Published in final edited form as:

Horm Behav. 2018 November ; 106: 62–73. doi:10.1016/j.yhbeh.2018.09.005.

Androgens modulate chronic intermittent hypoxia effects on brain and behavior

Brina Snyder^a, Phong Duong^a, Jenny Trieu^b, and Rebecca L. Cunningham^a

^aDepartment of Physiology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX

^bTexas College of Osteopathic Medicine, University of North Texas Health Science Center, Fort Worth, TX

Abstract

Sleep apnea is associated with testosterone dysregulation as well as increased risk of developing neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). A rodent model of the hypoxic events of sleep apnea, chronic intermittent hypoxia (CIH), has been previously documented to impair cognitive function and elevate oxidative stress in male rats, while simultaneously decreasing testosterone. Therefore, androgens may modulate neuronal function under CIH. To investigate the role of androgens during CIH, male rats were assigned to one of four hormone groups: 1) gonadally intact, 2) gonadectomized (GDX), 3) GDX + testosterone (T) supplemented, or 4) GDX + dihydrotestosterone (DHT) supplemented. Each group was exposed to either normal room air or CIH exposure for one week, followed by memory and motor task assessments. Brain regions associated with AD and PD (entorhinal cortex, dorsal hippocampus, and substantia nigra) were examined for oxidative stress and inflammatory markers, key characteristics of AD and PD. Gonadally intact rats exhibited elevated oxidative stress due to CIH, but no significant memory and motor impairments. GDX increased memory impairments, regardless of CIH exposure. T preserved memory function and prevented detrimental CIH-induced changes. In contrast, DHT was not protective, as evidenced by exacerbated oxidative stress under CIH. Further, CIH induced significant spatial memory impairment in rats administered DHT. These results indicate androgens can have both neuroprotective and detrimental effects under CIH, which may have clinical relevance for men with untreated sleep apnea.

Keywords

neurodegeneration; oxidative stress; dihydrotestosterone; testosterone; substantia nigra; hippocampus; entorhinal cortex; Alzheimer's disease; Parkinson's disease; astrocytes

CORRESPONDING AUTHOR: Rebecca L. Cunningham, UNT Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107, Rebecca.Cunningham@unthsc.edu Phone: 817-735-5080; Fax: 817-735-0480.

CONFLICT OF INTEREST:

The authors have declared that no conflict of interest exists.

INTRODUCTION

Sleep apnea (SA) is estimated to affect about a quarter of the United States population, and is often undiagnosed (Kapur et al., 2002; Peppard et al., 2013; Senaratna et al., 2017). One measure of the severity of SA is the apnea/hypopnea index (AHI), which quantifies the hourly rate an apnea or a hypopnea occurs during sleep. The severity of SA can be defined as mild for AHI = 5 – 15, moderate for AHI = 15 – 30, and severe for AHI > 30 (Ruehlman et al., 2009). In addition to hypoxic events and disrupted sleep patterns, patients with SA often experience a dysregulation in inflammation and oxidative stress (Betteridge, 2000; Bouloukaki et al., 2017; Gozal et al., 2008; Lavie, 2014; May and Mehra, 2014).

People diagnosed with SA are at higher risk to develop hypertension, metabolic disorders, and neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Grandner, 2017; Kapur et al., 2002; Lavie and Lavie, 2009; Saareanta et al., 2016; Shao et al., 2015; Stelmach-Mardas et al., 2017; Yeh et al., 2016). Key characteristics of all these disorders include inflammation, oxidative stress, and, in men, low testosterone (reviewed in (Dai et al., 2014)). The elevation of oxidative stress and inflammation occurs during early stages of SA. It is possible this initial rise in oxidative stress and inflammation may be contributing factors to later clinical outcomes and an increased risk for AD and PD.

Sex differences are observed in SA, which suggests a role for sex hormones. Regardless of ethnicity or race, men are more likely than women to be diagnosed with SA (Punjabi, 2008; Quintana-Gallego et al., 2004; Redline et al., 1997; Sawatari et al., 2016; Tenkorang et al., 2017). Aging is positively correlated with both incidence and severity of SA in men (Basoglu and Tasbakan, 2017). Interestingly, even though an increase in SA incidence is observed in post-menopausal women, severity is not associated with aging in women (Basoglu and Tasbakan, 2017; Young et al., 1993). Although circulating testosterone levels in men with SA remain higher than circulating testosterone in women, men with SA commonly report symptoms of low testosterone, which can be reversed by treatment of SA (Zhang et al., 2016). Therefore, androgens may be modulating underlying SA severity and neuropathology (Tenkorang et al., 2017),

Testosterone is chiefly known for promoting male secondary sex characteristics (Knussmann and Sperwien, 1988). More recently, physiological levels of testosterone have been linked with quality of life indices, such as maintaining memory (Janowsky, 2006; Zitzmann, 2006). While men do not experience a drastic decline of their primary sex hormone, testosterone, as women do with estradiol, they do experience a slow decline in androgens as they age (Araujo and Wittert, 2011). This loss of circulating testosterone can influence the brain, as circulating plasma testosterone concentration is positively correlated with androgen levels in the central nervous system (CNS) (Hojo and Kawato, 2018). Furthermore, low testosterone has been associated with a rise in cardiovascular, metabolic, and neurodegenerative disorders, prompting a recent surge in testosterone replacement therapy prescriptions (Pinsky and Hellstrom, 2010).

Prior studies have proposed that maintaining physiological levels of testosterone is neuroprotective against oxidative stress insults (Pike et al., 2008). However, the

neuroprotective effects of testosterone may be diminished in conditions of low physiological levels of testosterone (Cunningham et al., 2014). Further, once oxidative stress reaches a specific threshold, testosterone no longer acts a neuroprotectant and even low testosterone levels can exacerbate oxidative stress generation and damage (Holmes et al., 2016). Thus, conditions which alter the presence of oxidative stress may underlie the dichotomous roles observed for testosterone.

SA is a multifaceted disease, which can be caused by either central or biomechanical failure (Dempsey et al., 2010). The resulting outcomes of SA are repetitive apneas and hypopneas during sleep resulting in hypoxia, hypercapnea, fragmented sleep, and frequent arousals. The rodent model of chronic intermittent hypoxia (CIH) has been well established to study the effects of the repetitive hypoxic events experienced by patients with SA, by modeling mild, moderate, and severe AHI's (Gozal et al., 2001; Ma et al., 2008; Shell et al., 2016). Similar to what is experienced by men with SA, male rats undergoing CIH exhibit sustained mean arterial pressure, as well as elevated circulating oxidative stress and inflammation, at early stages (Gozal et al., 2003; Knight et al., 2011; Nair et al., 2011a; Shell et al., 2016; Snyder et al., 2017). Moreover, gonadally intact female rats do not become hypertensive in response to CIH, which is consistent with the clinical phenotype of women with SA (Hinojosa-Laborde and Mifflin, 2005). These findings underscore the hypothesis that sex hormones contribute to oxidative stress and inflammation associated with SA.

In addition to systemic effects, CIH can influence the CNS of male rats (Rosenzweig et al., 2014). Neuronal activation due to CIH has been documented in hypothalamic and brainstem nuclei responsible for homeostatic regulation (Cunningham et al., 2012; Knight et al., 2011). Recently, we reported elevated oxidative stress within the substantia nigra (SN), the entorhinal cortex (ETC), and the hippocampus of male rats after one week of CIH exposure at an AHI of 10 (Snyder et al., 2017). Damage to these brain regions has been implicated in pre-clinical stages of different neurodegenerative diseases, such as AD and PD (Braak and Del Tredici, 2015; Braak et al., 2003; Lee and Gilbert, 2016; Reitz and Mayeux, 2014). It is not yet known if the observed CIH-induced increase in oxidative stress and inflammation in these regions contributes to neuronal dysfunction resulting in memory or fine motor deficits, or to what extent the influence of sex hormones may be in repetitive hypoxic events.

Whereas estrogens have been well established as protective against cardiovascular (dos Santos et al., 2014; Hinojosa-Laborde and Mifflin, 2005) and neurodegenerative disorders (Aubrecht et al., 2014; Gillies et al., 2004), the role of androgens is less defined (Aubrecht et al., 2014; Barron and Pike, 2012; Grimm et al., 2016; Holmes et al., 2013; Lau et al., 2014). Both estrogens and androgens can mediate rapid and long-term effects within the CNS, such as calcium signaling, increasing spine density within hippocampal regions or altering DNA transcription (Acas-Fonseca et al., 2016; Cunningham et al., 2007; Handa et al., 2008; Jacome et al., 2016; Mahmoud et al., 2016; McEwen and Milner, 2017). Testosterone's effects may be partially explained by its metabolism into two additional bioactive hormones: 17 β -estradiol (E2), via the enzyme aromatase, and dihydrotestosterone (DHT), via 5 α -reductase (Osborne et al., 2009; Tamaya et al., 1993). These enzymes (i.e. 3 β -hydroxysteroid dehydrogenase, aromatase, and 5 α -reductase) are present in cortical, midbrain, and hindbrain structures (Chetyrkin et al., 2001; Hojo and Kawato, 2018).

Testosterone's metabolites activate different signaling cascades through their effects on the androgen receptor, resulting in testosterone initiating a broad range of cellular effects (Pike et al., 2008; Rupprecht, 2003; Valera et al., 1992).

The purpose of this study is to determine the interaction between androgens and CIH-induced oxidative stress on early inflammation and behavioral outcomes in male rats. We hypothesized elevated oxidative stress due to 12-day exposure to CIH would be sufficient to induce behavioral deficits in memory and motor tasks. Additionally, we hypothesized behavioral deficits would be exacerbated by androgen supplementation. To investigate the interaction between androgens and oxidative stress, male rats were separated into various androgen groups by gonadectomy and exogenous hormone administration followed by exposure to either CIH or normal room air conditions. Following seven days of CIH, all groups underwent five days of behavior testing to assess memory and motor skills, while continuing CIH treatment. To examine androgen and CIH induced oxidative stress, proteins known to be associated with oxidative stress, such as NADPH oxidase (NOX1) and calpain activity, were quantified in the CNS. Further, caspase-3 activity was examined to determine if androgens or CIH induced apoptosis in the CNS. Consistent with previously published literature, a dichotomous role for androgens in cognitive measures under oxidative stress conditions was observed.

METHODS

Animals:

100 adult Long-Evans male rats (250-275 g body weight, 50-57 d, Charles River) were individually housed in a temperature controlled environment until one-week post-surgery to allow for recovery from all surgical procedures. Following recovery, rats were pair-housed for the duration of the study. Lights were set on a 12:12 h reverse cycle with lights off at 0900 h. Food and water were provided *ad libitum*. To acclimatize rats to operator handling and reduce stress responses during behavior testing, all rats were handled 3 times per day beginning the morning of the eighth day after arrival. Handling continued 5 days a week until behavior testing commenced. All experiments were approved by the IACUC at UNT Health Science Center and conducted according to NIH guidelines on laboratory animals.

