HHS Public Access

Author manuscript

Physiol Behav. Author manuscript; available in PMC 2019 May 15.

Published in final edited form as:

Physiol Behav. 2018 May 15; 189: 64–73. doi:10.1016/j.physbeh.2018.03.007.

Chronic Intermittent Hypoxia Induces Hormonal and Male Sexual Behavioral Changes: Hypoxia as an Advancer of Aging

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Abstract

Sleep apnea is a common sleep disorder characterized by intermittent periods of low blood oxygen levels. The risk for sleep apnea increases with age and is more prevalent in men than women. A common comorbidity of sleep apnea includes male sexual dysfunction, but it is not clear if a causal relationship exists between sleep apnea and sexual dysfunction. Possible mechanisms that link these two disorders include oxidative stress and testosterone. Oxidative stress is elevated in clinical patients with sleep apnea and in rodents exposed to chronic intermittent hypoxia (CIH), an animal model for apnea-induced hypopnea. Further, oxidative stress levels increase with age. Therefore, age may play a role in sleep apnea-induced sexual dysfunction and oxidative stress generation. To investigate this relationship, we exposed gonadally intact 3 (young) and 12 (middle-aged) month old male F344/BN F1 hybrid male rats to 8 days of CIH, and then examined male sexual function. Plasma was used to assess circulating oxidative stress and hormone levels. Middle-aged male rats had lower testosterone levels with increased sexual dysfunction and oxidative stress, independent of CIH. However, CIH decreased testosterone levels and increased sexual dysfunction and oxidative stress only in young gonadally intact male rats, but not in gonadectomized young rats with physiological testosterone replacement. In sum, CIH had a greater impact on younger gonadally intact animals, with respect to sexual behaviors, testosterone, and oxidative stress. Our data indicate CIH mimics the effects of aging on male sexual behavior in young gonadally intact male rats.

Keywords

Testosterone; Oxidative Stress; Sex Dysfunction; Corticosterone; Male Sex Behavior

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Introduction

Sleep apnea is a common disorder that consists of interruptions in breathing that cause hypoxia, hypercapnia, and increased thoracic pressure during sleep [1, 2]. One measure of sleep apnea severity is the apnea/hypopnea index (AHI), which is the frequency of apneic/hypoxic events per hour while sleeping [3]. The American Academy of Sleep Medicine classification of AHI as mild, moderate, or severe sleep apnea is considered 5, 15, and >30 hypoxic events per hour, respectively [3]. Notably, age and sex impact sleep apnea. The risk for sleep apnea increases with age and occurs at a higher frequency in men than women [4–6]. Male sexual dysfunction is commonly associated with sleep apnea and can greatly lower quality of life [7, 8].

Although sleep apnea and sexual dysfunction are comorbidities, the exact mechanisms underlying sleep apnea and sexual dysfunction are unknown [9–12]. Possible theories for sleep apnea-induced sexual dysfunction include alterations in testosterone and oxidative stress levels. Clinical studies consistently observe decreased circulating testosterone in middle-aged men (~45 years old) with sleep apnea [12, 13]. Only a few pre-clinical studies on sleep apnea and sexual function have been conducted. Surprisingly, pre-clinical studies are not aligned with clinical findings with respect to testosterone levels. Studies using young mice exposed to chronic intermittent hypoxia (CIH), an animal model of apnea-induced hypopnea, found no effects on testosterone levels, even though CIH decreased sexual activity [14].

Unlike the contrast between clinical and pre-clinical studies on testosterone levels, there is agreement on sleep apnea and CIH-induced oxidative stress [15–20]. Oxidative stress has been linked to both sexual dysfunction [21–25] and sleep apnea in humans [17, 26–29]. A pre-clinical study observed that antioxidants decreased CIH-induced erectile dysfunction in male rats [30], supporting the concept that oxidative stress may play a role in sexual dysfunction in men with sleep apnea.

Aging also contributes to oxidative stress [31], and is a risk factor for sleep apnea. Several studies indicate the involvement of age in the elevated cardiovascular risk for individuals with sleep apnea. Individuals less than 65 years old with sleep apnea have the greatest cardiovascular risk, unlike individuals older than 65 years [32–36]. Similarly in pre-clinical studies, CIH appeared to “age” young (3–4 month) male rats by increasing mean arterial blood pressure to ranges observed in aged (22–24 month) male rats, but had no effect on blood pressure in aged rats [37]. These studies indicate that sleep apnea may have a greater impact on younger individuals compared to older individuals. It is possible that the age-associated increase in oxidative stress may blunt CIH’s effects in older individuals, due to a ceiling effect.

Although there have been some pre-clinical studies on CIH and age, no studies on CIH and age on sexual function have been conducted. This gap is concerning because the number of individuals over the age of 65 increased 213% from 1950 to 2010 in the US [38, 39]. Due to aging of the US population, it is important to have a better understanding of how aging may influence disease modalities, such as sleep apnea and sexual dysfunction. To examine the

role of age on sleep apnea and sexual dysfunction, we exposed sexually naïve young and middle-aged male rats to 8 days of CIH, a model of mild sleep apnea. After CIH, we examined sexual function, oxidative stress, and hormone levels.

Methods

Animals

Sexually naïve gonadally intact 3 (young) and 12 (middle-aged) month old male F344/BN F1 hybrid rats were obtained from the National Institute of Aging (through Envigo, Indianapolis, IN). Since two different age groups (young and middle-aged) are examined in this study, we used sexually naïve rats to remove the confound of sexual experience that can increase circulating testosterone and androgen sensitive accessory organ weights [40–43]. Due to the lack of standardized sexual experience across the different age groups, age-wise comparisons would not be appropriate if sexually experienced males were incorporated. Stimulus females were gonadally intact Sprague Dawley (215–225 g) also obtained from Envigo. Animals were doubled housed in a temperature-controlled room (23°C). Food and water were provided *ad libitum*. Lighting was maintained on a reverse 12:12 light/dark cycle, with lights off at 0900 h. All surgeries were performed under isoflurane (2–3%) anesthesia. Animal care and experimental procedures were performed in accordance with the National Institutes of Health and American Physiological Society's guidelines for animal care and use. The protocol was approved by the University of North Texas Health Science Center Institutional Animal Care and Use Committee.

