LAB IN BIO PSYC FINAL

PSYC 4606

Abstract

Investigation into Anabolic Steroid Use in Syrian Hamsters

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Introduction (EXP 1: Behavioral)

Aggression as generalized behavior is an important aspect of typical developmental behavior in early childhood and adolescence *(Tremblay, 2018).* However, the highly nuanced and varied phenotype of human aggression has created sociological disruption and conflict across current societies *(Killgore et al., 2021).* This problem of social disruption and interpersonal aggression is of particular concern in relation to Anabolic Androgen Steroids, or AAS, use in adolescent males. The percentage of adolescent males frequently using AAS has risen sharply in past decades and has since remained relatively steady; A 1995 paper reported stated that male adolescent AAS use in the Denver area was 4.0% (*Tanner et., 1995).* In 2017, using data from the National Youth Risk Behavior survey the current rate of AAS use was 3.46% among all adolescent males in the US *(Schneider, 2022).*

While typical developmental aggression in human males is generally expected to decrease as one passes adolescence *(Tremblay, 2018),* It is not known if chronic AAS use in adolescence produces long lasting changes in one's behavioral phenotype relating to aggression. Additionally, AAS use potentially increases rates of short-term aggressive behaviors in users *(Chegeni et al., 2021)*. It was previously found that short-term reversable aggressive effects (increase in aggression) found in hamsters administered AAS for up to 25 days *(Ricci et., al 2005).* The most prominent difference between hamsters administered AAS and controls were on day 11. Both the potentially short and long-term changes of male-based aggression have the potential to only worsen social disruption and interpersonal aggression. Behavioral changes may include increased temperament *(Midgley and Davies, 2001),* increased generalized aggression *(Hauger et al., 2021)* and increased or unprecedented aggression towards women *(Choi et al., 1994).*

While the exact underlying neural mechanisms undergoing changes are not yet known, chronic AAS use has been associated with increased levels of depression, hypomania, and mania (*Bertozzi et*

al., 2018). Additionally, disorders including muscle dysmorphia, schizophrenia-related disorders, somatoform disorder, eating disorders, and general mood disorders have also been linked to chronic AAS use *(Piacentio et al., 2015).* The link between AAS use and an increased aggressive phenotype has become a common occurrence and even has a term associated with it known as *roid rage (NIDA, 2021).* Roid rage is characterized as a sudden and often uncontrollable outburst of violent, aggressive behavior as a result of steroid use *(NIDA, 2021).*

AAS use has been of particular concern regarding athletes, including bodybuilders and those seeking muscular growth and body changes for physical appearance *(Anawalt, 2019).* Given that a high degree of male adolescents both partake in sports and undergo changes around the perception of their body in relation social norms and environments, the group has been of great interest when seeking to address AAS abuse *(Office of the Inspector General, 1991).* AAS has been associated with other and future drug use *(Nyberg et al., 2008).* Lastly there is concern whether or not the developing, plastic, brain of adolescents will cause an increased possibility of developing permanent or long- lasting neurological and behavioral changes *(Cunningham, 2013).*

The link between male-based aggression and increased testosterone levels has been well documented *(Batrinos, 2012).* An increase in testosterone associated with aggression is manifested in many different ways in modern societies. Aggressive sports, violent crimes, winning competitions, are some examples of activities which have been linked to increases in testosterone *(Batrinos, 2012).* The link between testosterone and aggression is also seen in how increases in testosterone are manifested in certain behaviors such as verbal abuse *(Mattsson et al., 1980),* increased temperament or decreased patience *(Daitzman et al., 1980),* and a general increase in violent behavior *(Ehrenkranz et al., 1974).*

Much of inter-male aggression consists of "play-fighting" which often involves targeting non vital parts of the body, wrestling, and a lack of sudden jabs or movements *(Cervantes et al., 2007). When* the intent of inter-male aggression is to inflict harm, it is characterized by sudden jabs or movements towards vital areas of the body, often preceded by a flaring or expansion of the body

(Cervantes et al., 2007). Syrian hamsters have repeatedly been used in experiments to study aggression given that they are solitary in nature and territorial (*Elidio et al., 2021).* Syrian hamsters are commonly observed in the R-I paradigm as an ethologically valid model of naturally occurring aggression *(Lerwill and Makings, 1971; Floody and Pfaff, 1977).*

In the resident intruder paradigm hamsters follow a relatively consistent pattern of interaction and aggression that can be broken down into three phases *(Lerwill and Makings, 1971)*. The introductory phase consists of the following behaviors in a rough sequence of events: both hamsters will attend, approach, nose, investigate, and sniff *(Lerwill and Makings, 1971*). The hamsters often stretch out their bodies in this phase. The second phase is the sparring phase and consists of both hamsters taking upright posture, until the intruder takes defensive posture *(Lerwill and Makings, 1971)*. Then, once a threat is detected, the intruder will try and evade *(Lerwill and Makings, 1971)*. The final stage, or chasing phase mainly consists of the intruder taking a fully submissive pose, followed by full aggressive posture from the resident which results in a chase between the two *(Lerwill and Makings, 1971).*

Introduction (EXP 2: Neurobiology)

Given that aggression is a social behavior, it is seen a subset of social behaviors as a whole *(D.I Rubenstein and D.R Rubenstein, 2013).* Additionally, the neural networks that regulate aggressive behavior are seen as a subset of the broader neural networks that modulate other social behaviors (Delville et al., 2003; Wommack and Delville, 2002). The overlap between aggression networks and other social networks is most prominent within the limbic system *(Delville et al., 2000; Sokolowski, and Corbin, 2012).* Several areas of the limbic system were discovered to have reciprocal connections with the anterior hypothalamus (AH), an area known for the regulation of offensive aggression in adult male golden hamsters *(Delville et al., 2000).* These connections were found using retrograde and anterograde tracing from the AH to surrounding limbic regions *(Delville et al., 2000).* c-Fos staining was applied to measure subsequent neuronal activity in relation to aggression *(Delville et al., 2000).* Areas shown to have reciprocal connections affecting the regulation of aggression in the AH include the medial amygdaloid nucleus, ventrolateral hypothalamus, bed nucleus of the stria terminalis and dorsolateral part of the midbrain

(Delville et al., 2000). Within this network, the modulation of offensive aggression has been specifically identified within the lateral portion of the anterior hypothalamus (LAH) *(Carrillo et al., 2011).*

Many studies have implicated the release of the peptide vasopressin (AVP) in increased levels of aggression (*Ferris, 2005; Ferris and Potegal, 1988; Delville et al., 1996).* The role vasopressin has in increasing aggression is seen within the LAH through LAH-AVP pathways *(Carrillo et al., 2011).* The AVP producing cells within the LAH effect aggression through expression *(Carrillo et al., 2011).* This effect is possibly achieved through LAH-AVP pathways modulating glutamate activity within the brain nucleus of stria terminalis (BNST) *(Carrillo et al., 2011).* While increased AVP levels have been shown to increase aggressive behavior, increases in the neurochemical serotonin (5-HT) have been implicated in a decrease in aggression *(Ferris et al., 1997).* Serotonin indirectly decreases aggression through the inhibition of AVP within the AH *(Ferris et al., 1997).* It is possible that 5-HT also has a direct effect on the neural mechanisms for aggression in the AH, meaning it would have a more direct role than simply influencing AVP levels *(Ferris et al., 1997).* Given the broad and varied roles both AVP and 5-HT have on CNS functions and subsequent behaviors it is difficult to attribute the effect serotonin has on aggression to a singular process (*Ferris et al., 1997).* In contrast to the role of serotonin, increased levels of dopamine are correlated with increased levels of aggression *(Harrison et al., 1997).* Heightened levels of dopamine were recorded in rodents before, during, and after fights in several studies *(Hadfield, 1983; Miczek et al., 1994; Tidey and Miczek, 1996).* However, within the AH and LAH, dopamine has a more varied role in the modulation of aggression *(Schwartzer and Melloni, 2010).* It is believed that D2 receptors within the LAH directly modulate adolescent anabolic-androgenic steroid (AAS) induced aggression, leading to an increase in aggressive behavior *(Schwartzer and Melloni, 2010).* It is possible that increased D2 activation (via AAS) increases aggression by inhibiting GABA mediation inhibition of AVP *(Schwartzer and Melloni, 2010).* Additionally, D5 receptors may also play a more indirect role in facilitating AAS induced aggression in the LAH, although further research is needed. *(Schwartzer and Melloni, 2010).*

