



Androgen abuse and the brain

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Purpose of review

The purpose of this review is to examine the recent evidence regarding the effects of exogenous androgens on the brain. Understanding these effects is of high importance, as the consequences of androgens on the reproductive and endocrine system are well documented, while fewer studies have focused on the neural and cerebral consequences of androgen use.

Recent findings

Supraphysiological doses of androgens have been shown to contribute to neurodegeneration, decreased brain-derived neurotrophic factor, increased inflammation and decreased neuronal density in animal studies, which may correspond to changes in mood, cognition and aggression. Findings from human studies suggest that similar behavioural and cognitive deficits may occur as a result of prolonged use of androgens. Additional evidence suggests that androgen use, particularly in high doses, may contribute to brain ageing and cerebrovascular problems.

Summary

Findings from recent human and animal studies indicate that androgen use likely contributes to brain alterations, which may cause the frequently observed deficits in cognitive and emotional functioning. Although exogenous testosterone in appropriate doses for therapeutic purposes likely have some neurobiological benefits for certain populations, supraphysiological doses may cause multiple mental and physical health problems, indicating a need for additional large-scale studies in humans.

Keywords

anabolic-androgenic steroids, androgens, neurodegeneration, substance abuse, testosterone

INTRODUCTION

Testosterone is the primary circulating androgen in men, which is also present in women, though in much lower concentrations. This hormone plays a critical role in regulating bone mass, fat distribution and sperm production, in addition to mood and cognition [1–3]. Thus, exogenous testosterone can be an important therapy, particularly for patients experiencing symptoms of hypogonadism or low testosterone [3,4]. However, when testosterone is used in supraphysiological doses and without medical supervision, a number of risks arise.

Androgens, or anabolic-androgenic steroids, include testosterone and synthetic derivatives primarily used to increase lean muscle mass, and enhance physical appearance and athletic performance. The current global prevalence is estimated to be 3.3%, though this is higher for males (6.4%) [5]. Consistent use of supraphysiological doses of testosterone has been associated with a myriad of physical and psychiatric consequences, including cardiovascular complications, anxiety and aggressive

behaviour [6–9]; however, relatively few studies focus on the brain, though this has increased in recent years.

The majority of research investigating neuropsychiatric effects of androgen use has largely focused on behavioural problems, with a significant focus on violence and aggression. Researchers have suggested these behaviours may reflect changes to brain structures often associated with executive function and

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Curr Opin Endocrinol Diabetes Obes 2021, 28:604–614

DOI:10.1097/MED.0000000000000675

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KEY POINTS

- Recent research investigating the effects of androgens on the brain covers a myriad of different approaches and methodologies, suggesting a number of potential underlying pathways and mechanisms for the known mood and behavioural consequences of long-term use.
- Androgens act on a number of brain regions and mechanisms to bring about deficits in cognitive and behavioural functioning.
- Findings from both human and animal studies suggest androgen use contributes to abnormalities in several brain regions, thereof the prefrontal cortex and hippocampus, as indicated by direct observation or cognitive assessment.
- Future research will benefit from continued use of novel and innovative techniques such as neuroimaging and machine-learning, and the use of postmortem brain tissue to solidify and build upon the findings presented here.

emotional control, including the prefrontal cortex (PFC), hippocampus and amygdala. However, the mechanisms by which androgens influence changes to these structures and subsequent behaviour are not yet well understood. In recent years, likely as a consequence of a steadily growing population of older androgen users, more studies gained interest in the long-term impact on neuronal and brain health. This review describes the published findings of the last year and a half on the effects of androgen use on the brain, including alterations in neurobiology and neurochemistry, as well as deficits in cognition.

MATERIALS AND METHODS

The studies included in this review were identified following the guidelines of the Preferred Reporting Items of Systematic Reviews and Meta-Analysis (PRISMA) [10]. In order to identify studies, a search was conducted in PubMed with search terms included in the supplementary material, <http://links.lww.com/COE/A27>.

The search was conducted on 8 March 2021, and was restricted to studies published after September 2019, yielding 688 records. The initial records identified were independently screened by both authors (M.S. and A.B.) using the online tool Rayyan [11]. The PRISMA flow diagram for study selection is shown in Fig. 1.

Studies were included if they evaluated the effects of exogenous administration of testosterone and synthetic derivatives on cognitive, brain health or

neurobiological outcomes, and were written in English. Both human and animal studies were included. Studies were excluded if brain health was not assessed (including studies investigating only psychiatric outcomes such as aggression, depression or anxiety). Studies were excluded if the exogenous testosterone used was in physiological levels (i.e. hormone replacement therapy), or for other medically supervised processes (i.e. sex transitioning).

RESULTS AND DISCUSSION

After screening and selection, 16 studies were identified to be included in this review: 11 animal trials and five human studies, including one case study. Thirteen of these studies investigate the effects of androgens on brain health, including neurodegeneration, brain ageing, cognition and neuroplasticity. Five studies investigate alterations to the brain that may influence aggressive and drug-seeking behaviours known to be affected by androgens, with two studies relevant to both themes.

Description of studies

Table 1 provides a summary of the included studies' methodologies and results. Figure 2 provides of visual summary of a selection of findings. All studies were published between 2019 and 2021, with studies coming from Europe ($n=7$), North America ($n=5$), Brazil ($n=2$), Egypt ($n=1$) and Iran ($n=1$). The sample size of animal models ranged from 20 to 149, and the sample size of studies in humans ranged from 1 to 229. The majority of studies included all male samples, with one animal model [12] and one human study [13] stating that females were included in the sample.

BRAIN HEALTH

Prolonged use of androgens has been found to have deleterious effects on brain health. A number of mechanisms and processes may be implicated, including neurodegeneration via neurotoxicity, increased inflammation and oxidative stress, cardiovascular disease and impaired neuroplasticity [14].