Animals were habituated to housing conditions for a week prior to a one-week habituation to the chronic intermittent hypoxia (CIH) apparatus under normoxic conditions. After CIH apparatus habituation, rats were exposed to either normoxia or CIH for 8 days with behavior testing on the last day. Male rats were randomly divided into different experimental groups with the experimental factors of age and hypoxia: Young gonadally intact Normoxia (n=10), Middle-Age gonadally intact Normoxia (n=12), Young gonadally intact CIH (n=13), and Middle-Age gonadally intact CIH (n=10).

To further examine the influence of testosterone, additional young male rats were included: gonadectomized (GDX) Normoxia (n=8), GDX + testosterone replacement treatment (TRT) Normoxia (n=8), GDX-CIH (n=8), and TRT-CIH (n=8). GDX male rats were implanted with either 2 cholesterol-filled or crystalline testosterone-filled (Steraloids, Newport, RI.) continuous release Silastic implants (1.47 mm i.d. × 1.96 mm o.d. × 10 mm length, Dow Corning, Midland, MI). Testosterone-filled capsules resulted in 2–3 ng/ml circulating testosterone (Figure 1B), consistent with the average testosterone levels throughout the diurnal pattern (in the absence of daily hormone peaks occurring at end of the light phase) [44–47]. Male rats were given a 1-week recovery period prior to CIH apparatus habituation. Stimulus females were ovariectomized (OVX) and implanted with 1 Silastic capsule (1.47 mm i.d. × 1.96 mm o.d. × 5 mm length, Dow Corning, Midland, MI) containing 10% crystalline estradiol benzoate (Sigma, St. Louis, MO). Female rats were given at least one-week recovery before behavior testing. Females were made sexually receptive via a subcutaneous injection of 500 μg of progesterone (Sigma, St. Louis, MO) four hours prior to behavioral testing [48, 49].

Mild Chronic Intermittent Hypoxia (CIH)

One week following arrival to animal facility, rats were placed in Oxycycler chambers (76.2 × 50.8 × 50.8 cm, BioSpherix, Lacona, NY, USA) to acclimate to the CIH equipment under normoxic (21% oxygen) conditions for one week. After habituation, CIH was conducted for 7 days during the light cycle (1700 to 0500 h), as previously described [16, 50, 51]. The mild CIH protocol consists of 8-minute cycles of 5 minutes of hypoxia (10% O_2) and 3 minutes of normoxia (21% O_2). Hypoxia is initiated by injecting nitrogen into the chamber, resulting in a nadir of 10% oxygen within 4 minutes. Reoxygenation is initiated by oxygen injected over 3 minutes, resulting in peak 21% oxygen within 2.5 minutes. This CIH protocol results in an apnea/hypopnea index (AHI) of 8.

Behavioral Tests

All behavioral tests were carried out between 1200 and 1500 h using dim red lighting and digitally recorded. All tests were conducted in a neutral cage environment (50 × 25 × 30 cm) with clean bedding. No male rat was tested with the same female twice. A rater, blind to the treatment groups, scored male sexual behaviors.

Male Sex Behaviors—Sexually naïve male rats were placed into a neutral testing arena with a sexually receptive female. The appetitive and consummatory sexual behaviors of the male rats were recorded for 10 minutes and quantified: frequencies and latencies for mounts, intromissions, and ejaculations. Appetitive behaviors included latencies to first mount (no penile insertion), a measure of sexual motivation. Consummatory behaviors included intromission (penile insertion) latencies, intromission frequencies, and ejaculatory behavior [49, 52–54]. Hit rate was calculated to determine copulatory efficiency: hit rate = intromission frequency/(intromission frequency + mount frequency) [55, 56].

Biochemical Assays

Plasma Collection—One day after behaviour testing, male rats were anesthetized with isoflurane (2–3%) and sacrificed by decapitation. Trunk blood was collected into chilled 7 mL EDTA tubes. The blood was centrifuged for 10 minutes (2,240 × g) at 4° C. Plasma was promptly separated and placed in microcentrifuge tubes for storage at −80° C until analysis. All samples were collected between 0800–1000 h to examine peak hormone levels occurring at the end of the light phase in gonadally intact rats [44, 45].

A. ELISAs: Samples were assayed by ELISA using a BioTek multi-reader. Plasma testosterone levels were determined by a Competitive Testosterone ELISA, using a polyclonal rabbit anti-testosterone antibody specific to mice and rats (BioVendor, Asheville, NC, USA), according to manufacturer's instructions. The intra-assay coefficient of variation was 6.50% and the inter-assay coefficient of variation was 11.3%. The sensitivity of this assay is 0.066 ng/ml at the 2 s.d. confidence limit. Specificity of this assay is as follows: testosterone (100%), 5α-DHT (69.6%), androstenedione (≤ 0.1%), androstanediol (≤ 0.1%), progesterone (≤ 0.1%), and androsterone (≤ 0.1%).

Plasma corticosterone levels were determined by a Competitive Corticosterone ELISA, using a polyclonal rabbit anti-corticosterone antibody specific to mice and rats (BioVendor,

Asheville, NC, USA), according to manufacturer's instructions. The intra-assay coefficient of variation was 8.9% and the inter-assay coefficient of variation was 7.2%. The sensitivity of this assay is 6.1 ng/ml at the 2 s.d. confidence limit. Specificity of this assay is as follows: corticosterone (100%), cortisol (2.3%), aldosterone (0.3%), testosterone (≤ 0.1%), progesterone (6.2%), and androsterone (≤ 0.1%).

Plasma oxytocin levels were determined by a Competitive Oxytocin EIA, using rabbit anti-peptide IgG compatible with human, mice, rats, and bovine oxytocin (Phoenix Pharmaceuticals, Burlingame, CA, USA), according to manufacturer's instructions. The intra-assay coefficient of variation was ≤ 10.0% and the inter-assay coefficient of variation was ≤ 15.0%. The sensitivity of this assay is 0.13 ng/ml at the 2 s.d. confidence limit. Specificity of this assay is as follows: oxytocin (100%), [Lys8]-vasopressin (0.0%), [Arg8]-vasopressin (0.0%), CRF (human, rat) (0.0%), and somatostatin (0.0%).

Basal plasma luteinizing hormone (LH) levels were assayed by Competitive LH ELISA [57–59], using a monoclonal mouse anti-LH antibody that recognizes human and rat LH (Enzo Life Sciences, Farmingdale, NY, USA), according to manufacturer's instructions. The intra-assay coefficient of variation was 5.3% and the inter-assay coefficient of variation was 15.5%. The sensitivity of this assay is 0.612 ng/ml at the 2 s.d. confidence limit. Specificity of this assay is as follows: LH (100%), FSH (≤0.004%), TSH (0.3%), and hCG (≤ 0.004%).