While AVP, 5-HT and dopamine all play a crucial role in the regulation of aggression, the levels and function of all three can be disrupted through the administration of AAS *(Schwartzer et al., 2009; Schwartzer and Melloni, 2010; Harrison et al., 2000);* Increased AAS administration has been linked to

increases in the levels of AVP within the AH *(Harrison et al., 2000).* The increased AVP levels were defined as increases in both fiber density and peptide content within the AH (*Harrison et al., 2000).* However, no changes in neuronal or network structure or mRNA expression were discovered (*Harrison et al., 2000).* While AAS administration increases AVP levels, it has the opposite effect on 5-HT levels *(Grimes and Melloni, 2002).* AAS treated hamsters showed a significant reduction in the amount of 5-HT immunoreactive varicosities and fibers in the AH, ventrolateral hypothalamus (VLH) and medial amygdala (MeA) (*Grimes and Melloni, 2002)*. This treatment naturally resulted in increases in aggressive behaviors among the hamsters tested. *(Grimes and Melloni, 2002).* Lastly, AAS administration has been shown to have a varied and complex effect on dopamine levels. Within the AH, AAS administration increases dopamine levels in the nucleus circularis (NC) and the medial supraoptic nucleus (mSON) *(Schwartzer and Melloni, 2010).* These nuclei have a role in the modulation of LAH activity, meaning these networks could be the foundation for the dopaminergic effect on AAS induced aggression *(Schwartzer and Melloni, 2010).* However, AAS administration has also shown a decrease in both D1 and D2 receptor density in the nucleus accumbens *(Wallin-Miller et al., 2018)*. While such a decrease is not a measure of direct peptide levels decreases in dopamine receptor levels resulting from AAS exposure have been consistently documented *(*Kindlundh et al., 2003; Martínez-Rivera et al., 2015). It can be postulated that the decrease in dopamine receptor density is due to an upregulation, or an agonist-induced increase, in dopamine levels *(Martinez et al., 2004).*

Methods:

Intact adolescent male hamsters (P25) were obtained from Harlan Sprague-Dailey Labs (Indianapolis, IN), individually housed in Plexiglas cages and maintained at ambient temperature on reverse light: dark cycle (14L:10D; lights on at 1900 h). Food and water were provided ad libitum. On P27, hamsters were weighed and randomly distributed into three groups. Group one (G1 = AAS-treated, n = 10) animals received daily subcutaneous (SC) injections of a cocktail of AAS consisting of 2 mg/kg testosterone cypionate, 2 mg/kg nandrolone deconate, and 1 mg/kg boldenone undecylenate (Sigma Chemical Co., St. Louis, MO) suspended in sesame oil (SO) for 30 consecutive days (P27–56). This treatment regime was designed to mimic a chronic “heavy use” regimen (Pope and Katz, 1988; 1994). As a control, a second

group of hamsters (G2 = vehicle, n = 10) were injected with SO alone. In addition, a third group of stimulus hamsters (G3 = intruders, n = 20) was used solely to test the aggressive behavior of G1 and G2 animals. On the day following the last injection (P56), animals from each group (G1 and G2) were tested for offensive aggression against animals from G3.

Aggression Testing:

Animals from G1 and G2 were tested for aggressive behavior using the resident/intruder paradigm, a well-characterized and ethologically valid model of offensive aggression in golden hamsters (Floody and Pfaff, 1977; Lerwill and Makaings, 1971). Briefly, animals from G1 and G2 were presented in their home cage with an intruder (G3 animal) of equal age and weight and the resident hamster (G1 and G2 animals) was scored for aggressive behavior (i.e., number of attacks and bites) during a 10-min test period by two independent observers blind to the experimental treatment. An attack was scored when the resident hamster took an upright position that was followed by a sparring behavior. A sparring was considered as the rapid movement of the resident and intruder hamsters' front paws towards each other. An attack was also considered when the resident hamster held the intruder in a pinned position. A bite was scored when the resident hamster's mouth was observed near the intruder's body that was followed by a sudden jump and attempt to flee from the intruder. All tests were performed during the first 4 h of the dark phase under dim red illumination. All encounters were videotaped for verification of the behavioral measurements.
The results were compared between treatment groups.

The increased bite behavior recorded in the R-I paradigm provides sufficient data to warrant the investigation of the neurobiological modulation of aggression in the AH. 5-HT and dopamine are suspected to modulate aggression in the AH. 5-HT and dopamine are the primary neurotransmitters suspected to alter in level and function due to AAS administration. Therefore, methodology for neurobiological experiments focus on 5-HT and dopamine changes in the AH, and the effect 5-HT changes have on the modulation of AVP in the AH.

In Syrian hamsters (Mesocricetus auratus), the adolescent period of development can be identified as the time between postnatal days 25 and 56 (P25/P56). Weaning generally occurs around P25 with the onset of puberty beginning around P40 (Miller et al., 1997). During this developmental time period,

hamsters wean from their dams, leave the home nest, establish new solitary nest sites, and learn to defend their territory and participate in social dominance hierarchies (Schoenfeld and Leonard, 1985; Whitsett, 1975). In Experiment 1, intact adolescent male hamsters (P25) were obtained from Harlan Sprague/Dawley Labs (Indianapolis, IN), individually housed in Plexiglas cages, and maintained at ambient temperature on a reverse light: dark cycle of (14L:10D; lights on at 19:00). Food and water were provided ad libitum. On P27, animals (n=20 total, i.e. two groups of n=10).

Serotonin Immunohistochemistry:

For immunohistochemical analysis, brains from animals in AAS (n=10) and sesame oil-treatment groups (n=10) were fixed by transcardial perfusion with 4% paraformaldehyde and then incubated in 30% sucrose/phosphate buffered saline (PBS, pH 7.4) overnight at 4 ºC for cryoprotection. A consecutive series of 35 mm coronal sections were cut on a sliding microtome, collected as free-floating sections in 1/PBS and labeled for 5-HT by single-label immunohistochemistry using a modification of an existing protocol (Ferris et al., 1997). Briefly, free floating sections were pretreated with 1% sodium borohydride followed by pre-incubation in 20% normal goat serum with 1% H2O2 and 0.3% Triton X-100. Sections were incubated in primary antiserum (1:1000) for 5-HT anti-rabbit (Protos Biotech, Ridgefield, NJ) with 2% NGS and 0.3% Triton X-100 over two nights at 4 ºC. After primary incubation, sections were incubated in secondary anti-rabbit followed by tertiary antisera (Vectastain ABC Elite Kit/rabbit, Burlingame, CA) and then labeled with diaminobenzidine (DAB, Vector Labs, Burlingame, CA). Sections were mounted on gelatin-coated slides, allowed to air dry, and dehydrated through a series of ethanol and xylene solutions. Then, slides were cover slipped using Cytoseal-60 mounting medium (VWR Scientific, West Chester, PA).