Neurodegeneration

In a study of rat cortical cultures, several commonly used and structurally different androgen compounds; trenbolone, testosterone, stanozolol and nandrolone, were shown to reduce neurite outgrowth [15^{*}]. This effect was not inhibited or suppressed by a selective androgen receptor antagonist, indicating that the impact on neurite outgrowth is

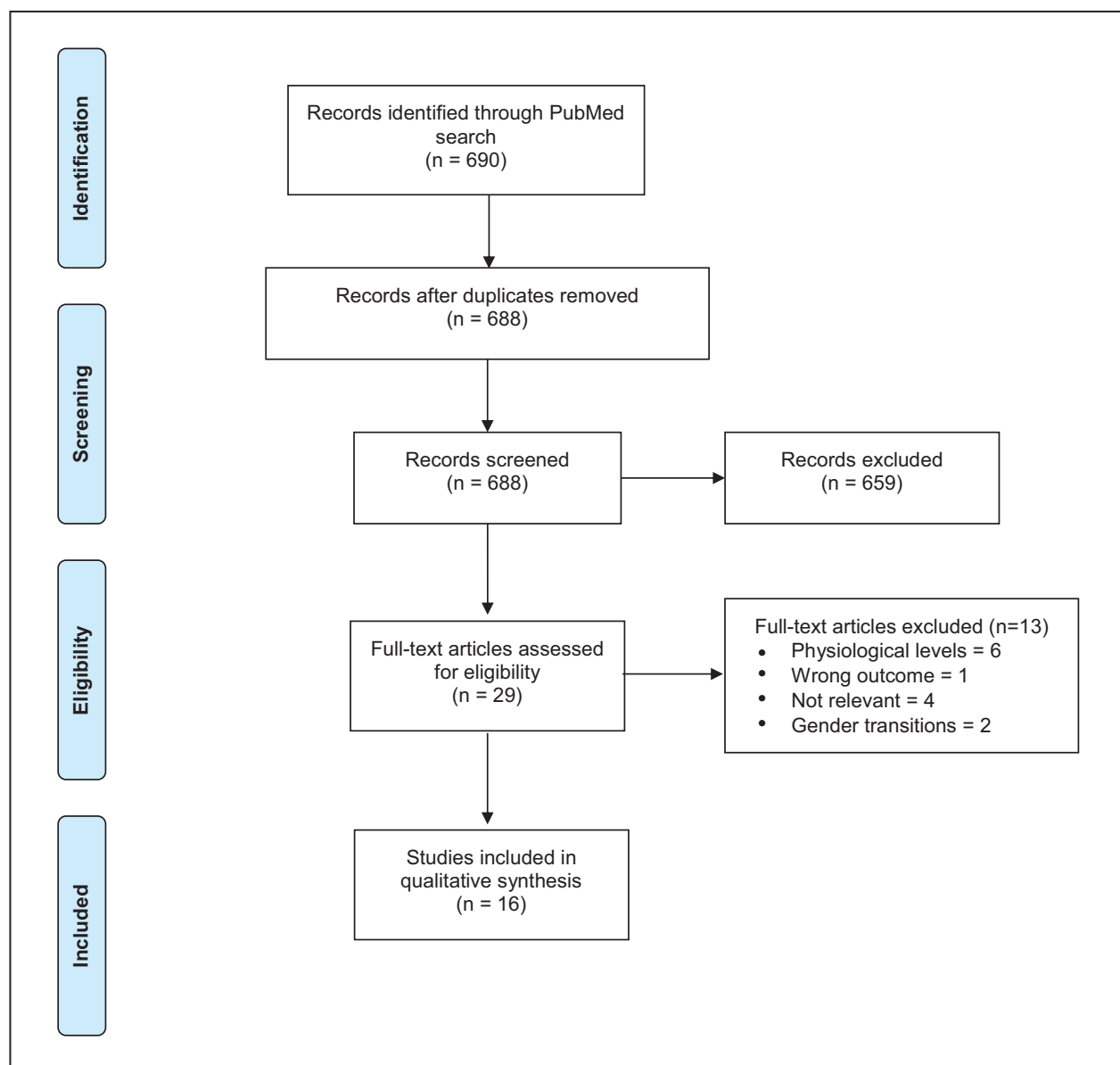


FIGURE 1. PRISMA flow diagram summarizing literature selection process.

caused by another mechanism. In addition, trenbolone seemed to cause the most harm, as it decreased the number of neurons, impaired mitochondrial function and downregulated *Tubb3* gene expression critical to nervous system development. The authors concluded that these neural alterations likely play a role in mental health problems common in androgen users.

Nandrolone was found to increase inflammatory markers, including tumour necrosis factor alpha ($\text{TNF-}\alpha$) and interleukin-1 beta ($\text{IL-1}\beta$) in the hippocampus and PFC in a study conducted in rats [16[¶]]. In addition, a combination of nandrolone and cannabis increased pro-inflammatory cytokines in both the hippocampus and PFC, suggesting

that polysubstance use may increase the risk of neurotoxic effects. This was also reflected in the results of oxidative stress markers in the hippocampus and PFC. Malondialdehyde was elevated as a result of both the nandrolone and combined cannabis/nandrolone conditions, while total nitrate levels were only significantly higher in the combined condition. Human studies using postmortem brain tissues suggest that similar processes might be involved in real-life human use. MicroRNAs (miRNAs), which have been associated with neuronal apoptosis and neuronal stress-induced adaptation, were assessed and compared among cocaine users, androgen users and ageing individuals. The findings showed that all miRNAs evaluated were