Plasma follicle stimulating hormone (FSH) levels were measured by Sandwich-ELISA, using a pre-coated microplate with an antibody specific for rat FSH (Elabscience Biotechnology Inc, Wuhan, China), according to manufacturer's protocol. The intra-assay coefficient of variation was 5.34% and the inter-assay coefficient of variation was 5.84%. The sensitivity of this assay is 1.88 ng/ml with a detection range of 3.13–200 ng/ml. Specificity of this assay recognizes rat FSH with no significant cross-reactivity or interference with analogues.

B. Oxidative Stress: Circulating oxidative stress levels were measured using a colorimetric OxiSelect Advanced Oxidative Protein Products (AOPP) assay (Cell Biolabs, San Diego, CA, USA) [16, 46], that detects proteins damaged by oxidative stress [60]. This assay quantifies oxidized proteins levels (μmol/L) in a sample relative to a known chloramine standard.

Statistical Analysis—All data were analyzed using IBM SPSS (SPSS v.23, IBM, 2015) software and presented as mean ± SEM. Measures were collected according to a 2 × 2 (Young and Middle Age × CIH and Normoxia) and (GDX and TRT × CIH and Normoxia) design and analyzed by ANOVA followed by Fisher Least Significant Difference (LSD) *post hoc*. P values ≤ 0.05 were designated as significant.

Results

Exposure to CIH alters hormone levels

Peak circulating total testosterone levels were assayed in gonadally intact young (3 month) and middle-aged (12 month) male rats (Figure 1 A). Testosterone levels (5.95 ng/ml ± 2.24

s.d.) in young gonadally intact males were consistent with the reported peak physiological testosterone levels in young male rats that range from 3.5–7 ng/ml [44, 45, 61]. As expected, age significantly affected testosterone levels ($F_{1,25} = 18.036$, $p \leq 0.05$), wherein middle-aged rats showed decreased total testosterone levels compared to young gonadally intact rats. Furthermore, hypoxia influenced testosterone levels ($F_{1,25} = 16.110$, $p \leq 0.05$), and there was a significant interaction between Age and Hypoxia ($F_{1,25} = 39.181$, $p \leq 0.05$). CIH decreased total testosterone in young gonadally intact males but did not alter testosterone levels in middle-aged males ($p = 0.053$). Further, no differences were observed between middle-aged CIH males and young CIH males ($p=0.064$). Total testosterone levels were examined in young GDX and TRT male rats (Figure 1 B). As expected, significantly lower testosterone levels were observed in GDX males compared to TRT young male rats ($F_{1,23} = 76.548$, $p \leq 0.05$). CIH did not affect testosterone levels in either GDX or TRT male rats ($F_{1,23} = 2.062$, $p = 0.164$). The gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are involved in the hypothalamic-pituitary-gonadal (HPG) axis negative feedback regulation of testosterone, were assayed in young and middle-aged gonadally intact males (Table 1). Gonadotropins in young gonadally intact males were consistent with reported levels in young male rats [62–68]. Neither CIH ($F_{1,18} = 0.493$, $p = 0.492$) nor age ($F_{1,18} = 1.039$, $p = 0.322$) altered basal LH levels in gonadally intact male rats. Similarly, FSH levels were not affected by either CIH ($F_{1,29} = 1.756$, $p = 0.195$) or age ($F_{1,29} = 0.706$, $p = 0.408$).

Unlike testosterone, age did not influence circulating levels of corticosterone ($F_{1,37} = 0.382$, $p = 0.54$; Figure 1 C), but hypoxia significantly increased corticosterone levels ($F_{1,37} = 10.141$, $p \leq 0.05$). Post hoc analysis showed CIH significantly increased corticosterone in middle-aged gonadally intact male rats, but not in young gonadally intact male rats ($p = 0.09$). No effects of hypoxia treatment on corticosterone levels were observed in GDX and TRT young male rats ($F_{1,25} = .049$, $p = 0.83$). However, there was a significant effect of hormone treatment on corticosterone levels in GDX and TRT young male rats ($F_{1,25} = 14.045$, $p \leq 0.05$; Figure 1 D), wherein TRT males had lower corticosterone levels than GDX males.

Only hypoxia, not age, significantly affected oxytocin levels ($F_{1,30} = 4.139$, $p \leq 0.05$) (Figure 2 A). Specifically, CIH significantly increased oxytocin in middle-aged gonadally intact rats, but did not alter oxytocin levels in young gonadally intact male rats. Since no significant differences were found in young gonadally intact male rats, oxytocin levels were not quantified in GDX and TRT male rats.

CIH increased circulating oxidative stress in young rats

Oxidative stress was quantified by using AOPP as a marker. There was a main effect for Hypoxia ($F_{1,28} = 7.241$, $p \leq 0.05$) and an interaction between Age and Hypoxia ($F_{1,28} = 5.511$, $p \leq 0.05$) on AOPP in gonadally intact young and middle-aged rats. Normoxic middle-aged rats had significantly elevated AOPP compared to normoxic young gonadally intact rats, and young animals exposed to CIH had significantly higher AOPP than normoxic young rats. No significant differences in AOPP were observed between young rats exposed to CIH and middle-aged rats, regardless of CIH exposure (Figure 2 B). Oxidative stress

levels were not altered by either hormone ($F_{1,17} = 0.335$, $p = 0.570$) or hypoxia ($F_{1,17} = 1.062$, $p = 0.317$) in young GDX and TRT male rats. However, there was a significant interaction between hormone and hypoxia ($F_{1,17} = 4.614$, $p \leq 0.05$). Only under normoxic conditions, TRT significantly increased oxidative stress levels compared to GDX (Figure 2 C).