TH Immunochemistry:

Following the behavioral test for aggression, hamsters were anesthetized with Ketamine/Xylazine (80 mg/12 mg) and transcardially perfused with a 4 °C saline rinse followed by 4% paraformaldehyde. Brains were removed, post-fixed for 90 min in perfusion fixative and cryoprotected in 30% sucrose in 0.1M phosphate buffered saline at 4 °C overnight. Brains were cut at 35 μm using a freezing microtome in serial, coronal sections and all subsequent immunohistochemical procedures were conducted at 21 °C unless

otherwise specified. Sections were washed 3 times for 10 min in 0.1 M phosphate buffered saline with 0.5% Triton-X (PBSTx), pretreated with 0.03% H2O2 in distilled water for 30 min, then rinsed thoroughly with 0.5% PBSTx. Sections were incubated in antibody buffer containing 10% normal goat serum in 0.5% PBSTx for 60 min. Primary antibody (TH, polyclonal, Chemicon; California) was prepared in antibody buffer diluted to a final concentration of 1:7500 and incubation with free-floating sections was carried out overnight at 4 °C on a rotation wheel. Sections were then rinsed 3 times for 10 min with 0.5% PBSTx, incubated for 60 min in biotinylated secondary goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA.) in 0.5% PBSTx and incubated for 90 min in Avidin–biotin-complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA.) in 0.5% PBSTx. The peroxidase reaction was revealed using 0.5% 3,3′-diaminobenzidine in distilled water as per manufacturer's recommendations (DAB Kit, Vectastain; Vector Laboratories, Burlingame CA.). The sections were mounted on gel-coated slides, air-dried, dehydrated through a series of alcohols, cleared with xylene and coverslipped with Cytoseal (Stephens Scientific, Kalamazoo, MI).

Image analysis:

The number of 5-HT immunoreactive (5-HT-ir) varicosities was determined within a specific brain area identified as the brain region of interest (ROI), the anterior hypothalamus, at low power (4 X) using a Nikon E600 microscope. At this magnification, a standard computer-generated parcel was drawn to outline the entire ROI. The AH brain region was assigned an ROI parcel, formatted in size specifically for that brain area. Then, under 20 X magnification images were thresholded at a standard RGB-scale level empirically determined by an observer unaware of the treatment conditions, such as to allow detection of stained 5-HT-ir elements with moderate to high intensity, while suppressing lightly stained elements. This thresholded value was then applied across subjects to control for changes in background staining and differences in foreground staining intensity between animals. The illumination was kept constant for all measurements. 5-HT-ir varicosities and fibers were identified by color as light brown (RBG #7E4E21). In each field, the varicosities were scored using a black sharpie to trace IR elements on a transparent plastic sheet (8.5” x 11”) with 6 demarcated 3.5” x 3.75” areas placed against the monitor of a 14” ASUS ROG Zephyrus laptop displaying the section being scored. Measurements at 20 X continued until 5-HT-ir

elements throughout the entire ROI were quantified. Three independent measurements were taken from 10 animals per treatment group. The number of 5-HT-ir varicosities and fibers was then averaged for each animal and used for statistical analysis.

Quantification of TH immunoreactive (TH-ir) cell bodies and fibers within the AH was performed manually (as outlined above for 5-HT-ir). The illumination was kept constant for all measurements. TH-ir cell bodies and fibers were identified by color as dark brown (RBG #38250E). In each field, the varicosities were scored using a black sharpie to trace IR elements on a transparent plastic sheet (8.5" x 11") with 6 demarcated 3.25" x 3.50" areas placed against the monitor of a 13" MacBook Pro displaying the section being scored. Measurements at 20 X continued until TH-ir elements throughout the entire ROI were quantified. Three independent measurements were taken from 10 animals per treatment group. The number of TH-ir cell bodies and fibers was then averaged for each animal and used for statistical analysis.

Statistics:

Animals were compared between groups (AAS vs SO) for both 5HT and TH fiber and varicosity counts. Data were analyzed using Student's t-test and alpha levels for significance were held at $p < 0.05$.

Hypothesis

Taken together, research indicates a link between AAS use in development and increased aggressive behaviors, the current study hypothesizes that Syrian hamsters (P27), administered AAS for consecutive 30 days during adolescent development will display an increase in the frequency of aggressive behaviors compared to Syrian hamsters treated with sesame oil. The hypothesis is based on the established link between increased levels of testosterone and increased levels of aggression as well the link between increased levels of testosterone during adolescent development. The aggressive phenotype was observed as the number of attacks and bites recorded. An attack was recorded when the resident hamster took an upright position that was followed by a sparring behavior. A bite was scored when the intruder produced a sudden jump attempting to flee.

The AAS induced alterations in the behavioral phenotypes of Syrian Hamsters likely reflect alterations in the neurobiology of the AH. Given previously cited work involving AAS administration and alterations in neurochemistry, it is evident that AAS exposure poses short and potentially long-term changes to both the level and function of AVP, 5-HT and dopamine within the brain. The current study hypothesizes that intact adolescent male hamsters (P27) treated with AAS for the first 14 days of the adolescent period will show a decrease in the amount of number of 5-HT-ir varicosities and fibers within 35 mm coronal AH slices when compared to adolescent male hamsters (P27) treated with sesame oil. It is further hypothesized that that intact adolescent male hamsters (P27) treated with AAS for first 14 days of the adolescent period will show an increase in the amount of TH immunoreactive (TH-ir) cell bodies and fibers in 35 µm coronal AH slices when compared to adolescent male hamsters (P27) treated with sesame oil. Quantification of TH immunoreactive cell bodies and fibers, and 5-HT varicosities and fibers, will be performed manually by an observer unaware of treatment conditions. Images will be analyzed under 20x magnification using a standardized RGB scale and illumination. Fibers and varicosities will be scored using a black sharpie against a transparent plastic sheet.

Results:

Animals treated with low doses of AAS during their adolescent development period showed significantly heightened measures of offensive aggression when presented with an intruder of equal size and weight (Figure 1). Hamsters treated with high-dose AAS during their adolescent development period produced significantly higher rates of bites ($t(20) = 2.71$, $p < 0.001$). Specifically, AAS-treated animals displayed an approximate 2.5 times increase in bites as compared to SO- treated animals (Figure 1). Although AAS treated animals showed heightened levels of aggressive behavior in the form of increased bite behavior, no significant changes were observed ($t = 2.71$, $p > 0.05$) in the frequency of attack behavior measured between the AAS-treated and vehicle-treated control animals (Figure 2).

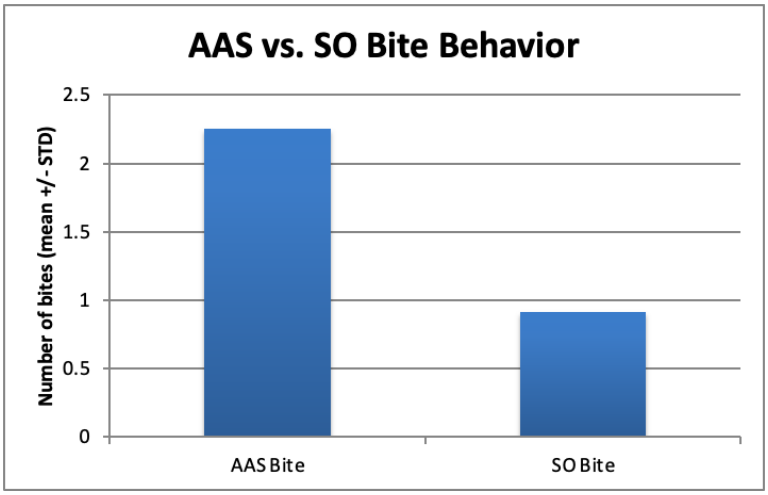

Figure 1. Adolescent AAS exposure increased bite behavior in the R-I paradigm compared to SO controls ($t(20)=2.71$, $p<0.001$).