Table 1. Summary of included studies population, methods and main findings

Author, year, location	Study population (age, weight)	N	Substance/dose/duration	Outcome(s) measured	Results
Animal studies					
Bontempi and Bonci [43 [■]]	Male mice (8–10 weeks)	N/A	Single intraperitoneal injection of either vehicle or T (10 mg/kg) or ND (10 mg/kg). Mice were anesthetized after 24 h.	Electrophysiology (synaptic transmission in putative VTA), locomotor activity, place preference, histological analysis.	Both T and ND strengthened excitatory synaptic transmission in dopaminergic neurons in putative VTA. This increase was associated with the activation of μ -opioid receptors, and an increase in β -endorphins released into the VTA. Blocking μ -opioid receptors in the VTA prevented two drug-seeking behaviours: locomotor activity and place preference.
Cattelan Souza <i>et al.</i> [29], Brazil	C57B/6J mice (3–6 months, 25–35 g)	48 (24 in independent group to evaluate hedonic alterations in the sucrose preference test)	Group 1: Vehicle + 1-MT, group 2: ND + vehicle, group 3: ND + 1-MT (1-MT dissolved in saline 0.9%), administered via intraperitoneal injection in 1 mg/kg (10 ml/kg volume injection) from day 23 to 28. ND: 10 mg/kg/day in a volume of 1 ml/kg dissolved in corn oil, administered in single daily subcutaneous injections for 28 days. Vehicle: daily oil injections for 28 days	Behavioural assessment: tail stress test, open-field test, sucrose preference test. Biochemical assays: hippocampus, striatum, and PFC (neurotrophins, KP, serotonergic systems, Na ⁺ , K ⁺ ,ATPase activity), blood (corticosterone levels)	ND led to depressive and anhedonic behaviour, decreased BDNF and neurotrophin-3 levels and reduced Na ⁺ , K ⁺ -ATPase activity in the hippocampus, striatum and PFC, decreased serotonin levels and increased 5-hydroxyindoleacetic acid levels. Treatment with 1-MT reversed these alterations induced by ND.
Damião <i>et al.</i> [12], Brazil	Swiss mice (90 days, 40–50 g)	60 (30 males and 30 females)	TC-group: T cyponate-0.8 mg/kg, S-group: stanozolol-1.8 mg/kg. One month of treatment with injections performed twice a week.	Neuronal density using simple random counting from the limbic, motor, and sensitive cortical areas, and the CA1, CA2 and CA3 hippocampal regions.	In male mice both AAS treatments caused ~16% decrease in neuronal density in the limbic area, and decreased numbers of neurons in CA1 ~24% in the S-group. Stanozolol contributed to greater reduction in neuronal density compared to TC in CA1. In females, TC caused a 26% loss in the limbic area, 30% in the motor area and 2.5% in the sensitive area. Hippocampal loss was 28, 29 and 18.5% in CA1, CA2 and CA3 areas. TC led to greater loss than S.
ElShamarka <i>et al.</i> [16 [■]], Egypt	Male Wistar rats (28 days, 75–90 g)	60 (15 in each group)	All administered subcutaneously for 30 days. Group 1: (controls) saline and peanut oil/benzyl alcohol at 90:10 v/v. Group 2: 20 mg/kg cannabis extract in isotonic saline. Group 3: 15 mg/kg ND in peanut oil/benzyl alcohol at 90:10 v/v. Group 4: cannabis (20 mg/kg) and ND (15 mg/kg)	Behavioural: Morris water maze, open field test, elevated as well as maze, defensive aggression test, irritability. Biochemical measurements: oxidative stress biomarkers in hippocampus and PFC, ELISA (TNF- α , IL-1 β , BDNF, cytochrome c), RNA/DNA. Histopathological examination.	Compared to controls, ND group demonstrated increased TNF- α , IL-1 β in both hippocampus and PFC, decreased BDNF in PFC, increased cytochrome c and caspase-8 in the hippocampus and PFC, and caspase-3 in the hippocampus. Combined Can/Nan induced learning and spatial memory deficits, hypocomotion, anxiety and aggression. The combined treatment led to deleterious effects in the hippocampal and PFC neural architecture and a decrease in BDNF. This group also demonstrated increased oxidative stress, elevated brain pro-inflammatory cytokines, and upregulated caspase-3, caspase-8, and caspase-9 mRNA expression and cytochrome c levels.

Table 1 (Continued)

Author, year, location	Study population (age, weight)	N	Substance/dose/duration	Outcome(s) measured	Results
Faitorelli <i>et al.</i> [30], Italy	Balb/c mice (27 months)	20 (five in each group)	Two groups received T; sedentary undergoing T administration, and treadmill training undergoing T administration (mice run on a treadmill 30 min a day, five days a week for 4 weeks at belt speed 8m/min). Both groups injected once a week with T (10 mg/kg in 200 µl peanut oil) for 4 weeks.	Hippocampal synapse morphology (ultrastructure of synapses in molecular stratum of Ammon's horn 1 and inner molecular layer of the hippocampal dentate gyrus), using the following measures: number of synapses/µm ³ of tissue, total area of contact zones/µm ³ of synaptic contact zone, and percentage of perforated synapses.	Exogenous T increases synaptic density in the inner molecular layer of the hippocampal dentate gyrus, independent of physical activity.
Lee <i>et al.</i> [38], USA	Male Syrian hamsters (postnatal day 21)	Treatment: 149, control: 27	Hamsters received daily injections [0.1–0.2 ml] of a mixture consisting of 2 mg/kg T cyponate, 2 mg/kg ND and 1 mg/kg dehydrotestosterone undecylenate dissolved in sesame oil for 30 days	Aggression, aggressive behaviour (with/without valproate doses), aggressive behaviour (with/without valproate doses, and with/without GABA receptor antagonist bicuculline). Social, comfort and motor behaviours.	Valproate selectively suppressed the aggressive phenotype in a dose-dependent manner, with the effective antiaggressive effects beginning at 5 mg/kg. Microinfusion of a GABA _A receptor antagonist (bicuculline) into the LAH reversed valproate's suppression of AAS-induced aggression. Starting at the 70 ng dose of bicuculline, animals expressed the highly aggressive behaviour typically observed following AAS exposure.
Moradpour <i>et al.</i> [27], Iran	Male Wistar rats (22 days)	64 (eight in each group)	Treatments began 7 days after surgery. ND and tacrolimus dissolved in Dimethyl sulfoxide (10% DMSO) as a vehicle were administered via intracerebroventricular injections. ND (100 µg/rat) was microinjected 30 min prior to behavioural assessments. Tacrolimus (2, and 20 µg/rat) was microinjected 20 min before ND in separate groups (n = 8 in all groups).	PAL, electrophysiological study of hippocampal slices.	ND impaired field excitatory postsynaptic potential long-term potentiation, and this effect was nullified by tacrolimus. ND decreased retrieval of PAL, and inhibiting calcineurin counteracted this impairing effect.
Morrison <i>et al.</i> [34], USA	Male Syrian hamsters (postnatal day 21)	34 (n = 6 controls)	Hamsters received daily injections [0.1–0.2 ml] for 30 consecutive days starting from postnatal day 27 of a mixture of three compounds: 2 mg/kg T cyponate, 2 mg/kg ND and 1 mg/kg dehydrotestosterone undecylenate dissolved in sesame oil.	(During withdrawal) Anxious behaviour, aggressive behaviour, LAH serotonin type-3 receptor signalling	During AAS withdrawal period, blocking LAH serotonin type-3 receptors increased aggression and decreased anxious behaviour, reversing the typical pattern of behaviour during AAS withdrawal.
Tabor <i>et al.</i> [23], Canada	Male Sprague Dawley rats (postnatal day 21)	82: Placebo + sham = 21, Placebo + RmTBI = 21, Met + sham = 20, Met + RmTBI = 20	Met, 1.5 mg/kg body weight/day in the drinking water starting at postnatal day 21 and maintained until euthanasia. Randomly assigned to receive three mild traumatic brain injuries or sham injuries with a lateral impact device	Behavioural test battery, postmortem MRI (markers of brain integrity), mRNA expression of markers for neurodevelopment, neuroinflammation, stress responses and repair processes	AAS exposure contributed alterations, including depression, aggression, and memory, and PFC atrophy, and amygdala enlargement, damaged white matter integrity in the corpus callosum. mRNA expression in the PFC and amygdala were altered by AAS. AAS and RmTBI did not produce cumulative deficits.
Wood and Serpa [28], USA	Male Long-Evans rats (5 weeks)	20 (10 vehicle/10 T)	7.5 g/kg T, 5 days/week, for at least 2 weeks, beginning at 5 weeks of age	Biconditional discrimination training (and extinction), novel object recognition.	T-treated rats performed worse than controls during the learning extinction period, suggesting T may impair cognitive flexibility.