CIH impaired male sex behaviors in young rats

All male rats were sexually naïve. Frequency and latencies (time to first behavior) for male sexual behaviors (mount, intromission, and ejaculation) were quantified in young (3 month), middle-aged (12 month) male rats, young GDX, and young TRT male rats (Figure 3). As expected, Age significantly impacted mount latencies ($F_{1,38} = 23.91$, $p \leq 0.05$) and mount frequencies ($F_{1,38} = 21.055$, $p \leq 0.05$), but Hypoxia did not affect mount latencies ($F_{1,38} = 0.821$, $p = 0.37$) and mount frequencies ($F_{1,38} = 0.97$, $p = 0.33$) in young intact and middle-aged gonadally intact male rats. Middle-aged rats showed increased mount latencies and decreased mount frequencies compared to young rats. CIH did not impact mounting behaviors (motivation) (Figure 3 A, C). Similarly, hypoxia did not affect mount latencies ($F_{1,24} = 1.929$, $p = 0.18$) or mount frequencies ($F_{1,25} = 0.202$, $p = 0.66$) in GDX and TRT male rats. As expected, hormone treatment did significantly influence mount latencies ($F_{1,24} = 2203.30$, $p \leq 0.05$) and mount frequencies ($F_{1,25} = 42.30$, $p \leq 0.05$) in GDX and TRT male rats. Significantly lower mount latencies were observed in TRT males compared to GDX young male rats. Furthermore, higher mount frequencies were observed in TRT males compared to GDX young male rats (Figure 3 B, D).

For intromission latency, there was a main effect for Age ($F_{1,38} = 23.111$, $p \leq 0.05$). Middle-aged rats have increased latency to first intromission compared to young intact rats, and young animals exposed to CIH had significantly higher intromission latency than normoxic young intact rats (Figure 4). No significant differences were found between young gonadally intact rats exposed to CIH and middle-aged rats (Figure 4 A). In TRT and GDX young male rats, hypoxia did not affect intromission latencies ($F_{1,25} = 0.459$, $p = 0.50$), whereas hormone treatment significantly decreased intromission latencies ($F_{1,25} = 131.35$, $p \leq 0.05$) (Figure 4 B).

However, for intromission frequencies both Age ($F_{1,36} = 41.685$, $p \leq 0.05$), Hypoxia ($F_{1,36} = 12.479$, $p \leq 0.05$) and an interaction between these two main effects ($F_{1,36} = 8.42$, $p \leq 0.05$) were significant in gonadally intact males. Young rats intromitted at a higher frequency than middle-aged rats, and CIH decreased the number of intromissions only in young gonadally intact rats (Figure 4 C). In TRT and GDX young male rats, hypoxia did not affect intromission frequencies ($F_{1,25} = 1.297$, $p = 0.27$), whereas hormone treatment significantly increased intromission frequencies ($F_{1,25} = 87.87$, $p \leq 0.05$) (Figure 4 D).

To examine copulatory efficiency, the hit rate was quantified. Age significantly impacted the hit rate ($F_{1,36} = 18.37$, $p \leq 0.05$), but Hypoxia did not significantly influence hit rate ($F_{1,36} = 3.01$, $p = 0.09$) in young and middle-aged gonadally intact male rats. Young male rats had better copulatory efficiency than middle-aged rats (Figure 5 A). Similarly, in TRT and GDX young male rats, hypoxia did not affect the hit rate ($F_{1,25} = 0.27$, $p = 0.61$), whereas hormone treatment significantly increased the hit rate ($F_{1,25} = 92.73$, $p \leq 0.05$) (Figure 5 B).

Only ejaculatory frequencies were analyzed, as no middle-aged or GDX rats ejaculated. Neither Age ($F_{1,38} = 3.26$, $p = 0.08$) nor hypoxia ($F_{1,38} = 0.004$, $p = 0.95$) significantly affected ejaculation frequencies in sexually naïve young and middle-aged gonadally intact male rats (Figure 5 C). In TRT and GDX young male rats, hypoxia did not affect ejaculation frequencies ($F_{1,25} = 0.036$, $p = 0.85$), whereas hormone treatment significantly increased ejaculation frequencies ($F_{1,25} = 18.01$, $p \leq 0.05$) (Figure 5 D).

Discussion

The prevalence of sleep apnea in the US is at least 20% [69, 70]. Strikingly, up to 90% of individuals with sleep apnea are undiagnosed [71–73]. An understudied, but common, co-morbidity of sleep apnea in men and women is sexual dysfunction [74, 75]. Currently, the relationship between sleep apnea and sexual dysfunction is unknown. Furthermore, as aging is associated with both increased prevalence of sleep apnea and sexual dysfunction, it is clinically important to determine if a causal relationship or interaction of age and sleep apnea on sexual function exists. This is the first study to examine the effects of CIH, an animal model of sleep apnea, on sexual function and oxidative stress in young and middle-aged male rats.

In this study we observed increased male sexual dysfunction in middle-aged rats compared to young gonadally male rats. The age-related sexual dysfunction in middle-aged rats was unaffected by CIH. However, CIH did induce sexual dysfunction in young gonadally intact male rats to levels observed in middle-aged male rats. This increase in male sexual behavior dysfunction was accompanied by an increase in circulating oxidative stress and a decrease in circulating testosterone levels. Indeed, CIH exposure in young male rats resulted in behavioral and hormonal profiles consistent with middle age. Therefore, in addition to prior reports of CIH “aging” the cardiovascular system in young (3–4 months) male rats to levels observed in aged (22–24 months) male rats [37], CIH “ages” the reproductive system.

To determine if CIH’s effects on sexual behavior is a direct effect or due to CIH decreasing testosterone levels in young gonadally intact male rats, we included young male rats that were testosterone deficient (GDX) or were given exogenous physiological testosterone replacement (TRT). Interestingly, CIH did not alter any tested parameters, such as corticosterone, AOPP, and male sex behaviors. These findings indicate that CIH induced sexual dysfunction is due to the loss of testosterone. Further, the presence of the exogenous physiological testosterone may be protective with respect to oxidative stress generation, as CIH did not increase oxidative stress in TRT male rats. This is consistent with our previous findings showing testosterone is a mild oxidative stressor, which can protect neurons against subsequent oxidative stress insults via a preconditioning mechanism [76, 77].

Several mechanisms could underlie the decline in testosterone levels in young gonadally intact male rats exposed to CIH. Since CIH increased corticosterone levels, it is possible the hypothalamic-pituitary-adrenal (HPA) axis, via corticosterone, decreased testosterone levels [78–80]. However, it is unlikely CIH-induced corticosterone affected testosterone levels via the hypothalamic-pituitary-gonadal (HPG) axis based on the lack of gonadotropin (LH and FSH) response to CIH. Another possible mechanism could be Leydig cell dysfunction. In

men, aging can negatively influence Leydig cell function, resulting in unchanged to elevated luteinizing hormone (LH) with decreased testosterone [81–83]. Similarly, several *in vivo* studies in rats found decreased testosterone levels with normal LH [62, 63, 84]. Furthermore, it is well established middle-aged Brown Norway male rats exhibit Leydig cell impairments, wherein approximately 50% testosterone loss is observed with unchanged LH levels [64, 85, 86]. Our results showing unchanged LH levels with decreased testosterone levels are consistent with these studies.