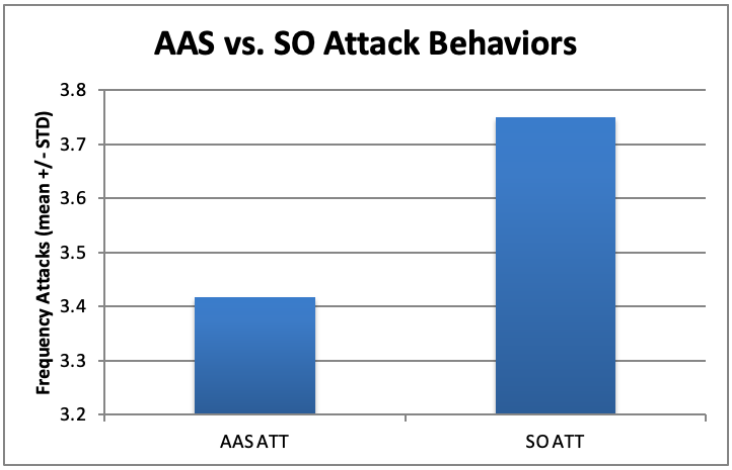

Figure 2. AAS administration did not increase frequency of bites compared to SO controls ($t(20)=2.71$, $p > 0.05$).

The AAS induced alterations in the behavioral phenotypes of Syrian Hamsters reflect changes to the neurobiological modulation of aggression. Using Student's t, 5HT and TH-ir were compared between adolescent hamsters treated with AAS and sesame oil (SO). Developmental exposure to AAS led to a significant decrease in 5HT-ir varicosities and fibers within the LAH as compared to the control animals ($t(20)= 2.71$, $p < 0.05$). Specifically, AAS-exposed hamsters exhibited significantly less 5HT-ir varicosities with a mean of 6.30 as compared to the SO-treated hamsters which had a mean of 10.20 (Figure 1). The average TH-ir cell body with proximal fiber count was 5.70 in the AAS group and 5.07 in the SO group (Figure 2). This difference in TH-ir fiber count was not statistically significant ($t(20)=2.71$, $p > 0.05$).

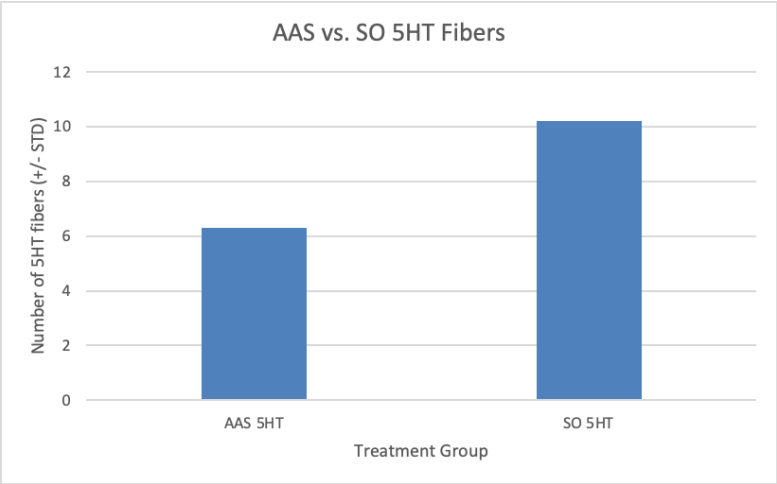

Figure 1. AAS decreased the number of 5-HT-ir fibers relative to the SO (sesame oil) controls ($t(20)= 2.71$, $p < .05$, Student t-test)

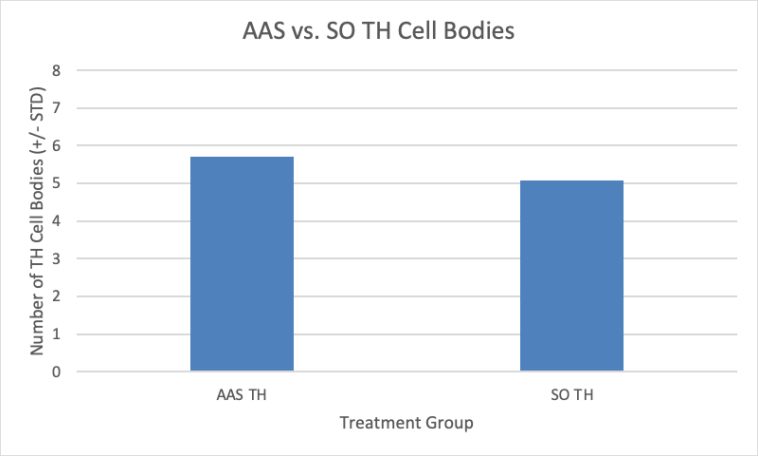

Figure 2. AAS did not produce changes in TH-ir cell bodies in the LAH ($t(20)=2.71$, $p>0.05$).

Discussion:

AAS are typically used for increased athletic performance or a more muscular physique. The adverse side effects of this abuse has been well documented; AAS use among adolescents has been shown to lead to increases in negative temperament (Midgley and Davies, 2001), generalized aggression (Hauger et al., 2021) and increased or unprecedented aggression towards women (Choi et al., 1994). Additionally, human aggression creates sociological disruption and conflict across current societies (Killgore et al., 2021). Given the established short-term and potentially long-term behavioral effects, along with the increased potential for societal problems of AAS exposure, there has been a push to not only address the outcomes but to understand the root causes of aggression to develop a more effective clinical response. Animal models have been crucial to expanding our understanding of AAS abuse and subsequent behaviors. Within the resident-intruder paradigm, Syrian Hamsters have produced reliable data concerning the aggression-stimulating effects of AAS (Elidio et al., 2021, Lerwill and Makings, 1971; Floody and Pfaff, 1977).

We hypothesized that Syrian Hamsters (P27), administered AAS for consecutive 30 days during adolescent development would display an increase in the frequency of aggressive behaviors compared to Syrian Hamsters treated with sesame oil. Our results show that Syrian Hamsters administered AAS did show a notable increase in the frequency of aggressive behaviors. However, this increase was only seen in phenotypic biting behavior and not in attack behaviors. Our findings that indicate AAS treatment does not facilitate an increase in attack behavior is interesting because it contradicts previous findings that show increases in attacks with AAS exposure (DeLeon et al., 2002; Harrison et al., 2000; Melloni et al., 1997; Melloni and Ferris, 1996). While the scoring criteria for an attack and bite do differ between studies, findings suggest increases in attacks and a bites within the R-I result from AAS experience (DeLeon et al., 2002; Harrison et al., 2000; Melloni et al., 1997; Melloni and Ferris, 1996). Our results that indicate AAS administration increases the frequency of biting behavior is not surprising as it corroborates previous findings (DeLeon et al., 2002; Harrison et al., 2000; Melloni et al., 1997; Melloni and Ferris, 1996). It is possible that differences in how attacks were scored played a role in our contradicting findings. Observations of biting behavior between studies are less varied than general attack behaviors, typically characterized as either flank or rump bites ((DeLeon et al., 2002; Harrison et al., 2000; Melloni et al., 1997; Melloni and Ferris, 1996). Differences in attack-based scoring criteria within the R-I paradigm is

