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Brain and cognition abnormalities in long-term anabolic-androgenic steroid users

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ABSTRACT

Background: Anabolic-androgenic steroid (AAS) use is associated with psychiatric symptoms including increased aggression as well as with cognitive dysfunction. The brain effects of long-term AAS use have not been assessed in humans.
Methods: This multimodal magnetic resonance imaging study of the brain compared 10 male weightlifters reporting long-term AAS use with 10 age-matched weightlifters reporting no AAS exposure. Participants were administered visuospatial memory tests and underwent neuroimaging. Brain volumetric analyses were performed; resting-state fMRI functional connectivity (rsFC) was evaluated using a region-of-interest analysis focused on the amygdala; and dorsal anterior cingulate cortex (dACC) metabolites were quantified by proton magnetic resonance spectroscopy (MRS).
Results: AAS users had larger right amygdala volumes than nonusers ($P = 0.002$) and reduced rsFC between right amygdala and frontal, striatal, limbic, hippocampal, and visual cortical areas. Left amygdala volumes were slightly larger in AAS users ($P = 0.061$) but few group differences were detected in left amygdala rsFC. AAS users also had lower dACC scyllo-inositol levels ($P = 0.004$) and higher glutamine/glutamate ratios ($P = 0.028$), possibly reflecting increased glutamate turnover. On a visuospatial cognitive task, AAS users performed more poorly than nonusers, with the difference approaching significance ($P = 0.053$).
Conclusions: Long-term AAS use is associated with right amygdala enlargement and reduced right amygdala rsFC with brain areas involved in cognitive control and spatial memory, which could contribute to the psychiatric effects and cognitive dysfunction associated with AAS use. The MRS abnormalities we detected could reflect enhanced glutamate turnover and increased vulnerability to neurotoxic or neurodegenerative processes, which could contribute to AAS-associated cognitive dysfunction.

1. Introduction

Illicit anabolic-androgenic steroids (AAS) use poses a growing public health problem worldwide, with 2.9–4.0 million individuals in the US alone having used these drugs (Pope et al., 2014). Virtually all of these users are male (Kanayama et al., 2007). Although used by elite athletes since the 1950s, AAS did not spread widely to the general population until the 1980s (Kanayama et al., 2008). Thus, even the oldest AAS users, who initiated AAS use as youths in the 1980s, are mostly under age 50 today. As the leading edge of this AAS-user population passes through middle age, adverse general health effects of long-term AAS exposure are becoming increasingly apparent (Pope et al., 2014).

AAS are known to cause *acute psychiatric effects* such as aggression (Copeland et al., 2000; Perry et al., 2003; Pope et al., 2014), violence, including increased partner violence (Beaver et al., 2008; Choi and Pope, 1994; Middleman et al., 1995; Pope and Katz, 1990; Skarberg et al., 2010; Thiblin and Parlklo, 2002), and impulsive behaviors including risky sexual and other behaviors (Hildebrandt et al., 2014; Middleman et al., 1995; Midgley et al., 2000). We (Kouri et al., 1995; Pope et al., 2000) and others (Su et al., 1993; Yates et al., 1999) have documented such effects in controlled human studies. AAS also increase aggressive behaviors in adolescent and adult rodents (Kalinine et al., 2014; Melloni and Ferris, 1996), which may be associated with reduced glutamate uptake and increased *N*-methyl-D-aspartate (NMDA) receptor activity (Kalinine et al., 2014).

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AAS also may cause *chronic cognitive effects*. Recently, we reported (Kanayama et al., 2013) that long-term AAS users exhibited deficits on two tests of visuospatial memory from the widely used CANTAB battery (Cambridge Cognition, 2015), and the severity of these deficits was associated with lifetime dose of AAS used. One of these tests, Paired Associates Learning, has previously been shown to predict the development of dementia (Swainson et al., 2001). Consistent with our human findings, rodent studies have shown that AAS exposure can impair performance on the Morris water maze test of spatial learning and memory (Magnusson et al., 2009; Novaes Gomes et al., 2014; Pieretti et al., 2013; Tanehkar et al., 2013). Impaired inhibitory control and attention also were recently reported in men actively taking AAS, with greater impairment found in adolescent-than adult-onset AAS users (Hildebrandt et al., 2014).