Interestingly, prior studies found corticosterone can cause Leydig cell dysfunction and decrease testosterone levels without affecting LH levels via a down-regulation of 17β-hydroxysteroid dehydrogenase (17β -HSD), the final step of testosterone synthesis in Leydig cells [87–92]. Therefore, it is possible CIH-induced corticosterone release is acting directly on Leydig cells, resulting in a decline in testosterone synthesis.

Another mechanism by which CIH could decrease testosterone is oxidative stress. Multiple studies have shown oxidative stress can induce Leydig cell dysfunction, resulting in low testosterone production [93–99]. Interestingly, corticosterone can also increase neuronal oxidative stress generation [100–104]. Both corticosterone and oxidative stress can be decreased by oxytocin [105–114]. CIH increased oxytocin, indicating oxytocin release may be a protective mechanism in response to CIH. Our results show age and CIH are associated with significant reductions in testosterone levels. In young gonadally intact male rats CIH alone can mimic the effect of age on both testosterone and male sexual behaviors. Interestingly, increased oxidative stress, low testosterone, and male sexual dysfunction are shared outcomes between middle-aged rats and CIH exposed young male rats. Therefore, these results suggest CIH-induced oxidative stress is one of the primary factors involved in CIH-induced reproductive aging.

In conclusion, these results indicate oxidative stress generated by CIH could be an important mechanistic link between sleep apnea associated sexual dysfunction. Since young male rats exposed to CIH had similar sexual dysfunction and oxidative stress levels as middle-aged rats, CIH appears to “age” young male rats. Based on these results, a causal relationship exists between sleep apnea and sexual dysfunction.

Acknowledgments

We would like to thank Jessica Proulx and Drs. Marilyn Y. McGinnis, Augustus Lumia, J. Thomas Cunningham, and Styliani Goulopoulou for their excellent technical assistance and advice. This work was supported by the following NIH grants: NS088514 to R.L.C. and AG049255 to D.A.S. and R.L.C.

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Highlights

- Middle-aged male rats had increased sexual dysfunction and oxidative stress, independent of CIH.
- CIH increased sexual dysfunction and oxidative stress only in young male rats.
- CIH “ages” the reproductive system.

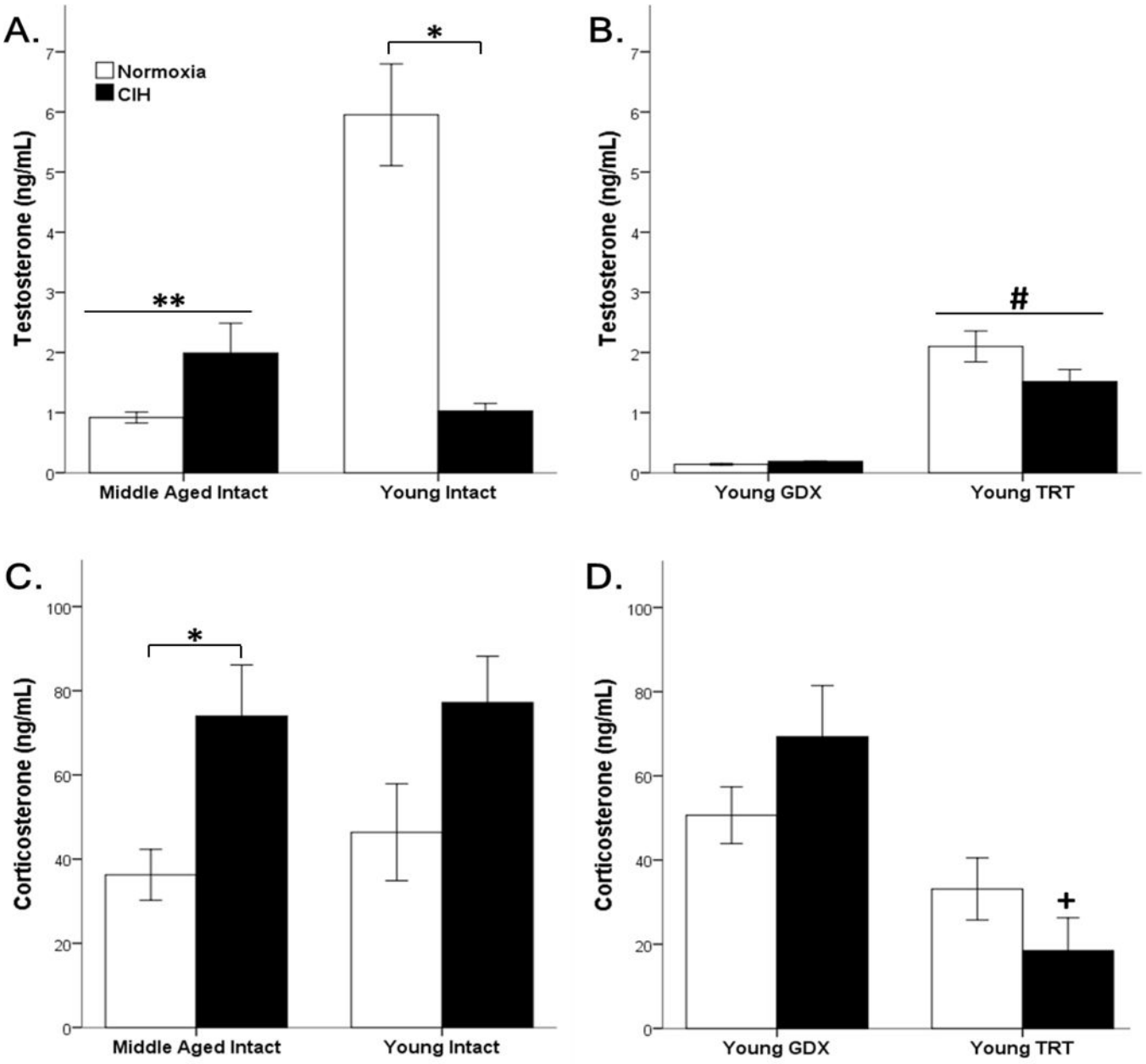

Figure 1. CIH and age altered steroid hormones

Middle-aged rats have significantly lower circulating testosterone levels than young intact males. 8-day exposure to CIH significantly decreased testosterone in young intact male rats (A). TRT significantly increased testosterone levels compared to GDX males (B). Although age did not impact corticosterone, CIH significantly increased circulating corticosterone levels in middle-aged intact rats (C). Lower corticosterone levels were observed in TRT compared to GDX rats, specifically in the CIH treatment group (D). Fisher's LSD *post hoc* analyses: * $p \leq 0.05$ normoxia vs. CIH; ** $p \leq 0.05$ vs. young normoxia; # $p \leq 0.05$ vs. GDX; + $p \leq 0.05$ vs. GDX CIH.