clear as previous studies have scored attack behavior based solely on whether the resident attacks the lateral portions of the intruder's body (Grimes et al., 2006). Indeed, our attack criteria was solely based on sparring and pining behaviors (Lerwill and Makings, 1971). In general, our findings of increased bite behaviors resulting from AAS exposure align with previous literature, showing that AAS increases aggression, giving support for our hypothesis (DeLeon et al., 2002; Harrison et al., 2000; Melloni et al., 1997; Melloni and Ferris, 1996; Grimes et al., 2006). It is interesting that one study administered 4 types of AAS over a 6-month period in several doses in gonadally intact mice and found no link between aggression and administration (Bronson, 1996). Another study that administered nandrolone decanoate in mice found a decrease in aggressive behavior (Moya-Albiol et al., 1996). Martínez-Sanchis and colleagues corroborated previous findings when they reported that there was no recorded increase in aggressive behavior in mice after they were administered testosterone propionate repeatedly over 10 weeks (Martínez-Sanchis et al., 1997; Lumia et al., 1994). The lack of a link between AAS and aggression in mice, and our current failure to observe an AAS induced increase in attacks, suggest that the aggression-stimulating effects of AAS are complex.

Taken together, our findings provide further support for a well-established link between AAS use and aggression. The underlying physiological changes resulting from AAS exposure in our study could be used to investigate AAS and aggression. Future research should be directed toward understanding the neurobiology of aggression and how AAS interacts with the neurochemical mechanisms and developing brain regions of adolescents. One brain region that is important for regulating aggressive behavior is the Anterior Hypothalamus (AH) (Goodson et al., 2012). The nonapeptide Arginine Vasopressin (AVP) is responsible for the facilitation or promotion of aggression within the AH (Ferris et al., 1997). Data also suggests serotonin to be the primary neurotransmitter responsible for inhibiting aggression in the AH by actively inhibiting AVP (Ferris et al., 1997). Given the data of previous investigations, dopamine has also been implicated in the AH, having a more varied role (Schwartzer and Melloni, 2010). Gaining a deeper understanding of aggression regulation on a mechanistic level within the AH could provide valuable insight into broader interactions between brain regions regulating aggression and how such interactions manifest into behavioral phenotypes of aggression (Gouveia et al., 2019).

Research investigating the neurobiology of aggression has been well established for years *(De Leon et al., 2002).* Many studies investigating aggression using animal models have sought to differentiate the difference between defensive and offensive aggression in relation to neurobiological mechanisms *(De Leon et al., 2002; Jackson et al., 2005; Bhatt et al., 2005).* Studies investigating aggression in humans differ from animal studies such that the behaviors are better described as proactive or reactive, rather than simply offensive or defensive *(Boccadoro et al., 2021; Walters et al., 2005).* This shift in focus is reflective of the increasing complexity of aggressive phenotypes as they are scaled to a human and societal context *(Georgiev et al., 2013).* This increasing complexity often encourages human studies to remain largely behavioral in their methodology or paradigm *(Boccadoro et al., 2021; Walters et al., 2005).* While humans may present a relatively complex representation of aggression in relation to other animals, aggression is an evolutionary conserved behavior *(Waltes et al., 2016).* This conserved state makes aggression difficult to research and therefore gain a greater understanding, as aggression encompasses a wide variety of behaviors necessary for survival and evolutionary fitness *(Tremblay, Hartup, Archer, 2005).* Such behaviors include eating, competing for mates, protecting themselves and family from predators, and territorial protection or acquisition. (*Tremblay, Hartup, Archer, 2005).* Current research does not investigate aggression with the overall objective of eliminating aggressive behaviors, but rather with the aim of identifying ways in which aggressive is maladaptive in specific environments *(Flanigan, Russo, 2019; Connor et al., 2019).* Understanding how maladaptive aggression is manifested, modulated, and represented provides a foundation for clinical and societal interventions *(Flanigan, Russo 2019).*

Alterations in the behavioral phenotype of aggression are consistent with neurobiolgocal changes in the brain. The changes in 5-HT likely reflect decreased inhibition of AVP and its ability to increase aggressive responding. While no significant changes in dopamine levels were found more research is needed to determine the complex role dopamine has in the modualtion of aggression. The criterion for social behaviors is both varied and complex (Grant, 1963). Given this, aggressive behavior can be defined as a subset of social behaviors (D.I Rubenstein and D.R Rubenstein, 2013). The neural circuitry regulating aggressive behavior is a subset of broader neural circuitry regulating social behaviors, particularly within the limbic system (Delville et al., 2003; Wommack and Delville, 2002; Delville et al., 2000; Sokolowski, and Corbin, 2012). The modulation of offensive aggression has been identified within the LAH as part of a

broader network containing reciprocal connections between the AH, the medial amygdaloid nucleus, ventrolateral hypothalamus, bed nucleus of the stria terminalis and the dorsolateral part of the midbrain (Carrillo et al., 2011; Delville et al., 2000). Increases in AVP levels causing an increase in aggressive phenotypes have been well documented (Ferris, 2005; Ferris and Potegal, 1988; Delville et al., 1996). Increases in 5-HT levels causing a decrease in aggression has been well documented (Grimes and Melloni, 2002). Dopamine has also been known to have a role in the modulation of aggressive behavior, although a more varied and complex one (Schwartzer and Melloni, 2010). D5 receptors within the LAH regulate AAS induced aggression, leading to an increase in aggressive behavior. A possible explanation for the role of D2 receptors have in modulating aggression is that D2 receptors inhibit the GABA mediation inhibition of AVP (Schwartzer and Melloni, 2010). The role D5 receptors have on the modulation of aggression within the LAH is still largely unknown, although it is hypothesized that any possible role is indirect (Schwartzer and Melloni, 2010). AAS administration can disrupt both the level and function of AVP, 5-HT, and dopamine within the LAH aggression network (Schwartzer et al., 2009; Schwartzer and Melloni, 2010; Harrison et al., 2000).

This study found that AAS administered to intact adolescent male hamsters significantly decreased 5-HT levels in the AH and that AAS administration had no significant effect on dopamine levels in the AH. The decreased levels of 5-HT are consistent with previous findings (Grimes and Melloni, 2002). Given the varied role of 5-HT within the brain, it is difficult to attribute a singular mechanistic change to the decrease in aggressive phenotypes. However, it is possible 5-HT inhibits AVP expression within the AH (Ferris et al., 1997). The decreased AVP levels cause a decrease in aggressive behavior. Thus, if AAS decreases 5-HT levels, and therefore increases AVP levels, aggression levels increase. It is also posited that 5-HT has a more direct role in influencing aggression in the AH in addition to its AVP inhibitory action (Ferris et al., 1997). Our findings that show no significant changes in dopamine levels in the AH is not consistent with previous literature. Schwartzer and Melloni reported that AAS administration increases dopamine levels in the AH, specifically in the nucleus circularis and the medial supraoptic nucleus (Schwartzer and Melloni, 2010). However, dopamine has been known to have a varied and complex role in the modulation of aggressive behavior (Schwartzer and Melloni, 2010). Schwartzer JJ and colleagues reported that AAS administration caused an increase in D2-ir receptors in the LAH (Schwartzer JJ et al., 2009). This

relationship is inconsistent with the findings of Schwartzer and Melloni, 2010. If a decrease in dopamine receptor density is due to an upregulation, or an agonist-induced increase, in dopamine levels, then an increase in dopamine receptor density could be due to a downregulation in dopamine levels (Martinez et al., 2004). Additionally, Birgner and colleagues reported that AAS administration increased D1 receptor density in the amygdala but a decrease in the hippocampus levels (Birgner et al., 2008). While these findings are also inconsistent with our data showing no changes in dopamine levels the variation in findings is indicative of the varied and complex relationship between AAS administration and dopamine receptor dynamics and dopamine levels.