Table 1 (Continued)

Author, year, location	Study population (age, weight)	N	Substance/dose/duration	Outcome(s) measured	Results
Zelleröth <i>et al.</i> [15], Sweden	Rat cortical cultures (embryonic day 17)	10 brains (pregnant Wistar dams - euthanized on embryonic day 17)	Cortical cells were administered with 10 ³ or 100 µmol/l of AAS compounds (T, ND, stanzolol and trenbolone) every 24 h for three continuous days. AAS was administered with 10 µmol/l of flutamide by following the same 3-day repeated treatment regime. All chemical treatments were repeated at least three times (n = 3–6).	Androgen receptor expression, neurite outgrowth, cell viability, beta-III tubulin	Trenbolone, T, and ND in the highest concentration reduced neurite outgrowth. Trenbolone downregulated the Tubb3 gene expression and decreased the number of neurons and mitochondrial function.
Human studies					
Bjørnebekk <i>et al.</i> [24], Norway	Male weightlifters (controls: 35.0 ± 8.8, AAS users: 36.2 ± 9.4)	229 (n = 130 with a history of prolonged AAS use, n = 99 with no history of AAS use)	Self-reported history of AAS use (at least one year of cumulative use)	Rates of change in brain age gap, as determined using machine learning algorithm and chronological age.	AAS users had higher brain age gap than weightlifting controls. Accelerated brain aging was demonstrated with longer exposure to AAS.
Choulerton <i>et al.</i> [25], UK	34-year-old man recreational bodybuilder admitted with ischemic stroke	1	3 months of AAS use (T propionate and trenbolone acetate, 25 and 50 mg, respectively, per day) and 1 month of postcycle therapy (tamoxifen 20–40 mg and clomiphene citrate 25–50 mg)	Medical history, physical examination (blood tests, ECG, MRI, chest X-ray, CT angiogram)	Hypothesized a transient prothrombotic state due to AAS use with paradoxical embolization due to an atrial septal defect
Hauger <i>et al.</i> [26], Norway	Male weightlifters (AAS dependents: 38.7 ± 9.5, AAS nondependents: 38.3 ± 11.6, nonusers: 36.5 ± 8.8)	174 (AAS dependents = 58, AAS nondependents = 38, nonusers = 78)	Self-reported history of AAS use (mean weekly dose nondependents 692.0 (536.6), mean weekly dose dependents = 1196.9 (664.8))	Executive function, ADHD symptoms, anxiety and depression, neuropsychological battery	AAS dependents exhibited poorer executive functions and more psychological distress and ADHD symptoms compared to nonusers. Nondependent users demonstrated poorer function in some areas (impulse inhibition, working memory) compared to nonusers.
Sessa <i>et al.</i> [17], Italy	Adult men (AAS: 33.28 ± 4.68, cocaine: 29.42 ± 6.1, aging: 77.28 ± 3.25, controls: 44.85 ± 5.75)	28 (seven AAS, seven cocaine, seven ageing and seven controls)	Postmortem toxicological positive test for AAS	miRNAs: hsa-miR-21-5p, hsa-miR-34a-5p, hsa-miR-124-5p, hsa-miR-132-3p and hsa-miR-144-3p	All included miRNAs were overexpressed in all groups (except controls)
Vaskinn <i>et al.</i> [13], Norway	Weightlifters (Controls female: 28.4 ± 4.5, male: 31.8 ± 9.5, AAS female: 28.7 ± 7.5, male: 33.2 ± 8.3, AAS dependent female: 34.0 ± 7.4, male: 33.4 ± 8.6)	Used AAS: 34M/9F, AAS dependent: 44M/7F, weightlifting comparison: 69M/16F	Self-administration; mean (SD) years of AAS use, AAS group: 6.5 (5.4), AAS dependent: 10.0 (6.1)	Theory of mind; assessed with the Movie for the Assessment of Social Cognition (MASC); total ToM, cognitive ToM, affective ToM, overmentalizing/undermentalizing errors)	AAS dependents performed significantly worse than weightlifting controls. Sex and sex x group interaction effects were not significant.

1-MT, 1-methyl-DL-tryptophan; BDNF, brain-derived neurotrophic factor; GABA, gamma aminobutyric acid; KP, kynurenine pathway; LAH, latero-anterior hypothalamus; Met, metandione; ND, nandrolone; IL-1β, interleukin-1 beta; PAL, passive avoidance learning and memory; PFC, prefrontal cortex; Rmtβ1, repetitive mild traumatic brain injury; T, testosterone; TNF-α, tumour necrosis factor alpha; VTA, ventral tegmental area.