Surgical procedures:

To investigate the contributions of androgens on the effects of CIH, rats were randomly assigned to one of 4 hormonal groups: gonadally intact control with a cholesterol-filled Silastic capsule implant (INTACT), gonadectomized control with a cholesterol-filled Silastic capsule implant (GDX), gonadectomized with two testosterone-filled Silastic capsule implants (T), or gonadectomized with one dihydrotestosterone-filled Silastic capsule implant (DHT). For all surgery procedures, rats were lightly anesthetized with isoflurane (2-3%). All rats underwent either gonadectomy or sham surgery. Hormone replacement was achieved with subcutaneous Silastic capsule implants (1.47 mm i.d. × 1.96 mm o.d. × 10 mm length, Dow Corning, Midland, MI) filled with either crystalline testosterone, dihydrotestosterone, or cholesterol (Steraloids, Newport, R.I.), as previously described (Wilson et al., 2018). We have previously observed depletion of circulating testosterone and significant reduction in

androgen-responsive tissue weights one week post-gonadectomy in young adult male rats (Garza-Contreras et al., 2017), whereas implantation of two testosterone capsules maintained physiological levels of testosterone and tissue weight in male rats (Nguyen et al., 2007; Wilson et al., 2018). Testosterone's metabolite, DHT, has a higher affinity for the androgen receptor, is more potent, and is biologically available in lower concentration than testosterone (Gao et al., 2005; Toorians et al., 2003). Consistent with prior studies examining physiological DHT levels in adult male rats (Saksena and Lau, 1979), one Silastic capsule of DHT, in the current study, resulted in plasma DHT levels (mean 65.93 ± 26.92 pg/ml) similar to DHT levels in gonadally intact male rats (mean: 54.56 ± 32.27 pg/ml) and testosterone-implanted gonadectomized rats (mean: 70.77 ± 22.35 pg/ml). This allowed us to investigate the contribution of physiological levels of the non-aromatizable androgen, DHT, under CIH and normoxic (NORM) conditions.

Chronic intermittent hypoxia protocol:

Following pair-housing, rats from each hormone group were randomly assigned to receive either CIH or NORM exposure during sleep, resulting in 8 treatment groups: INTACT-NORM (n = 26), INTACT-CIH (n = 26), GDX-NORM (n = 10), GDX-CIH (n = 8), T-NORM (n = 6), T-CIH (n = 8), DHT-NORM (n = 8), & DHT-CIH (n = 8). Home cages were placed into Oxycycler chambers ($76.2 \times 50.8 \times 50.8$ cm, BioSpherix, Lacona, NY, USA) and rats were allowed to acclimatize to the Oxycycler under NORM conditions for one week. Upon initiation of the CIH protocol, oxygen was reduced from 21% to 10%, then returned to 21% in 8 min cycles (AHI = 8) from 2100 – 0500 for 12 days, as previously described (Wilson et al., 2018).

Sample collection:

Between 0900 and 1100 on the thirteenth day, rats were deeply anesthetized with isoflurane, then euthanized by decapitation. Trunk blood was collected in chilled EDTA coated tubes, then allowed to sit on ice and centrifuged to collect plasma for biochemical analysis as previously described (Snyder et al., 2017). Additionally, brains were rapidly harvested, placed on ice, and immediately sliced into 2 mm coronal sections using a commercially available matrix (Ted Pella, Inc., Redding, CA). To ensure specificity of the different brain regions, the SN (−5.30 mm from Bregma) was collected by micro-punch using blunt 20 gauge needles attached to 1 ml syringes, and the ETC (−5.30 mm from Bregma) and dorsal hippocampus (−4.52 mm from Bregma) were dissected. All tissue samples were placed in 1.7 ml microcentrifuge tubes and then flash frozen on dry ice. Samples were stored at -80° C for later analysis.

Tissue Homogenization:

Each tissue sample was homogenized in a RIPA homogenization cocktail according to previously published methods (Snyder et al., 2017). Protein concentrations were determined by Pierce BCA assay according to manufacturer's protocol (ThermoFisher Scientific). Lysate was aliquoted and stored in microcentrifuge tubes at -80° C for further analysis.

Advanced oxidative protein products (AOPP) assay:

Circulating oxidative stress was assayed using the Advanced Oxidative Protein Products assay kit from Cell Biolabs, Inc. as previously described (Snyder et al., 2017; Wilson et al., 2018). Values are expressed as a percent of the mean INTACT-NORM control values for each run using the formula

$$\text{Percent of control} = ((\text{sample uM concentration} / \text{mean of INTACT-NORM uM concentrations})) * 100.$$

Hormone measurement:

Circulating total dihydrotestosterone was assayed by ELISA using a commercially available kit (NBP2-67999, Novus Biologicals, LLC, Littleton, CO) according to manufacturer's instructions. The intra-assay coefficient of variation is 5.08% and the inter-assay coefficient of variation is 4.93%. The sensitivity of this assay is 23.44 pg/ml at the 2 s.d. confidence limit. Reported values are mean $\pm$ s.d. (pg/ml).

Western blot:

Equal volumes of denatured tissue samples containing 20 ug protein were loaded into a BioRad 4-20% polyacrylamide gel. They underwent electrophoresis at 25 mA in a Tris-glycine running buffer followed by overnight transfer onto a PVDF membrane at 60mA. Following 30 min washing, membranes were blocked for 30 min with 5% nonfat milk in TBS-Tween (TBST) at room temperature. Membranes were then transferred to 1% nonfat milk TBST solutions containing specific primary antibodies (NOX1, Santa Cruz sc-25545 1:200; spectrin, EMD Millipore MAB1622 1:5000; GFAP, Sigma-Aldrich G3893 1:2000; GAP-DH, GeneTex GT627408 1:10,000) and incubated overnight at 4°C. In contrast to other primary antibodies, solutions containing primary antibody for cd11b (Novus Biologicals NB110-89474 1:500) were prepared in 5% milk TBST solution and allowed to incubate at RT for 1 h according to manufacturer's protocol (Novus Biologicals). Afterwards, membranes were washed in 10 min increments for 30 min, and then incubated in 1% milk TBST secondary antibody solutions (goat anti-rabbit 1:5000, goat anti-mouse 1:10,000 or 1:2000) at room temperature for 1 h. Protein bands were visualized using West Pico enhanced chemiluminescence detection assay (Thermoscientific) on an Syngene G:Box system using FlourChem HD2 AIC software as previously described (Garza-Contreras et al., 2017). NIH Image J software (version 1.50i) was used to quantify band densitometry. Values from AR, NOX1, and GFAP were normalized to GAP-DH values. For spectrin cleavage analysis, bands representing cleavage by either calpain (145 kDa) or caspase-3 (120 kDa) were normalized to total spectrin (240 kDa) values.

Cytokine multiplexing:

Plasma, cell lysate (90ug), and brain tissue homogenate (120ug) samples were used to quantify secreted cytokines using the Bioplex Rat Th1/Th2 12-Plex kit available from Biorad and fluorescence was measured on a Luminex platform (MAGPIX, Luminex Corporation, Austin, TX; table 1) (Snyder et al., 2017). Activated astrocytes have been observed to increase secretion of IL-1 β , IL-6, TNF- α , and GM-CSF under inflammatory conditions (Block et al., 2005; Choi et al., 2014; Zhang et al., 2014). These same proteins

are documented to recruit and activate pro-inflammatory macrophages (M1) (Crain et al., 2013; Fie et al., 2015; Komohara et al., 2016; Zhang et al., 2012), while IL-10, IL-13, and IL-4 cytokines are released to recruit and activate anti-inflammatory macrophages (M2). Homeostasis often depends upon maintaining the proper ratio of inflammatory responses. Conversely, degenerative processes accompany the dysregulation of pro- and anti-inflammation. Therefore, cytokine concentrations from this assay were averaged into M1-activating (IL-1 β , IL-6, TNF- α , and GM-CSF) and M2-activating (IL-10, IL-13, and IL-4) cytokine groups for statistical analysis of inflammatory dysregulation. Values are reported as mean M1 or M2 concentration $\pm$ s.d. (pg/ml).

Morris Water Maze:

The Morris Water Maze (MWM) was used to explore the hippocampal-dependent cognitive behavioral effects of CIH and hormone administration. Rats with damage to hippocampal neurons exhibit impairments in spatial memory retention (Vorhees and Williams, 2006). Beginning at 0900, on the eighth day of CIH, all rats were trained to swim to a visible platform in a pool filled with room temperature water and to remain on the platform for 20 s, until removed by the operator. Morris Water Maze training began the following morning at 0900. Training consisted of 3 trials per day for 3 days with a 10 min inter-trial interval per rat. For each trial, a rat was placed into the pool filled with opaque water at a randomly assigned point. Each point was equidistant from a platform (target), which was hidden 1 cm below the surface of the water. Rats were then allowed 90 s to locate the target. The trial ended when either the rat located the target and climbed onto it, or 90 s had passed, whichever occurred first. Latency and distance to locate the target was recorded using ANY-maze software (v. 5.14, Stoelting Co.). Rats which did not locate the target during a particular trial were guided to the target by means of the operator tapping on the target until the rat swam to it. Once the target was located, each rat was allowed to sit on the platform and observe visual cues placed on the walls to aid in formation of spatial memory for 20 s. After the 20 s passed, the rat was removed from the water maze and placed into a carrier to dry and await the next trial. The target remained in the same location throughout all 3 days of training.

At 0900 on the 12th day of CIH, each rat was administered a probe trial to test for spatial memory retention. During the probe trial, the underwater platform was removed. Each rat was placed into the water at one of the pre-determined random entry points and allowed 30 s to swim to the target location and search for the platform. At the end of 30 s, the platform was returned to its original location and the rat once again had 20 s to sit on the platform to reduce stress from failure to locate the platform in subsequent behavior testing. Rats were then returned to their home cages for 1 h to rest prior to further behavioral testing. Latency to the target and distance traveled during the 30 s, was recorded and used for statistical analysis as indicators of spatial memory retention and gross motor function, respectively.

(Modified) Open field assay:

Significant cell loss in the SN results in movement disorders (Braak et al., 2003). To assess fine motor skills, an open field apparatus (16" (W) $\times$ 16" (D) $\times$ 15" (H), San Diego Instruments) was modified with a wire mesh platform raised 2 cm above the floor. Laser

beams were placed both above and below the plane of the platform to record horizontal movement and falls through the mesh platform. Movements were recorded by PAS software (PAS v. 1.0.0.0, San Diego Instruments). The operator manually recorded the number of nose-pokes through the wire mesh platform and vertical exploratory events (rearing), both assisted (defined by placement of a forepaw on a maze wall or a hind paw on the solid floor of the maze) and unassisted. The number of nose-pokes was subtracted from the fall count to provide an accurate count of falls for statistical analysis. Each rat was placed into the center of the open field on the mesh platform and allowed 5 min to explore the space. Following testing, rats were returned to their home cages for at least 1 h prior to further assessments. The number of falls and the number of rearing events (assisted, unassisted, and total) were quantified and used for statistical analysis.