While the human brain substrates for these AAS effects have yet to be elucidated, the findings reviewed above suggest that attentional regions of the brain associated with threat reactivity and regulation, such as the amygdala, the hippocampus, and the dorsal anterior cingulate cortex (dACC), may be particularly vulnerable to chronic AAS use. The amygdala is involved in threat processing and aggression (Siever, 2008). The rat amygdala is androgen-sensitive (Cooke et al., 1999; Lynch and Story, 2000) and androgen administration to male rats induces amygdala neurogenesis and neuronal soma and astrocyte volume and complexity increases (Fowler et al., 2003; Cooke et al., 1999; Johnson et al., 2008, 2012). Functional MRI (fMRI) studies in healthy men report positive associations between amygdala reactivity to angry or fearful faces and levels of the endogenous AAS testosterone (Derntl et al., 2009). Similarly, testosterone administration to healthy men acutely increased amygdala reactivity to angry faces (Goetz et al., 2014). Further, amygdala volume increases have been associated with aggressive behavior among substance users (Schiffer et al., 2011). Collectively these findings suggest that AAS could increase amygdala volume and possibly catalyze or enable aggression behaviors. The hippocampus is involved in spatial memory processes (Squire, 1992). In rats, AAS induce hippocampal apoptosis (Ma and Liu, 2015; Tugyan et al., 2013), and inhibit hippocampal neurogenesis (Brannvall et al., 2005; Novaes Gomes et al., 2014), suggesting that AAS could reduce hippocampal volume, which could be a basis for the AAS-associated spatial memory impairments observed in human and animal studies. The dACC is a cognitive control region involved in attentional processes (Bush and Shin, 2006), which as noted above are abnormal in human AAS users (Hildebrandt et al., 2014). Abnormal dACC activation has been documented in alcohol-dependent subjects performing a spatial working memory fMRI task (Vollstädt-Klein et al., 2010), suggesting that visuospatial dysfunction among AAS users could be related to dACC dysfunction.

Although case reports have documented cerebrovascular problems associated with human AAS use (Akhter et al., 1994; Shimada et al., 2012), no systematic neuroimaging studies have yet assessed human brain effects of long-term AAS use. Accordingly, we acquired from long-term AAS users and nonusers 3 T structural magnetic resonance imaging (MRI), resting state functional connectivity (rsFC) MRI (which maps brain regions thought to be functionally coupled and inherently organized at rest (Greicius, 2008)), and proton magnetic resonance spectroscopy (MRS, which evaluates neurochemistry). We also administered the two computerized tests of visuospatial memory revealing deficits in AAS users in our prior study (Kanayama et al., 2013). Because it is technically challenging to acquire high-quality 3 T MRS spectra from the small, irregularly-shaped amygdala and hippocampus, due in part to partial volume effects (contamination by adjacent structures), we acquired MRS spectra from the dACC, from which MRS data be more reliably acquired. Also, as discussed above, this region may contribute to deficits associated with AAS use. We used MRI to determine whether long-term AAS use is associated with abnormal amygdala and hippocampal volumes and connectivities. Also, given rodent studies suggesting that AAS may enhance the effects of glutamate neurotransmission (Kalinine et al., 2014; Orlando et al., 2007), we used MRS to determine whether long-term AAS use is associated with dACC glutamate abnormalities.

2. Methods and materials

2.1. Participants

Study participants were drawn from a pool of about 150 experienced male weightlifters aged 35–55 who had been evaluated in 2011–2014 in a large ongoing study of the cardiac effects of long-term AAS use (Weiner et al., 2013). Since virtually all AAS users are weightlifters, we initially recruited these men by advertising in gymnasiums frequented by AAS users and nonusers (Kanayama et al., 2003; Pope et al., 2012). Participants received a comprehensive interview covering athletic, medical, and psychiatric histories, and alcohol and drug use history, with detailed questions about AAS use (types of drugs used, doses, and durations), and questions about use of other appearance- and performance-enhancing drugs. Individuals reporting either (a) long-term AAS use ($\geq$2 years of cumulative lifetime AAS exposure) or (b) no AAS use were selected for further study. To assess the validity of participants' self-reports, we tested hair and urine samples for AAS and other drugs of abuse, and performed measures of body muscularity to detect possible surreptitious AAS users. Individuals with inconsistencies between these measures and their self-reports were excluded from study participation. We have detailed these procedures previously (Pope et al., 2012). Participants provided informed consent both for the prior cardiac and present imaging studies, which were approved by the McLean Hospital Institutional Review Board.

2.2. Study procedures

We selected 10 long-term AAS users and 10 nonusers from our pool described above to return for a single three-hour midday visit, without specific control for time or dietary factors. We first reviewed participants' interim history since their initial evaluation, and then screened them for recent alcohol (Alco-Sensor IV; Intoximeters, Saint Louis, Missouri) and standard drugs of abuse (AmediCheck 12-Panel Drug Test Cups; Amedica Biotech, Hayward, CA). They then received the Pattern Recognition Memory (PRM) and Paired Associates Learning (PAL) tests from the widely-used computerized (touch-screen) CANTAB battery (Cambridge Cognition, 2015). The PRM tests visual memory (Sahakian et al., 1988) by presenting two sets of 12 visual patterns, each followed by a recognition phase during which participants are shown two patterns and asked to touch the one they have previously seen. The PAL tests visuospatial memory and new learning (Robbins et al., 1997) by presenting several white boxes that serially "open" to reveal underlying patterns. Participants are shown each pattern and asked to touch the box covering that pattern. We selected these tests because they revealed deficits in AAS users in our previous study (Kanayama et al., 2013). We also administered the short (35-word) version of the North American Adult Reading Test (NAART35; Uttl, 2002) to estimate intellectual ability.

Following cognitive testing, participants underwent neuroimaging using a Siemens TIM Trio 3 T scanner (Erlangen, Germany) and a 32-channel head coil. A clinical brain scan including a high-resolution T1-weighted, dual echo MPRAGE3D anatomical whole-brain image (repetition time (TR) = 2.1 s, echo