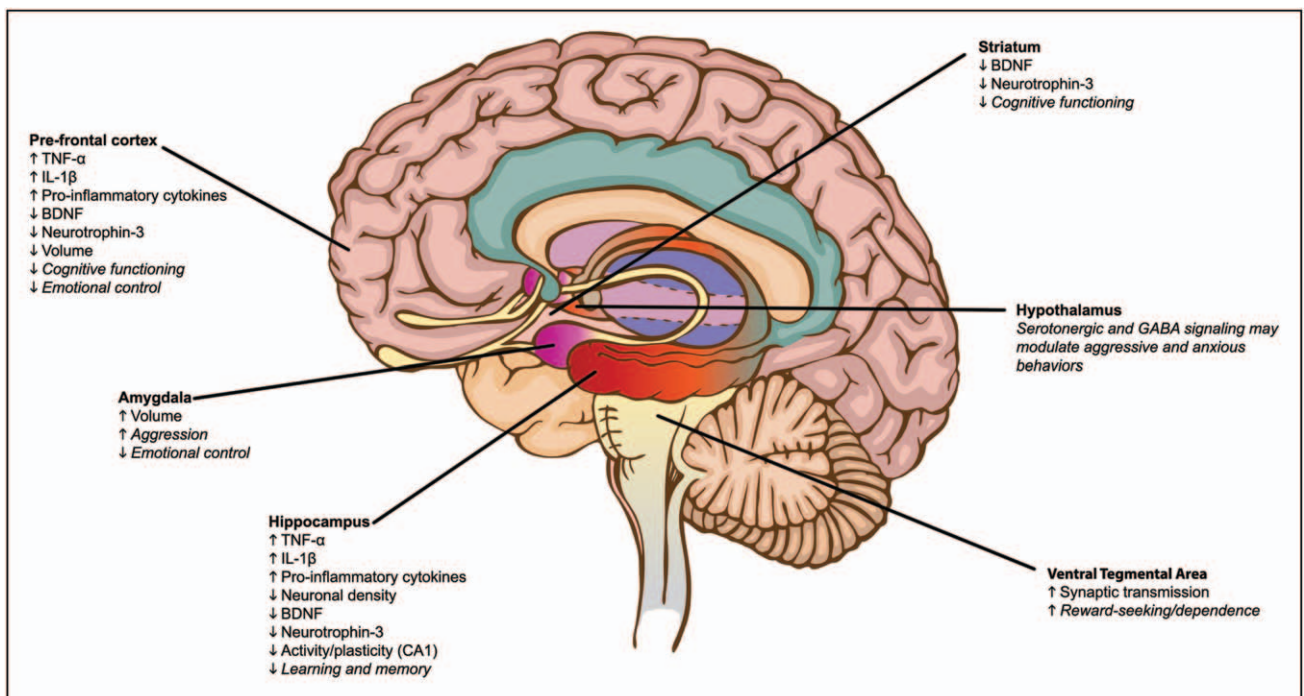


FIGURE 2. Overview of effects of AAS on brain regions and structures from included studies. BDNF, brain-derived neurotrophic factor; GABA, gamma aminobutyric acid; IL-1 β , interleukin-1 beta; TNF- α , tumour necrosis factor alpha.

overexpressed in all groups, relative to controls [17[¶]]. Furthermore, certain miRNAs, such as miR-144, were elevated in androgen users compared with the other groups. This miRNA has been found to play a critical role in ageing [18] and is overexpressed following traumatic brain injuries [19]. This method has been used in numerous studies of more typical substances of abuse, namely cocaine [20]. Furthermore, miRNAs are known to be altered by brain injuries [21], and postmortem studies may provide valuable insights into the consequences of androgens.

Brain volumes and age

Previous neuroimaging findings suggest that androgen use may contribute to brain ageing, as users may demonstrate similar structural changes seen in those at risk for developing dementia [22]. In recent research, a study in rats found that androgen use was associated with PFC atrophy, amygdala enlargement and damaged white matter integrity in the corpus callosum [23]. However, mild traumatic brain injuries with androgen use did not produce cumulative deficits.

Furthermore, a study of 99 weightlifters and 130 androgen users suggests that prolonged use can accelerate brain aging [24[¶]]. Biological brain age was estimated based on machine learning models, and the difference between chronological and biological age

indicated the brain age gap. Androgen users had a higher brain age gap than controls, and longer exposure to these substances corresponded with accelerated brain ageing.

Cerebrovascular

A recent case study demonstrated the cardiovascular risks of androgen use [25]. A 34-year-old male recreational bodybuilder was admitted with an ischemic stroke. The patient reported 3 months of androgen use and 1 month of postcycle therapy. The team hypothesized androgens use led to a transient prothrombotic state with paradoxical embolization due to an atrial septal defect identified on bubble echocardiogram.

Cognition

Cognitive functioning comprises several domains, such as memory, language, attention and executive functions. Studying these functions can provide insight about the health of the specific brain regions implicated for each domain. For example, deficits in executive functioning may imply alterations in the PFC, while difficulties with learning and memory can suggest problems in the hippocampus.

Several studies assessed the risks androgens pose to cognitive functioning, in both animals and humans. One study found that dependent androgen

users demonstrated greater executive function deficits and more attention-deficit hyperactivity disorder (ADHD) symptoms compared with weightlifters that did not use androgens [26]. Non-dependent androgen users demonstrated worse impulse inhibition and working memory relative to nonusers. Furthermore, in a related study, both male and female weightlifters who were dependent on androgens demonstrated impaired theory of mind, suggesting that prolonged androgen use may also impair social cognition [13]. However, it is important to note the cross-sectional nature of these studies, making it difficult to establish causality, and it is possible that reduced executive functioning and impaired theory of mind could be a premorbid risk factor for use and dependence.

In animal models, rats which were administered nandrolone decanoate demonstrated decreased retrieval of passive avoidance learning and memory (PAL). The researchers also suggest a putative mechanism; as administration of tacrolimus, a potent immunosuppressant and inhibitor of calcineurin, alleviated the impairment effect of nandrolone decanoate on PAL [27]. Similarly, Wood and Serpa [28] found that rats treated with testosterone for at least 2 weeks performed worse than controls during the extinction phase of biconditional discrimination, which corresponds to the human Stroop test, suggesting that testosterone may impair cognitive flexibility. In assessing the consequences of poly-substance use, El-Shamarka *et al.* [16[¶]] found that cannabis and nandrolone in combination led to learning and spatial memory deficits and hypo-locomotion.