Novel object assay:

The lateral ETC is involved in development of short-term contextual and episodic memory, and has been documented to be damaged during pre-clinical phases of AD (Deshmukh and Knierim, 2011; Khan et al., 2014). Damage to the lateral ETC results in rats spending less time with a novel object than a familiar object in the novel object assay, as previously reported (Wilson et al., 2013), indicating ETC-compromised rats do not retain detailed, short-term, contextual memory, as compared to rats with an intact ETC. Two objects were placed into adjacent corners of an open field (24" (W) × 24" (D) × 12" (H)) for habituation (Wilson et al., 2013). Activity in the open field was monitored and recorded on ANY-maze software (v. 5.14, Stoelting Co.). This test consisted of 2 trials, one hour apart. In the first trial, both objects were similar in size and nature. The rat was placed into the open field facing the wall opposite the two objects to avoid object bias and allowed to explore the space and the objects for 5 min. At the end of 5 min, the rat was removed from the open field and returned to a carrier to rest for 1 h. Prior to the second trial, one object was replaced with a novel object, different in both size and shape from the first two objects. The rat was then placed back into the field in the same location as before and allowed 3 min to explore the field. At the end of 3 min, the rat was returned to the carrier for transport back to its home cages. The distance traveled and the time spent with each object were recorded and used for statistical analysis. Results for object preference are shown as the percent of time spent with the novel object using the formula:

$$\text{Percent of time with novel object} = ((\text{time (s) spent with novel object}) / \text{time (s) spent with both objects}) * 100$$

Values equaling 50% indicate the same amount of time was spent with both objects and no object preference was observed. Values over 50% indicate a preference for the novel object and values under 50% indicate a preference for the familiar object.

Statistical analysis:

IBM SPSS (SPSS v. 24, IBM, 2016) was used for statistical analysis. Two-way ANOVAs were run on all assays with CIH and hormone status as fixed factors. Fisher LSD post hoc analysis was performed to determine differences between treatment groups. Results are shown as mean ± s.e.m., unless otherwise stated. Statistical significance was at $p < 0.05$.

RESULTS

CIH increases plasma oxidative stress

Oxidative stress, as measured by Advanced Oxidative Protein Products (AOPP), was significantly, but independently, affected by both hormonal status ($F_{3,90} = 3.58$, $p = 0.05$, $\eta^2 = 0.09$) and CIH exposure ($F_{1,90} = 6.30$, $p = 0.05$, $\eta^2 = 0.06$) (figure 1). LSD post-hoc analysis revealed GDX rats had significantly lower oxidative stress than all other groups, regardless of CIH exposure. Although an AHI = 8 was used in this study, as opposed to AHI = 10 in our previous study to avoid possible ceiling effects, we observed similar findings due to CIH on oxidative stress (Snyder et al., 2017). Gonadally intact male rats experienced a significant 20% elevation of oxidative stress due to CIH in comparison to INTACT-NORM rats (figure 1). Further, CIH significantly increased oxidative stress 40% above NORM oxidative stress levels in DHT rats. Previous studies in our lab have shown that CIH (AHI = 8) can decrease circulating testosterone levels by as much as 80% (Wilson et al., 2018), which is commonly reported in clinical populations (Barrett-Connor et al., 2008; Luboshitzky et al., 2002; Zhang et al., 2016). To control for CIH-induced testosterone loss, a subset of rats received exogenous testosterone (T) at physiological levels (Damassa et al., 1977; Wilson et al., 2018). Rats receiving T maintenance did not experience increased in oxidative stress due to CIH. Similarly, CIH did not significantly influence oxidative stress in GDX rats.

CIH alters behavioral outcomes in rats given DHT

During the probe trial of the Morris Water Maze that examines spatial memory retention, the latency to locate the target was significantly affected by both CIH ($F_{1,60} = 16.58$, $p = 0.05$, $\eta^2 = 0.12$) and hormonal status ($F_{3,60} = 14.55$, $p = 0.05$, $\eta^2 = 0.32$) (figure 2a). GDX rats took significantly longer to reach the target area than any other group, and these results were not affected by CIH. CIH significantly increased the latency to locate the target only in the DHT group.

CIH did not affect the swimming distance by any treatment group ($F_{1,57} = 0.22$, $p = 0.64$, $\eta^2 = 0.05$) (figure 2b). This indicates the observed differences in probe latency were not due to deficits in swimming ability among the groups. Additionally, there were no significant differences due to CIH in mean latency during the training sessions on day 2 ($F_{1,92} = 1.67$, $p = 0.20$, $\eta^2 = 0.02$) or day 3 ($F_{1,92} = 0.00$, $p = 0.96$, $\eta^2 = 0.00$; data not shown). Therefore, although GDX rats had overall impaired memory retention, CIH induced overnight spatial memory retention deficits only in DHT rats.

The novel object assay evaluates episodic memory retention. In this study, the percent of time spent with the novel object was significantly influenced by the rat's hormonal status ($F_{3,75} = 4.60$, $p = 0.05$, $\eta^2 = 0.19$), but not CIH ($F_{1,75} = 0.97$, $p = 0.33$, $\eta^2 = 0.01$; figure 3a). Under NORM and CIH conditions, DHT rats spent significantly more time with the novel object than the original object than all other hormone groups. A non-significant ($p = 0.09$) interaction was observed between CIH and hormone status, in which GDX rats were the only group to exhibit a change in preference for the novel object following CIH exposure. There were no significant differences in distance traveled between any treatment

group (figure 3b), demonstrating neither hormone status ($F_{3, 91} = 1.19$, $p = 0.32$, $\eta^2 = 0.04$) nor CIH ($F_{1, 91} = 1.10$, $p = 0.30$, $\eta^2 = 0.01$) caused gross motor impairments.

To investigate if CIH impaired fine motor skills, a modified version of the open field assay was used. The number of falls through a mesh platform was significantly affected by hormonal status ($F_{3, 83} = 4.76$, $p = 0.05$, $\eta^2 = 0.14$). Specifically, DHT rats experienced more falls than any other group, regardless of CIH exposure (figure 3c). No differences due to CIH or hormonal status were observed in total rearing (CIH: $F_{1, 92} = 0.85$, $p = 0.36$, $\eta^2 = 0.01$; hormone: $F_{3, 92} = 1.52$, $p = 0.22$, $\eta^2 = 0.05$; data not shown) or independent rearing (CIH: $F_{1, 92} = 2.06$, $p = 0.16$, $\eta^2 = 0.02$; hormone: $F_{3, 92} = 0.96$, $p = 0.41$, $\eta^2 = 0.03$; data not shown).

CIH differentially alters protein expression in a regionally-specific manner

Brain tissue from each hormone group was further examined for evidence of oxidative stress, apoptosis, and inflammation in the SN, ETC, and hippocampus. To examine oxidative stress, we quantified protein expression of NADPH oxidase (NOX-1) and the 145 kDa fragment of spectrin cleavage by calpain. The 120 kDa fragment of spectrin cleavage by caspase-3 was used to quantify apoptosis.

Significant differences in the calpain-cleaved 145 kDa fragment of spectrin were observed in all three brain regions (figure 4). Within the SN, calpain activity was significantly affected by both CIH ($F_{1, 26} = 4.63$, $p = 0.05$, $\eta^2 = 0.04$) and hormonal status ($F_{3, 26} = 24.14$, $p = 0.05$, $\eta^2 = 0.63$) (figure 4a & 4b). CIH significantly increased calpain activity only in INTACT rats. DHT administration resulted in significantly elevated calpain activity in comparison with all other hormone groups, regardless of CIH exposure. Apoptosis was not evident in the SN based on the lack of 120 kDa caspase-3 cleaved bands. This result suggests CIH increases oxidative stress in the SN of INTACT rats, but not apoptosis in the SN after 12 days of CIH exposure.

In both the ETC ($F_{3, 22} = 6.65$, $p = 0.05$, $\eta^2 = 0.41$) and hippocampus ($F_{3, 25} = 31.31$, $p = 0.05$, $\eta^2 = 0.77$), calpain activity was significantly affected by hormonal status. In both regions, DHT rats had significantly more calpain activity, as indicated by the 145 kDa band (figure 4c & 4e). In the hippocampus, T also significantly increased calpain activity compared to INTACT (figure 4e). Unlike what is observed in the SN, CIH did not have an effect on calpain activity in the ETC or hippocampus (figure 4a, 4c, & 4e). These results indicate that androgens modulate oxidative processes in the brain in a regionally specific manner.

Caspase-3 cleaved 120 kDa fragments are detectable in both the ETC and hippocampus, unlike what is observed in the SN. In the hippocampus ($F_{3, 25} = 5.62$, $p = 0.05$, $\eta^2 = 0.40$), but not in the ETC ($F_{3, 35} = 1.50$, $p = 0.23$, $\eta^2 = 0.11$), caspase-3 activity was significantly affected by hormonal status. DHT caused a significant increase in the 120 kDa caspase-3 fragment of spectrin in the hippocampus compared to all other groups (figure 4a & 4f). CIH did not cause significant differences in the 120 kDa fragment in either the hippocampus ($F_{1, 25} = 0.00$, $p = 0.98$, $\eta^2 = 0.00$) or the ETC ($F_{1, 35} = 0.00$, $p = 0.95$, $\eta^2 = 0.00$) of any

hormone groups (figure 4a, 4d, & 4f). Similar to what was observed in the SN, it appears caspase-3 is not activated in response to 12 days of CIH exposure.

Similar to what was observed with calpain activity, NOX1 expression was affected by androgens in a regionally specific manner. In the SN, NOX1 expression was not altered in response to either CIH ($F_{1, 24} = 0.03$, $p = 0.86$, $\eta^2 = 0.00$) or hormone status ($F_{3, 24} = 2.37$, $p = 0.10$, $\eta^2 = 0.21$) (figure 5a & 5b). In the ETC ($F_{3, 20} = 109.20$, $p = 0.05$, $\eta^2 = 0.94$) and hippocampus ($F_{3, 28} = 16.29$, $p = 0.05$, $\eta^2 = 0.63$), NOX1 expression was significantly affected by hormone status (figures 5d & 5e, 5g & 5i), but not by CIH. In both regions, DHT rats had significantly higher NOX1 expression than all other groups (figure 5e & 5h). T significantly increased NOX1 expression in the ETC, but not the hippocampus, compared to INTACT rats, although to a much lesser extent than DHT (figure 5e).