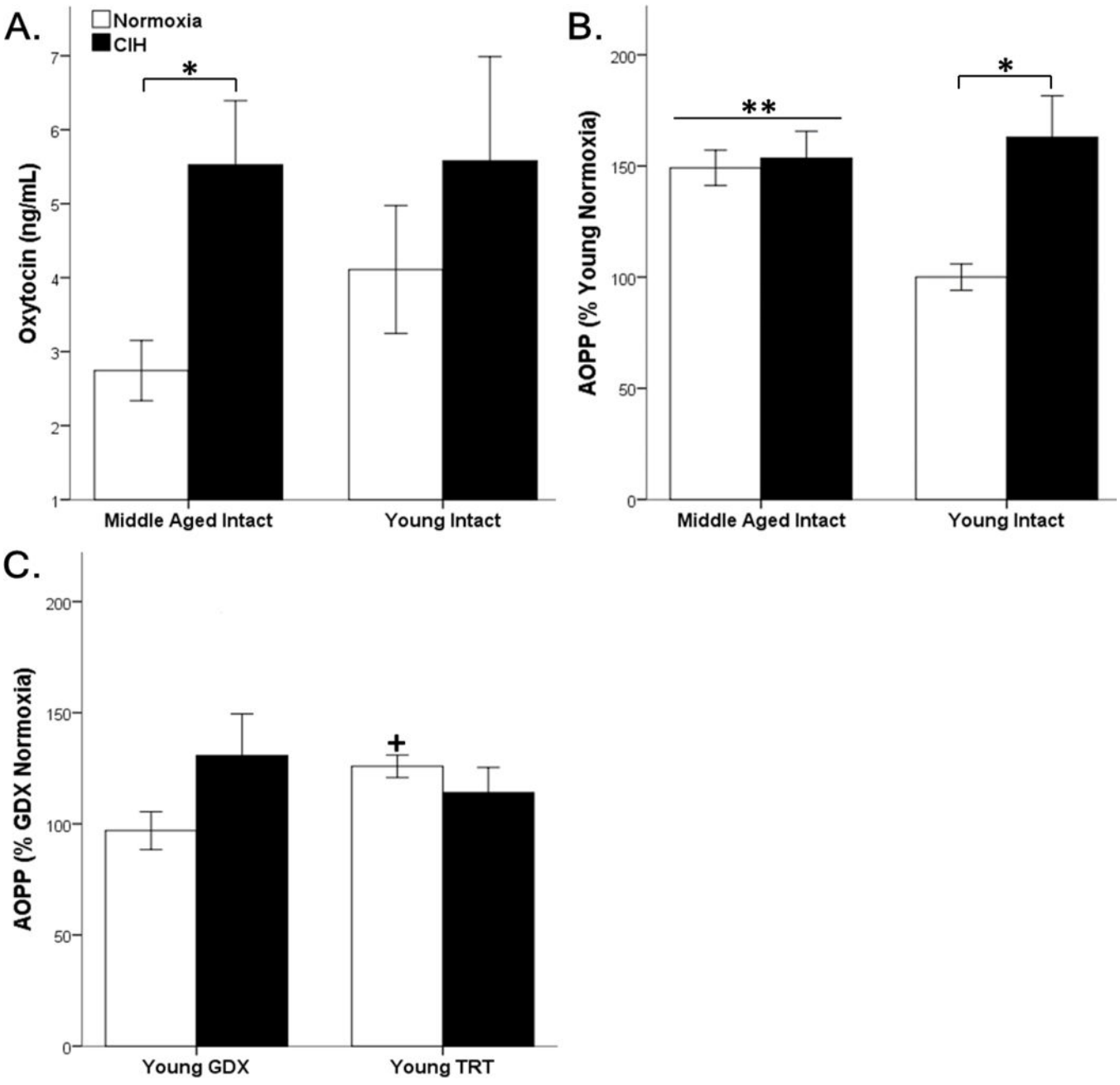

Figure 2. CIH and age altered circulating hormones and oxidative stress

Age did not impact oxytocin, CIH significantly increased oxytocin levels in middle-aged intact rats (A). Middle-aged rats have significantly higher circulating oxidative stress (AOPP) than young intact males. CIH significantly exacerbated oxidative stress in young rats but not middle-aged rats (B). CIH did not impact oxidative stress in GDX and TRT rats. TRT, itself, increased oxidative stress, but only in a normoxic environment (C). Fisher's LSD *post hoc* analyses: * $p \leq 0.05$ normoxia vs. CIH; ** $p \leq 0.05$ vs. young normoxia; + $p \leq 0.05$ vs. GDX CIH.