Neuroplasticity

Neuroplasticity refers to all modifications that occur within the central nervous system through a number of mechanisms, including changes in synapses and dendritic spines, neurotropic factors and cell viability. However, continued use of androgens may impede these processes.

The hippocampal region has an impressive capacity for structural reorganization, and plays a major role in learning and memory, and is thus of particular interest for studies investigating the effects of androgens on the brain. Damião *et al.* [12] tested the effects of two different steroid treatments, testosterone cypionate and stanozolol, on neuronal density in the limbic, motor and sensitive cortical areas, as well as the CA1, CA2 and CA3 hippocampal regions, in mice of both sexes. In male mice, both testosterone cypionate and stanozolol contributed to statistically significant decreases in neuronal density in the limbic area, while only

stanozolol led to a statistically significant decrease in neuronal density in CA1. The effects were even more pronounced in female mice, wherein testosterone cypionate decreased neuronal density in all areas and regions studied, and stanozolol led to a decrease in the CA2 and CA3 regions. Similarly, in an electrophysiological study of hippocampal slices from male rat, it was found that intracerebroventricular injections of nandrolone decanoate diminished CA1 activity and plasticity [27]. In addition, the research group found that this effect was mediated by calcineurin, a serine/threonine phosphatase, which has been associated with a decline in cognitive performance. Administration of nandrolone also seem to contribute to behaviour indicative of depression and anhedonia, as a study in mice suggests [29]. The findings may be related to the observed decreased brain-derived neurotrophic factor (BDNF) and neurotrophin-3 in the hippocampus, striatum and PFC, along with decreasing serotonin levels. Similarly, El-Shamarka *et al.* [16[¶]] found that a combined cannabis/nandrolone protocol decreased BDNF in PFC and hippocampus, and nandrolone alone decreased BDNF in the PFC. The combined protocol in this study also contributed to increased anxiety and aggression.

Conversely, for specific populations, age groups and doses, exogenous testosterone may be beneficial. Fattoretti *et al.* [30] investigated the effects of physical activity and exogenous testosterone in old mice (27 months, which should resemble a human age of at least 80 years [31]). Testosterone was administered once a week at a dose of 10 mg/kg for 4 weeks to groups of mice with and without an exercise regimen. Testosterone treatment increased synaptic density in the inner molecular layer of the hippocampal dentate gyrus, independent of physical activity [30].

THE BRAIN AND AGGRESSION

Behavioural side effects of androgen use, particularly aggression, have been well documented. However, the precise mechanisms underlying these behavioural consequences following androgen use remain somewhat ambiguous [32]. Neuronal activity in the latero-anterior hypothalamus (LAH) has previously been associated with aggressive behaviour in animals treated with androgens [33]. Building upon these findings, Morrison *et al.* [34^{¶¶}] found that serotonergic factors could be involved as blocking serotonin type-3 receptors in this region increased aggression, but decreased anxious behaviour in adolescent Syrian hamsters during androgen withdrawal. Typically, during androgen withdrawal, anxiety increases and aggression declines, suggesting that serotonin neural

signalling within the LAH contributes to shifting between these behaviours.

In addition, previous findings suggest that gamma aminobutyric acid (GABA)-ergic mechanisms in the LAH are involved in androgen-induced aggressive behaviour [35–37]. In a recent study, valproate, an anticonvulsant shown to manage pathological aggression and enhance GABA activity in the brain, attenuated androgen-induced aggression in a dose-dependent manner [38]. This effect was reversed following infusion of a GABA_A receptor antagonist into the LAH, implying a role of GABA neural signalling within the LAH in androgen-induced aggressive behaviour.

Furthermore, additional brain structures are likely implicated in androgen-induced aggression. Previously, a decrease in amygdala volume has been associated with increased aggressive behaviours [39]. However, Tabor *et al.* [23] found that rats treated with androgens demonstrated increased aggression and enlarged amygdala volumes. Notably, a previous study in humans also identified right amygdala enlargement in androgen users [40], though the sample size of this study was small and more recent findings have found no differences in amygdala volume between androgen users and controls [41]. In addition, polysubstance use may contribute to aggressive behaviour, as rats treated with combined cannabis and nandrolone demonstrated increased aggression, but those exposed to either substance alone did not [16[•]]. This may be a result of the increased pro-inflammatory cytokines seen in this treatment group, which have been frequently associated with aggression [42].

Finally, androgen dependence is of growing concern, as those who use androgens for a longer period and in higher doses tend to experience worse side effects. In mice, a single injection of either testosterone or nandrolone strengthened excitatory synaptic transmission in ventral tegmental area dopaminergic neurons, which mediates reward and drug-seeking behaviour [43^{••}]. However, blocking opioid receptors in this region countered two drug-seeking behaviours, suggesting that androgens indirectly stimulate the reward centre by activating endogenous opioid signalling, which may mediate the addictive effects of androgens.

COMMENTARY

During the last year and a half, 16 original publications have directly or indirectly addressed cerebral consequences of high-dose androgen use. Despite a rather limited number of publications, this period has offered some high-quality articles with key novel findings. An interesting observation is the

depth and variety of methods these studies represent, actually no study are the other alike. Rather than replicating previous findings, researchers in this novel field instead seem to seek new discoveries, which is natural as the opportunity is still there. Overall, the findings from studies looking at different aspects of brain health go in the same direction where high-dose, long-term androgen use seems to have a negative impact on brain health. Methods applied range from cell culture studies, animal behavioural, histological and electrophysiological studies, human postmortem studies, a medical case report and large-scale cognitive and neuroimaging studies. Some findings are of particular interest. First, from cell cultures, it was shown that different androgenic compounds might differently affect cell viability and survival, wherein trenbolone was worse in all areas. Trenbolone is repeatedly mentioned by users as a substance causing particular harm, and it would be interesting to see such findings replicated in human real-life use. Moreover, we hope more laboratories will continue the use of postmortem brain tissue, and the use of machine learning and brain age prediction on brain imaging or other data.