Androgens modulate astrocyte presence in CNS regions

Since our current study utilized CIH (AHI = 8) for 12 days, instead of 7 days, we wanted to determine if a macrophage presence was evident. Thus, we examined markers of activated macrophages and microglia (cd11b) and astrocytes (GFAP). We found no evidence of cd11b staining in any of the brain regions of any treatment group (data not shown). However, GFAP protein was detectable in all 3 brain regions, indicating the presence of astrocytes (figures 5c, 5f, & 5i). GFAP protein expression was observed to be significantly altered by hormonal status in all brain regions. In the SN ($F_{3, 27} = 4.52$, $p = 0.05$, $\eta^2 = 0.33$), GDX rats exhibited significantly more GFAP expression than all other groups (figure 5c). In the ETC ($F_{3, 20} = 26.39$, $p = 0.05$, $\eta^2 = 0.78$), DHT rats had significantly more GFAP expression than all other groups, whereas rats with T exhibited less GFAP expression when compared with INTACT rats (figure 5f). In the hippocampus ($F_{3, 25} = 16.59$, $p = 0.05$, $\eta^2 = 0.64$), GDX and T rats had significantly lower GFAP expression than INTACT or DHT rats did (figure 5i). GFAP expression was not altered by CIH in any brain region (SN: $F_{1, 20} = 0.00$, $p = 0.95$, $\eta^2 = 0.00$; ETC: $F_{1, 20} = 0.07$, $p = 0.79$, $\eta^2 = 0.00$; HIPP: $F_{1, 25} = 1.57$, $p = 0.22$, $\eta^2 = 0.02$).

CIH induces inflammatory dysregulation in a region-specific manner

In plasma, pro-inflammatory M1 cytokine expression was found to be significantly altered by hormonal status ($F_{3, 20} = 16.69$, $p = 0.05$, $\eta^2 = 0.88$), but not CIH ($F_{1, 20} = 0.09$, $p = 0.77$, $\eta^2 = 0.00$). GDX and T rats exhibited significantly lower M1 cytokines than INTACT rats (table 1). T rats also had significantly lower circulating M1 cytokines than DHT rats. Correspondingly, anti-inflammatory M2 cytokine expression was significantly affected by hormonal status ($F_{3, 20} = 19.47$, $p = 0.05$, $\eta^2 = 0.71$), but not CIH ($F_{1, 20} = 2.07$, $p = 0.17$, $\eta^2 = 0.03$). Post hoc analysis revealed DHT rats had higher M2 levels than either the GDX or T hormone groups. Additionally, INTACT rats exhibited significantly more M2 cytokines than all other groups.

Similar to what was observed with oxidative stress, regional CNS differences were observed in inflammation. In the SN, a significant interaction between CIH and hormone status on M1 cytokines was observed ($F_{3, 26} = 4.74$, $p = 0.05$, $\eta^2 = 0.10$), with main effects of both CIH ($F_{1, 26} = 4.42$, $p = 0.05$, $\eta^2 = 0.03$) and hormone ($F_{3, 26} = 33.63$, $p = 0.05$, $\eta^2 = 0.69$) (table 1). In this brain region associated with movement disorders, DHT rats had significantly more

M1 cytokine expression than all other hormone groups. Although there was a significant overall effect of CIH exposure to elevate M1 cytokine expression and a significant interaction between CIH and hormone status, post hoc analysis revealed no significant differences due to CIH within any of the hormone groups. M2 cytokine expression in the SN was also significantly affected by hormonal status ($F_{3, 26} = 5.53$, $p = 0.05$, $\eta^2 = 0.35$) but not CIH ($F_{1, 26} = 0.48$, $p = 0.50$, $\eta^2 = 0.01$). DHT rats had significantly less M2 expression, than all other groups. In the SN, DHT appears to promote a pro-inflammatory environment by increasing M1 cytokines and simultaneously decreasing M2 cytokines.

In the ETC, both M1 and M2 activating cytokines were significantly affected by hormonal status (M1: $F_{3, 19} = 7.31$, $p = 0.05$, $\eta^2 = 0.01$; M2: $F_{3, 19} = 8.22$, $p = 0.05$, $\eta^2 = 0.49$) (table 1). In this region, INTACT rats exhibited significantly higher M1 and M2 cytokines than all other groups, similar to what was observed in plasma. There was no effect of CIH on cytokine expression in this region (M1: $F_{1, 19} = 0.15$, $p = 0.71$, $\eta^2 = 0.00$; M2: $F_{1, 19} = 0.78$, $p = 0.39$, $\eta^2 = 0.02$).

In the hippocampus, a significant effect of hormone status (M1: $F_{3, 18} = 3.94$, $p = 0.05$, $\eta^2 = 0.27$) (table 1) was observed, which resulted in T treated rats exhibiting less M1 cytokines than INTACT or GDX rats. Although a significant overall effect of CIH was not observed in the hippocampus ($F_{1, 19} = 2.46$, $p = 0.13$, $\eta^2 = 0.06$), a significant interaction between hormone status and CIH was observed ($F_{3, 18} = 3.80$, $p = 0.05$, $\eta^2 = 0.26$). CIH suppressed M1 cytokines in the hippocampus of INTACT rats but elevated M1 cytokine expression in T-treated rats. Similar to the effect on M1 cytokines, a significant main effect of hormone status on M2 cytokines was observed ($F_{3, 19} = 4.01$, $p = 0.05$, $\eta^2 = 0.29$), but not a main effect of CIH ($F_{1, 19} = 2.47$, $p = 0.13$, $\eta^2 = 0.06$). This resulted in T treated rats expressing significantly less M2 cytokines than INTACT or GDX rats, regardless of CIH exposure.

DISCUSSION

We hypothesized androgens influence the behavioral outcomes, oxidative stress, and inflammatory effects of CIH in male rats. This study used a slightly lower hypoxic exposure (AHI = 8) than our previous study (AHI = 10) to avoid a possible ceiling effect due to superimposing androgens (Snyder et al., 2017). However, our results indicate CIH caused a similar increase in circulating oxidative stress (figure 1), as well as regional differences in the brain (figures 4 & 5) in agreement with our prior observations (Snyder et al., 2017). Androgens appear to contribute to diametric mechanisms under CIH. To our knowledge, this is the first study investigating the effects of CIH on the Long-Evans rat strain. The main findings of our study include: 1) CIH did not influence oxidative stress in rats with maintained testosterone levels, 2) CIH only increased circulating oxidative stress in gonadally intact and DHT male rats, 3) CIH induced spatial memory impairment in DHT rats, and 4) DHT increased susceptibility to oxidative stress and M1 pro-inflammatory cytokines in the CNS (table 1, figures 1,2, 4 & 5). These findings suggest androgen signaling, in the absence of aromatized estrogenic signaling, may render male rats susceptible to the deleterious effects of CIH.

Our data show that both CIH and androgens can increase circulating and brain region specific oxidative stress. CIH increased circulating oxidative stress (as measured by AOPP) in male rats, but not in hormone-deficient rats. Prior studies in our lab demonstrate testosterone itself is a mild oxidative stressor, which may contribute to preconditioning protective qualities (Holmes et al., 2013; Wilson et al., 2018). The contribution of testosterone to oxidative stress was corroborated in this study by the significant drop in basal oxidative stress that was observed in gonadectomized male rats under normal room air conditions (figure 1). Rats that received androgen supplementation following gonadectomy did not experience a drop in basal levels of oxidative stress. Therefore, we wanted to determine if the androgen pathway may be involved in the oxidative stress response to CIH. Indeed, significantly increased oxidative stress due to CIH was observed in DHT rats, indicating the involvement of androgens.

To further examine what impact CIH and androgens had on the brain, we investigated the response of oxidative stress-related proteins (NOX1 and calpain-cleavage of spectrin) in the ETC, hippocampus, and the SN, brain regions affected by AD and PD. DHT, and to some extent T, increased oxidative stress markers in all brain regions examined. The reduced response to T compared with DHT is most likely due to partial aromatization of T to estradiol. Surprisingly, we found NOX1 expression was not responsive to CIH in any of the brain regions examined. It is possible that the lower AHI used in our study may underlie the difference from other studies that have reported a role of NOX1 in CIH-induced oxidative stress generation (Nair et al., 2011b; Sun et al., 2012). However, it is more probable a ceiling effect was reached, especially in the DHT group in which CIH was not able to further elevate NOX1 expression in the SN. Although protein expression of NOX1 was unaffected by CIH, NOX1 activity may have been influenced, which will be assessed in future studies.

The possibility NOX1 activity is affected by CIH is consistent with our data using the oxidative stress marker, calpain-cleavage of spectrin, which was elevated by CIH in the SN of gonadally intact male rats but did not further increase protein expression in the DHT group. The significant increase in calpain-cleavage observed in the SN, but not the other regions examined, may partially be due to the abundance of catecholaminergic neurons present within the SN, which are sensitive to oxidative stress (Lotharius et al., 2002). The neuronal composition of the SN is relatively homogeneous, in which 95% of the cells are dopaminergic neurons (Lacey et al., 1989). Due to the metabolism of the primary neurotransmitter (Fornstedt, 1990), dopamine, in the SN, this brain nuclei operates under elevated levels of oxidative stress. Therefore, the SN may have a lower oxidative stress threshold than the other brain regions studied. In contrast, the ETC and hippocampus are composed of a wider variety of cell types, which may partially compensate for the oxidative effects of CIH in those regions (Ferrante et al., 2016; Insausti, 1993).

Both androgens and CIH contribute to neuronal oxidative stress pathways. Our current study suggests DHT also plays an integral role in basal inflammatory signaling in male rats (table 1), particularly in generating pro-inflammatory M1 cytokines. Interestingly, the DHT group was observed to increase both oxidative stress and M1 cytokines within the brain regions investigated (figures 4 & 5, table 1). M1 signaling, in turn, can further increase oxidative stress (Montine et al., 2002). The memory deficits observed in the DHT group, following

CIH, may be attributable to the observed increases in oxidative processes and pro-inflammatory signaling. An accumulation of oxidative stress within neurons can be damaging and trigger apoptotic pathways (Valko et al., 2007). Oxidative stress has been implicated in pro-inflammatory responses to CIH (Lavie, 2014; Semenza and Prabhakar, 2015). The cyclic relationship between oxidative stress and inflammation may partially explain why both elevated oxidative stress and inflammation are reported to contribute to neurodegenerative diseases (Barnham et al., 2004; Chao et al., 2014; Jenner, 2003; Lucas et al., 2006).

In our previous study, Snyder, et al., 7 days of CIH at AHI = 10 resulted in an elevation of pro-inflammatory M1 cytokines compared to anti-inflammatory M2 cytokines in the plasma, SN, and ETC in Sprague-Dawley rats (Snyder et al., 2017). Contrary to those observations, significant effects due to 12 days CIH at AHI = 8 were not observed in the circulation of INTACT Long-Evans rats, possibly due to the use of a milder AHI (table 1). Similarly, CIH did not elevate inflammation in the SN or the ETC, although an interaction between CIH and hormone status suggesting T prevents an elevation of M1 cytokines was observed in the SN. In contrast, a pro-inflammatory response due to CIH was observed in the hippocampus of INTACT rats. Further study is needed to fully deduce the regional effects of inflammatory signaling during CIH.