While the lack of change in dopamine levels does not support our hypothesized increase in TH-immunoreactive cells bodies and fibers, the increase in 5-HT levels in combination with valid behavioral paradigms provide strong support for the link between developmental AAS exposure and an increase in aggressive phenotypes. The AAS induced increase in AVP provides strong evidence for the importance AVP-LAH pathways have in the modulation of offensive aggression (Carrillo et al., 2011). A possible explanation for the lack of change in dopamine levels is due to interactions between dopamine, GABA, 5-HT; Increased D2 activation (via AAS) increases aggression by inhibiting GABA mediation inhibition of AVP (Schwartzer and Melloni, 2010). If reciprocal connections mediate these mechanistic actions, it is possible that the 5-HT inhibition of AVP causes dysregulation within this system. Our data indicating the modulatory effects AAS has on 5-HT could initiate such changes. Additionally, the modulation of 5-HT and subsequent interactions with AVP supports our hypothesis. Our study provides further evidence of the importance the LAH-AVP pathway and 5-HT-AVP mechanistic interactions have in the regulation of aggression. AAS changes to the level and function of neurotransmitters within these systems supports previous concern over AAS use among developmental populations. Future studies should investigate the role dopamine has in the modulation of LAH reciprocal connections with surrounding regions, and the regulation of mechanisms within the LAH and AH.

Sources:

Albert, D. J., Walsh, M. L., & Jonik, R. H. (1993). Aggression in humans: what is its biological foundation?. *Neuroscience and biobehavioral reviews*, *17*(4), 405–425. https://doi.org/10.1016/s0149-7634(05)80117-4

Anawalt, Bradley D. "Diagnosis and Management of Anabolic Androgenic Steroid Use." *The Journal of clinical endocrinology and metabolism* vol. 104,7 (2019): 2490-2500. doi:10.1210/jc.2018-01882

Batrinos, Menelaos L. (2012). "Testosterone and aggressive behavior in man." *International journal of endocrinology and metabolism* vol. 10,3 (2012): 563-8. doi:10.5812/ijem.3661

Bertozzi, G., Sessa, F., Albano, G. D., Sani, G., Maglietta, F., Roshan, M., Volti, G. L., Bernardini, R., Avola, R., Pomara, C., & Salerno, M. (2018). The Role of Anabolic Androgenic Steroids in Disruption of the Physiological Function in Discrete Areas of the Central Nervous System. *Molecular neurobiology*, *55*(7), 5548–5556. https://doi.org/10.1007/s12035-017-0774-

Bhatt, S., Zalcman, S., Hassanain, M., & Siegel, A. (2005). Cytokine modulation of defensive rage behavior in the cat: role of GABAA and interleukin-2 receptors in the medial hypothalamus. *Neuroscience*, *133*(1), 17–28. https://doi.org/10.1016/j.neuroscience.2005.01.065

Birgner, C., Kindlundh-Högberg, A. M., Oreland, L., Alsiö, J., Lindblom, J., Schiöth, H. B., & Bergström, L. (2008). Reduced activity of monoamine oxidase in the rat brain following repeated nandrolone decanoate administration. *Brain research*, *1219*, 103–110. https://doi.org/10.1016/j.brainres.2008.05.014

Boccadoro, S., Wagels, L., Henn, A. T., Hüpen, P., Graben, L., Raine, A., & Neuner, I. (2021). Proactive vs. Reactive Aggression Within Two Modified Versions of the Taylor Aggression Paradigm. *Frontiers in behavioral neuroscience*, *15*, 749041. https://doi.org/10.3389/fnbeh.2021.749041

Bronson F. H. (1996). Effects of prolonged exposure to anabolic steroids on the behavior of male and female mice. *Pharmacology, biochemistry, and behavior*, *53*(2), 329–334. https://doi.org/10.1016/0091-3057(95)02030-6

M. Carrillo, L.A. Ricci, R.H. Melloni. (2011). Glutamate-vasopressin interactions and the neurobiology of anabolic steroid-induced offensive aggression, Neuroscience, Volume 185, 2011, Pages 85-96, ISSN 0306-4522, https://doi.org/10.1016/j.neuroscience.2011.03.056.

Cervantes, M. C., Taravosh-Lahn, K., Wommack, J. C., & Delville, Y. (2007). Characterization of offensive responses during the maturation of play-fighting into aggression in male golden hamsters. *Developmental psychobiology*, *49*(1), 87–97. https://doi.org/10.1002/dev.20183

Chegeni, R., Pallesen, S., McVeigh, J., & Sagoe, D. (2021). Anabolic-androgenic steroid administration increases self-reported aggression in healthy males: a systematic review and meta-analysis of experimental studies. *Psychopharmacology*, *238*(7), 1911–1922. https://doi.org/10.1007/s00213-021 05818-7

Choi, P. Y., & Pope, H. G., Jr (1994). Violence toward women and illicit androgenic-anabolic steroid use. *Annals of clinical psychiatry : official journal of the American Academy of Clinical Psychiatrists*, *6*(1), 21–25. https://doi.org/10.3109/10401239409148835

Connor, D. F., Newcorn, J. H., Saylor, K. E., Amann, B. H., Scahill, L., Robb, A. S., Jensen, P. S., Vitiello, B., Findling, R. L., & Buitelaar, J. K. (2019). Maladaptive Aggression: With a Focus on Impulsive Aggression in Children and Adolescents. *Journal of child and adolescent psychopharmacology*, *29*(8), 576–591. https://doi.org/10.1089/cap.2019.0039

Cuijpers, P., Sijbrandij, M., Koole, S. L., Andersson, G., Beekman, A. T., & Reynolds, C. F., 3rd (2014). Adding psychotherapy to antidepressant medication in depression and anxiety disorders: a meta-analysis. *World psychiatry : official journal of the World Psychiatric Association (WPA)*, *13*(1), 56–67. https://doi.org/10.1002/wps.20089

Cunningham, R. L., Lumia, A. R., & McGinnis, M. Y. (2013). Androgenic anabolic steroid exposure during adolescence: ramifications for brain development and behavior. *Hormones and behavior*, *64*(2), 350–356. https://doi.org/10.1016/j.yhbeh.2012.12.009

Daitzman, R., & Zuckerman, M. (1980). Disinhibitory sensation seeking, personality and gonadal hormones. *Personality and Individual Differences*, *1*(2), 103–110. https://doi.org/10.1016/0191-8869(80)90027-6

DeLeon, K. R., Grimes, J. M., & Melloni, R. H., Jr (2002). Repeated anabolic-androgenic steroid treatment during adolescence increases vasopressin V(1A) receptor binding in Syrian hamsters: correlation with offensive aggression. *Hormones and behavior*, *42*(2), 182–191. https://doi.org/10.1006/hbeh.2002.1802

DeLeon, K. R., Grimes, J. M., Connor, D. F., & Melloni, R. H., Jr (2002). Adolescent cocaine exposure and offensive aggression: involvement of serotonin neural signaling and innervation in male Syrian hamsters. *Behavioural brain research*, *133*(2), 211–220. https://doi.org/10.1016/s0166-4328(02)00004-9

Delville, Y., Mansour, K. M., & Ferris, C. F. (1996). Serotonin blocks vasopressin-facilitated offensive aggression: interactions within the ventrolateral hypothalamus of golden hamsters. *Physiology & behavior*, *59*(4-5), 813–816. https://doi.org/10.1016/0031-9384(95)02166-3

Delville, Y., De Vries, G. J., & Ferris, C. F. (2000). Neural connections of the anterior hypothalamus and agonistic behavior in golden hamsters. *Brain, behavior and evolution*, *55*(2), 53–76. https://doi.org/10.1159/000006642

Yvon Delville, J.Tracey David, Kereshmeh Taravosh-Lahn, Joel C Wommack (2003). Stress and the development of agonistic behavior in golden hamsters, Hormones and Behavior, Volume 44, Issue 3, 2003, Pages 263-270, ISSN 0018-506X, https://doi.org/10.1016/S0018-506X(03)00130-2.