It is also worth noticing how animal and human findings of executive abilities nicely complement each other, thereby increasing the validity of these findings. This was also partly the case in the neuroimaging studies, wherein higher brain age gap or smaller prefrontal volumes were seen after long-term androgen use in both humans and rats. One contradictory finding stands out suggesting that androgen use might increase synaptic density in the dentate gyrus in old mice, and it is intriguing to speculate if debuting and use in an older age where endogenous testosterone levels are low are less harmful. Otherwise, we have seen glimpses that androgen use might impact the immune system increasing inflammation, findings of interest to study further, as they might relate to other medical and psychological effects of androgen use. A case study, although being a single event, provides us a reminder that the cardiovascular disease associated with androgen use, influences brain health and poses a risk for stroke. Actually, albeit the cardiovascular consequences of androgens are profound, few have given attention to vascular impacts, as they relate to the brain, a relationship of great importance particularly, as it relates to brain ageing [44].

Lastly, it is worth highlighting one study providing mechanistic insight into the rewarding properties of androgens. In a set of experiments, Bontempi and Bonci [43^{••}] found that the drug seeking and rewarding effects of androgens were

not mediated by androgen receptors, but indirectly by opioid receptors on dopaminergic neurons, a finding that might explain the addictive nature of androgens. Understanding the mechanisms underlying why some users lose control of use is of utmost importance, and provides hope for future medical intervention to aid in treatment of androgen-dependence.

CONCLUSION

Published findings on the consequences of androgens on the brain from the last 18 months comprise a wide range of areas of interest. The impact of these substances on cognition and behaviour are likely brought about by a myriad of alterations to specific brain regions, primarily the cerebral cortex, hippocampus, amygdala and hypothalamus. Furthermore, high-dose androgen use appears to contribute to increased inflammation and oxidative stress, and impaired neuroplasticity. However, a large portion of these findings come from animal models, with varying species and doses, making it difficult to extrapolate these findings to humans.

Acknowledgements

None.

Financial support and sponsorship

This research was funded by the South-Eastern Norway Regional Health Authority [Grant Nos. 2013087, 2016049, 2017025, 2018075 and 2020088 (to A.B.)] and by internal research grants from the Division on Mental Health and Addiction (to A.B.).

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- ■ of outstanding interest

1. Tchernof A, Brochu D, Maltais-Payette I, *et al.* Androgens and the regulation of adiposity and body fat distribution in humans. *Compr Physiol* 2018; 8:1253–1290.
 2. Harada N. Role of androgens in energy metabolism affecting on body composition, metabolic syndrome, type 2 diabetes, cardiovascular disease, and longevity: lessons from a meta-analysis and rodent studies. *Biosci Biotechnol Biochem* 2018; 82:1667–1682.
 3. Huo S, Scialli AR, McGarvey S, *et al.* Treatment of men for 'low testosterone': a systematic review. *PLoS One* 2016; 11:e0162480.
 4. Elliott J, Kelly SE, Millar AC, *et al.* Testosterone therapy in hypogonadal men: a systematic review and network meta-analysis. *BMJ Open* 2017; 7:e015284.
 5. Sagoe D, Molde H, Andreassen CS, *et al.* The global epidemiology of anabolic-androgenic steroid use: a meta-analysis and meta-regression analysis. *Ann Epidemiol* 2014; 24:383–398.
 6. Baggish AL, Weiner RB, Kanayama G, *et al.* Cardiovascular toxicity of illicit anabolic-androgenic steroid use. *Circulation* 2017; 135:1991–2002.
 7. Piacentino D, Kotzalidis GD, Del Casale A, *et al.* Anabolic-androgenic steroid use and psychopathology in athletes. A systematic review. *Curr Neuropharmacol* 2015; 13:101–121.
 8. Vorona E, Nieschlag E. Adverse effects of doping with anabolic androgenic steroids in competitive athletics, recreational sports and bodybuilding. *Minerva Endocrinol* 2018; 43:476–488.
 9. Chegeni R, Pallesen S, McVeigh J, Sagoe D. Anabolic-androgenic steroid administration increases self-reported aggression in healthy males: a systematic review and meta-analysis of experimental studies. *Psychopharmacology* 2021; 238:1911–1922.
 10. Page MJ, McKenzie JE, Bossuyt PM, *et al.* The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021; 372:n71.
 11. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan: a web and mobile app for systematic reviews. *Syst Rev* 2016; 5:210.
 12. Damião B, Rossi-Junior WC, Guerra FDR, *et al.* Anabolic steroids and their effects of on neuronal density in cortical areas and hippocampus of mice. *Braz J Biol* 2021; 81:537–543.
 13. Vaskinn A, Hauger LE, Bjørnebekk A. Theory of mind in users of anabolic androgenic steroids. *Psychopharmacology* 2020; 237:3191–3199.
 14. Pomara C, Neri M, Bello S, *et al.* Neurotoxicity by synthetic androgen steroids: oxidative stress, apoptosis, and neuropathology: a review. *Curr Neuropharmacol* 2015; 13:132–145.
 15. Zelleroth S, Nylander E, Örtenblad A, *et al.* Structurally different anabolic androgenic steroids reduce neurite outgrowth and neuronal viability in primary rat cortical cell cultures. *J Steroid Biochem Mol Biol* 2021; 210:105863.
- This study investigates the influence of various synthetic compounds and testosterone on cortical cultures, and finds neurotoxic effects, which vary in degree depending on the substance, a relevant finding for future studies.
16. El-Shamarka ME, Sayed RH, Assaf N, *et al.* Combined neurotoxic effects of cannabis and nandrolone decanoate in adolescent male rats. *Neurotoxicology* 2020; 76:114–125.
- This study highlights the consequences of polysubstance use, which is quite common among AAS users, through a number of mechanisms, and takes several approaches including oxidative stress and inflammation in addition to behavioural and cognitive deficits.
17. Sessa F, Salerno M, Cipolloni L, *et al.* Anabolic-androgenic steroids and brain injury: miRNA evaluation in users compared to cocaine abusers and elderly people. *Aging* 2020; 12:15314–15327.
- This study is of interest, as miRNA is not a commonly used marker in androgen abuse and brain health research and the comparisons among AAS users, cocaine users and ageing individuals provide valuable insight into the potential consequences of high doses of AAS. The study also indicates the value of larger studies investigating these novel markers.
18. Persengiev SP, Kondova II, Bontrop RE. The impact of microRNAs on brain aging and neurodegeneration. *Curr Gerontol Geriatr Res* 2012; 2012:359369.
 19. Sun L, Zhao M, Zhang J, *et al.* MiR-144 promotes β -amyloid accumulation-induced cognitive impairments by targeting ADAM10 following traumatic brain injury. *Oncotarget* 2017; 8:59181–59203.
 20. Bali P, Kenny PJ. Transcriptional mechanisms of drug addiction. *Dialogues Clin Neurosci* 2019; 21:379–387.
 21. Najem D, Rennie K, Ribocco-Lutkiewicz M, *et al.* Traumatic brain injury: classification, models, and markers. *Biochem Cell Biol* 2018; 96:391–406.
 22. Kaufman MJ, Kanayama G, Hudson JI, Pope HG Jr. Supraphysiologic-dose anabolic-androgenic steroid use: a risk factor for dementia? *Neurosci Biobehav Rev* 2019; 100:180–207.
 23. Tabor J, Wright DK, Christensen J, *et al.* Examining the effects of anabolic-androgenic steroids on repetitive mild traumatic brain injury (RmTBI) outcomes in adolescent rats. *Brain Sci* 2020; 10:258–274.
 24. Bjørnebekk A, Kaufmann T, Hauger LE, *et al.* Long-term anabolic androgenic steroid use is associated with deviant brain aging. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging* 2021; 6:579–589.
- This study utilizes machine learning techniques, which have not been used previously in this area of research, to determine the aging effects of AAS.
25. Choulerton J, Guha N, Squires R. Anabolic steroid use and ischaemic stroke in a young fitness enthusiast. *BMJ Case Rep* 2021; 14:e234241.
 26. Hauger LE, Westlye LT, Bjørnebekk A. Anabolic androgenic steroid dependence is associated with executive dysfunction. *Drug Alcohol Depend* 2020; 208:107874.
 27. Moradpour F, Moazedi AA, Pourmotabbed A, Zarei F. Calcineurin is involved in retrieval of passive avoidance memory and synaptic plasticity impairment induced by Nandrolone administration in adolescent male rats. *Neurobiol Learn Mem* 2019; 163:107032.
 28. Wood RI, Serpa RO. Anabolic-androgenic steroid abuse and cognitive impairment: testosterone IMPAIRS biconditional task performance in male rats. *Behav Brain Res* 2020; 379:112339.
 29. Cattelán Souza L, de Brito MLO, Jesse CR, *et al.* Involvement of kynurenine pathway in depressive-like behaviour induced by nandrolone decanoate in mice. *Steroids* 2020; 164:108727.
 30. Fattoretti P, Malatesta M, Mariotti R, Zancanaro C. Testosterone administration increases synaptic density in the gyrus dentatus of old mice independently of physical exercise. *Exp Gerontol* 2019; 125:110664.