Unpublished *in vitro* data from our lab using a neuronal cell line did not find a neuronal inflammatory response to oxidative stress (hydrogen peroxide). However, an overall inflammatory response to CIH was evident in the SN as well as in the hippocampus of INTACT and T treated rats. The inflammatory response is most likely mediated by non-neuronal cells in the CNS. Protein analysis did not provide evidence of either microglia or macrophages, but there were regional differences in a marker of astrocyte expression (figure 5). These results indicate the initial inflammatory response observed may be mediated by astrocytes. Based on previous publications which indicate astrocytes modulate protective responses to early hypoxic insults (Acas-Fonseca et al., 2016; González-Reyes et al., 2017; Hirayama and Koizumi, 2017), the flux of astrocytes observed within each brain region after 7 days of CIH may be indicative of protective mechanisms. Regional astrocytes can serve to tune neuronal responses within each system, which could underlie SA comorbidities, such as neurodegenerative diseases (AD and PD).

In this study we observed differences in behavioral responses to CIH and androgens. We did not see any overt motor impairments (figure 2 & 3). Behavioral deficits associated with PD are not observed until 70-80% cell loss within the SN (Braak et al., 2003; Crocker, 1997), which is consistent with our behavioral data. However, we did observe fine motor impairments in the DHT group, regardless of CIH exposure. The DHT group consistently experienced more falls in the modified open field than any other group (figure 3). Although oxidative stress and M1 signals were elevated by CIH in the SN, apoptosis induced cell death was not evident in the SN at this time point (figure 4). Our study indicates oxidative stress due to DHT or a more androgenic profile may increase motor function susceptibility to future impairments, such as long term CIH (sleep apnea) exposure.

The behavior most impacted by CIH in this study was memory. Unlike PD that requires significant cell loss in the SN before onset of behavioral impairment, patients with AD begin to exhibit clinical symptoms when a substantial number of their hippocampal cells have been compromised (Braak and Braak, 1994). Patients with SA are at a higher risk to develop cognitive disorders, such as AD and vascular dementia (Buratti et al., 2014; Gagnon et al., 2014). In the current study, DHT elevated oxidative stress in both the ETC and hippocampus, irrespective of CIH exposure, as well as increased apoptosis in the hippocampus (figures 4 & 5). This elevation of oxidative stress indicates androgens can increase the sensitivity of neurons to a subsequent oxidative stressor, such as CIH.

Gozal, et al., reported cognitive impairment in the Morris Water Maze in male rats exposed to 2 weeks of CIH treatment (Gozal et al., 2001). The lack of significant impairment in the probe latency of INTACT rats in our study (figure 2) may be due to the low AHI (8) utilized for 12 days. It is likely that CIH exposure longer than 12 days or a more severe AHI would result in significant impairment in gonadally intact male rats. In this study, CIH induced significant cognitive impairments in the latency to locate the target during the probe trial of the Morris Water Maze in DHT rats, and thus androgens may be involved in the SA-associated comorbidity of AD. This possibility is supported by the elevated risk for women with PCOS (characterized by elevated androgens) to be diagnosed with both SA and AD (reviewed in (Snyder and Cunningham, 2018)).

Unlike the results obtained in the Morris Water Maze, administration of DHT significantly increased the amount of time spent with a novel object as opposed to a familiar object. These effects were not affected by CIH. The different type of memory processes evaluated by each behavior assay may provide insight into the types of memory functions which are most susceptible to the repetitive hypoxic insults of SA. The results from this study suggest overnight spatial and working memory consolidation could be more sensitive to hypoxic insults during sleep than short term episodic memory for novel encounters. However, it is more probable that the lack of CIH effect on DHT treated rats in the novel object assay could be due to DHT itself. DHT has been documented to increase novelty seeking behaviors (Mosher et al., 2018), therefore any differences due to CIH may be masked by the DHT-promoting novelty variable in this assay. The current protocol for the novel object task may be inappropriate for assaying memory in androgenic rats. Future experiments with DHT treatment may require a longer habituation phase to reduce the novelty aspect. Overall, these findings are consistent with literature demonstrating that both testosterone and estrogens are protective in the CNS and facilitate memory, while DHT can contribute to damaging processes on occasion (Aubrecht et al., 2014; Beer et al., 2006; Bimonte-Nelson et al., 2003; Frye et al., 2008; Osborne et al., 2009).

Our findings are of clinical relevance to men with SA. Although exogenous testosterone (presumably via estrogenic signaling) in this study prevented CIH-induced oxidative stress and memory deficits, androgenic signaling by DHT exacerbated these measures. Men have higher androgen concentrations and are more likely to be diagnosed with SA than women (Basoglu and Tasbakan, 2017; Dempsey et al., 2010). The discrepancy in diagnosis between the sexes is unlikely to be exclusively due to a genetic influence based on prior studies of women with polycystic ovarian syndrome. Women with PCOS are more likely to have SA,

hypertension, and cognitive disorders. These observations suggest elevated androgenic signaling contributes to risk (Helvacı, 2017; Randeve et al., 2012). Therefore, androgen-induced upregulation of oxidative stress and inflammation can create a vicious cycle that may underlie the comorbidities of SA.

Of concern, is that these effects of CIH were observed after only 12 days. Many people with SA remain undiagnosed (Kapur et al., 2002; Lindberg et al., 2017). Among the population diagnosed for SA, the recommendation for treatment of mild SA is optional, and large portions of the clinical population with more severe diagnoses elect to halt compliance (Aurora et al., 2015; Epstein et al., 2009). These clinical outcomes result in a large segment of the population with untreated SA, which may have more severe long-term health ramifications than commonly believed.

In spite of efforts to develop treatments for AD and PD, there is a general lack of information about early mechanisms which lead to neurodegenerative diseases. This deficiency in information is accompanied by a scarcity of models with which to effectively study early neurodegenerative mechanisms. The majority of neurodegenerative disorders develop sporadically. The results from this study suggest early processes initiated during mild stages of SA could have serious longitudinal consequences on brain and behavior. Consequently, the CIH model provides valuable insight into how early mechanisms of SA could be a vital point for therapeutic intervention to prevent early neurodegenerative mechanisms.

CONCLUSIONS

In male rats, maintaining physiological testosterone levels was protective against CIH-induced oxidative stress. However, increased CIH-induced oxidative stress was observed in rats with physiological levels of the non-aromatizable androgen, DHT. Thus, the negative effects of androgens in an oxidative stress environment may be mediated through androgen receptor signaling. In cases of SA, observed sex differences may be due to a negative interaction between oxidative stress and androgens. Therefore, men who have been diagnosed with SA in conjunction with elevated oxidative stress may be susceptible to neurodegenerative pathophysiology.

ACKNOWLEDGEMENTS

The authors would like to thank E. Nicole Wilson for her technical assistance.

FUNDING

The work was supported by the National Institutes of Health (NIH) [R01 NS0091359] to RLC; and UNT Health Science Center, Neurobiology of Aging NIH training grant [T32 AG 020494] to BDS.

References

- Acáz-Fonseca E, Avila-Rodriguez M, Garcia-Segura LM, Barreto GE, 2016 Regulation of astroglia by gonadal steroid hormones under physiological and pathological conditions. *Prog Neurobiol* 144, 5–26. [PubMed: 27283249]

- Araujo AB, Wittert GA, 2011 Endocrinology of the aging male. Best practice & research. Clinical endocrinology & metabolism 25, 303–319. [PubMed: 21397200]
- Aubrecht TG, Jenkins R, Magalang UJ, Nelson RJ, 2014 Influence of Gonadal Hormones on the Behavioral Effects of Intermittent Hypoxia in Mice. American journal of physiology. Regulatory, integrative and comparative physiology, ajpregu.00379.02014.
- Aurora RN, Collop NA, Jacobowitz O, Thomas SM, Quan SF, Aronsky AJ, 2015 Quality Measures for the Care of Adult Patients with Obstructive Sleep Apnea. Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine 11, 357–383. [PubMed: 25700878]
- Barnham KJ, Masters CL, Bush AI, 2004 Neurodegenerative diseases and oxidative stress. Nature reviews. Drug discovery 3, 205–214. [PubMed: 15031734]
- Barrett-Connor E, Dam TT, Stone K, Harrison SL, Redline S, Orwoll E, 2008 The association of testosterone levels with overall sleep quality, sleep architecture, and sleep-disordered breathing. The Journal of clinical endocrinology and metabolism 93, 2602–2609. [PubMed: 18413429]
- Barron AM, Pike CJ, 2012 Sex hormones, aging, and Alzheimer's disease. Frontiers in bioscience (Elite edition) 4, 976–997. [PubMed: 22201929]
- Basoglu OK, Tasbakan MS, 2017 Gender differences in clinical and polysomnographic features of obstructive sleep apnea: a clinical study of 2827 patients. Sleep & breathing = Schlaf & Atmung.
- Beer TM, Bland LB, Bussiere JR, Neiss MB, Wersinger EM, Garzotto M, Ryan CW, Janowsky JS, 2006 Testosterone loss and estradiol administration modify memory in men. The Journal of urology 175, 130–135. [PubMed: 16406889]
- Betteridge DJ, 2000 What is oxidative stress? Metabolism 49, 3–8.
- Bimonte-Nelson HA, Singleton RS, Nelson ME, Eckman CB, Barber J, Scott TY, Granholm AC, 2003 Testosterone, but not nonaromatizable dihydrotestosterone, improves working memory and alters nerve growth factor levels in aged male rats. Exp Neurol 181, 301–312. [PubMed: 12782002]
- Block F, Dihne M, Loos M, 2005 Inflammation in areas of remote changes following focal brain lesion. Prog Neurobiol 75, 342–365. [PubMed: 15925027]
- Bouloukaki I, Mermigkis C, Tzanakis N, Kallergis E, Moniaki V, Mauroudi E, Schiza SE, 2017 Evaluation of Inflammatory Markers in a Large Sample of Obstructive Sleep Apnea Patients without Comorbidities. Mediators of Inflammation 2017.
- Braak H, Braak E, 1994 Morphological criteria for the recognition of Alzheimer's disease and the distribution pattern of cortical changes related to this disorder. Neurobiol Aging 15, 355–356; discussion 379–380. [PubMed: 7936061]
- Braak H, Del Tredici K, 2015 The preclinical phase of the pathological process underlying sporadic Alzheimer's disease. Brain 138, 2814–2833. [PubMed: 26283673]
- Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E, 2003 Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24, 197–211. [PubMed: 12498954]
- Buratti L, Viticchi G, Falsetti L, Cagnetti C, Luzzi S, Bartolini M, Provinciali L, Silvestrini M, 2014 Vascular impairment in Alzheimer's disease: the role of obstructive sleep apnea. J Alzheimers Dis 38, 445–453. [PubMed: 23985418]
- Chao Y, Wong SC, Tan EK, 2014 Evidence of inflammatory system involvement in Parkinson's disease. Biomed Res Int 2014, 308654. [PubMed: 25050341]
- Chetyrkin SV, Hu J, Gough WH, Dumaual N, Kedishvili NY, 2001 Further characterization of human microsomal 3alpha-hydroxysteroid dehydrogenase. Archives of biochemistry and biophysics 386, 1–10. [PubMed: 11360992]
- Choi SS, Lee HJ, Lim I, Satoh J, Kim SU, 2014 Human Astrocytes: Secretome Profiles of Cytokines and Chemokines. PLoS One 9.
- Crain JM, Nikodemova M, Watters JJ, 2013 Microglia express distinct M1 and M2 phenotypic markers in the postnatal and adult central nervous system in male and female mice. J Neurosci Res 91, 1143–1151. [PubMed: 23686747]
- Crocker AD, 1997 The regulation of motor control: an evaluation of the role of dopamine receptors in the substantia nigra. Rev Neurosci 8, 55–76. [PubMed: 9402645]

