha et al., 2007; Rojas-Ortiz et al., 2006). Given the incidence of AAS use among that adolescent population, it is surprising that relatively little, if any, preclinical research has focused on

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elucidating the bio-behavioral effects of adolescent AAS exposure on anxiety-like behaviors, and *none* that have focused on the long-term consequences of withdrawal from repeated, early-onset AAS exposure.

For over a decade we have used pubertal male Syrian hamsters (*Mesocricetus auratus*) as an adolescent animal model to investigate the effects of adolescent AAS exposure on the neurobiology of aggressive behavior (see Melloni and Ricci, 2010 for a comprehensive review). Interestingly, recent reports have shown a neurobiological relationship between aggressive behavior and anxiety (Apter et al., 1990; Fehon et al., 2001; Guillot and Chapouthier, 1996; Veenema and Neumann, 2007; Veenema et al., 2007). Closer examination of the neural networks regulating these behaviors reveal overlapping neural loci, supporting the notion that aggression and anxiety share a common neuroanatomy (Delville et al., 2000; Ernst and Fudge, 2009; Ferris, 2000; Newman et al., 1999; Pratt, 1992) (see Fig. 1). In particular, the anterior hypothalamus (AH), central amygdala (CeA), frontal cortex (FC), lateral septum (LS), and medial amygdala (MeA) appear to be sites of overlap for neural inputs that influence *both* aggressive behavior (Delville et al., 2000; Nelson and Trainor, 2007) and anxiety (Pratt, 1992). Findings from a number of our previous studies identified the serotonin (5HT) neural system in several of these brain sites as important points of convergence for developmental and neuroplastic alterations modulating adolescent AAS-induced aggression. In brief, reductions in 5HT afferent development were observed in two of these sites, i.e., the AH and MeA, in highly-aggressive, adolescent AAS-treated animals (Grimes and Melloni, 2002; Grimes et al., 2007). However, at 21 days of AAS withdrawal, AAS-treated animals were no longer aggressive (Carrillo et al., 2011; Grimes and Melloni, 2006; Grimes et al., 2006), yet reductions in 5HT afferent development to these regions remained (Grimes and Melloni, 2006), an indication that adolescent AAS exposure produced lasting reductions in 5HT signaling that did not correlate with offensive aggression. From these data we hypothesized that perhaps behaviors other than aggression that are influenced by 5HT would emerge during withdrawal from adolescent AAS exposure as a result of this persistent reduction in 5HT signaling. For instance, in humans abnormalities in the 5HT neural system have also been implicated in generalized anxiety disorder, panic disorder, post traumatic stress disorder, depression and obsessive compulsive disorder (Deakin, 1998a, 1998b; File, 2001; Graeff et al., 1996; Kennett et al., 1987; Lesch, 1991; Murphy et al., 1989; Southwick et al., 1999). Likewise in animals, reductions in 5HT signaling have been implicated in the development of anxiety (Collinson and Dawson, 1997; Cowen, 1991; Holmes et al., 2003). Given our previous findings that adolescent AAS exposure reduced the development of the 5HT neural system in several brain areas serving both anxiety and offensive aggression (i.e. most notably the AH and MeA) (Grimes and Melloni, 2002; Grimes et al., 2007), we questioned that reduced 5HT signaling may also modulate anxiety-like behaviors during AAS withdrawal. To date, however, it is unknown whether adolescent AAS exposure has any effects on anxiety-like response during AAS withdrawal. Moreover, there is no clear evidence as to whether 5HT signaling plays a significant role in any anxiety-provoking effects of adolescent AAS exposure.

To investigate the relationship between withdrawal from adolescent AAS exposure, 5HT neural development/signaling and anxiety-like behaviors, we used pubertal male hamsters as an adolescent animal model. First, to determine whether adolescent AAS exposure produced alterations in anxiety-like response during AAS withdrawal, AAS-treated hamsters were tested for anxiety-like behaviors 21 days following the cessation of adolescent AAS administration (i.e., the 21st day of withdrawal — when AAS-treated animals no longer display the aggressive phenotype (Carrillo et al., 2011; Grimes and Melloni, 2006; Grimes et al., 2006)). Then, to determine whether adolescent AAS exposure altered the development of the 5HT neural systems in brain sites implicated in *both* anxiety and aggression, immunohistochemistry was utilized to visualize and quantify 5HT afferent fibers and varicosities in AAS-treated hamsters. Finally, using an experimental design similar to the one we employed previously to highlight the role of 5HT signaling in adolescent AAS-induced offensive aggression, we tested whether the selective serotonin

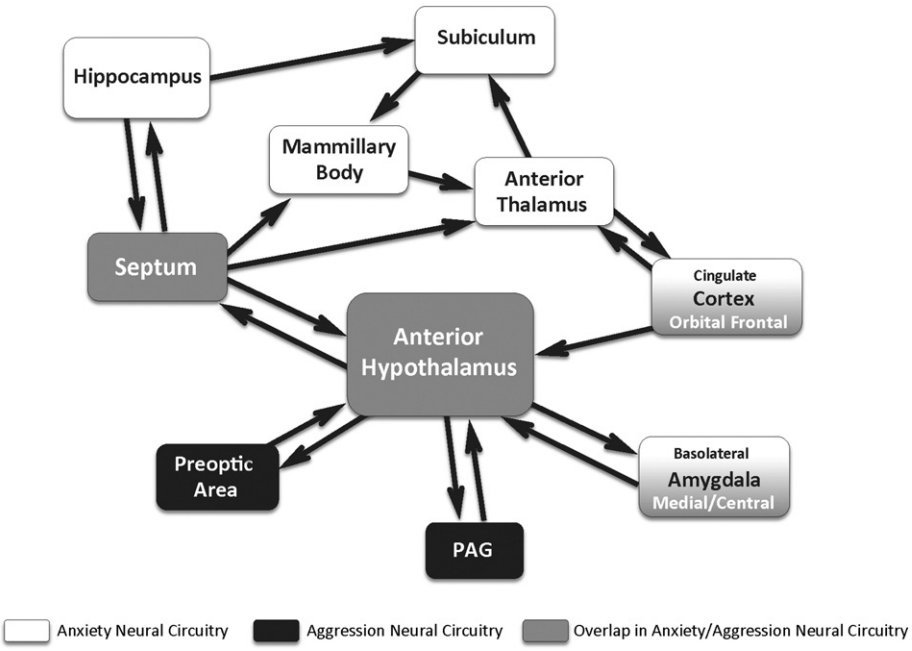

Fig. 1. Conceptual model of the overlap in neural circuits regulating both offensive aggression and anxiety, identifying the anterior hypothalamus, central amygdala, frontal cortex, medial amygdala and lateral septum as brain regions modulating both aggression and anxiety.
Adapted from Delville et al. (2000) and Nelson and Trainor (2007).