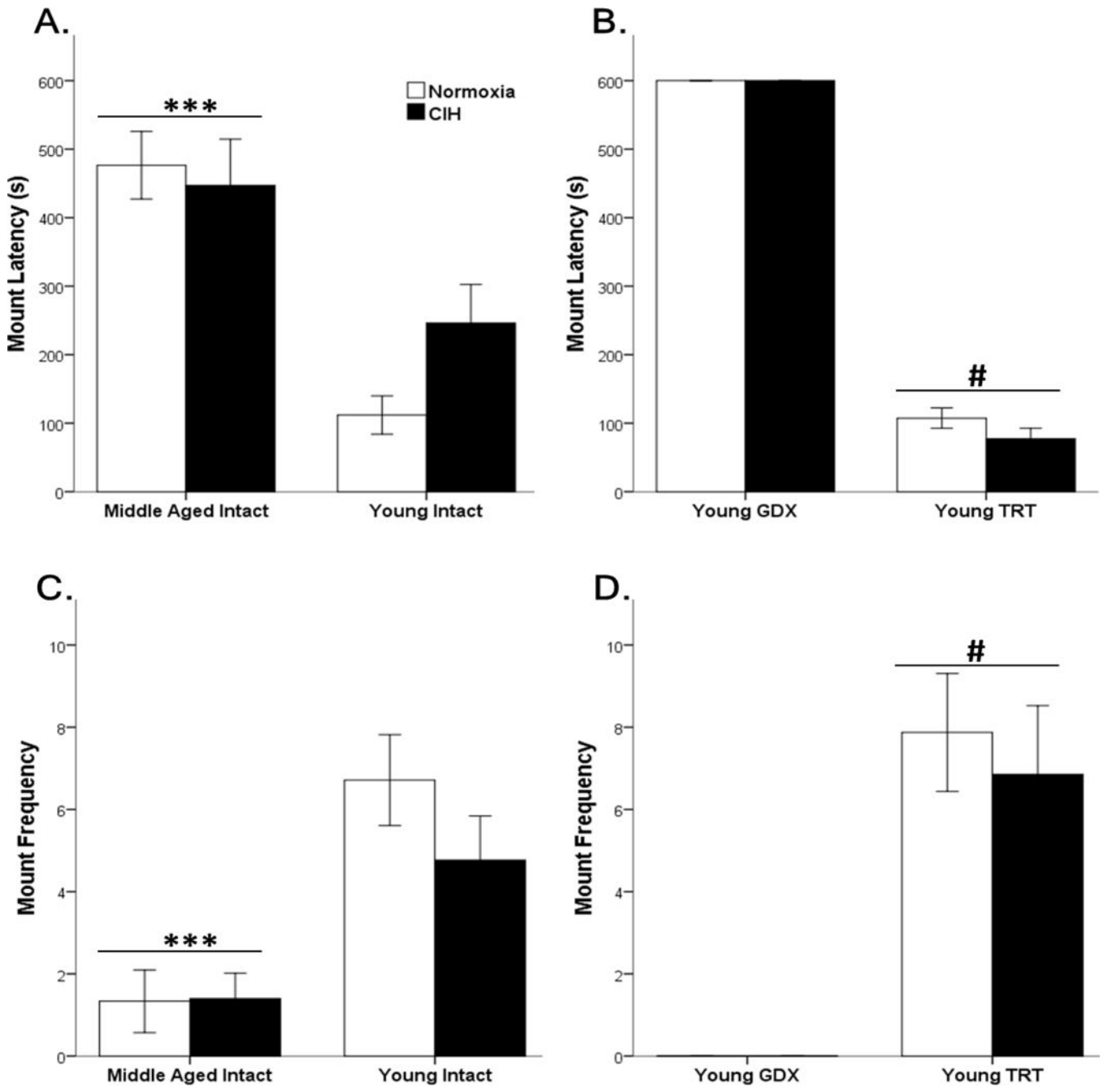

Figure 3. Age impaired appetitive mounting behaviors

Middle-aged rats had significantly longer mount latency (A) and lower mount frequency (C) than young intact male rats. GDX rats did not exhibit mounting sexual behaviors, whereas testosterone replacement in GDX rats decreased mount latency (B) and increased mount frequency (D). No effects of CIH were observed. Fisher's LSD *post hoc* analyses: *** $p \leq 0.05$ vs. young intact rats; # $p \leq 0.05$ vs. young GDX.