- Cunningham JT, Knight WD, Mifflin SW, Nestler EJ, 2012 An Essential role for DeltaFosB in the median preoptic nucleus in the sustained hypertensive effects of chronic intermittent hypoxia. *Hypertension* 60, 179–187. [PubMed: 22689746]
- Cunningham RL, Claiborne BJ, McGinnis MY, 2007 Pubertal exposure to anabolic androgenic steroids increases spine densities on neurons in the limbic system of male rats. *Neuroscience* 150, 609–615. [PubMed: 17980492]
- Cunningham RL, Singh M, O'Bryant SE, Hall JR, Barber RC, 2014 Oxidative stress, testosterone, and cognition among Caucasian and Mexican-American men with and without Alzheimer's disease. *J Alzheimers Dis* 40, 563–573. [PubMed: 24496073]
- Dai DF, Chiao YA, Marcinek DJ, Szeto HH, Rabinovitch PS, 2014 Mitochondrial oxidative stress in aging and healthspan. *Longevity & healthspan* 3, 6. [PubMed: 24860647]
- Damassa DA, Smith ER, Tennent B, Davidson JM, 1977 The relationship between circulating testosterone levels and male sexual behavior in rats. *Hormones and behavior* 8, 275–286. [PubMed: 881168]
- Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP, 2010 Pathophysiology of sleep apnea. *Physiol Rev* 90, 47–112. [PubMed: 20086074]
- Deshmukh S, Knierim J, 2011 Representation of Non-Spatial and Spatial Information in the Lateral Entorhinal Cortex. *Frontiers in Behavioral Neuroscience* 5. [PubMed: 21720525]
- dos Santos RL, da Silva FB, Ribeiro RF Jr., Stefanon I, 2014 Sex hormones in the cardiovascular system. *Hormone molecular biology and clinical investigation* 18, 89–103. [PubMed: 25390005]
- Epstein LJ, Kristo D, Strollo PJ Jr., Friedman N, Malhotra A, Patil SP, Ramar K, Rogers R, Schwab RJ, Weaver EM, Weinstein MD, 2009 Clinical guideline for the evaluation, management and long-term care of obstructive sleep apnea in adults. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine* 5, 263–276. [PubMed: 19960649]
- Ferrante M, Tahvildari B, Duque A, Hadzipasic M, Salkoff D, Zagha EW, Hasselmo ME, McCormick DA, 2016 Distinct Functional Groups Emerge from the Intrinsic Properties of Molecularly Identified Entorhinal Interneurons and Principal Cells. *Cerebral cortex (New York, N. Y. : 1991)*.
- Fornstedt B, 1990 5. Role of catechol autooxidation in the degeneration of dopamine neurons. *Acta neurologica Scandinavica* 82, 12–14.
- Frye CA, Koonce CJ, Edinger KL, Osborne DM, Walf AA, 2008 Androgens with activity at estrogen receptor beta have anxiolytic and cognitive-enhancing effects in male rats and mice. *Hormones and behavior* 54, 726–734. [PubMed: 18775724]
- Gagnon K, Baril AA, Gagnon JF, Fortin M, Decary A, Lafond C, Desautels A, Montplaisir J, Gosselin N, 2014 Cognitive impairment in obstructive sleep apnea. *Pathologie-biologie* 62, 233–240. [PubMed: 25070768]
- Gao W, Bohl CE, Dalton JT, 2005 Chemistry and Structural Biology of Androgen Receptor. *Chemical reviews* 105, 3352–3370. [PubMed: 16159155]
- Garza-Contreras J, Duong P, Snyder BD, Schreihöfer DA, Cunningham RL, 2017 Presence of Androgen Receptor Variant in Neuronal Lipid Rafts. *eNeuro* 4.
- Gillies GE, Murray HE, Dexter D, McArthur S, 2004 Sex dimorphisms in the neuroprotective effects of estrogen in an animal model of Parkinson's disease. *Pharmacol Biochem Behav* 78, 513–522. [PubMed: 15251260]
- González-Reyes RE, Nava-Mesa MO, Vargas-Sánchez K, Ariza-Salamanca D, Mora-Muñoz L, 2017 Involvement of Astrocytes in Alzheimer's Disease from a Neuroinflammatory and Oxidative Stress Perspective. *Frontiers in Molecular Neuroscience* 10. [PubMed: 28197071]
- Gozal D, Daniel JM, Dohanich GP, 2001 Behavioral and anatomical correlates of chronic episodic hypoxia during sleep in the rat. *J Neurosci* 21, 2442–2450. [PubMed: 11264318]
- Gozal D, Row BW, Kheirandish L, Liu R, Guo SZ, Qiang F, Brittian KR, 2003 Increased susceptibility to intermittent hypoxia in aging rats: Changes in proteasomal activity, neuronal apoptosis and spatial function. *Journal of Neurochemistry* 86, 1545–1552. [PubMed: 12950463]
- Gozal D, Serpero LD, Sans Capdevila O, Kheirandish-Gozal L, 2008 Systemic inflammation in non-obese children with obstructive sleep apnea. *Sleep Med* 9, 254–259. [PubMed: 17825619]
- Grandner MA, 2017 Sleep, Health, and Society. *Sleep medicine clinics* 12, 1–22. [PubMed: 28159089]

- Grimm A, Biliouris EE, Lang UE, Gotz J, Mensah-Nyagan AG, Eckert A, 2016 Sex hormone-related neurosteroids differentially rescue bioenergetic deficits induced by amyloid-beta or hyperphosphorylated tau protein. *Cellular and molecular life sciences : CMLS* 73, 201–215. [PubMed: 26198711]
- Handa RJ, Pak TR, Kudwa AE, Lund TD, Hinds L, 2008 An alternate pathway for androgen regulation of brain function: activation of estrogen receptor beta by the metabolite of dihydrotestosterone, 5alpha-androstane-3beta,17beta-diol. *Hormones and behavior* 53, 741–752. [PubMed: 18067894]
- He D, Kou X, Luo Q, Yang R, Liu D, Wang X, Song Y, Cao H, Zeng M, Gan Y, Zhou Y, 2015 Enhanced M1/M2 macrophage ratio promotes orthodontic root resorption. *Journal of dental research* 94, 129–139. [PubMed: 25344334]
- Helvacı N, 2017 Polycystic ovary syndrome and the risk of obstructive sleep apnea: a meta-analysis and review of the literature. 6, 437–445.
- Hinojosa-Laborde C, Mifflin SW, 2005 Sex differences in blood pressure response to intermittent hypoxia in rats. *Hypertension* 46, 1016–1021. [PubMed: 16157795]
- Hirayama Y, Koizumi S, 2017 Hypoxia-independent mechanisms of HIF-1alpha expression in astrocytes after ischemic preconditioning. *Glia* 65, 523–530. [PubMed: 28063215]
- Hojo Y, Kawato S, 2018 Neurosteroids in Adult Hippocampus of Male and Female Rodents: Biosynthesis and Actions of Sex Steroids. *Frontiers in endocrinology* 9, 183. [PubMed: 29740398]
- Holmes S, Abbassi B, Su C, Singh M, Cunningham RL, 2013 Oxidative stress defines the neuroprotective or neurotoxic properties of androgens in immortalized female rat dopaminergic neuronal cells. *Endocrinology* 154, 4281–4292. [PubMed: 23959938]
- Holmes S, Singh M, Su C, Cunningham RL, 2016 Effects of Oxidative Stress and Testosterone on Pro-Inflammatory Signaling in a Female Rat Dopaminergic Neuronal Cell Line. *Endocrinology* 157, 2824–2835. [PubMed: 27167771]
- Insausti R, 1993 Comparative anatomy of the entorhinal cortex and hippocampus in mammals. *Hippocampus* 3 Spec No, 19–26. [PubMed: 8287096]
- Jacome LF, Barateli K, Buitrago D, Lema F, Frankfurt M, Luine VN, 2016 Gonadal Hormones Rapidly Enhance Spatial Memory and Increase Hippocampal Spine Density in Male Rats. *Endocrinology* 157, 1357–1362. [PubMed: 26844375]
- Janowsky JS, 2006 The role of androgens in cognition and brain aging in men. *Neuroscience* 138, 1015–1020. [PubMed: 16310318]
- Jenner P, 2003 Oxidative stress in Parkinson's disease. *Ann Neurol* 53 Suppl 3, S26–36; discussion S36–28. [PubMed: 12666096]
- Kapur V, Strohl KP, Redline S, Iber C, O'Connor G, Nieto J, 2002 Underdiagnosis of sleep apnea syndrome in U.S. communities. *Sleep & breathing = Schlaf & Atmung* 6, 49–54. [PubMed: 12075479]
- Khan UA, Liu L, Provenzano FA, Berman DE, Profaci CP, Sloan R, Mayeux R, Duff KE, Small SA, 2014 Molecular drivers and cortical spread of lateral entorhinal cortex dysfunction in preclinical Alzheimer's disease. *Nat Neurosci* 17, 304–311. [PubMed: 24362760]
- Knight WD, Little JT, Carreno FR, Toney GM, Mifflin SW, Cunningham JT, 2011 Chronic intermittent hypoxia increases blood pressure and expression of FosB/DeltaFosB in central autonomic regions. *American journal of physiology. Regulatory, integrative and comparative physiology* 301, R131–139.
- Knusmann R, Sperwien A, 1988 Relations between anthropometric characteristics and androgen hormone levels in healthy young men. *Annals of human biology* 15, 131–142. [PubMed: 3281575]
- Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya M, 2016 Contribution of Macrophage Polarization to Metabolic Diseases. *Journal of atherosclerosis and thrombosis* 23, 10–17. [PubMed: 26412584]
- Lacey MG, Mercuri NB, North RA, 1989 Two cell types in rat substantia nigra zona compacta distinguished by membrane properties and the actions of dopamine and opioids. *J Neurosci* 9, 1233–1241. [PubMed: 2703874]
- Lau CF, Ho YS, Hung CH, Wuwongse S, Poon CH, Chiu K, Yang X, Chu LW, Chang RC, 2014 Protective effects of testosterone on presynaptic terminals against oligomeric beta-amyloid peptide in primary culture of hippocampal neurons. *Biomed Res Int* 2014, 103906. [PubMed: 25045655]