31. Dutta S, Sengupta P. Men and mice: relating their ages. *Life Sci* 2016; 152:244–248.
 32. Bertozzi G, Sessa F, Albano GD, *et al.* The role of anabolic androgenic steroids in disruption of the physiological function in discrete areas of the central nervous system. *Mol Neurobiol* 2018; 55:5548–5556.
 33. Morrison TR, Sikes RW, Melloni RH. Anabolic steroids alter the physiological activity of aggression circuits in the lateral anterior hypothalamus. *Neuroscience* 2016; 315:1–17.
 34. Morrison TR, Ricci LA, Puckett AS, *et al.* Serotonin type-3 receptors differentially modulate anxiety and aggression during withdrawal from adolescent anabolic steroid exposure. *Horm Behav* 2020; 119:104650.
- This is the first study to demonstrate the significant role serotonergic neural signalling within the LAH plays in shifting between aggressive and anxious behaviours during AAS withdrawal.
35. Morrison TR, Ricci LA, Melloni RH Jr. γ -Aminobutyric acid neural signaling in the lateroanterior hypothalamus modulates aggressive behavior in adolescent anabolic/androgenic steroid-treated hamsters. *Behav Pharmacol* 2014; 25:673–683.
 36. Grimes JM, Ricci LA, Melloni RH Jr. Glutamic acid decarboxylase (GAD65) immunoreactivity in brains of aggressive, adolescent anabolic steroid-treated hamsters. *Horm Behav* 2003; 44:271–280.
 37. Schwartzer JJ, Ricci LA, Melloni RH Jr. Interactions between the dopaminergic and GABAergic neural systems in the lateral anterior hypothalamus of aggressive AAS-treated hamsters. Netherlands: Elsevier Science; 2009.
 38. Lee TJ, Zanello AF, Morrison TR, *et al.* Valproate selectively suppresses adolescent anabolic/androgenic steroid-induced aggressive behavior: implications for a role of hypothalamic γ -aminobutyric acid neural signaling. *Behav Pharmacol* 2021; 32:295–307.
 39. Rosell DR, Siever LJ. The neurobiology of aggression and violence. *CNS Spectr* 2015; 20:254–279.
 40. Kaufman MJ, Janes AC, Hudson JI, *et al.* Brain and cognition abnormalities in long-term anabolic-androgenic steroid users. *Drug Alcohol Depend* 2015; 152:47–56.
 41. Bjørnebekk A, Walhovd KB, Jørstad ML, *et al.* Structural brain imaging of long-term anabolic-androgenic steroid users and nonusing weightlifters. *Biol Psychiatry* 2017; 82:294–302.
 42. Takahashi A, Flanigan ME, McEwen BS, Russo SJ. Aggression, social stress, and the immune system in humans and animal models. *Front Behav Neurosci* 2018; 12:56.
 43. Bontempi L, Bonci A. μ -Opioid receptor-induced synaptic plasticity in dopamine neurons mediates the rewarding properties of anabolic androgenic steroids. *Sci Signal* 2020; 13:eaba1169.
- This study demonstrates the potential role of opioid receptors in the VTA in the addictive behaviour following AAS use, an important mechanism to understand as those with a dependence often experience worse side effects.
44. Dobbie S, Seshadri S, Beiser A, *et al.* Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. *Neurology* 2011; 77:461–468.