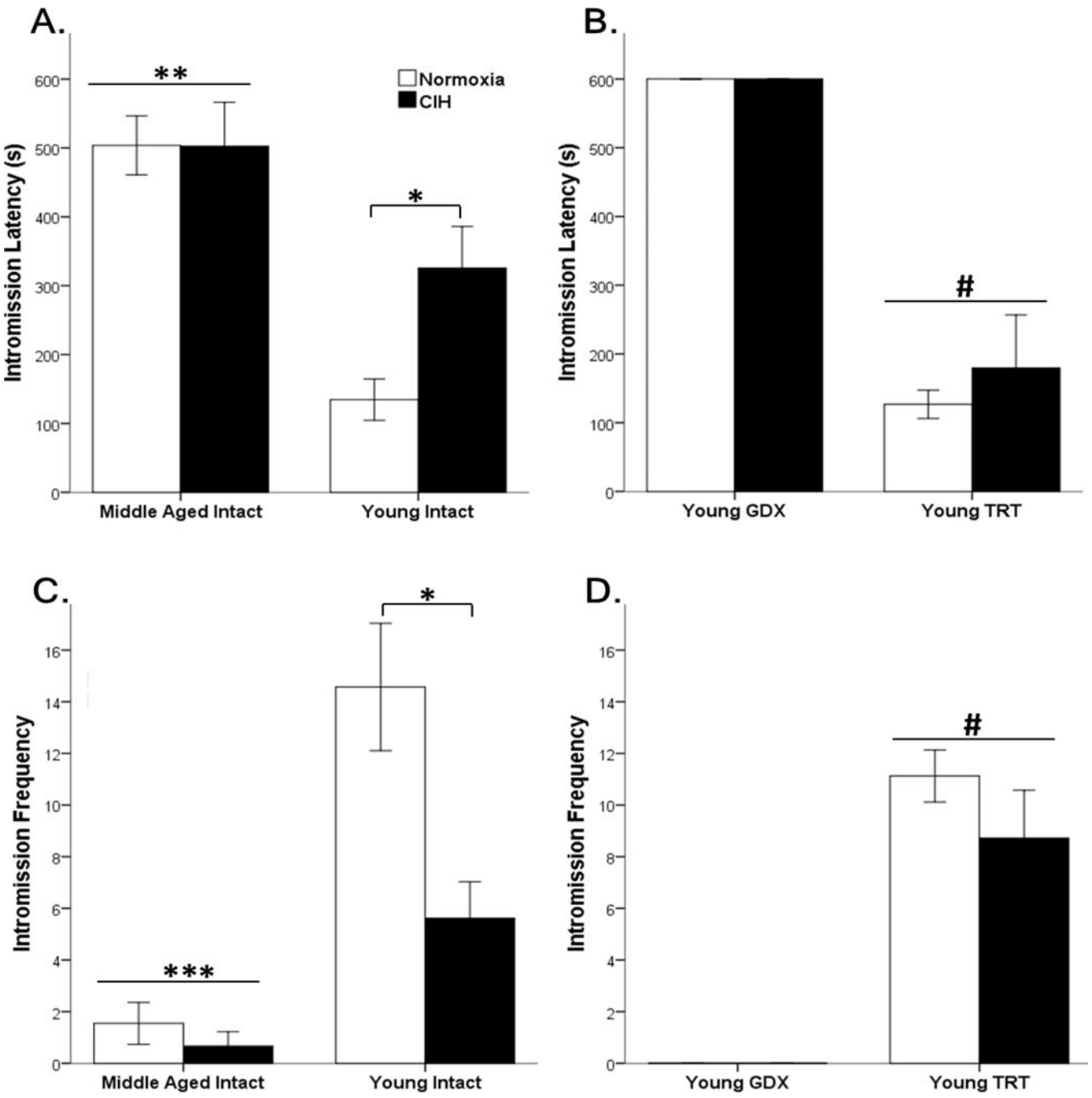

Figure 4. CIH and age impaired consummatory intromission behaviors

Middle-aged rats had significantly longer intromission latency (A) and fewer intromissions (C) than young intact male rats. CIH did not alter behavior in middle-aged rats. In young intact male rats, CIH increased intromission latencies and decreased the frequencies of intromissions. GDX rats did not exhibit consummatory sexual behaviors, whereas testosterone replacement in GDX rats decreased intromission latency (B) and increased intromission frequency (D). Fisher's LSD *post hoc* analyses: * $p \leq 0.05$ normoxia vs. CIH;

** $p \leq 0.05$ vs. young normoxia; *** $p \leq 0.05$ vs. young intact rats; # $p \leq 0.05$ vs. young GDX.

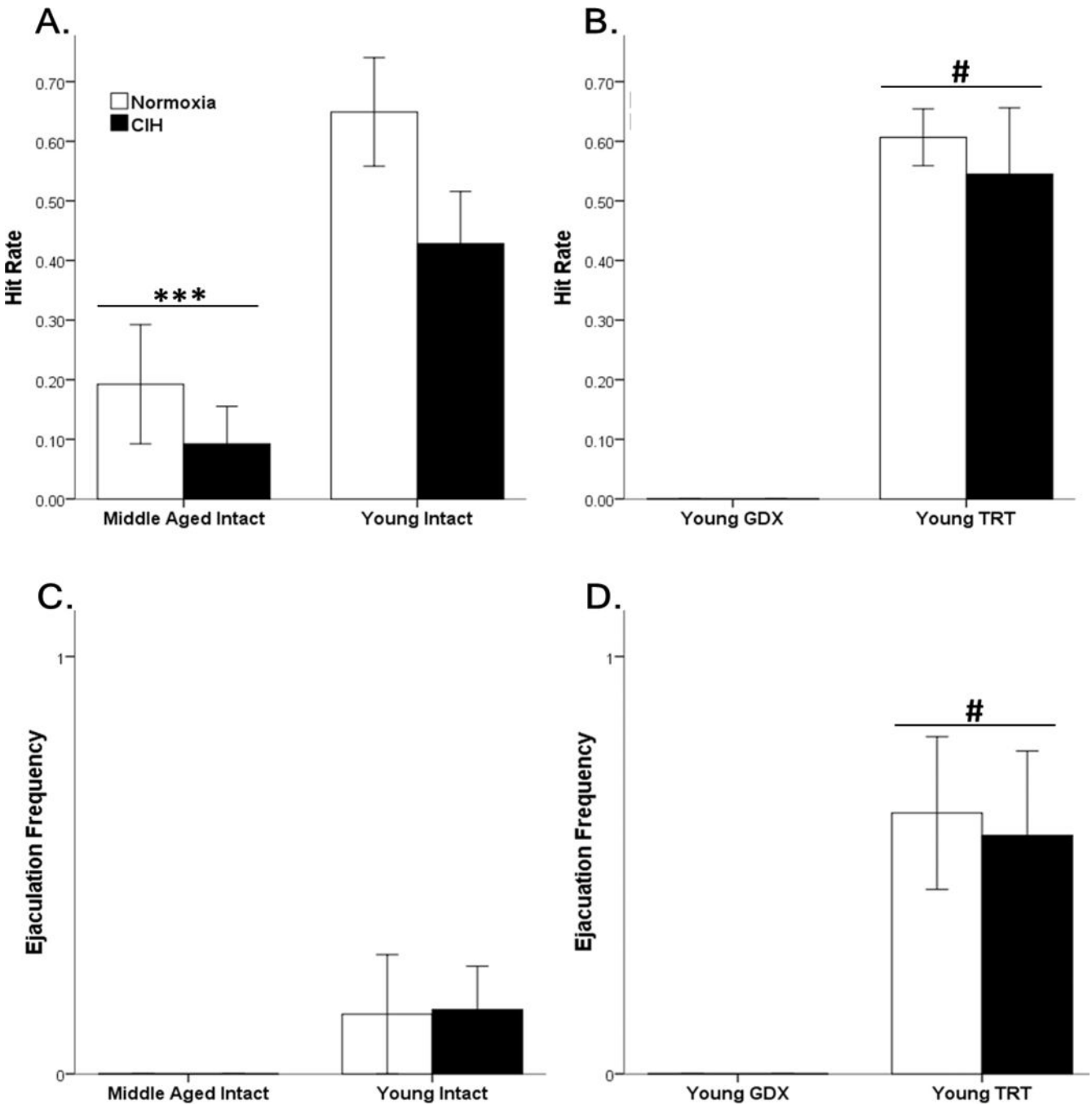

Figure 5. Age impaired consummatory efficiency and ejaculations

Age decreased hit rate compared to young intact males (A). GDX rats did not exhibit sexual behaviors, whereas testosterone replacement in GDX rats increased the hit rate (B). Middle-aged rats did not ejaculate during the testing period (C). Testosterone replacement in GDX rats increased ejaculation frequency (D). Fisher's LSD *post hoc* analyses: *** $p \leq 0.05$ vs. young intact males; # $p \leq 0.05$ vs. young GDX.

Table 1

Circulating gonadotropin levels (ng/mL) in gonadally intact male rats.

Group	LH	FSH
Normoxia		
Middle-aged Intact	52.25 ± 3.79	16.14 ± 3.56
Young Intact	48.96 ± 1.50	18.05 ± 4.98
CIH		
Middle-aged Intact	49.49 ± 4.22	19.09 ± 5.41
Young Intact	49.41 ± 3.58	20.79 ± 8.27