- Lavie L, 2014 Oxidative stress in obstructive sleep apnea and intermittent hypoxia - Revisited - The bad ugly and good: Implications to the heart and brain. *Sleep Med Rev*.
- Lavie L, Lavie P, 2009 Molecular mechanisms of cardiovascular disease in OSAHS: the oxidative stress link. *Eur Respir J* 33, 1467–1484. [PubMed: 19483049]
- Lee A, Gilbert RM, 2016 Epidemiology of Parkinson Disease. *Neurologic clinics* 34, 955–965. [PubMed: 27720003]
- Lindberg E, Benediktsdottir B, Franklin KA, Holm M, Johannessen A, Jogi R, Gislason T, Real FG, Schlunssen V, Janson C, 2017 Women with symptoms of sleep-disordered breathing are less likely to be diagnosed and treated for sleep apnea than men. *Sleep Med* 35, 17–22. [PubMed: 28619177]
- Lotharius J, Barg S, Wiekop P, Lundberg C, Raymon HK, Brundin P, 2002 Effect of mutant alpha-synuclein on dopamine homeostasis in a new human mesencephalic cell line. *The Journal of biological chemistry* 277, 38884–38894. [PubMed: 12145295]
- Luboshitzky R, Aviv A, Hefetz A, Herer P, Shen-Orr Z, Lavie L, Lavie P, 2002 Decreased pituitary-gonadal secretion in men with obstructive sleep apnea. *The Journal of clinical endocrinology and metabolism* 87, 3394–3398. [PubMed: 12107256]
- Lucas SM, Rothwell NJ, Gibson RM, 2006 The role of inflammation in CNS injury and disease. *Br J Pharmacol* 147 Suppl 1, S232–240. [PubMed: 16402109]
- Ma S, Mifflin SW, Cunningham JT, Morilak DA, 2008 Chronic intermittent hypoxia sensitizes acute hypothalamic-pituitary-adrenal stress reactivity and Fos induction in the rat locus coeruleus in response to subsequent immobilization stress. *Neuroscience* 154, 1639–1647. [PubMed: 18554809]
- Mahmoud R, Wainwright SR, Galea LA, 2016 Sex hormones and adult hippocampal neurogenesis: Regulation, implications, and potential mechanisms. *Frontiers in neuroendocrinology*.
- May AM, Mehra R, 2014 Obstructive sleep apnea: role of intermittent hypoxia and inflammation. *Seminars in respiratory and critical care medicine* 35, 531–544. [PubMed: 25333334]
- McEwen BS, Milner TA, 2017 Understanding the broad influence of sex hormones and sex differences in the brain. *J Neurosci Res* 95, 24–39. [PubMed: 27870427]
- Montine TJ, Milatovic D, Gupta RC, Valyi-Nagy T, Morrow JD, Breyer RM, 2002 Neuronal oxidative damage from activated innate immunity is EP2 receptor-dependent. *J Neurochem* 83, 463–470. [PubMed: 12423256]
- Mosher LJ, Godar SC, Morissette M, McFarlin KM, Scheggi S, Gambarana C, Fowler SC, Di Paolo T, Bortolato M, 2018 Steroid 5 α -reductase 2 deficiency leads to reduced dominance-related and impulse-control behaviors. *Psychoneuroendocrinology* 91, 95–104. [PubMed: 29544191]
- Nair D, Dayyat EA, Zhang SX, Wang Y, Gozal D, 2011a Intermittent hypoxia-induced cognitive deficits are mediated by NADPH oxidase activity in a murine model of Sleep Apnea. *PLoS ONE* 6.
- Nair D, Zhang SX, Ramesh V, Hakim F, Kaushal N, Wang Y, Gozal D, 2011b Sleep fragmentation induces cognitive deficits via nicotinamide adenine dinucleotide phosphate oxidase-dependent pathways in mouse. *Am J Respir Crit Care Med* 184, 1305–1312. [PubMed: 21868506]
- Nguyen MM, Tamashiro KL, Melhorn SJ, Ma LY, Gardner SR, Sakai RR, 2007 Androgenic influences on behavior, body weight, and body composition in a model of chronic social stress. *Endocrinology* 148, 6145–6156. [PubMed: 17884946]
- Osborne DM, Edinger K, Frye CA, 2009 Chronic administration of androgens with actions at estrogen receptor beta have anti-anxiety and cognitive-enhancing effects in male rats. *Age (Dordrecht, Netherlands)* 31, 191–198.
- Peppard PE, Young T, Barnett JH, Palta M, Hagen EW, Hla KM, 2013 Increased prevalence of sleep-disordered breathing in adults. *Am J Epidemiol* 177, 1006–1014. [PubMed: 23589584]
- Pike CJ, Nguyen TV, Ramsden M, Yao M, Murphy MP, Rosario ER, 2008 Androgen cell signaling pathways involved in neuroprotective actions. *Hormones and behavior* 53, 693–705. [PubMed: 18222446]
- Pinsky MR, Hellstrom WJ, 2010 Hypogonadism, ADAM, and hormone replacement. *Therapeutic advances in urology* 2, 99–104. [PubMed: 21789087]
- Punjabi NM, 2008 The Epidemiology of Adult Obstructive Sleep Apnea. *Proceedings of the American Thoracic Society* 5, 136–143. [PubMed: 18250205]

- Quintana-Gallego E, Carmona-Bernal C, Capote F, Sanchez-Armengol A, Botebol-Benhamou G, Polo-Padillo J, Castillo-Gomez J, 2004 Gender differences in obstructive sleep apnea syndrome: a clinical study of 1166 patients. *Respiratory medicine* 98, 984–989. [PubMed: 15481275]
- Randeva HS, Tan BK, Weickert MO, Lois K, Nestler JE, Sattar N, Lehnert H, 2012 Cardiometabolic Aspects of the Polycystic Ovary Syndrome. *Endocrine Reviews* 33, 812–841. [PubMed: 22829562]
- Redline S, Tishler PV, Hans MG, Tosteson TD, Strohl KP, Spry K, 1997 Racial differences in sleep-disordered breathing in African-Americans and Caucasians. *Am J Respir Crit Care Med* 155, 186–192. [PubMed: 9001310]
- Reitz C, Mayeux R, 2014 Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. *Biochemical pharmacology* 88, 640–651. [PubMed: 24398425]
- Rosenzweig I, Williams SC, Morrell MJ, 2014 The impact of sleep and hypoxia on the brain: potential mechanisms for the effects of obstructive sleep apnea. *Current opinion in pulmonary medicine* 20, 565–571. [PubMed: 25188719]
- Ruehland WR, Rochford PD, O'Donoghue FJ, Pierce RJ, Singh P, Thornton AT, 2009 The new AASM criteria for scoring hypopneas: impact on the apnea hypopnea index. *Sleep* 32, 150–157. [PubMed: 19238801]
- Rupprecht R, 2003 Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties. *Psychoneuroendocrinology* 28, 139–168. [PubMed: 12510009]
- Saareanta T, Hedner J, Bonsignore MR, Riha RL, McNicholas WT, Penzel T, Anttalainen U, Kvamme JA, Pretl M, Sliwinski P, Verbraecken J, Grote L, 2016 Clinical Phenotypes and Comorbidity in European Sleep Apnoea Patients. *PLoS One* 11, e0163439. [PubMed: 27701416]
- Saksena SK, Lau IF, 1979 Variations in serum androgens, estrogens, progestins, gonadotropins and prolactin levels in male rats from prepubertal to advanced age. *Experimental aging research* 5, 179–194. [PubMed: 160322]
- Sawatari H, Chishaki A, Ando SI, 2016 The Epidemiology of Sleep Disordered Breathing and Hypertension in Various Populations. *Current hypertension reviews* 12, 12–17. [PubMed: 26778203]
- Semenza GL, Prabhakar NR, 2015 Neural Regulation of Hypoxia-Inducible Factors and Redox State Drives the Pathogenesis of Hypertension in a Rodent Model of Sleep Apnea. *J Appl Physiol* (1985), 117, 00162 02015.
- Senaratna CV, Perret JL, Lodge CJ, Lowe AJ, Campbell BE, Matheson MC, Hamilton GS, Dharmage SC, 2017 Prevalence of obstructive sleep apnea in the general population: A systematic review. *Sleep Medicine Reviews* 34, 70–81. [PubMed: 27568340]
- Shao C, Jiang JB, Wu HC, Wu SB, Yu BY, Tang YD, 2015 Clinical assessment and polysomnographic study of sleep apnea in a Chinese population of snorers. *Journal of Zhejiang University. Science. B* 16, 215–223. [PubMed: 25743123]
- Shell B, Faulk K, Cunningham JT, 2016 Neural Control of Blood Pressure in Chronic Intermittent Hypoxia. *Current hypertension reports* 18, 19. [PubMed: 26838032]
- Snyder B, Cunningham RL, 2018 Sex differences in sleep apnea and comorbid neurodegenerative diseases. *Steroids* 133, 28–33. [PubMed: 29258810]
- Snyder B, Shell B, Cunningham JT, Cunningham RL, 2017 Chronic intermittent hypoxia induces oxidative stress and inflammation in brain regions associated with early-stage neurodegeneration. *Physiological Reports* 5, e13258–n/a. [PubMed: 28473320]
- Stelmach-Mardas M, Mardas M, Iqbal K, Kostrzevska M, Piorunek T, 2017 Dietary and cardio-metabolic risk factors in patients with Obstructive Sleep Apnea: cross-sectional study. *PeerJ* 5, e3259. [PubMed: 28649465]
- Sun W, Yin X, Wang Y, Tan Y, Cai L, Wang B, Cai J, Fu Y, 2012 Intermittent hypoxia-induced renal antioxidants and oxidative damage in male mice: hormetic dose response. *Dose-response : a publication of International Hormesis Society* 11, 385–400. [PubMed: 23983666]
- Tamaya T, Misao R, Nakagawa M, Itoh T, Wada K, Fujimoto J, Imai A, 1993 Estrogen and androgen receptors in aorta of the rabbit and regulation by estrogen and androgen. *Research communications in chemical pathology and pharmacology* 82, 3–11. [PubMed: 8272573]