Department of Health and Human Services, Office of the Inspector General, Richard, P Kusserow. (1991). Adolescent Steroid Use. *United States Department of Health and Human Services. Washington D.C, United States. https://oig.hhs.gov/oei/reports/oei-06-90-01080.pdf*

Jackson, D., Burns, R., Trksak, G., Simeone, B., DeLeon, K. R., Connor, D. F., Harrison, R. J., & Melloni, R. H., Jr (2005). Anterior hypothalamic vasopressin modulates the aggression-stimulating effects of adolescent cocaine exposure in Syrian hamsters. *Neuroscience*, *133*(3), 635–646. https://doi.org/10.1016/j.neuroscience.2005.02.047

Ehrenkranz, J., Bliss, E., & Sheard, M. H. (1974). Plasma testosterone: correlation with aggressive behavior and social dominance in man. *Psychosomatic medicine*, *36*(6), 469–475. https://doi.org/10.1097/00006842197411000-00002

Elidio, H., Coelho, J., da Silva, L., & Dos Santos, I. B. (2021). Housing Density and Aggression in Syrian Hamsters. *Journal of the American Association for Laboratory Animal Science : JAALAS*, *60*(5), 506–509. https://doi.org/10.30802/AALAS-JAALAS-21-000020

Ferris, C. F., Melloni, R. H., Jr, Koppel, G., Perry, K. W., Fuller, R. W., & Delville, Y. (1997). Vasopressin/serotonin interactions in the anterior hypothalamus control aggressive behavior in golden hamsters. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *17*(11), 4331–4340. https://doi.org/10.1523/JNEUROSCI.17-11-04331.1997

Ferris C. F. (2005). Vasopressin/oxytocin and aggression. *Novartis Foundation symposium*, *268*, 190–253.

Flanigan, M. E., & Russo, S. J. (2019). Recent advances in the study of aggression. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *44*(2), 241–244. https://doi.org/10.1038/s41386-018-0226-2

Floody, O. R., & Pfaff, D. W. (1977). Communication among hamsters by high-frequency acoustic signals: III. Response evoked by natural and synthetic ultrasounds. *Journal of Comparative and Physiological Psychology*, *91*(4), 820–829. https://doi.org/10.1037/h0077360

Georgiev, A. V., Klimczuk, A. C., Traficonte, D. M., & Maestripieri, D. (2013). When violence pays: a cost-benefit analysis of aggressive behavior in animals and humans. *Evolutionary psychology : an international journal of evolutionary approaches to psychology and behavior*, *11*(3), 678–699.

Gobrogge, K. L., Liu, Y., Jia, X., & Wang, Z. (2007). Anterior hypothalamic neural activation and neurochemical associations with aggression in pair-bonded male prairie voles. *The Journal of comparative neurology*, *502*(6), 1109–1122. https://doi.org/10.1002/cne.21364

Goodson, J. L., Kelly, A. M., Kingsbury, M. A., Thompson, R. R., (2012). *An aggression-specific cell type in the anterior hypothalamus of finches.* Proceedings of the National Academy of Sciences of the United States of America. https://www.pnas.org/doi/10.1073/pnas.1207995109

Gouveia, F. V., Hamani, C., Fonoff, E. T., Brentani, H., Alho, E., de Morais, R., de Souza, A. L., Rigonatti, S. P., & Martinez, R. (2019). Amygdala and Hypothalamus: Historical Overview With Focus on Aggression. *Neurosurgery*, *85*(1), 11–30. https://doi.org/10.1093/neuros/nyy635

Grimes, J. M., Ricci, L. A., & Melloni, R. H., Jr (2006). Plasticity in anterior hypothalamic vasopressin correlates with aggression during anabolic-androgenic steroid withdrawal in hamsters. *Behavioral neuroscience*, *120*(1), 115–124. https://doi.org/10.1037/0735-7044.120.1.115

Grant, E.C. (1963). An Analysis of the Social Behaviour of the Male Laboratory Rat, *Behaviour*, *21*(3-4), 260-281. doi: https://doi.org/10.1163/156853963X00194

Grimes, J. M., & Melloni, R. H., Jr (2002). Serotonin modulates offensive attack in adolescent anabolic steroid-treated hamsters. *Pharmacology, biochemistry, and behavior*, *73*(3), 713–721. https://doi.org/10.1016/s0091-3057(02)00880-8

Hadfield M. G. (1983). Dopamine: mesocortical vs nigrostriatal uptake in isolated fighting mice and controls. *Behavioural brain research*, *7*(3), 269–281. https://doi.org/10.1016/0166-4328(83)90019-0

Harrison, A. A., Everitt, B. J., & Robbins, T. W. (1997). Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: interactions with dopaminergic mechanisms. *Psychopharmacology*, *133*(4), 329–342. https://doi.org/10.1007/s002130050410

Harrison, R. J., Connor, D. F., Nowak, C., Nash, K., & Melloni, R. H., Jr (2000). Chronic anabolic-androgenic steroid treatment during adolescence increases anterior hypothalamic vasopressin and aggression in intact hamsters. *Psychoneuroendocrinology*, *25*(4), 317–338. https://doi.org/10.1016/s0306-4530(99)00057-8

Hauger, L. E., Havnes, I. A., Jørstad, M. L., & Bjørnebekk, A. (2021). Anabolic androgenic steroids, antisocial personality traits, aggression and violence. *Drug and alcohol dependence*, *221*, 108604. https://doi.org/10.1016/j.drugalcdep.2021.108604

Kanayama, G., Brower, K. J., Wood, R. I., Hudson, J. I., & Pope, H. G., Jr (2009). Anabolic-androgenic steroid dependence: an emerging disorder. *Addiction (Abingdon, England)*, *104*(12), 1966–1978. https://doi.org/10.1111/j.1360-0443.2009.02734.x

Killgore, W., Cloonan, S. A., Taylor, E. C., Anlap, I., & Dailey, N. S. (2021). Increasing aggression during the COVID-19 lockdowns. *Journal of affective disorders reports*, *5*, 100163. https://doi.org/10.1016/j.jadr.2021.100163

K. R., Grimes, J. M., & Melloni, R. H., Jr (2002). Repeated anabolic-androgenic steroid treatment during adolescence increases vasopressin V(1A) receptor binding in Syrian hamsters: correlation with offensive aggression. *Hormones and behavior*, *42*(2), 182–191. https://doi.org/10.1006/hbeh.2002.1802

Kristin E. Schneider, Lindsey Webb, Denali Boon & Renee M. Johnson (2022) Adolescent Anabolic- Androgenic Steroid Use in Association With Other Drug Use, Injection Drug Use, and Team Sport Participation, Journal of Child & Adolescent Substance Abuse, DOI: 10.1080/1067828X.2022.2052219