- Tenkorang MA, Snyder B, Cunningham RL, 2017 Sex-related differences in oxidative stress and neurodegeneration. *Steroids*.
- Toorians AW, Kelleher S, Gooren LJ, Jimenez M, Handelsman DJ, 2003 Estimating the contribution of the prostate to blood dihydrotestosterone. *The Journal of clinical endocrinology and metabolism* 88, 5207–5211. [PubMed: 14602751]
- Valera S, Ballivet M, Bertrand D, 1992 Progesterone modulates a neuronal nicotinic acetylcholine receptor. *Proc Natl Acad Sci U S A* 89, 9949–9953. [PubMed: 1409725]
- Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J, 2007 Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology* 39, 44–84. [PubMed: 16978905]
- Vorhees CV, Williams MT, 2006 Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature protocols* 1, 848–858. [PubMed: 17406317]
- Wilson DIG, Langston RF, Schlesiger MI, Wagner M, Watanabe S, Ainge JA, 2013 Lateral Entorhinal Cortex is Critical for Novel Object-Context Recognition. *Hippocampus* 23, 352–366. [PubMed: 23389958]
- Wilson EN, Anderson M, Snyder B, Duong P, Trieu J, Schreihof DA, Cunningham RL, 2018 Chronic Intermittent Hypoxia Induces Hormonal and Male Sexual Behavioral Changes: Hypoxia as an Advancer of Aging Physiology & Behavior. *Physiology & behavior, in press*.
- Yeh NC, Tien KJ, Yang CM, Wang JJ, Weng SF, 2016 Increased Risk of Parkinson's Disease in Patients With Obstructive Sleep Apnea: A Population-Based, Propensity Score-Matched, Longitudinal Follow-Up Study. *Medicine* 95. [PubMed: 27334457]
- Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S, 1993 The occurrence of sleep-disordered breathing among middle-aged adults. *The New England journal of medicine* 328, 1230–1235. [PubMed: 8464434]
- Zhang HL, Hassan MY, Zheng XY, Azimullah S, Quezada HC, Amir N, Elwasila M, Mix E, Adem A, Zhu J, 2012 Attenuated EAN in TNF-alpha deficient mice is associated with an altered balance of M1/M2 macrophages. *PLoS One* 7, e38157. [PubMed: 22666471]
- Zhang XB, Lin QC, Zeng HQ, Jiang XT, Chen B, Chen X, 2016 Erectile Dysfunction and Sexual Hormone Levels in Men With Obstructive Sleep Apnea: Efficacy of Continuous Positive Airway Pressure. *Archives of sexual behavior* 45, 235–240. [PubMed: 26370402]
- Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ, 2014 An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci* 34, 11929–11947. [PubMed: 25186741]
- Zitzmann M, 2006 Testosterone and the brain. *The aging male : the official journal of the International Society for the Study of the Aging Male* 9, 195–199.

Figure 1:

CIH and hormone status independently impacted circulating oxidative stress as measured by Advanced Oxidative Protein Products (AOPP) assay. Gonadectomized (GDX) male rats had significantly lower oxidative stress than all other hormone groups. INTACT rats exposed to CIH experienced a significant increase in oxidative stress, which was not observed in rats administered exogenous testosterone (T) or GDX. CIH further increased oxidative stress in rats administered dihydrotestosterone (DHT). *Results were determined by two-way ANOVA followed by LSD post hoc analysis. Results are shown as mean $\pm$ s.e.m. $p < 0.05$; * vs. normoxic, # vs. all other groups*

Figure 2:

CIH caused significant impairment in the probe trial of the Morris Water Maze. **A)** GDX male rats exhibited longer latency to reach the target during the probe trial, regardless of CIH exposure. Male rats administered DHT exhibited longer latency to reach the target following CIH administration. **B)** Gross motor ability was not impaired by CIH or hormone status, as seen by the distance traveled during the probe trial. This suggests the observed differences were not due to swimming ability. *Results were determined by two-way ANOVA followed by LSD post hoc analysis. Results are shown as mean $\pm$ s.e.m. $p < 0.05$; * vs. normoxic, # vs. all other groups*

Figure 3:

DHT impacts behavior in the novel object and open field assays. **A)** Dashed line represents equal preference for novel and familiar objects. Means above the line exhibit a preference for the novel object. Only the GDX rats exhibited a change in preference for the novel object following CIH exposure. The percent of time spent with a novel object was not affected by CIH exposure. Rats which were administered DHT exhibited a higher percent of time spent with the novel object than all other groups. **B)** Gross motor ability was not impaired by CIH or hormone status, as seen by the distance traveled during the novel object assay. **C)** Rats administered DHT had impaired fine motor ability to balance on a wire mesh platform in the modified open field assay. CIH exposure did not affect results. *Results were determined by two-way ANOVA followed by LSD post hoc analysis. Results are shown as mean $\pm$ s.e.m. $p < 0.05$; # vs. all other groups*

Figure 4:

Oxidative stress and apoptosis occurs differently within brain regions. **A)** Representative images of western blots of tissue homogenate from the substantia nigra (SN), entorhinal cortex (ETC), and hippocampus (HIPP) of INTACT, GDX, T, or DHT supplemented rats exposed to normoxic (N) or CIH (C) conditions. Full-length spectrin is 240 kDa, calpain-cleaved fragments (oxidative stress) are 145kDa, and caspase-3-cleaved fragments (apoptosis) are 125 kDa. Densitometry for the fragments was normalized to the full length (cleaved fragment/full length). **B)** In the SN, a significant increase in CIH-induced calpain cleavage was observed in INTACT male rats. Elevated calpain cleavage was observed in rats administered DHT, regardless of CIH exposure. No caspase-3 cleavage was evident in the SN. **C)** In the ETC, elevated calpain cleavage was observed in rats administered DHT, regardless of CIH exposure. **D)** Although caspase-3 cleavage was evident in the ETC, no differences due to CIH or hormone were observed. **E)** Androgen (T and DHT)

administration significantly increased calpain cleavage, regardless of CIH exposure, with more calpain cleavage present following DHT administration than after testosterone administration. **F)** Increased caspase-3 cleavage in the hippocampus was observed in rats administered DHT. *Results were determined by two-way ANOVA followed by LSD post hoc analysis. Results are shown as mean $\pm$ s.e.m. $p < 0.05$; * vs. normoxic, ** vs. intact, # vs. all other groups*

Figure 5:

Hormonal status, but not CIH, causes a change in oxidative stress and astrocytes in brain regions. **A)** Representative western blot results from the substantia nigra of INTACT, GDX, T, and DHT supplemented male rats exposed to normoxic (N) or CIH (C) conditions. Proteins probed for were GFAP (51kDa), an astrocyte marker, NOX1 (49 kDa), a marker of oxidative stress, and GAP-DH (37 kDa). Protein densitometries were normalized to GAP-DH densitometry readings for analysis. **B)** T-treated rats exhibited lower NOX1 expression in the SN than DHT administered rats. **C)** GDX rats exhibited significantly higher GFAP expression than all other groups. **D)** Representative western blot results from the entorhinal cortex of INTACT, GDX, T, and DHT supplemented male rats exposed to CIH. **E)** Androgens elevate NOX1 expression in the ETC of male rats. Higher NOX1 expression is present in the ETC of rats administered T compared to INTACT rats. Administration of DHT resulted in higher NOX1 expression in the ETC than all other groups. **F)** Decreased GFAP expression is observed in the ETC of T-treated rats. Significantly higher GFAP

expression is observed in the ETC of DHT-treated rats when compared to all other groups.

G) Representative western blot results from the hippocampus of INTACT, GDX, T, and DHT supplemented male rats exposed to CIH. **H)** DHT-treated rats exhibit significantly more NOX1 expression in the hippocampus than either INTACT or GDX male rats. **I)** GDX and T rats exhibited significantly less GFAP expression than gonadally INTACT or DHT rats. *Results were determined by two-way ANOVA followed by LSD post hoc analysis. Results are shown as mean $\pm$ s.e.m. $p < 0.05$; ** vs. INTACT, + vs. DHT, # vs. all other groups*

Table 1:

comparison of average pro-inflammatory M1 cytokines (IL-1 β , IL-6, TNF- α , and GM-CSF) and anti-inflammatory M2 cytokines (IL-4, IL-10, and IL-13) between hormone groups exposed to CIH or normoxic conditions.

Sample	hormone	M1 cytokines		sig.	M2 cytokines		sig.
		NORM	CIH		NORM	CIH	
plasma	INTACT	681.12 $\pm$ 305.41	534.08 $\pm$ 251.88		373.03 $\pm$ 216.81	250.97 $\pm$ 110.59	#
	GDX	236.95 $\pm$ 127.96	133.34 $\pm$ 49.27	**	77.39 $\pm$ 45.27	39.91 $\pm$ 26.07	
	T	29.43 $\pm$ 58.87	0.00 $\pm$ 0.00	**	10.19 $\pm$ 20.38	0.00 $\pm$ 0.00	
	DHT	382.62 $\pm$ 82.12	581.64 $\pm$ 344.90		194.24 $\pm$ 40.39	182.57 $\pm$ 42.98	#
substantia nigra	INTACT	17.80 $\pm$ 2.64	20.99 $\pm$ 1.77		3.34 $\pm$ 0.42	3.07 $\pm$ 1.26	
	GDX	21.40 $\pm$ 0.64	23.35 $\pm$ 2.49		2.54 $\pm$ 2.36	3.18 $\pm$ 2.17	
	T	20.56 $\pm$ 3.12	16.54 $\pm$ 2.98		4.47 $\pm$ 2.46	2.21 $\pm$ 1.61	
	DHT	32.64 $\pm$ 11.58	45.59 $\pm$ 4.52	#	0.29 $\pm$ 0.37	0.57 $\pm$ 0.47	#
entorhinal cortex	INTACT	342.98 $\pm$ 182.03	426.48 $\pm$ 119.87	#	400.63 $\pm$ 186.46	491.60 $\pm$ 98.01	#
	GDX	119.32 $\pm$ 6.26	92.62 $\pm$ 22.06		145.97 $\pm$ 20.16	86.25 $\pm$ 34.89	
	T	279.27 $\pm$ 175.15	124.27 $\pm$ 122.07		333.85 $\pm$ 213.34	107.71 $\pm$ 143.74	
	DHT	174.73 $\pm$ 38.18	205.41 $\pm$ 57.09		241.61 $\pm$ 75.35	263.45 $\pm$ 64.80	
hippocampus	INTACT	120.18 $\pm$ 21.83	74.90 $\pm$ 19.61	*	163.03 $\pm$ 43.25	80.81 $\pm$ 35.21	
	GDX	117.51 $\pm$ 21.12	84.97 $\pm$ 35.66		160.54 $\pm$ 33.05	98.83 $\pm$ 79.65	
	T	52.43 $\pm$ 3.86	78.46 $\pm$ 4.86	*	31.79 $\pm$ 10.26	82.72 $\pm$ 25.18	
	DHT	81.25 $\pm$ 5.05	81.30 $\pm$ 21.01		89.21 $\pm$ 30.22	75.52 $\pm$ 24.40	

p 0.05;

* vs. normoxic,

** compared to INTACT,

compared to testosterone,

compared to all other groups