Kindlundh, A. M., Lindblom, J., & Nyberg, F. (2003). Chronic administration with nandrolone decanoate induces alterations in the gene-transcript content of dopamine D(1)- and D(2)-receptors in the rat brain. *Brain research*, *979*(1-2), 37–42. https://doi.org/10.1016/s0006-8993(03)02843-9

Lumia, A. R., Thorner, K. M., & McGinnis, M. Y. (1994). Effects of chronically high doses of the anabolic androgenic steroid, testosterone, on intermale aggression and sexual behavior in male rats. *Physiology & behavior*, *55*(2), 331–335. https://doi.org/10.1016/0031-9384(94)90142-2

Martinez, D., Broft, A., Foltin, R. W., Slifstein, M., Hwang, D. R., Huang, Y., Perez, A., Frankle, W. G., Cooper, T., Kleber, H. D., Fischman, M. W., & Laruelle, M. (2004). Cocaine dependence and d2 receptor availability in the functional subdivisions of the striatum: relationship with cocaine-seeking behavior. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *29*(6), 1190–1202. https://doi.org/10.1038/sj.npp.1300420

Martínez-Sanchis, S., Salvador, A., Moya-Albiol, L., González-Bono, E., & Simón, V. M. (1998). Effects of chronic treatment with testosterone propionate on aggression and hormonal levels in intact male mice. *Psychoneuroendocrinology*, *23*(3), 275–293. https://doi.org/10.1016/s0306-4530(98)00005-5

Martínez-Rivera, F. J., Natal-Albelo, E. J., Martínez, N. A., Orozco-Vega, R. A., Muñiz-Seda, O. A., & Barreto-Estrada, J. L. (2015). The effect of the anabolic steroid, nandrolone, in conditioned place preference and D1 dopamine receptor expression in adolescent and adult mice. *Behavioural processes*, *113*, 81–85. https://doi.org/10.1016/j.beproc.2015.01.008

Mattsson, A., Schalling, D., Olweus, D., Löw, H., & Svensson, J. (1980). Plasma testosterone, aggressive behavior, and personality dimensions in young male delinquents. *Journal of the American Academy of Child Psychiatry*, *19*(3), 476–490. https://doi.org/10.1016/s0002-7138(09)61065-7

Melloni, R. H., Jr, Connor, D. F., Hang, P. T., Harrison, R. J., & Ferris, C. F. (1997) . Anabolic-androgenic steroid exposure during adolescence and aggressive behavior in golden hamsters. *Physiology & behavior*, *61*(3), 359–364. https://doi.org/10.1016/s0031-9384(96)00373-3

Melloni, R. H., Jr, & Ferris, C. F. (1996). Adolescent anabolic steroid use and aggressive behavior in golden hamsters. *Annals of the New York Academy of Sciences*, *794*, 372–375. https://doi.org/10.1111/j.1749-6632.1996.tb32546.x

Miczek, K. A., DeBold, J. F., & van Erp, A. M. (1994). Neuropharmacological characteristics of individual differences in alcohol effects on aggression in rodents and primates. *Behavioural pharmacology*, *5*(4 And 5), 407–421. https://doi.org/10.1097/00008877-199408000-00004

Midgley, S. J., Heather, N., & Davies, J. B. (2001). Levels of aggression among a group of anabolic- androgenic steroid users. *Medicine, science, and the law*, *41*(4), 309–314. https://doi.org/10.1177/002580240104100407

Moya-Albiol, Luis & Martínez-Sanchis, Sonia & Salvador, Alicia. (1996). Effects of Chronic Administration With High doses of testosterone propionate. Psicológica. 17. 503-517.

NIDA. (2021). How does anabolic steroid misuse affect behavior? *National Institute on Drug Abuse* https://nida.nih.gov/publications/research-reports/steroids-other-appearance-performance-enhancing drugs-apeds/how-does-anabolic-steroid-misuse-affect-behavior

Piacentino, D., Kotzalidis, G. D., Del Casale, A., Aromatario, M. R., Pomara, C., Girardi, P., & Sani, G. (2015). Anabolic-androgenic steroid use and psychopathology in athletes. A systematic review. *Current neuropharmacology*, *13*(1), 101–121. https://doi.org/10.2174/1570159X13666141210222725

Reebye P. (2007). Developmental Origins of Aggression. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, *16*(3), 137–139.

Daniel I. Rubenstein, Dustin R. Rubenstein. (2013). Social Behavior, Editor(s): Simon A Levin, Encyclopedia of Biodiversity (Second Edition), Academic Press, 2013, Pages 571-579, ISBN 9780123847201. https://doi.org/10.1016/B978-0-12-384719-5.00126-X.

Schwartzer, J. J., Ricci, L. A., & Melloni, R. H., Jr (2009). Adolescent anabolic-androgenic steroid exposure alters lateral anterior hypothalamic serotonin-2A receptors in aggressive male hamsters. *Behavioural brain research*, *199*(2), 257–262. https://doi.org/10.1016/j.bbr.2008.11.048

Schwartzer, J. J., & Melloni, R. H., Jr (2010). Dopamine activity in the lateral anterior hypothalamus modulates AAS-induced aggression through D2 but not D5 receptors. *Behavioral neuroscience*, *124*(5), 645–655. https://doi.org/10.1037/a0020899

Skårberg, K., Nyberg, F., & Engström, I. (2008). The development of multiple drug use among anabolic- androgenic steroid users: six subjective case reports. *Substance abuse treatment, prevention, and policy*, *3*, 24. https://doi.org/10.1186/1747-597X-3-24

Sokolowski, K., & Corbin, J. G. (2012). Wired for behaviors: from development to function of innate limbic system circuitry. *Frontiers in molecular neuroscience*, *5*, 55. https://doi.org/10.3389/fnmol.2012.00055

Tanner, S. M., Miller, D. W., & Alongi, C. (1995). Anabolic steroid use by adolescents: prevalence, motives, and knowledge of risks. *Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine*, *5*(2), 108–115. https://doi.org/10.1097/00042752-199504000-00007

Tidey, J. W., & Miczek, K. A. (1996). Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. *Brain research*, *721*(1-2), 140–149. https://doi.org/10.1016/0006-8993(96)00159-x

Tremblay, R E., Hartup, WW., Archer, J (2005). Developmental Origins of Aggression. The Guilford Press. https://books.google.com/books?hl=en&lr=&id=XmSfJEl2v4sC&oi=fnd&pg=PA3&ots=MvhTHk0V9d&sig=P8HminhAYg0rTrM6aVr_beUO41M#v=onepage&q&f=false

Wallin-Miller, K. G., Kreutz, F., Li, G., & Wood, R. I. (2018). Anabolic-androgenic steroids (AAS) increase sensitivity to uncertainty by inhibition of dopamine D1 and D2 receptors. *Psychopharmacology*, *235*(4), 959–969. https://doi.org/10.1007/s00213-017-4810-7

Walters, G. D. (2005). Proactive and Reactive Aggression: A Lifestyle View. In J. P. Morgan (Ed.), *Psychology of aggression* (pp. 29–43). Nova Science Publishers.

Waltes, R., Chiocchetti, A. G., & Freitag, C. M. (2016). The neurobiological basis of human aggression: A review on genetic and epigenetic mechanisms. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*, *171*(5), 650–675. https://doi.org/10.1002/ajmg.b.32388

Joel C. Wommack, Yvon Delville (2002) Chronic social stress during puberty enhances tyrosine hydroxylase immunoreactivity within the limbic system in golden hamsters, Brain Research, Volume 933, Issue 2, 2002, Pages 139-143, ISSN 0006-8993, https://doi.org/10.1016/S0006-8993(02)02311